UNIVERSITY OF CALIFORNIA, SAN DIEGO

Ablation of adult neurogenesis in the dentate gyrus does not produce disruptions in place learning for adjacent spatial locations

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in

Biology

by

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2014

The thesis of Yu-Ling An is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2014

I dedicate this thesis to Mom, Dad, Steven, and Angie.

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ABSTRACT OF THE THESIS

Ablation of adult neurogenesis in the dentate gyrus does not produce disruptions in place learning for adjacent spatial locations

by

Yu-Ling An

Master of Science in Biology

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Professor Jill K. Leutgeb, Chair

The process of pattern separation is essential for a more accurate encoding of memories so that newly encoded information would not overwrite similar previously stored information. The dentate gyrus (DG) of the mammalian hippocampus, a highly plastic region with adult neurogenesis, has been long suggested to play a critical role in this process. Adult-born granule cell neurons integrate into the pre-existing network throughout life, and are known to exhibit high plasticity. As a result, this population has been proposed to function in pattern separation by expanding the capacity for plasticity in the DG. The primary aim of this study is to test this hypothesis by testing if adult-born neurons in the DG are necessary to perform a spatial discrimination task, which had previously been shown to be dentate dependent. A GFAP-TK transgenic rat model was used, in which neurogenesis could be ablated with the administration of the drug valganciclovir. This was corroborated through doublecortin

immunohistochemistry (98 ± 0.5% reduction in GFAP-TK animals with drug administration in comparison to controls). These animals were compared to two control groups: wildtype animals with drug administration and GFAP-TK animals without drug administration. This study also involved dorsal DG (dDG) lesioned animals to confirm the task was dentate dependent in the conditions under which this study was conducted. Results show similar performance between animals with and without intact NG, suggesting that the ablation of immature neurons from the DG does not produce disruptions in place learning for spatial locations.

I:

Introduction

The process of pattern separation, in which overlapping or similar inputs during learning are orthogonized (Leutgeb, et al., 2007; Colgin, et al., 2008), is essential to allow for more accurate encoding of memories. This reduction of interference between similar inputs allows for rapid learning without catastrophic interference so that newly encoded information would not overwrite similar previously stored information and instead, would be distinctly represented (Norman, et al., 2002). This essential feature of episodic memory has been hypothesized to require the hippocampus (Yassa, et al., 2011).

The hippocampus is comprised of a tri-synaptic pathway involving the dentate granule cells, CA3 pyramidal neurons, and CA1 pyramidal cells. The entorhinal cortex provides the major cortical input into the DG through the perforant pathway (West, et al., 1991). This information is then fed forward to the CA3 by the powerful and sparse mossy fiber projection, originating from the DG (West, et al., 1991). This, combined with the low probability that the same set of CA3 cells will always receive inputs from a similar set of DG granule neurons, suggest a role of this network in pattern separation (Jung and McNaughton, et al., 1993; Rolls and Kesner, et al., 2006).

There have been many studies, both behavioral and physiological, which have explored and validated this statement. The first study, through examining CA1 and CA3 place cell firing patterns in a cue mismatch paradigm using two different environments, found larger changes in CA3 place fields, indicative of pattern separation (Leutgeb, et al., 2004). Another study used the technique of immediate early gene brain imaging to compare the overlap of CA1 and CA3 in response to environmental changes (Vazdarjanova, et al., 2004). When the environmental change

was large, overlap in CA1 was greater than CA3, consistent with the proposed involvement of CA3 in pattern separation.

There are findings that suggest the DG plays a critical role in pattern separation. Through an electrophysiological study, it was found through a morph experiment, where the animal explored shapes that gradually changed from a circle to a square or vice versa, that the firing rates of place cells in the DG show remapping through the task, suggesting that small changes in spatial input will produce highly divergent output (Leutgeb, et al. 2007). Additionally, lesion studies in rats have shown that the DG is required for spatial pattern separation. (Gilbert, et al., 2001; Morris, et al., 2012). These results support the definition of pattern separation and the proposed role of the DG in pattern separation.

The DG is one of the most plastic regions in the mammalian brain, and is one of the two regions that undergo adult neurogenesis (Zhao, et al., 2008; Piatti and Ewell, et al., 2013). These adult-born granule cell neurons integrate into the pre-existing network, and continuously modify the network (Sahay, et al., 2011; Marin-Burgin, et al., 2012) of the adult DG. Due to this integration of adult-born neurons into the existing neural circuit, the adult DG is a heterogeneous structure where neurons of different ages reside (Piatti, et al., 2006).

This population of neurons is known to exhibit plasticity during a specific window of their maturation, and as a result, have been proposed to function in pattern separation (Sahay, et al., 2011). The neuronal phenotype of this population is determined at one week old, and neuronal maturation occurs over the course of several weeks until these neurons reach complete maturation between six to eight weeks after

birth (Esposito, et al., 2005; Zhao, et al., 2006; Tonil, et al., 2008). During this maturation window, these neurons exhibit high plasticity that is distinct from mature granule cell neurons (Schmidt-Hieber, et al., 2004; Marin-Burgin, et al., 2012). It is hypothesized that adult neurogenesis expands the capacity for plasticity in the DG, as the mature population does not exhibit the same level of activity of the immature neuron population. However, the contribution and purpose of the adult-born population of neurons is still largely unknown.

There have been many studies which have produced results consistent with the hypothesis that these adult-born neurons are required for pattern separation. A delayed non-matching and screen spatial discrimination task (Clelland, et al., 2009) and contextual fear discrimination learning task (Tronel, et al., 2010; Sahay, et al., 2011; Nakashiba, et al., 2012), which was previously shown to require dentate granule neuron mediated pattern separation (McHugh, et al., 2004), demonstrated that mice without neurogenesis performed at a lower level than their control counterparts. Another study, which tested spatial pattern separation performance through a delayed non-matching to place task using an eight arm radial maze, showed that mice without neurogenesis were impaired when the separation between the arms was low (Clelland, et al., 2009). Alternatively, mice with selectively increased neurogenesis showed an improvement in pattern separation in a contextual fear discrimination task (Sahay, et al., 2011).

All these studies suggest the population of immature adult-born neurons is the main contributors to pattern separation. Therefore, the primary aim of this study is to test this hypothesis and determine if adult-born neurons in the DG are necessary to

perform a spatial discrimination task at a low degree of spatial separation in a task previously shown to be dentate dependent (Morris, et al., 2012). This paper will present the effects of neurogenesis on pattern separation when neurogenesis in the DG is selectively ablated.

To ablate neurogenesis, the rat model described in Snyder, et al., 2011 was used. These animals were genetically modified to express the herpes simplex virus thymidine kinase (TK) under control of the GFAP promoter. When administered the drug valganciclovir, mitotic cells are specifically targeted for elimination, and spares post-mitotic cells. It was shown in Snyder, et al., 2011 that 99% of immature neurons expressing doublecortin (DCX) were ablated without effects on the general health of the animal when valganciclovir was administered to GFAP-TK animals.

This study also involves dorsal DG (dDG) lesioned animals to confirm the task used is dentate dependent in the conditions under which this study was conducted. The neurotoxin colchicine was administered through an intracranial injection to selectively ablate the granule neurons in the dorsal dentate gyrus (Goldschmidt, et al., 1982).

II:

Results

After the pre-training treatments of BrdU, valganciclovir administration, and food restriction (Figure 1), the animals began the behavioral paradigm place-learning task. The animals were first habituated to the environment, and then trained and observed as they performed the task under the adjacent arm spatial separation condition as described in Morris, et al., 2012.

The animals were evaluated on the number of trials it took to reach criterion of learning in this task. The animal is trained every day until the criterion is reached. Each day, the animal performs the task, consisting of 10 trials. Completion of training is defined as completing two consecutive days in which the animal completes the task with a score of at least 90% correct. The trials to criterion include all trials completed to reach the criterion.

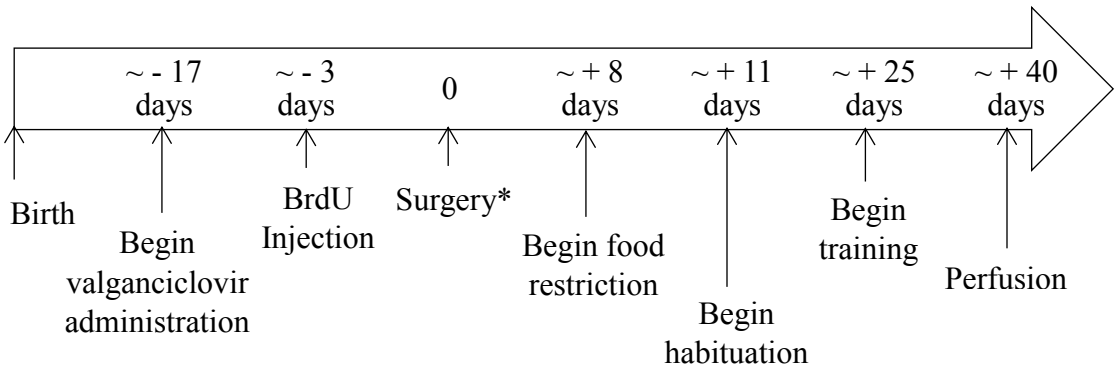
The animals involved were categorized into three groups: valganciclovir treated GFAP-TK (v-GFAP-TK) transgenic animals (N = 8), control animals (N = 6), and dDG lesion animals (N = 7). The control animals consisted of two groups: valganciclovir treated wild type (v-Wildtype) and GFAP-TK animals. The performance of these two control subgroups was analyzed to determine if valganciclovir administration affected performance (Figure 2). The comparison of the mean (±SE) trials to criterion showed that there was no significant difference between these two groups, and as a result, the performance of these two groups were consolidated and represented together as the control group in this study. Additionally, these findings provide evidence that the performance of the animals in this study was not attributed to nor was affected by valganciclovir administration.

Through comparing the mean (±SE) trials to criterion (Figure 3), the control and v-GFAP-TK groups showed no significant difference in their performance in this task. The animals with neurogenesis performed at the same level as those with neurogenesis ablated. The dDG lesion group, however, performed differently, with higher mean trials to criterion. This dDG lesion group demonstrated a deficit in performance through needing more trials to complete the training. These results agree with those presented in Morris, et al., 2012.

After the behavioral task was completed, the animals were perfused and immunohistological procedures were used to stain the tissue. Doublecortin (DCX) staining of the tissue, used to label proliferating, immature neurons up to three weeks of age (Esposito, et al., 2005), confirmed the expected function of the valganciclovir drug. Through stereological methods, it was confirmed that the administration of valganciclovir to the GFAP-TK group ablated 98.3 ± 0.5% of the adult-born neuron population in the entire DG region in comparison to the control group (Figure 4).

The immunohistological BrdU stain was used to label neurons that were proliferating at the time of the injections, which were administered four weeks before the training began. These were immature, four weeks old neurons when training began, and upon perfusion, were five to six weeks old (Figure 1). Neurons reach complete maturation between six to eight weeks after birth, and since DCX staining only labels neurons up to three weeks of age, it was crucial to perform BrdU as well to label these older immature neurons. Administration of valganciclovir to the GFAP-TK group ablated 82.8 ± 2.3% of this group of neurons in comparison to the control group (Figure 5).

Statistical tests were performed to determine the statistical significance of the data. Bartlett's test was performed first to determine if the variance was equal or unequal. The knowledge of the nature of the variance was required to complete unpaired t-tests for the data. In Figure 3, three groups of animals are analyzed. The significance across the three groups was normalized using the Bonferroni-Holm correction.



* This procedure was only performed on animals in the dDG lesion group

Figure 1. The outline of the experimental paradigm. Each subject in this study experienced the events of this timeline, set relative to the date of surgery or when the animal turns 17 weeks of age, which is the approximate age of the animal at the time of surgery. The events outlined are approximate and may vary up to ±5 days.

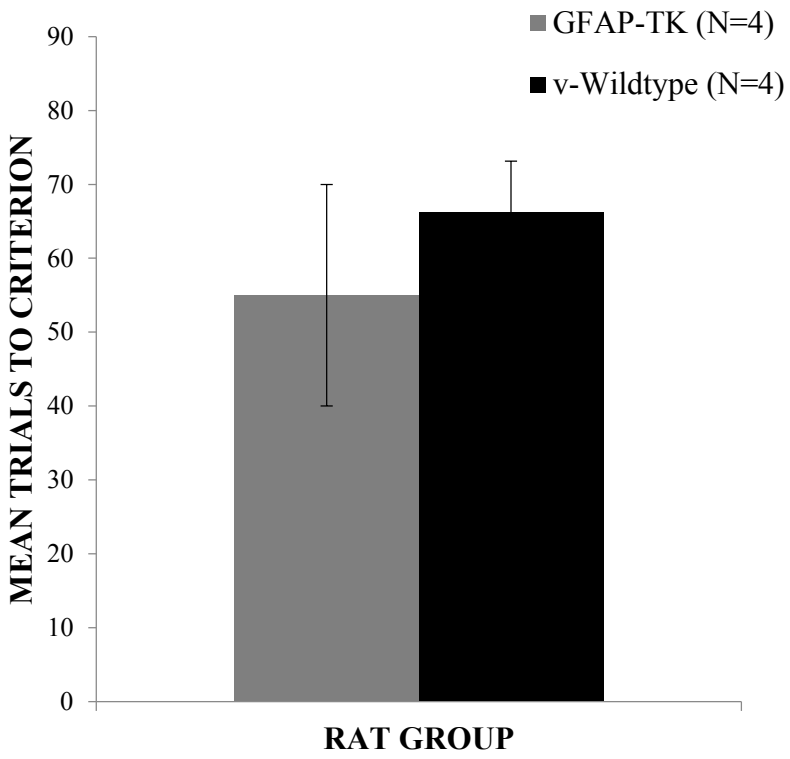


Figure 2. Valganciclovir administration shows no effect on performance. A preliminary sampling of the control group shows no significant difference (P = 0.52) between the mean (±SE) trials to criterion for GFAP-TK transgenic and v-Wildtype Long Evans male rats on the adjacent arm spatial separation condition of the place-learning task.

A.

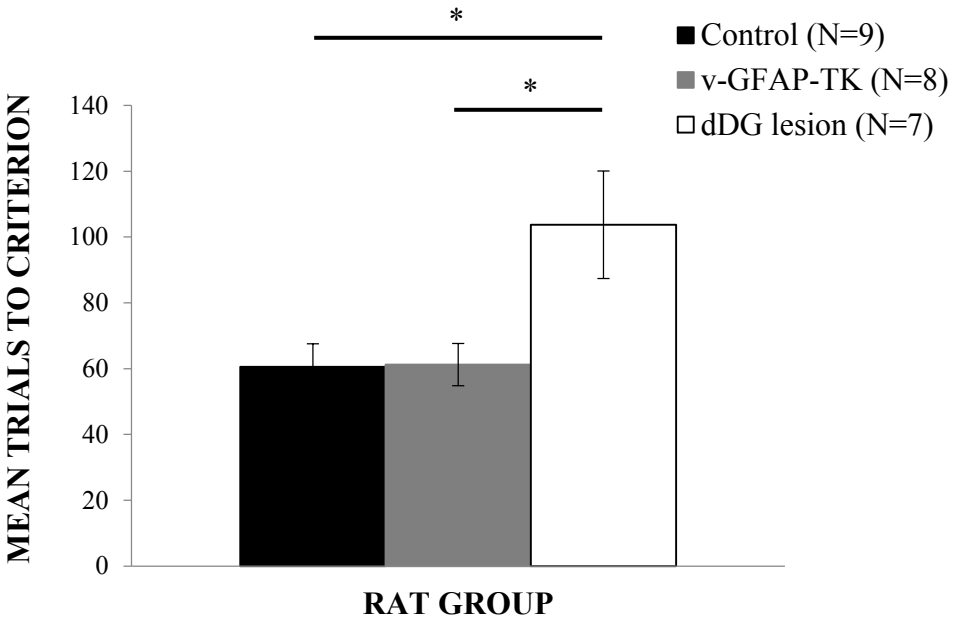
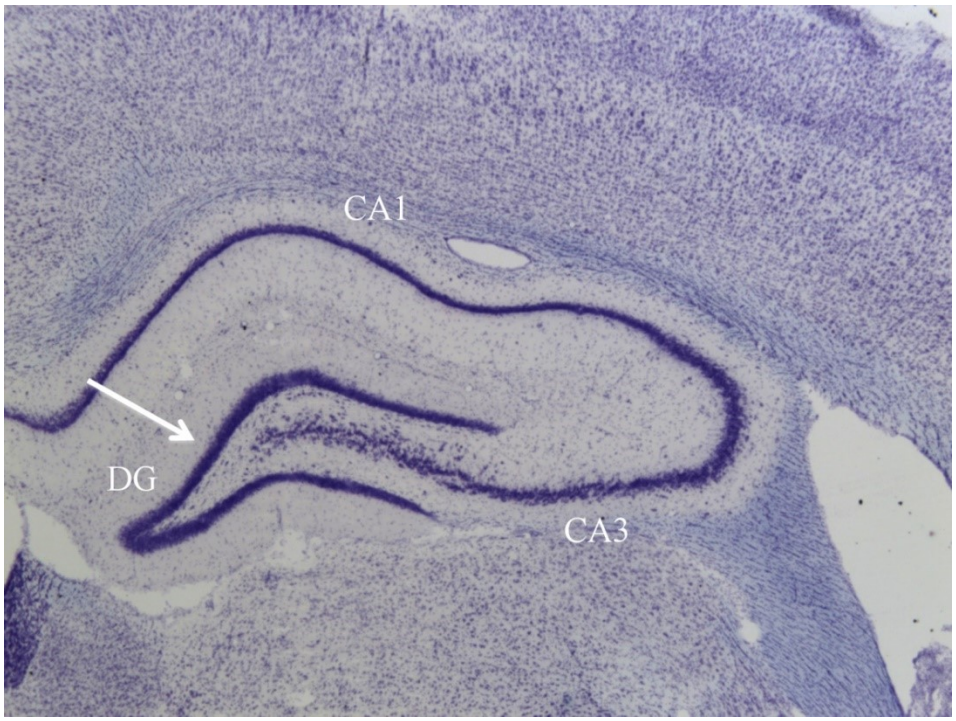


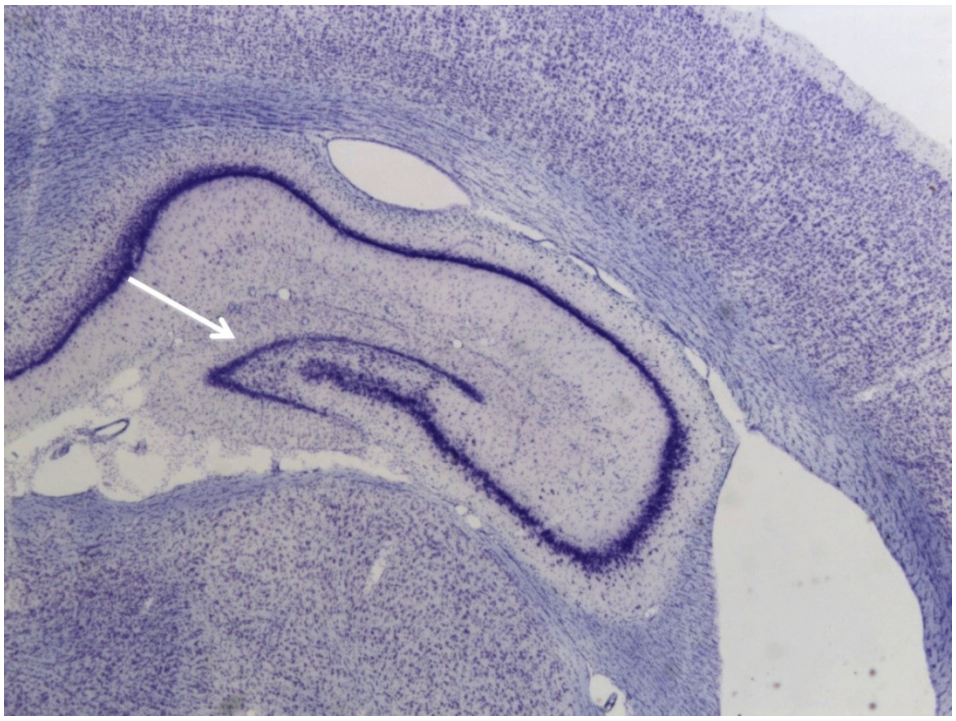
Figure 3. v-GFAP-TK animals show no significant difference in the trials needed to reach criterion when compared to the control animals, but both are significantly different from the dDG lesion group.

- **A.** The mean (±SE) trials to criterion for dDG lesion, control, and v-GFAP-TK transgenic Long Evans male rats on the adjacent arm spatial separation condition of the place-learning task. The dDG lesion animals performed significantly more different from the control and v-GFAP-TK animal groups ($P = 0.045$ using the Bonferroni-Holm correction).
- **B.** Cresyl violet staining of an animal without a dDG lesion. The dentate granule layer is intact and fully present.
- **C.** Cresyl violet staining of an animal with a dDG lesion. The dentate granule layer is sparser and there is a visual difference between this and an animal without a lesion. This demonstrates that the colchicine neurotoxin lesion was successful.

B.



C.



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A.

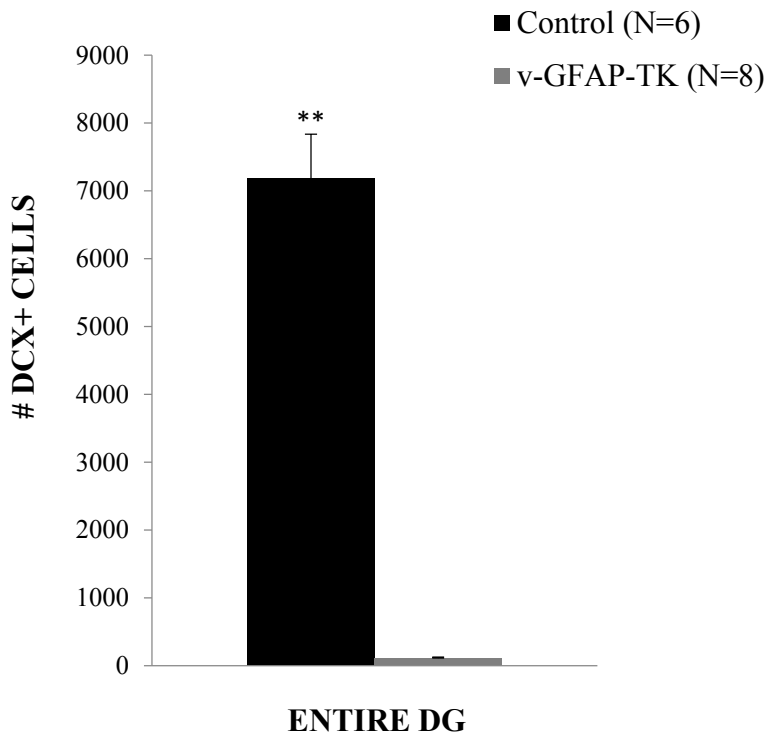
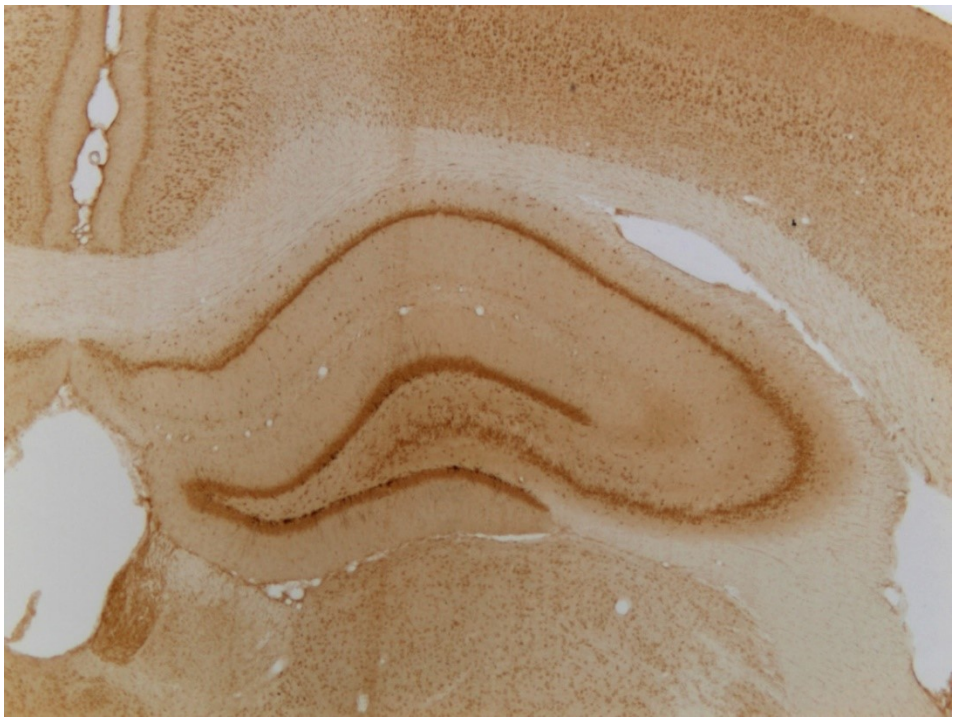


Figure 4. Neurogenesis is ablated in v-GFAP-TK transgenic Long Evans male rats.

- **A.** The mean (±SE) number of DCX^+ cells in various regions of the DG show a 98.3 ± 0.5% ablation of DCX^+ cells in v-GFAP-TK animals compared to the control animals (P = 0.0033). Only a subsample of animals involved in the study is represented, as the dDG lesion group is not represented. The quantification was performed stereologically.
- **B.** DCX staining of a control animal at 2.5x magnification.
- **C.** DCX staining of the same section of a control animal at 20x magnification, confirming the presence of the adult-born neuron population. An example of a DCX^+ cell is indicated.
- **D.** DCX staining of a v-GFAP-TK animal at 2.5x magnification.
- **E.** DCX staining of the same section of a v-GFAP-TK animal at 20x magnification, confirming the lack of the population of adult-born neurons.

B.



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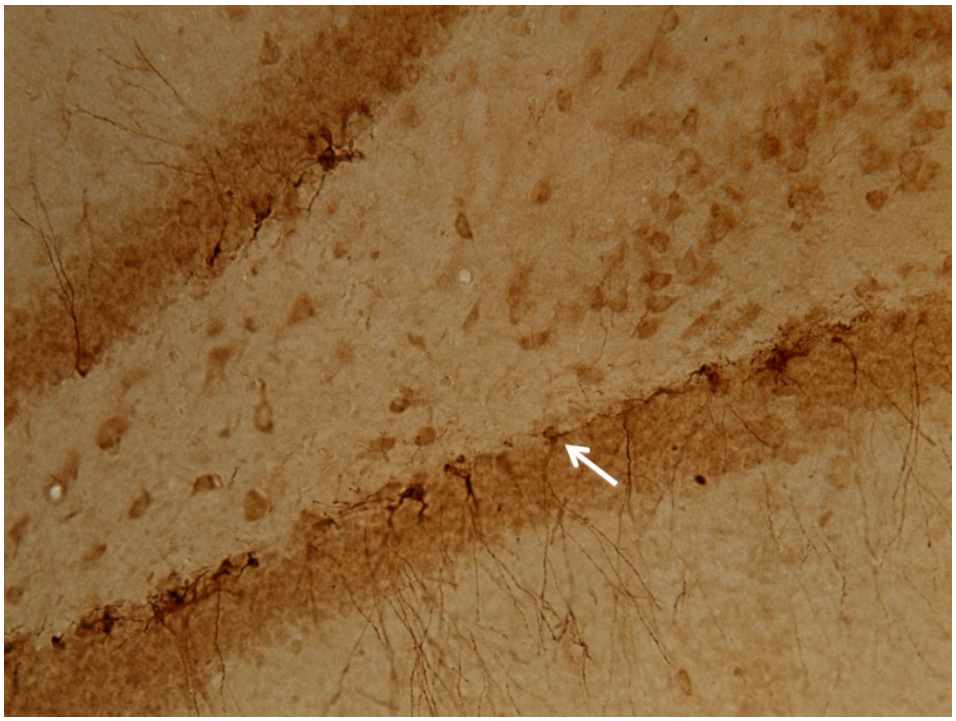

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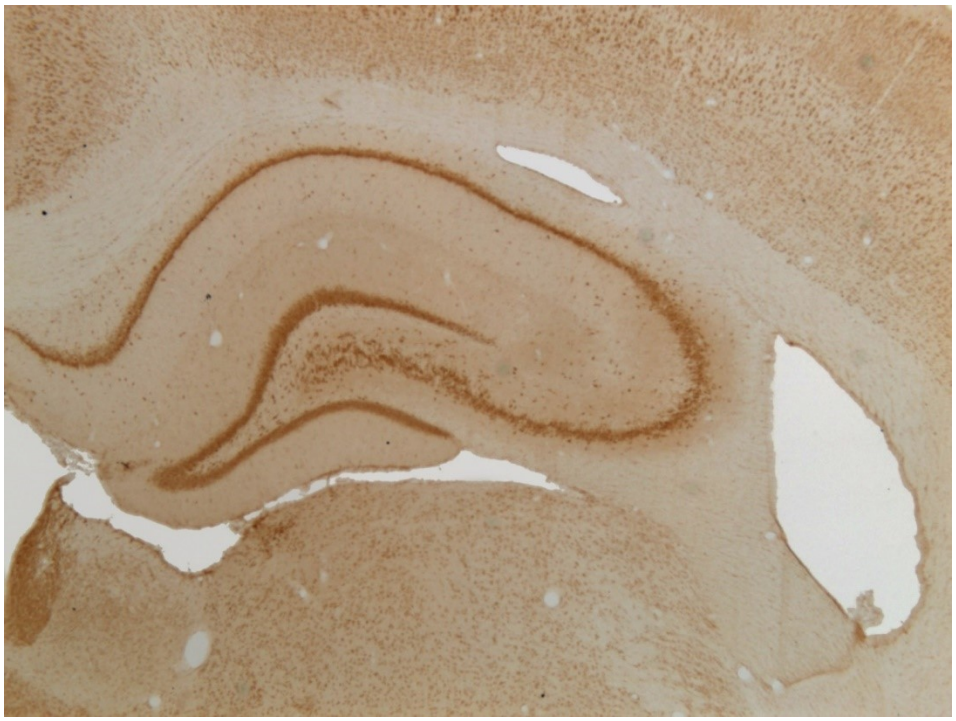

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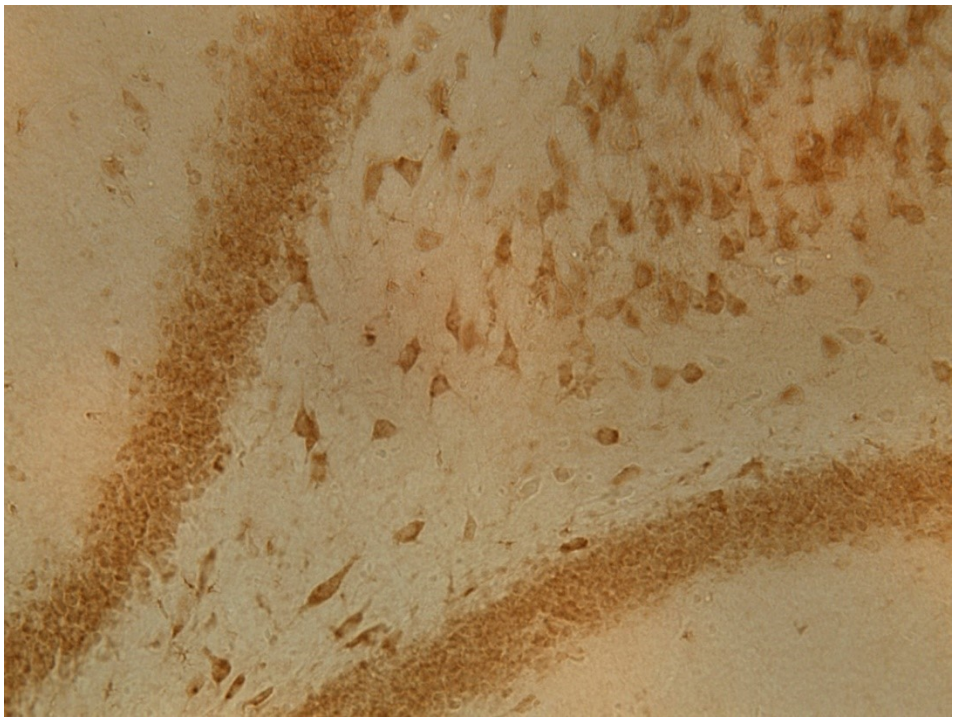

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A.

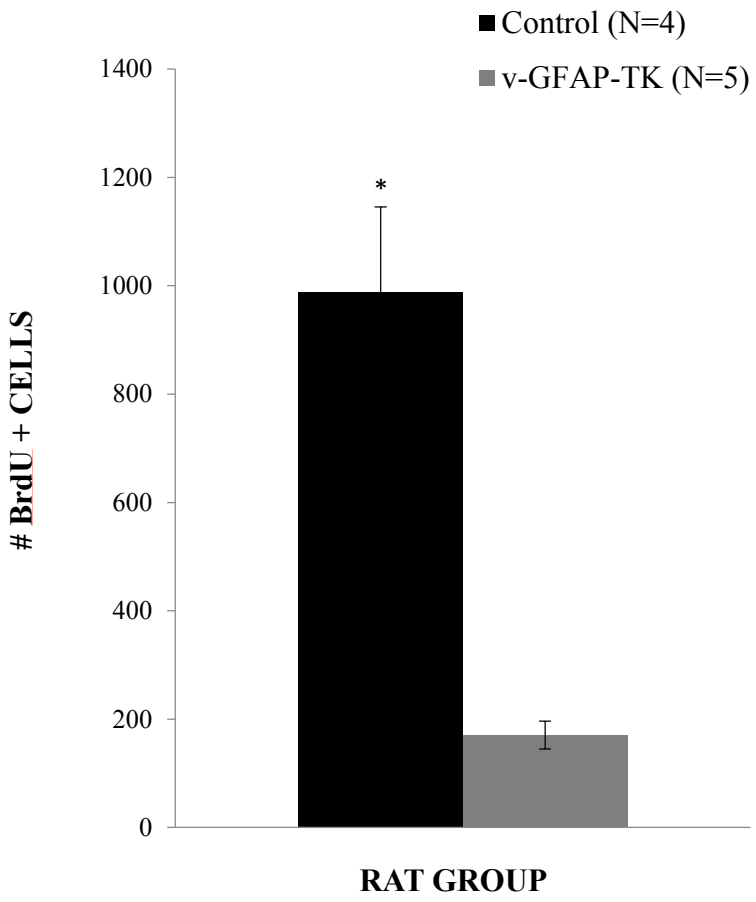
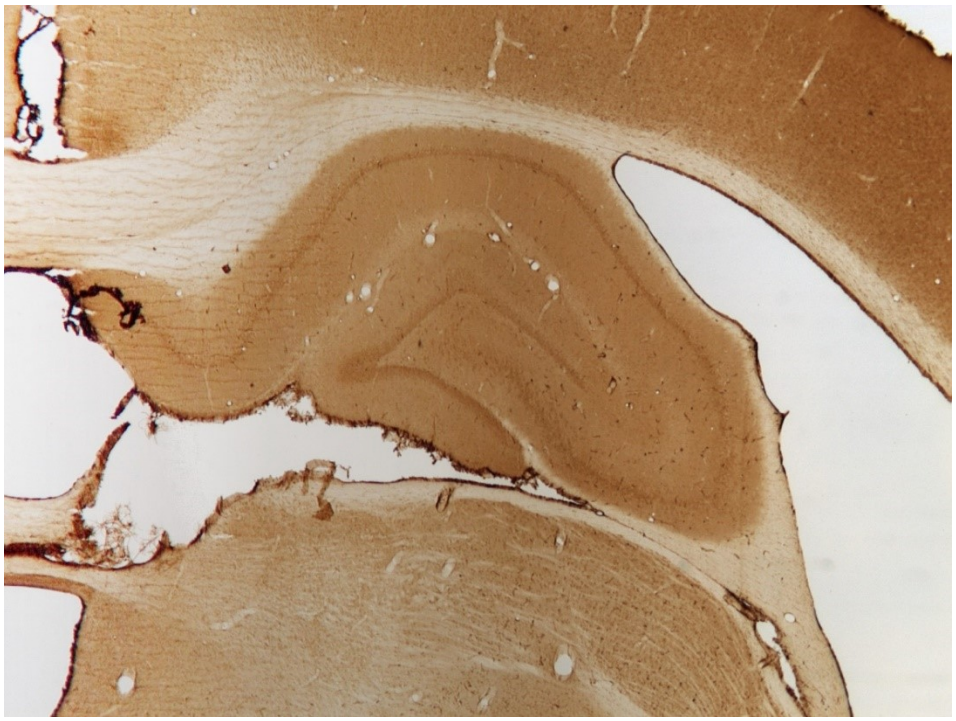


Figure 5. Population of older immature neurons at the time of training.

- **A.** The mean (±SE) number of $BrdU^+$ cells in the granule cell layer of the DG. These cells were five to six weeks old at the time of perfusion and were labeled two weeks after administration of valganciclovir begun. The comparison of this population of immature neurons show incomplete ablation (82.8 ± 2.3%) of neurogenesis two weeks after administration of valganciclovir began. This is a subsample of animals involved in the study, however, there is sufficient data to demonstrate a significant difference between the two groups (P = 0.0125). The quantification was performed stereologically.
- **B.** BrdU staining of a control animal at 2.5x magnification.
- **C.** BrdU staining of the same section of a control animal at 10x magnification, confirming the presence of the population of adult-born neurons
- **D.** BrdU staining of a v-GFAP-TK animal at 2.5x magnification.
- **E.** BrdU staining of the same section of a v-GFAP-TK animal at 10x magnification, confirming the lack of the population of adult-born neurons.

B.



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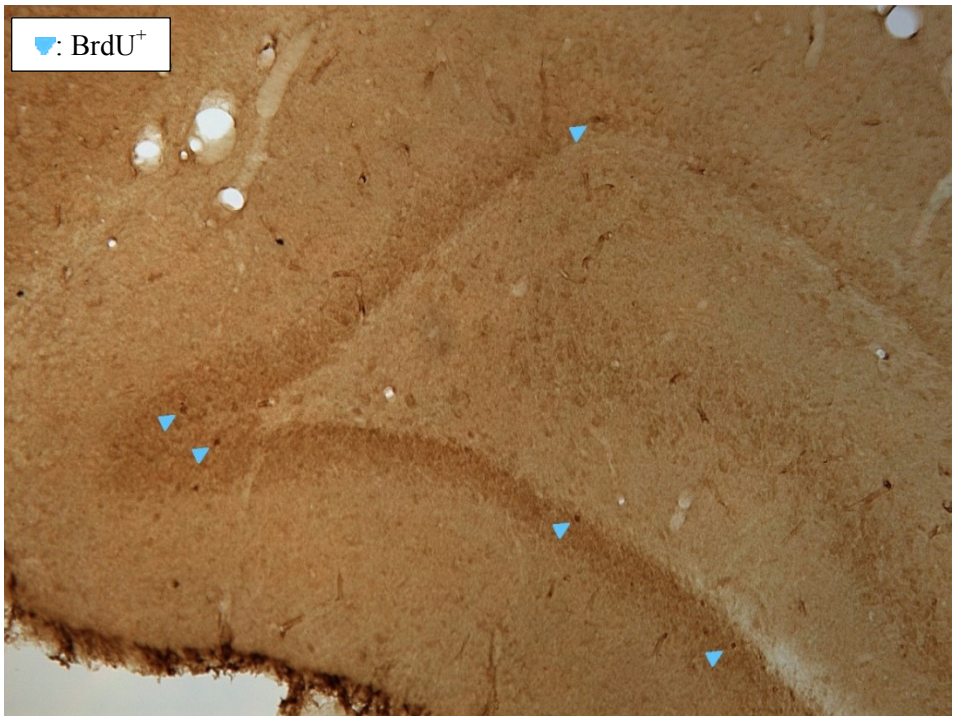

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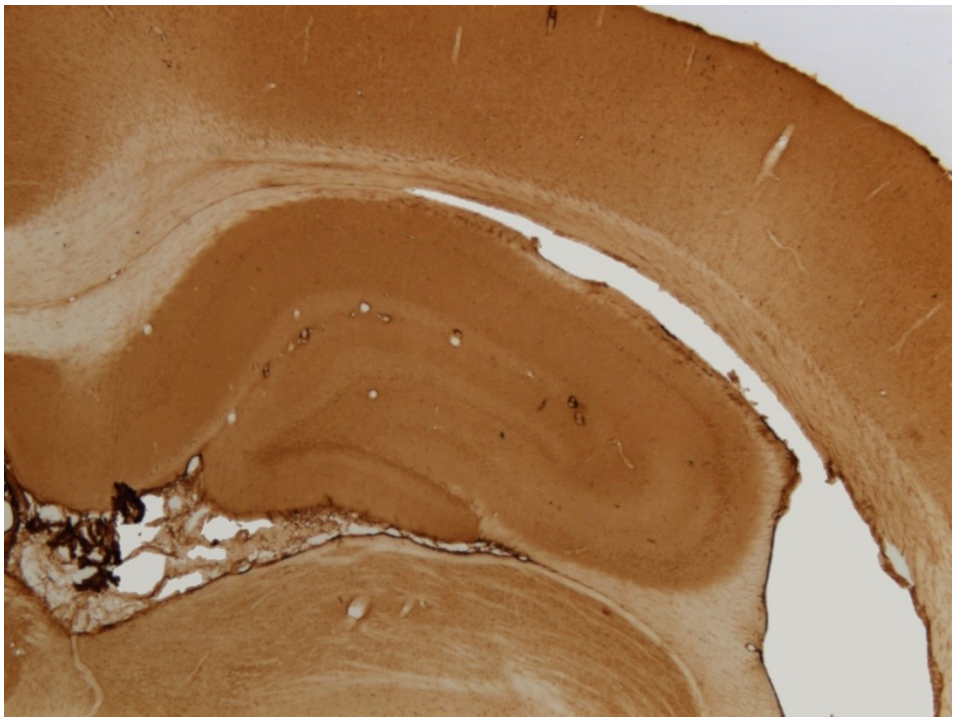

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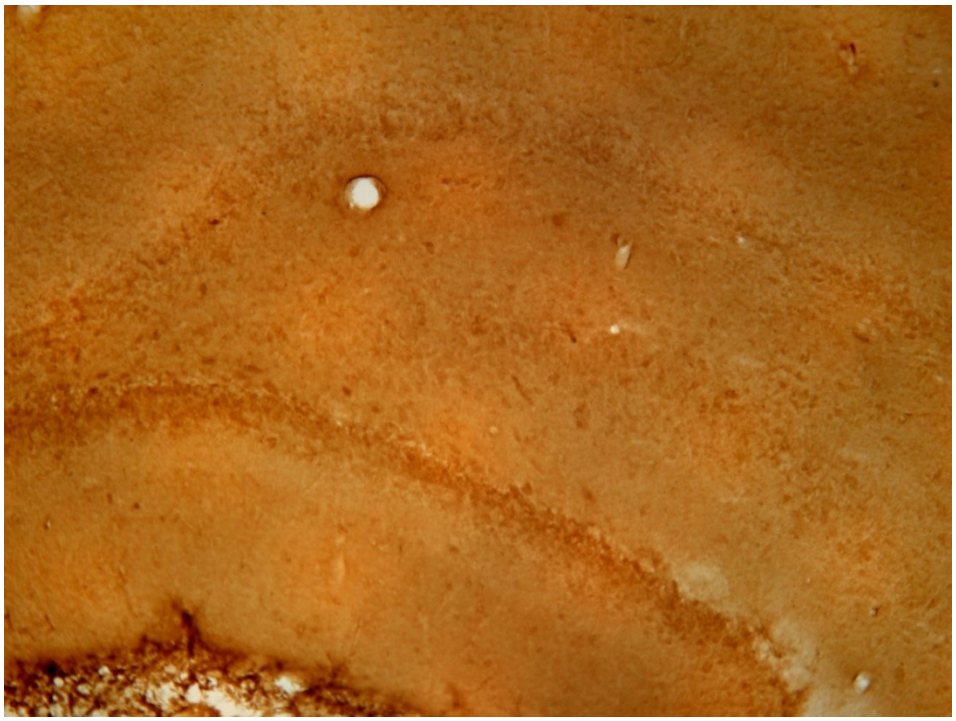

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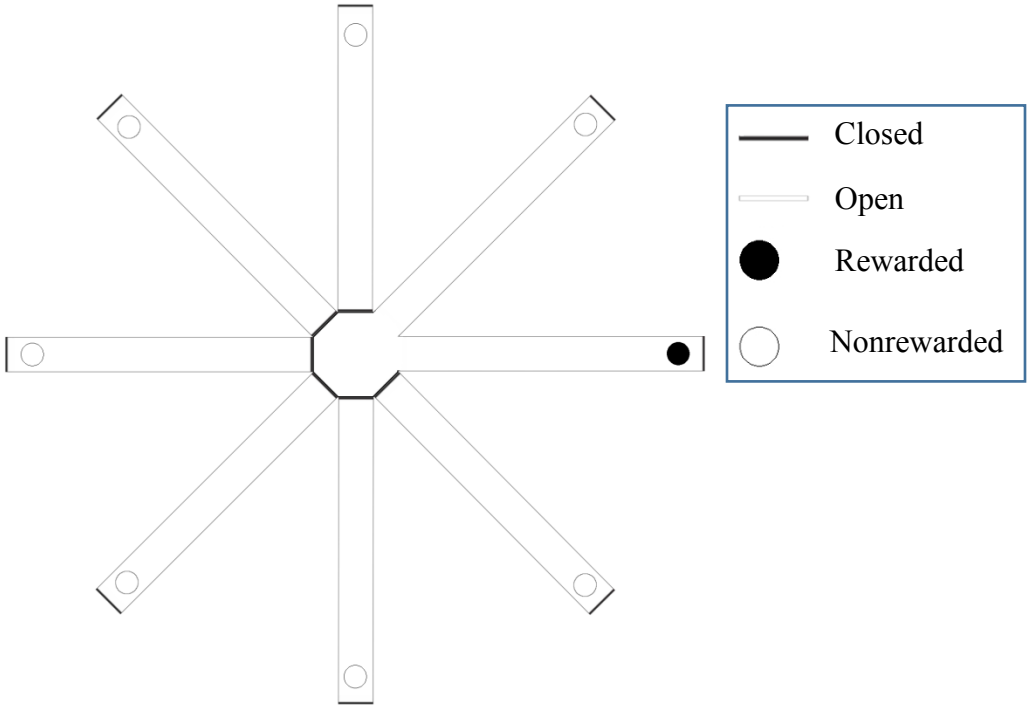

Figure 6. A representation of the eight arm radial maze in the adjacent arm separation. This is one of the two formations of the radial maze for this study. The other formation would involve the other arm adjacent to the reward arm being open instead.

III:

Discussion

The results present no significant differences in performance between control and v-GFAP-TK animal groups, suggesting that the adult-born neurons generated through neurogenesis are not necessary for performing pattern separation. These results refute the current hypothesis that neurogenesis in the DG are the main contributors to pattern separation (McHugh, et al., 2004; Zhao, et al., 2006; Toni, et al., 2008; Clelland, et al., 2009; Sahay, et al., 2011; Nakashiba, et al., 2012; Marin-Burgin, et al., 2012; Tronel, et al., 2012). The absence of neurogenesis correlated with the lack of performance deficit compared to control animals suggests that while this adult-born cell population may play a role in the ability to pattern separate, it is not the main contributor to this function.

DCX labeling has confirmed the presence of neurogenesis the control animal group, and the absence of neurogenesis in v-GFAP-TK groups. This data corroborates the previous results, presented in Snyder, et al. 2011 in a mouse model, that administering valganciclovir to the GFAP-TK transgenic animal group results in the ablation of neurogenesis, but valganciclovir will otherwise have no effect on non-transgenic animals. However, since DCX only labels immature neurons up to three weeks of age (Piatti, et al., 2011), it was necessary to quantify $BrdU^+$ neurons as well. BrdU labels the proliferating neurons at the time of the injection (Kuhn, et al., 1996). Due to the timing of the BrdU injection into the animals, neurons that were $BrdU^+$ were in an advanced stage of maturation during the training period, and would be between five to six weeks old at the time of perfusion. This population of $BrdU^+$ neurons is important to consider because even though some neurons may have reached

complete maturation at six weeks old at the end of the task (Esposito, et al., 2005), others may still exhibit characteristics of immature neurons.

The decreased population of adult born neurons and neurons in their final stage of maturation during the training period in v-GFAP-TK animals would suggest a decreased level of performance (McHugh, et al., 2004; Zhao, et al., 2006; Toni, et al., 2008; Clelland, et al., 2009; Sahay, et al., 2011; Nakashiba, et al., 2012; Marin-Burgin, et al., 2012; Tronel, et al., 2012). However, the results from this place learning paradigm suggest otherwise. Since the experimental paradigm is similar to that described in Morris, et al., 2012, incongruences in the execution of training may account for these unexpected results. In this study, the additional group of dDG lesion animals was used to refute this possibility. In Morris, et al., 2012, dDG lesion animals were shown to perform more poorly compared to the control animals, which was corroborated in this study (Figure 3). As a result, the paradigm used in this study is confirmed to be a dentate dependent task. Since all animals are trained using the same protocol in this study, the results from this study is unlikely attributed to a deviation from this dentate dependent task as described in Morris, et al., 2012.

The results of this study align with those presented in Groves, et al., 2013, which also suggested that neurogenesis has no effect on spatial pattern separation. The same GFAP-TK rat model was used to ablate neurogenesis in that study, and although the drug used was different, neurogenesis was ablated to the same extent as the data presented in this study. Similarly to the subjects in this study, the animals were administered the drug for six weeks prior to the onset of training. The animals perform a radial arm maze task similar to what was used in this study, as well as other tasks

that have been proven to be dentate dependent. However, the results presented in Groves, et al., 2013 are slightly incomplete. Although the tasks presented have been proven to require the dentate gyrus in other publications, Groves, et al., 2013 does not include a lesion model to confirm the same held true for the experimental paradigm presented. As a result, these findings are preliminary and require further verification.

However, it is worth noting that following the ablation of neurogenesis, network changes in the dentate gyrus may compensate for the loss of this population, and restore long-term potentiation (LTP) (Singer, et al., 2011). Deficits in the dentate gyrus and a loss of LTP resulted when the animal is observed four weeks after the administration of the neurogenesis ablating drug began, and this loss in LTP was shown to be specific to this population of newborn neurons through recordings. However, after some time, homeostatic changes in the mature dentate network may compensate for the absence of immature neurons. The complete recovery of LTP was observed in the absence of neurogenesis ten weeks past the initial administration of the neurogenesis ablating drug.

The results presented in Singer, et al., 2011 present insight into the results of this study as well as the results of Groves, et al., 2013. Approximately five weeks of valganciclovir administration was completed before the training began in this study. It is possible that the five weeks of valganciclovir administration prior to the onset of training was sufficient time for the DG network to begin rewiring to accommodate the absence of neurogenesis. If Singer is correct, this may serve to explain why no differences in performance in this spatial differentiation task was observed between animals with neurogenesis ablated and controls.

To account for this possibility, another animal group that will begin valganciclovir administration approximately three weeks prior to the onset of the training, and would have been administered the drug for approximately five weeks at the time of training completion and perfusion, should be included in this study. This new group would begin training three weeks after drug administration begins because in this study, comparison between the $BrdU^+$ (Figure 5) and DCX^+ (Figure 4) show that at two weeks of valganciclovir administration, neurogenesis is still not completely ablated. There is no definitive data that provides insight on the length of drug administration necessary to almost completely ablate neurogenesis, however the data presented in these figures as well as in Singer, et al., 2011 suggest it is between two and four weeks. As a result, immunohistochemistry results would be necessary to confirm the precise level of ablation of these newborn neurons. During the training, the animals would approach four weeks of valganciclovir administration, which was shown in Singer, et al., 2011 to be the time at which an animal expresses a diminished LTP due to the loss of this population.

The role of neurogenesis is widely debated, and perhaps a conclusion has not been reached due to the different protocols used to ablate neurogenesis in different studies, as well as other variables involved such as age and species. Singer, et al., 2011 presented findings that suggested the dentate network could rewire in at most six weeks. If the results of this study are attributed to the recovery of LTP, this process would be shortened to as little as two weeks. Studies are inconsistent across the field in the way neurogenesis is ablated, the age of animal subjects, and most importantly, the latency between the onset of neurogenesis ablation and training. If a mere two

weeks is sufficient for a network to rewire and compensate for the loss of adult-born neurons, it's no wonder the same task could present different results. Understanding this timeline could provide insight into the experimental design of future projects and perhaps begin to pave the way to a more united conclusion across researchers regarding the role of adult-born neurons in spatial discrimination and other dentate dependent tasks.

IV:

Materials and Methods

Subjects

Male Long-Evans rats were used as subjects, GFAP-TK strain transgenic animals and wild type animals. Each animal was individually housed in a plastic container in the vivarium of the building. The vivarium was maintained on a scheduled light cycle, and all the testing was conducted during the light phase. The experimenter is blind to the genotypes of the animals while performing this study. The animals participate in this task individually. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Behavioral apparatus

An eight-arm radial maze was used as the testing apparatus. The maze consisted of an octagonal central platform 28 cm in diameter with eight arms extending outward from the central platform. Each arm was 80cm long and 11.5 cm wide. Access to the arms was controlled using transparent Plexiglas panels which were 0.5 cm thick and extended 29 cm above the maze surface. Metal bowls, attached to the distal ends of the arms using Velcro, measured 5 cm in diameter and extended 2.5 cm above the maze surface.

During the task, the Plexiglas panels were lowered or raised by the experimenter to control access to the arms, out of sight from the animal. An opaque cylindrical bucket, 25 cm in diameter and 25 cm in height, was raised and lowered by the experimenter to block the view of the maze while manipulations of the maze were being performed.

The maze was located in the center of a windowless room with a variety of distal cues. The experimenter remained in the testing room and in sight of the animal during testing. The experimenter served as one of the distal cues in the room, and as a result, remained and moved in a set route when in sight of the animal.

Experimental Paradigm (Figure 1)

Valganciclovir Treatment

The animals were orally administered a dosage of the antiviral prodrug valganciclovir daily. Each dosage contained 7.5 mg of valganciclovir, made following the protocol outlined in Snyder, et al., 2011, in a mixture of food pellets and peanut butter. The administration of the drug began when the animal was 15 weeks old to selectively block neurogenesis in the DG when administered to GFAP-TK transgenic animals. The administration of the drug begins two weeks prior to the BrdU injection to ensure a minimal proliferating neuron population at the time of the BrdU injection.

Surgical procedures

dDG lesion: Neurotoxic lesions were administered to a group of subjects (N=7) through a stereotaxial injection of the drug colchicine, targeting granule cells in the dorsal dentate gyrus. Buprenorphine HCl (0.02mg/kg; sc) was used an analgesic prior to surgery. The procedure consisted of bilateral infusions of colchicine (2.5 mg/ml in phosphate buffer 0.1M) delivered to the dDG using a 1µl-Hamilton syringe. The syringe was positioned with the opening facing the midline prior to injection. The final volume of each intracranial infusion was 40 nL with an infusion rate of 12 nL/ min. The surgical coordinates for each bilateral injection site to perform the dDG lesion were as follows: anteroposterior

(A.P.): -2.8, -4, -5.2; mediolateral (M.L.): ±0.9, ±2.1, ±3.2; and dorsoventral (D. V.): -3.7, -3.6, -3.7, respectively. A latency of 8 minutes followed each infusion of the drug to prevent the reflux of the drug with the exit of the needle.

BrdU: The animals are anesthetized with isofluorane and then injected with a sterile BrdU solution containing 200 mg BrdU/kg rat weight in the intraperitoneal space. To make the solution, the workspace was first sterilized with 70% EtOH. The appropriate amount of BrdU was dissolved in warm (50°C) sterile saline (10 mg BrdU/mL). 1N NaOH (7μL/mL of BrdU and saline solution) was then added to complete the BrdU solution. The temperature of the BrdU solution was decreased to room temperature prior to injection.

Food Restriction

Food and water were provided *ad libitum* until two days post-surgery. From that point onward, animals had limited access to water and were food restricted to 80-85% of the standard baseline weight to increase the animals' motivation to pursue food rewards during the study.

Habituation

Animals begin the habituation phase a week and a half after food restriction begins. At this time, the animals have been restricted to 80-85% of the baseline weight. Habituation lasts a week and a half regardless of if animals complete the habituation task early.

During habituation, the three experimental arms were unrestricted. The animal was permitted to individually explore the entire maze regardless of which arms were restricted. Each habituation period lasted 10 minutes, or until the animal completes the

task by eating all the rewards. Cocoa Puffs cereal (General Mills, Minneapolis, MN) was distributed evenly across the three arms and in the metal bowls of the three arms to ensure that the animal recognizes that they will only explore those three arms and that the Cocoa Puff is only found in the metal bowl at the end. As this phase progresses and the animal becomes more comfortable with the environment, the experimenter gradually places fewer and fewer Cocoa Puffs until Cocoa Puffs are only placed in the metal bowls.

The habituation phase is completed when the animal retrieves the food reward from the metal bowl of all three of the arms, and does not visit any extraneous arms in 5 minutes or less. A week and a half is allocated for habituation, however not every animal will require the same amount of time to reach the habituation criterion. If an animal completes habituation early, it will rest until the last day of the habituation phase. Each animal will undergo one last habituation task to ensure his readiness for training the following day.

Training

Every animal is tested in the adjacent condition. An arm is designated as the rewarded arm, and the arms to the immediate left and right of the rewarded arm are designated as the nonrewarded arms (Figure 6). The positions of these three arms and their identities are conserved for all the animals.

The animals are trained every day until criterion is met. Each day, the animal is evaluated on their performance on ten trials. This set of 10 trials consist of equal number of combinations of the rewarded and one of the nonrewarded arms, and these

combinations are randomized to ensure the animal does not perform out of habit, but instead uses the environmental cues to guide his decision.

Prior to the first trial of the day for an animal, the entire maze is in the default condition, with all the Plexiglas panels intact and cleaned with 70% alcohol. The 70% alcohol is necessary to prevent the animal from completing the task using olfactory cues rather than spatial cues. The animal is then placed on the center platform and the opaque cylindrical bucket is placed over the animal for 30 seconds. During these 30 seconds, the experimenter places one Cocoa Puff in the metal bowl of the reward arm and the Plexiglas panels are then removed from the reward arm and the designated nonrewarded arm for the specific trial.

The bucket is removed after 30 seconds and the animal is allowed to make his one choice between the rewarded and nonrewarded arm. The animal can retrieve a reward if he chooses the rewarded arm, but not if he chooses the nonrewarded arm. The animal is not permitted to change his arm choice after he places all four paws on an arm. After the animal makes his choice and returns to the platform, the experimenter places the bucket over the animal for 30 seconds. During these 30 seconds, the experimenter would revert the maze back to the default condition and customizes the maze for the next trial. At the end of the 10 trials, the experimenter reverts the maze back into the default condition during the 30 seconds the animal is in the bucket, and the animal is removed from the maze and placed back into the home cage at the conclusion of the 10 trials.

The remaining animals during the training phase are kept in their home cages in the experimental room, and white towels are draped over the cages. The animals are trained in the same order they were habituated in.

The following experimental conditions were always conserved: the room used, the position of the maze, position of the experimenter, the position of the distal cues, and the time delays between trials.

Histological procedures

After an animal has reached criterion in training, it is perfused the following day. During perfusion, the animal receives an overdose of Equithesin and was perfused intracardially with saline and 4% paraformaldehyde. The protocols followed are the same as those described in Leutgeb, et al., 2007, with the only difference being the usage of paraformaldehyde instead of formaldehyde for improved immunohistochemistry results. The brain tissue is then extracted for further processing. The brain tissue was immersed in a solution of 30% glucose in 1x phosphate buffered saline (PBS) for approximately 3 days until the tissue was denser than the solution. The tissue was then frozen and sectioned into 40-μm sections using a microtome, and were stored in a solution of 2% sodium azide in 1x PBS until immunohistochemistry procedures were performed. The following histological procedures were completed by using a sample of one in six sections of tissue.

Doublecortin (DCX) Immunostaining

On the first day of the protocol, the sample of tissues were first washed in 1x PBS (3 x 5 mins) to remove the 2% sodium azide in 1x PBS solution. The tissues were

then treated with 0.6% H_2O_2 in 1x PBS (1 x 15 mins), washed again with 1x PBS (3 x 5 mins), and then treated with a blocking solution (5% goat serum and 0.25% Triton X-100 in 1x PBS) (1 x 60 mins). The tissues were them removed from the blocking solution and placed into the primary antibody solution (1/1000 DCX antibody in 1x PBS) overnight. On the second day of the protocol, the tissues were washed in 1x PBS (3 x 8 mins) and then placed into the secondary antibody solution (1/1000 rabbit antibody in 1x PBS) overnight. On the third day of the protocol, the tissues were washed in 1x PBS (3 x 8 mins), treated with ABC (1/1000 in 1x PBS), washed again with 1x PBS (3 x 8 mins), and were treated with a DAB solution (DAB in 1x PBS) until visual labeling of DCX^+ cells appeared. Stereological counts of DCX^+ cells were performed on one in six sections of the DG and normalized to represent the entire volume of the DG.

5-Bromo-2'-deoxyuridine (BrdU) Immunostaining

On the first day of the protocol, the sample of tissues were first washed in 1x PBS (3 x 5 mins) to remove the 2% sodium azide in 1x PBS solution. The tissues were then treated with 0.6% H_2O_2 in 1x PBS (1 x 15 mins), washed in 1x PBS (3 x 5 mins), and then treated with 2N HCl (HCl in distilled water) at 37°C (1 x 30 mins). The tissues were then removed from the 2N HCl and treated with a 0.1M Borate Buffer (pH=8.5; sodium borate in distilled water) (1 x 2 mins). Thorough washes in 1x PBS (6 x 15 mins) followed, after which the tissue was placed into a blocking solution (5% goat serum and 0.25% Triton X-100 in 1x PBS) (1 x 60 mins). The tissues were them removed from the blocking solution and placed into the primary antibody solution (1/500 DCX antibody in 1x PBS) overnight. On the second day of the protocol, the

tissues were washed in 1x PBS (2 x 15 mins), rinsed in the blocking solution (1 x 15 mins), and then placed into the secondary antibody solution (1/1000 biotinated rat antibody in 1x PBS) overnight. On the third day of the protocol, the tissues were washed in 1x PBS (3 x 8 mins), treated with ABC (1/1000 in 1x PBS) (1 x 60 mins), washed again with 1x PBS (3 x8 mins), and were treated with a DAB solution (DAB in 1x PBS) until visual labeling of $BrdU^+$ cells appeared. Stereological counts of $BrdU^+$ cells were performed on one in six sections of the DG and normalized to represent the entire volume of the DG.

Cresyl Violet

The sample of tissues was chronologically mounted onto glass slides and was allowed to dry overnight. The following day, the slides were first soaked in double distilled water (5 minutes) before dehydration. During dehydration, the slides were dipped ten times in each 70%, 80%, 90%, 100%, and 100% solutions of EtOH in double distilled water, starting with the lowest dilution of alcohol and progressing to the highest. The slides were then soaked in xylene (2 minutes) and then removed to rehydrate. During rehydration, the slides were dipped ten times in each 100%, 100%, 90%, 80%, 70%, and 50% solutions of EtOH in double distilled water. The slides were then soaked in double distilled water (2 minutes) and then removed and placed into the cresyl violet solution. The slides remained in the cresyl violet solution until the desired staining was achieved

References

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Jr., Frangniere, A., Tyers, P., Jessberger, S., Saksida, L. M., Barker, R. A., Gage, F. H., and Bussey, T. J. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **325**: 210-213

Colgin, L. L., Moser, E. I., and Moser, M. B. (2008) Understanding memory through hippocampal remapping. *Trends in Neurosciences* **31**: 469-477

Esposito, M. S., Piatti, V. C., Laplagne, D. A., Morgenstern, N. A., Ferrari, C. C., Pitossi, F. J., and Schinder, A. J. (2005). Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *The Journal of Neuroscience* **25**: 10074-10086

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

Goldschmidt, R. B., and Steward, O. (1982). Neurotoxic effects of colchicine: differential susceptibility of CNS neuronal populations. *Neuroscience* **7**: 695-714

Groves, J. O., Leslie, I., Huang, G. J., McHugh, S. B., Taylor, A., Mott, R., Munafo, M., Bannerman, D. M., and Flint, J. (2013). Ablating adult neurogenesis in the rat has no effect on spatial processing: evidence from a novel pharmacogenetic model. *PLOS Genetics* **9**: 1-16

Kuhn, H.G., Dickinson-Anson, H., and Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *The Journal of Neuroscience* **16(6)**: 2027-2033

Leutgeb J. K., Leutgeb S., Moser M., Moser E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* **16**: 961-966

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., and Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science* **27**: 1295-1298

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

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., and Tonegawa, S. (2007) Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* **317**: 94-99

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., and Tonegawa, S. (2012). Young dentate cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell* **149**: 188-201

Norman, K. A., and O'Reilly, R. C. (2002) Modeling hippocampal and neocortical contributions to recognition memory: a complementary learning systems approach. *Psychological Review* **110**: 611-646

Piatti, V. C., Davies-Sala, M. G., Esposito, M. S., Mongiat, L. A., Trinchero, M. F., and Schinder, A. F. (2011). The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. *The Journal of Neuroscience* **31(21)**: 7715-7728

Piatti, V. C., Esposito, M. S., and Schinder, A. F. (2006). The Timing of Neuronal Development in Adult Hippocampal Neurogenesis. *The Neuroscientis* **12**: 463-467

Piatti, V. C., Ewell, L. A., and Leutgeb, J. K. (2013). Neurogenesis in the dentate gyrus: carrying the message or dictating the tone. *Frontiers in Neuroscience* **7**: 1-11

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

Sahay, A., Wilson, D. A., and Han, R. (2011). Pattern Separation: A Common Function for New Neurons in Hippocampus and Olfactory Bulb. *Neuron* **70**: 582-588

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

Singer, B. H., Gamelli, A. E., Fuller, C. L., Temme, S. J., Parent, J. M., and Murphy, G. G. (2011). Compensatory network changes in the dentate gyrus restore long-term potentiation following ablation of neurogenesis in young-adult mice. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 5437-5442

Snyder, J. S., Grigereit, L., Brewer, M., Pickel, J., and Cameron, H. A. (2011). A transgenic rat model for reducing adult neurogenesis. *Society for Neuroscience* **2011**

Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., Cameron, H. A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behavior. *Nature* **476**: 458-461

Toni, N., Laplagne, D. A., Zhao, C., Lombardi, G., Ribak, C. E., Gage, F. H., and Schinder, A. F. (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature Neuroscience* **11**: 901-907

Tronel, S., Belnoue, L., Grosjean, N., REvest, J. M., Piazza, P. V., Koehl, M., and Abrous, D. N. (2010). Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus* **22**: 292-298

Vazdarjanova, A., and Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *The Journal of Neuroscience* **24**: 6489-6496

Zhao, C., Deng, W., and Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* **132**: 645-660

Zhao, C., Teng, E. M., Summers Jr, R. G., Ming, G., and Gage, F. H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *The Journal of Neuroscience* **26**: 3-11