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Authors

Kesner, Raymond P
Xu, Hui
Sommer, Taylor
et al.

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The Role of Postnatal Neurogenesis in Supporting Remote Memory and Spatial Metric Processing

Raymond P. Kesner,^{1*} Xu Hui,¹ Taylor Sommer,¹ Casey Wright,¹ Vanessa R. Barrera,² and Michael S. Fanselow²

ABSTRACT: In this study, we determined the contribution of juvenile neurogenesis to the performance of mice on a remote memory for temporally based association task and in a novelty based spatial pattern separation task. This was accomplished by mating homozygous DNMT1-loxP mice with heterozygous GFAP-Cre mice and comparing Cre+ (no postnatal neurogenesis) to Cre− (wild type) littermate offspring. The results indicate that Cre+ mice are impaired relative to Cre− mice in the remote memory for a temporal based association task and in a novelty based spatial pattern separation task. These results support the temporal integration model of Aimone et al., [(2006) *Nat Neurosci* 9:723–727] and provide further support for an important role for postnatally born neurons in spatial pattern separation. In contrast, Cre+ mice are not impaired relative to Cre− mice in an object-context recognition task and a spatial location recognition task. These latter data suggest that postnatally derived neurons in the dentate gyrus (DG) do not support all spatial and object recognition functions of the DG. © 2014 Wiley Periodicals, Inc.

KEY WORDS: pattern separation; object recognition; spatial recognition; DNA methyltransferase; 1-c knockout mice; cued recall task

INTRODUCTION

It has been suggested that postnatal neurogenesis within the dentate gyrus (DG) plays an important role in mediating cued recall based remote memory (Aimone et al., 2006), spatial pattern separation (Clelland et al., 2009; Tronel et al., 2012), object recognition when contextual cues are available (Dees and Kesner, 2013), and place recognition (Goodman et al., 2010). Aimone et al. (2006) have suggested that one of the cognitive functions for neurogenesis within the DG derives from the continual influx of newly formed granular cells into existing hippocampal circuitry which gives rise to a continually changing population of hyperexcitable immature granule cells. Specifically, they proposed that young granule cells mediate a temporal integration process that operates to form associations among temporally contiguous events. In other

words, events that occur close in time may be encoded by a similar set of young granule cells, whereas events that occur farther apart in time may be encoded and represented by different cell populations allowing for the formation and separation of distinct memory representations (Deng et al., 2010). To test the Aimone model, Morris et al. (2013) have developed a behavioral model demonstrating that in a cued recall task, control but not DG lesioned rats can remember an association between two spatial locations within a 3 min time frame 7 days after exposure.

Because the DG lesions impair granule cells and indirectly impair neurogenesis, it was not possible to be certain whether or not postnatal neurogenesis contributed to the underlying etiology for the observed remote memory deficit. Thus, the first aim of this study was to examine DNA methyltransferase 1-c knockout mice (DNMT1-cKO) with a marked reduction in all postnatal juvenile neurogenesis (Cushman et al., 2012) on the cued recall remote memory task. The DG is characterized by a period of pronounced neurogenesis starting 2 weeks after birth until ~2 months of age (Cushman et al., 2012). During this period of juvenile neurogenesis, there is a significant increase in the number of granule cells in the dentate. After this period, new cells are continually added but at a slow rate without appreciable change in the volume of the dentate. Therefore, it is important to recognize that the dentate contains three developmentally diverse populations of granule cells: mature prenatally born, mature postnatally born, and immature postnatally born. Each of these three populations appears to differentially contribute to behavior (Cushman et al., 2012). The DNMT1-cKO is a developmental model that specifically targets postnatally derived cells because of the delayed onset of GFAP expression. Therefore, both the mature and immature postnatally derived neurons are absent in this developmental model of juvenile neurogenesis (Cushman et al., 2012).

With respect to spatial pattern separation, it has been shown that rats with dDG lesions display deficits (Gilbert et al., 2001; Goodrich-Hunsaker et al., 2008). Based on the observation that neurogenesis occurs in the DG and that new DG granule cells can be formed across time, it has been proposed that the DG contributes to a spatial pattern separation mechanism (Aimone, et al., 2006). Thus far, it has been shown in

¹ Department of Psychology, University of Utah, Salt Lake City, Utah;
² Departments of Psychology and Psychiatry, University of California Los Angeles, Los Angeles, California.

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*Correspondence to: Raymond P. Kesner, Department of Psychology, University of Utah, 380 South 1530 East, Rm 502, Salt Lake City, UT 84112, USA. E-mail: ray.kesner@psych.utah.edu

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FIGURE 1. Pictures of the objects used in the five experiments. For the cued recall remote memory task (1) see Picture 1, for the cued recall remote memory task (2) see Picture 2, for the metric spatial processing task see Pictures 3 and 4, for the object recognition task see Pictures 5, 6, and 7, and for the place recognition task see pictures 8 and 9. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mice that disruption of neurogenesis resulted in spatial pattern separation deficits (Clelland et al., 2009; Tronel et al., 2012). These data suggest that postnatally born neurons in the DG may contribute to the operation of spatial pattern separation. Thus, the second aim of this study was to examine DNMT1-cKO mice (Cushman et al., 2012) on a spatial pattern separation task.

Previous research has shown that rats with lesions of the DG are impaired in object recognition (Deese and Kesner, 2013), and place recognition (Lee et al., 2005). Based on the above mentioned evidence, the third and fourth aim of this study was to examine DNA methyltransferase 1-c knockout mice (Cushman et al., 2012) in a clear box to examine object recognition and place recognition.

MATERIALS AND METHODS

Subjects

Twenty-three male mice were bred at the University of California, Los Angeles and shipped to University of Utah after weaning. They were kept on a 12-h light/dark cycle and experimentation was conducted during the light portion of the light/dark cycle. The animals also had unlimited access to food and water. Three to four mice were housed in plastic cages. All animal care and experimental testing procedures conformed to IACUC standards and protocols.

Juvenile neurogenesis was eliminated by generating a conditional knockout of the DNA methyltransferase type 1 (DNMT1) gene in mGFAP expressing cells as previously

described (DNMT1-cKO see Cushman et al., 2012). Loss of DNMT1 is lethal to actively dividing cells but this deletion is restricted to GFAP-positive cells. Since adult neuroprogenitors express GFAP, adult neurogenesis is prevented (Garcia et al., 2004). mGFAP-Cre mice from line 73.12 (Garcia et al., 2004) were crossed with floxed DNMT1 mice (Cushman et al., 2012) and backcrossed to C57Bl6 for greater than 10 generations. Experimental mice were the offspring of female DNMT1-loxP/mGFAP-Cre⁻ negative and male DNMT1-loxP/mGFAP-Cre⁺ positive mice. Thus, we had DNMT1-cKO (Cre⁺) and littermate controls (Cre⁻). A complete characterization of these mice can be found in Cushman et al. (2012). In brief, sensory motor function (SHIRPA test battery) is normal, and there is no impairment on simple cued or contextual fear conditioning tasks. Gross brain and body weight are normal, as is cytoarchitecture. The number of granule cells in DG and olfactory bulb are normal up to 2 weeks of age, but no new granule cells are added to these structures after that point. Thus, the manipulation spares prenatal neurogenesis but abolishes all postnatal neurogenesis in these structures. Thus by 3 months of age, the total number of granule cells in DG and olfactory bulb is 75% and 55% of control, respectively. There is no detectable change in the number of astrocytes. There were 10 Cre⁺ mice and 13 Cre⁻ mice at 34–86 days of age at the beginning of the experiment. All mice were tested on each of the tasks.

Apparatus

The apparatus for these experiments consisted of one large clear Plexiglas box 40 × 40 cm² with clear walls and a dark grey floor. The box was placed on a square white table 60 ×

Cued Recall for Spatial Location

FIGURE 2. Schematic of the cued recall remote memory task (2). Presentation of A and B on Day 1 and C and D on Day 2 with a 30 s ISI and for the test 2 or 3 days later a 2 min cue exposure was followed 10 s later by the test.

60 cm²) and totally surrounded by a black curtain. Four distinct 2D black and white cues 12 × 12 cm² were placed 12 cm away from each side of the box. Objects were made from various washable, nonporous materials (plastic, metal, glass, etc.), 2–7 cm in height and had various color and pattern schemes to ensure each object was visually distinct (See Fig. 1). To prevent odor cues, all apparatus and objects were disinfected and deodorized with HDQ (a sanitizing agent) after each use.

Behavioral Procedures

Experiments consisted of one task that utilized a cued recall based remote memory procedure (Morris et al., 2013), and three exploratory paradigms based on the inherent tendency of rodents to differentially explore novel stimuli over familiar stimuli including spatial pattern separation as indicated by metric distance recognition (Goodrich-Hunsaker et al., 2008), object recognition (Dees and Kesner, 2013; Spanswick and Sutherland, 2010), and spatial recognition (Lee et al. 2005). The experimental paradigms used for each task are shown in Figures 2–4.

Exploration was determined as any investigative behavior where mice had active and direct contact with an object. Such behaviors included head orientation and sniffing within <1.0 cm of the object, pawing the objects, biting the objects,

and even crawling over the objects. Exploration was recorded with an overhead video camera, and the duration of exploration was measured with a stopwatch. The week before testing, all subjects received daily handling sessions for 7 days. The first few days, the mice were touched in their home cage. The next 5 days, the mice were picked up by the tail placed on a table and stroked for 1 min and on Day 8 the mice were given an opportunity to habituate to the apparatus. Each experimental session presented the subject with new object sets, and tests were separated by a minimum 48-h interval. All four testing paradigms were conducted in the apparatus (see Figs. 2–5). The order of the presentation of these experiments is as listed above, since order effects are not normally observed in exploration tasks. A cued recall procedure is used for the remote memory task. In the metric, object recognition, and place recognition test paradigms, there is a study phase and a test phase based on novelty detection that is interpreted to reflect recognition memory.

Cued Recall Remote Memory

In this study, DNMT1-cKO mice (Cushman et al., 2012) were tested on a cued recall remote memory task. Before testing, each animal was allowed to individually explore the test apparatus for 5 min. No objects were present during the

habituation phase. The parameters that were selected were based on a previous study with rats (Morris et al., 2013). Testing began the following day. The same object was used, but the location of the objects was varied. The task consisted of a study phase and a test phase. A pictorial representation of the procedure is shown in Figure 1. The study phase was conducted across 2 consecutive days (Day 1 and Day 2) and consisted of two 5 min exploration sessions separated by a 3 min intersession interval (ISI) per day. On Day 1, the animal was placed in the box and allowed to explore the object positioned at spatial location A for 5 min. Following the exploration period, a black container was placed over the mouse for the 3 min ISI. After this interval, the black container was removed from the box the animal was placed in the test chamber, and allowed to explore the object positioned at spatial location B for 5 min. The same procedure was used on Day 2 of the study phase; however, the object was positioned at spatial location C and D, respectively. The test phase was conducted 7 days after the first study phase (Day 1) and was conducted across two consecutive days (Day 8 and Day 9). On the first day of the test phase (Day 8), mice were placed in the box and allowed to explore an object positioned at spatial location A or C. A and C were used as cues for 1 min followed by a 3 min ISI. After this interval, animals were given a 5-min preference test between the same objects in spatial location B and D (positioned 106 cm apart). Even though the configuration changed during the recall test, the locations of the objects were not different from the locations experienced during the study phase (see Fig. 2). The same procedure was used on the second day of the test phase (Day 9). The presentation order of spatial location A and C was counterbalanced across subjects and across days. The start location was held constant across all sessions and phases. To account for individual activity levels of each mouse, a preference ratio for time spent exploring objects positioned in spatial location B and D was calculated for each animal. Exploration was defined as active and direct contact with an object such as sniffing and pawing the objects. When cued with A, the preference ratio was $(B-D)/(B+D)$. When cued with C, the preference ratio was $(D-B)/(D+B)$. Positive preference ratio scores (above zero) indicated a preference for the paired temporal associate (B when cued with A; D when cued with C). Negative preference ratio scores (below zero) indicated a preference for the spatial location that was not previously paired with the cue (D when cued with A; B when cued with C). A score of zero indicated no preference for B or D.

The results indicated that both groups had a discrimination ratio of 0.09. Because the Cre⁻ and Cre⁺ mice could not learn the task using the parameters that resulted in good remote memory in rats, the parameters were changed to enhance the association process between A and B on Day 1 and C and D on Day 2 with a 30 s ISI and for the test 2 or 3 days later with a 2 min cue exposure that was followed 10 s later by the test. The reason for reducing the time of exposure to cue A or Cue C is that the mice explore the object location mostly during the first 2 min, and thus, the 2 min was selected because it represented the optimal time for cue presentation.

FIGURE 3. Schematic of the metric spatial processing task. Delay 2 min between study and test phase.

We did not use the same object (see Fig. 1) and because the performance was not stellar with no differences between Cre⁺ and Cre⁻ mice, we made drastic changes in the time parameters. These changes suggest that the first test had little if any influence on the second test.

Metric Spatial Processing

In this study, DNMT1-cKO mice (Cushman et al., 2012) were tested on a spatial pattern separation task. Mice were placed in center of the box and presented with two different objects 25 cm apart for 15 min of free exploration of the apparatus, stimulus objects, and distal environmental cues (study phase). A black container was placed over the mouse for 2 min (Delay) followed after removal of the black container by a 5 min Test Phase during which identical copies of the same objects were moved closer to each other so that the objects were 8 cm apart. A pictorial representation of the procedure is shown in Figure 3. Re-exploration is based on an index of detecting a distance (metric) change. A Discrimination Ratio was calculated: $[\text{Test Phase exploration of both objects (A)} - \text{last 5 min of study phase exploration of both objects (B)}] / \text{Total Exploration (A+B)}$.

FIGURE 4. Schematic of the object recognition task. Delay 2 min between study and test phase.

FIGURE 5. Schematic of the place recognition task. Delay 2 min between study and test phase.

Object Recognition

In this study, DNMT1-cKO mice (Cushman et al., 2012) were tested in the same box for object recognition. Mice were placed in center of the box and presented with two different objects 15 cm apart for 15 min of free exploration of the box (study phase). A black container was placed over the mouse for 2 min (Delay) followed by a 5 min Test Phase during which one object was replaced with a new object unfamiliar to the mouse to measure object recognition (novel) and the other object was exchanged with an identical copy (familiar). The container was removed, and the mouse was allowed to re-explore for 5 min (Test Phase). A pictorial representation of the procedure is shown in Figure 4. To take into account activity level of each mouse for the recall of recognition information, the following Discrimination Ratio was calculated: $[\text{Exploration of Novel (A)} - \text{Exploration of Familiar (B)}] / \text{Total Exploration (A+B)}$.

Place Recognition

In this study, DNMT1-cKO mice (Cushman et al., 2012) were tested in a place recognition task. The Study Phase is the

same as in the Object Recognition task, but following a 2 min delay in the Test Phase one of the objects was located in a different position with a 15 cm distance to measure place recognition (novel) and the other object was exchanged with an identical copy (familiar). A pictorial representation of the procedure is shown in Figure 5. The Discrimination Ratio was calculated with the same equation used in the Object Recognition task. The time frame for the order of the different experiments was as follows: habituation (Days 1–7), cued recall remote memory 1 (Days 8–17), cued recall remote memory 2 (19–24), metric spatial processing (Day 26), object recognition (Day 28), and place recognition (Day 30). There were 2 days between experiments.

Histology

To verify the absence of postnatally born granule cells, BrdU was injected at the end of behavioral testing. The mice were between 98 and 150 days of age. The BrdU parameters were based on Stone et al. (2011) who used 50 mg/kg per injection rather than 200 mg/kg per injection. The mice received 10 injections of BrdU (50 mg/kg) two times per day separated by 8 h for 5 consecutive days. The total amount of BrdU injection was slightly higher than in the (Cushman et al., 2012) paper. Two weeks after BrdU injections, the mice were anesthetized and transcardially perfused using 0.1M phosphate buffered saline for 5 min. This was followed by 4% paraformaldehyde for 7 min. The head was removed and brain extracted. Perfused brains were post-fixed with 4% paraformaldehyde in 0.1M phosphate buffered saline for 24 h at 4°C. They were then cryoprotected in 10% sucrose in 0.1M phosphate buffered saline overnight and stored at 4°C. The same procedure was conducted for two additional days using 20% and 30% sucrose in 0.1M phosphate buffered saline. The brains were then flash frozen using powdered dry ice and stored at –80°C until ready for sectioning. Frozen brains were cut into 40-μm coronal sections, serially collected, and placed in 0.1M phosphate buffered saline at 4°C. Antigen retrieval for Bromodeoxyuridine was conducted on sections and counterstained with a DAB substrate kit (Vector Laboratories). Primary antibody used for immunohistochemistry was sheep anti-BrdU (1:450, Maine Biotechnology services) and rabbit anti-sheep (1:400, Vector Laboratories) as secondary.

FIGURE 6. Mean discrimination ratio and ($\pm$ SE) for Cre– and Cre+ mice in the cued recall for spatial location task.

RESULTS

Cued Recall Remote Memory

The results of the remote memory processing test are shown in Figure 6 and indicate that the Cre+ mice display an impairment in remote memory, whereas the Cre– mice show intact remote memory. A one way ANOVA with groups (Cre– and Cre+) as the between factor revealed a significant group effect ($F = 5.5$, df 1, 22, $P = 0.029$). The results imply that the

FIGURE 7. Mean discrimination ratio and ($\pm$ SE) for Cre- and Cre+ mice in the metric spatial processing task.

Cre+ mice are clearly impaired in displaying remote memory. Based on 10 min of total exploration of the two objects on Day 1 for the Cre- mice, the mean level of exploration was 23.0 s with a standard error (SE) of 4.1 s, and for the Cre+ mice, the mean level of exploration was 16.9 s with an SE of 4.3 s. Based on 10 min of total exploration of the two objects on Day 2 for the Cre- mice, the mean level of exploration was 30.0 s with an SE of 4.9 s, and for the Cre+ mice, the mean level of exploration was 19.8 s with an SE of 3.6 s. The difference between the Cre- and Cre+ mice was not significant. Even though the Cre- mice were a bit more active than the Cre+ mice, the differences were not significant. Cue mean exploration for Day 4 Cre- mice was 2.7 s and for Cre+ mice was 2.5 s; for Day 5 Cre- mice was 2.3 s and Cre+ mice 2.7 s. Preference for the previous association recall cue mean exploration for Day 4 Cre- mice was 10.43 s and for

the nonassociation recall cue mean exploration for Day 4 Cre- mice was 7.4 s; preference for the previous association recall cue mean exploration for Day 5 Cre+ mice was 7.40 s and for the nonassociation recall cue mean exploration for Day 5 Cre+ mice was 6.65 s.

Metric Spatial Processing

The results of the metric processing tests are shown in Figure 7 and indicate that the Cre- mice show good place (metric based) recognition memory, whereas the Cre+ mice showed impaired place (metric based) recognition memory. A one way ANOVA with groups (Cre- and Cre+) as the between factor revealed a significant group effect ($F = 10.01$, $df 1, 21$, $P = 0.0047$). A t -test was used to determine whether the Cre+ mice were significantly below zero, suggesting a potential problem in detecting the metric change. The t -test revealed a significant effect with $df = 9$, $t = 2.3$, $P < 0.025$, one tailed test. Also, the Cre- mice were significant above zero with a $df = 12$, $t = 2.19$, $P < 0.025$ one tailed test, suggesting the detection of the metric change. The results imply that the Cre+ mice are clearly impaired when the distance metric for spatial location is important and the Cre- mice clearly detected the metric change. Based on the first 5 min total exploration during the study phase, for the Cre- mice the mean level of exploration was 19.1 s with an SE of 5.4 s and for the Cre+ mice the mean level of exploration was 24.7 s with an SE of 5.7 s. The difference between Cre- and Cre+ mice was not significant. Mean cue exploration for the second study phase Cre- mice was 18.9 s and for Cre+ mice was 19.8 s; for the test phase Cre- mice was 19.8 s and Cre+ mice was 15.5 s.

Object Recognition

The results for the object recognition tests are shown in Figure 8 and indicate that the Cre- and Cre+ mice show excellent object recognition memory. A one way ANOVA with groups (Cre- and Cre+ mice) as the between factor revealed a nonsignificant group effect ($F = 1.98$, $df 1, 22$, $P = 0.175$). The results suggest that juvenile neurogenesis does not play a role in object recognition. Based on 15 min of total exploration during the study phase, for the Cre- mice the mean level of exploration was 38.9 s with an SE of 7.6 s and for the Cre+ mice the mean level of exploration was 54.8 s with an SE of 11.1 s. Even though the Cre+ mice were a bit more active than the Cre- mice, the difference was not significant. Mean cue exploration for the Cre- mice was 3.0 s for the familiar object and for the Cre+ mice was 3.1 s; for the novel object Cre- mice was 11.0 s and Cre+ was 8.1 s.

FIGURE 8. Mean discrimination ratio and ($\pm$ SE) for Cre- and Cre+ mice in the object recognition task.

Place Recognition

The results for the place recognition tests are shown in Figure 9 and indicate that the Cre- and Cre+ show good place recognition memory. A one way ANOVA with groups (Cre- and Cre+) revealed a nonsignificant group effect ($F = 0.001$, $df 1, 22$, $P = 0.975$). The results suggest that juvenile neurogenesis does not play a role in object recognition. Based on 15 min of

FIGURE 9. Mean discrimination ratio and ($\pm$ SE) for Cre $-$ and Cre $+$ mice in the place recognition task.

total exploration during the study phase, for the Cre $-$ mice the mean level of exploration was 27.9 s with an SE of 6.4 s and for the Cre $+$ mice the mean level of exploration was 27.7 s with an SE of 5.6 s. The difference between Cre $-$ and Cre $+$ mice was not significant. Mean cue exploration for the familiar location for the Cre $-$ mice was 7.9 s and for the novel location for Cre $-$ mice was 11.5 s; for the familiar object for the Cre $+$ mice was 9 s and for the Cre $+$ mice was 13.5 s.

Histological Results

Figure 10 shows the presence of BRDU labeled cells of representative Cre $+$ and Cre $-$ mice. Although BRDU positive cells were clearly apparent in the DG of all Cre $-$ mice, they were totally absent in Cre $+$ animals. The complete absence of BRDU labeling in the Cre $+$ mice is entirely consistent with earlier characterization of this mutant using unbiased stereology (Cushman et al., 2012).

FIGURE 10. BRDU labeling of newly generated granule cells. The top two panels (A) show an absence of labeling in representative sections taken from two different Cre $+$ mice. The lower two panels (B) are representative sections taken from two different Cre $-$ mice. BrdU-labeled cells are indicated by arrows. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

This study sought to determine whether postnatally born granule cells within DG mediate the formation of temporal associations for proximal spatial events using a cued-recall of sequence paradigm for different spatial locations. In this task, mice were allowed to explore identical objects placed in designated spatial locations across 2 days (e.g., Day 1: A and B; Day 2: C and D) with a 30 s ISI. Two days later, animals were given a brief cue (A or C) followed by a preference test between spatial location B and D. The data revealed that during the preference test, control mice (Cre $-$) showed a significant preference for the spatial location previously paired with the cue (a within day over a between day preference) suggesting that control mice (Cre $-$) formed a stronger temporal association for proximal rather than distal spatial events. In comparison to controls, mice with juvenile neurogenesis knock-out (Cre $+$) did not show a preference for either spatial location during the preference test. This finding suggests that postnatally generated granule cells are necessary for the formation of temporal associations between spatial events presented closer in time. The data are consistent with the Morris et al. (2013), finding of a similar cued recall disruption, but in that case with DG lesions that do not discriminate between pre and postnatally derived neurons in rats and the use of somewhat different parameters.

Previous research suggests that rodents display a natural tendency to detect changes in the environment as evidenced by increased exploration for novel topological and metric changes compared to configurations that were previously encountered. Although this finding is somewhat unexpected based on results from standard preference tasks, the task used in this study is not a typical preference task; rather, it is a cued-recall task with a preference test portion. Based on this distinction, it is possible that a cued-recall based pattern completion process can account for the observed preference for the cued location in control animals. The major question is how to explain the preference pattern observed in the present experiment and the role of juvenile neurogenesis in disrupting this preference function. It has been suggested that temporal remote memories are initially processed in the DG and likely via DG-based neurogenesis. The information is then stored in CA3 (Aimone et al., 2010) and/or based on Kesner's (2013) view stored in the neocortex with the likelihood that CA3 will receive stored neocortical input from the entorhinal cortex via the perforant path. The CA3 is known to mediate pattern completion (Nakazawa et al., 2002; Gold and Kesner, 2005; Hunsaker and Kesner, 2013). In the present spatial cued recall paradigm, the animal could use a spatial cued based pattern completion process to generate the observed preference for the temporally paired spatial location, i.e., when initially location A is temporally paired with location B, then because of spatial pattern completion there is likely to be a preference for B rather than D. Similarly, when initially location C is temporally paired with location D, then because of spatial pattern completion there is likely to be a

preference for D rather than B. In rats this pattern completion preference is even evident when the choice is between the cued recall spatial location and a novel spatial location (Morris et al., 2013). Thus, a spatial pattern completion process can account for the preference for the cued location in control rats. Because DG lesions can disrupt the storage process of spatial information into CA3 or neocortex (Dees and Kesner, 2013), the spatial pattern completion process is disrupted resulting in a lack of preference in this paradigm.

Thus, our cued-recall findings provide support for the temporal integration theory proposed by Aimone and coworkers (Aimone et al., 2006; Deng et al., 2010). The temporal integration theory is largely based on computational evidence that indicates that newly generated granule cells, at different stages of development, may differentially contribute to hippocampal dependent learning and memory by forming associations among temporally proximal events (Aimone et al., 2010; Deng et al., 2010). Specifically, Aimone et al. (2010) suggest that young granule cells may support a pattern integration process such that temporally proximal events are encoded by a similar set of new cells and different cell populations represent temporally distal events. Because all postnatal neurogenesis is eliminated in the current animal model, there would be no unique cohort of cells that had a fixed temporal relationship with any environmental experience. Therefore, temporal integration theory predicts that the current genetic manipulation would cause a major impairment in tests requiring a temporal linkage between stimuli as was observed in the cued recall task.

The results of the present experiments show that the Cre+ mice are impaired in spatial pattern separation as measured by the failure to detect changes in spatial locations using an exploratory behavior paradigm. This finding is similar to the Goodrich-Hunsaker et al. (2008) study with dorsal DG lesions in rats. The results of an impairment in spatial pattern separation are also consistent with previous research using different models to inhibit neurogenesis (Clelland et al., 2009). Thus, it appears that postnatally born DG granule cells influence a spatial pattern separation process.

The results of the present experiment indicate that there are no deficits in object recognition for Cre+ mice relative to Cre- mice. There are other studies with no deficits in object recognition with short-term delays in mice and rats lacking adult-born neurons (Goodman et al., 2010; Jessberger et al., 2009). However, Wei et al. (2011) showed that both juvenile and adult mice were impaired on an object recognition test, which differs from the observations made in the present study. It should be noted that in the present study a 2 min delay between the study and test phases was used, whereas Wei et al. (2011) had a 24 h delay between the study and test phases. A 2-min delay interval is usually interpreted as reflecting short-term or working memory, whereas a 24-h delay interval is interpreted as reflecting long-term memory. As a result, one cannot compare results of the present and Wei et al. (2011) study. However, in the TSd5Dn/Dnj mice with excessive inhibition in the DG, we were able to show that in an object recognition task there were no deficits at 5 min delay, but a

deficit at 24 h. (Smith et al., 2014). Also in the Deese and Kesner (2013) study, DG lesions in rats do not disrupt object recognition at a 10 min delay, but there is a deficit at 24 h. These data imply that the present data are consistent with a lack of impairment with short time intervals.

In a second study, Martinez-Canabal et al. (2013) showed that following injections of temozolomide, an antiproliferation agent, juvenile, and adult neurogenesis mice acquired a water maze spatial navigation task as readily as vehicle treated mice, but on subsequent recall probes there were problems only for the juvenile neurogenesis, but not adult neurogenesis or vehicle treated mice they were less precise in searching for the platform, displayed a decrease in the number of platform crosses, and they spend less time in a circular zone centered on the platform location. This study suggests that there is a problem in recall for remembering a spatial location in the water maze. We did find a deficit in the present cue-recall remote experiment, but it is very difficult to compare the data of the Martinez-Canabal et al. (2013) and the present experiment.

The results of the present experiment also indicate that there are no deficits in spatial location recognition in Cre+ mice relative to Cre- mice. These data are not consistent with observation of spatial location recognition deficits in rats with DG lesions (Lee et al., 2005). There are no available data with short-term tests of spatial location recognition in mice with a loss of postnatal neurogenesis. It is clear that damage to juvenile neurogenesis does not always produce deficits that can be observed with DG lesions, suggesting that prenatal born granule cells support object context recognition. This is consistent with other literature, for a more detailed analysis of this issue, see Frankland (2013). It is important to note that there are no significant differences between the Cre+ and Cre- mice in any of the four tasks in terms of the overall exploratory behavior during the study phase. Also, comparing the Cre+ vs. the Cre- mice the pattern of results suggest that sensory and motivational factors cannot easily account for a deficit pattern in the cued recall memory and metric spatial processing tasks compared to intact processing of information in the object and place recognition tasks. It should be noted that according to Cushman (2012) because DNMT1-cKO mice do not undergo the normal period of juvenile neurogenesis that occurs from 2 weeks to 60 days after birth. Therefore, as adults they have 25% fewer granule cells in the dentate. Thus, there is a possibility that the deficits observed in the present study may be due to a loss of creating new granule cells that contribute to dentate development. Despite this, the Cre+ mice displayed intact place recognition, which assesses overall dentate function (Lee et al., 2005).

In summary, the present results indicate that mice with a complete deletion of all postnatal neurogenesis in the DG are impaired relative to wild type littermates, who display intact neurogenesis in a remote memory for temporal based associations task and in a novelty based spatial pattern separation task. These results support the temporal integration model of Aimone et al., 2006 and provide further support for an important role for postnatal neurogenesis in spatial pattern separation. In contrast, Cre+ mice are not impaired relative to

Cre— mice in an object-context recognition task and a spatial location recognition task. These latter data are consistent with the suggestion that DG granule cells from different stages of development support different spatial and contextual processing functions of the DG.

REFERENCES

- Aimone JB, Wiles J, Gage FH. 2006. Potential role for adult neurogenesis in the encoding of time in new memories. *Nat Neurosci* 9:723–727.
- Aimone JB, Deng W, Gage FH. 2010. Adult neurogenesis: Integrating theories and separating functions. *Trends Cogn Sci* 14:325–337.
- Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, Salsida LM, Barker RA, Gage F, Bussey TJ. 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325:210–213.
- Cushman JD, Maldonado J, Kwon EE, Garcia AD, Fan G, Imura T, Sofroniew MV, Fanselow MS. 2012. Juvenile neurogenesis makes essential contributions to adult brain structure and plays a sex-dependent role in fear memories. *Front Behav Neurosci* 6:1–17.
- Dees RL, Kesner RP. 2013. The role of the dorsal dentate gyrus in object and object-context recognition. *Neurobiol Learn Mem* 106:112–117.
- Deng W, Aimone JB, Gage FH. 2010. New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci* 11:339–350.
- Frankland PW. 2013. Neurogenic evangelism: Comment on Urbach et al. (2013). *Behav Neurosci* 127:126–129.
- Gilbert PE, Kesner RP, Lee I. 2001. Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus* 11:626–636.
- Garcia AD, Imura T, Busg TG, Sofroniew MV. 2004. GFAP-express progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat Neurosci* 7:1233–1241.
- Gold AE, Kesner RP. 2005. The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat. *Hippocampus* 15:808–814.
- Goodman T, Trouche S, Massou I, Verret L, Zerwas M, Rouillet P, Rampon C. 2010. Young hippocampal neurons are critical for recent and remote spatial memory in adult mice. *Neuroscience* 171:769–778.
- Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP. 2008. The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behav Neurosci* 22:16–26.
- Hunsaker M, Kesner RP. 2013. The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neurosci Biobehav Rev* 37:36–58.
- Jessberger S, Clark RE, Broadbent NJ, Clemenson Jr GD, Consiglio A, Chichung Lie D, Squire LR, Gage FH. 2009. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem* 16:147–154.
- Kesner RP. 2013. A process analysis of the CA3 subregion of the hippocampus. *Front Cell Neurosci*, doi: 10.3389/fncel.0078.
- Lee I, Hunsaker M, Kesner RP. 2005. The role of hippocampal subregions in detecting spatial novelty. *Behav Neurosci* 119:145–153.
- Martinez-Canabal A, Akers KG, Josselyn S, Frankland PW. 2013. Age-dependent effects of hippocampal neurogenesis suppression on spatial learning. *Hippocampus* 23:66–74.
- Morris AM, Curtis BJ, Churchwell JC, Maasberg DW, Kesner RP. 2013. Temporal associations for spatial events: The role of the dentate gyrus. *Behav Brain Res* 256:250–256.
- Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel ME, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, Tonegawa S. 2002. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* 297:211–218.
- Smith GK, Kesner RP, Korenberg JR. 2014. Dentate gyrus mediates cognitive function in the Ts65Dn/DnJ mouse model of down syndrome. *Hippocampus* 24:354–362.
- Spannwick SC, Sutherland RJ. 2010. Object/context-specific memory deficits associated with loss of hippocampal granule cells after adrenalectomy in rats. *Learn Mem* 17:241–245.
- Stone SS, Teixeira CM, Zaslavsky K, Wheeler AL, Martinez-Canabal A, Wang AH, Sakaguchi M, Lozano AM, Frankland PW. 2011. Functional convergence of developmentally and adult-generated granule cells in dentate gyrus circuits supporting hippocampus-dependent memory. *Hippocampus* 21:1348–1362.
- Tronel S, Belnoue L, Grosjean N, Revest JM, Piazza PV, Koehl M, Abrous DN. 2012. Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus* 22:292–298.
- Wei L, Meaney MJ, Duman RS, Kaffman A. 2011. Affiliative behavior requires juvenile, but not adult neurogenesis. *J Neurosci* 31:14335–14345.