Title
Seizure-induced Increased Neurogenesis Occurs in the Dentate Gyrus of Aged Sprague-Dawley Rats.

Journal
Aging and disease, 2(4)

ISSN
2152-5250

Authors
Shapiro, Lee A
Wang, Lulu
Upadhyaya, Pooja
et al.

Publication Date
2011-08-30

Aging and Disease
www.aginganddisease.org
Volume 2, Number 4; 286-293, August 2011

Original Article

Seizure-induced Increased Neurogenesis Occurs in the Dentate Gyrus of Aged Sprague-Dawley Rats

Lee A. Shapiro[1], *, Lulu Wang[2], Pooja Upadhyaya[2] and Charles E. Ribak[2]

[1]Dept. of Surgery and Neurosurgery, College of Medicine, Texas A&M Health Science Center, Scott & White Hospital, Central Texas Veterans Health Care System, Temple, TX 76504, USA
[2]Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, CA 92697, USA

[Received June 14, 2011; Revised August 18, 2011; Accepted August 18, 2011]

ABSTRACT: Neurogenesis in the hippocampal dentate gyrus persists throughout the lifespan of mammals, however, the rate of neurogenesis decreases as the animal ages. Although seizures increase neurogenesis in young adult brains, this relationship has not been shown in aged animals. Using doublecortin (DCX) immunocytochemistry, the number of DCX-labeled cells in the dentate gyrus from aged rats (23 months of age) was assessed 30 days following pilocarpine-induced seizures and was compared to the number obtained from age-matched control rats. DCX-labeled cells were located in the subgranular zone, at the border between the hilus and the granule cell layer, and within the granule cell layer in both epileptic and control aged brains. When comparing the aged epileptic rats to age-matched controls, there was a significant increase in the number of DCX-labeled cells that was almost four and a half-fold. Therefore, aged rats also display an increase in adult neurogenesis following seizures.

Key words: Adult neurogenesis; dentate gyrus; subgranular zone; doublecortin; hippocampus; seizures; pilocarpine

Granule cells are continuously generated in the rodent adult dentate gyrus [1-3]. The newly generated cells have features similar to existing cells and some become integrated into hippocampal circuitry [4-6]. The rate of neurogenesis can be affected by a number of factors such as stress, exercise, an enriched environment and seizures [7-9].

The increased neurogenesis that occurs following seizures leads to several neuroplastic changes to the newly generated granule cells. These include ectopic granule cell migration into the hilus and the formation of hilar basal dendrites [7,10,11]. The hilar ectopic granule cells become integrated into the excitatory circuitry of the dentate gyrus [12,13], and display increased excitability [14]. Although hilar basal dendrites are commonly found on newly generated granule cells, they are mostly transient in normal conditions, whereas seizures increase their frequency [11], persistence [15,16] and the formation of synaptic input to them [17]. Of those synapses that are formed on them, greater than 90% are excitatory, or non-GABAergic [18], thus substantially increasing recurrent excitation as compared to normal granule cells. Therefore, these two cellular changes associated with increased neurogenesis following seizures contribute to neuronal circuits that appear to be proconvulsant.

In aged rodents, a decreased rate of neurogenesis is found in the dentate gyrus when compared to young rodents [3,8]. The substantial decrease with age is due to a reduction in the rate of proliferation of neural progenitor cells [3,19,20], in the number of neural progenitor cells [21], in the number of neurons generated by progenitor cells relative to glial cells [22], and in the proportion of progenitor cells that adopt a neuronal phenotype [4,8]. However, aged mice that are exercised or placed into an enriched environment show an increase

***Correspondence should be addressed to:** Dr. Lee A. Shapiro, Dept. of Surgery, College of Medicine, Texas A&M Health Science Center, Temple, TX 76504, USA. Email: lshapiro@medicine.tamhsc.edu

ISSN: 2152-5250

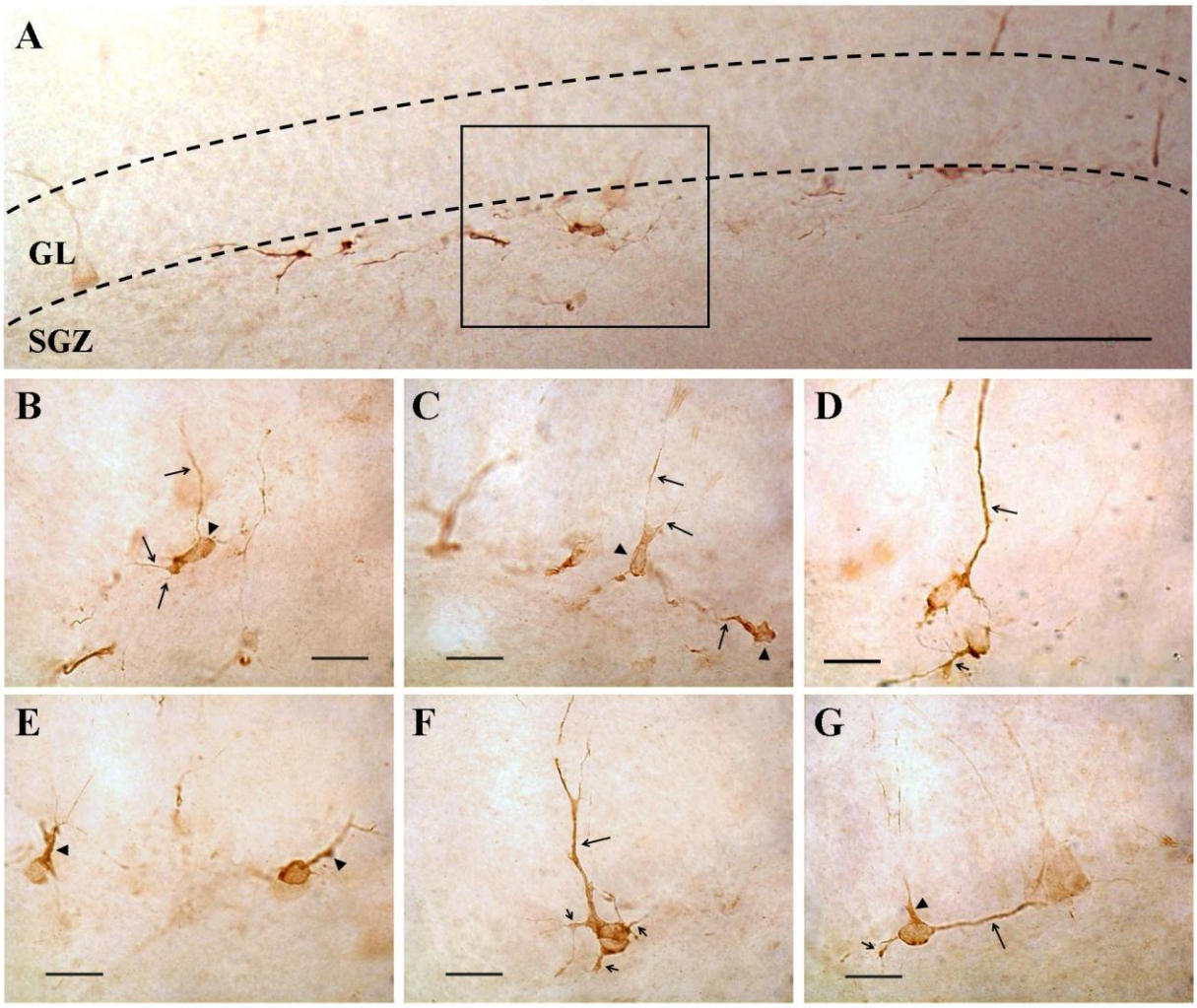

Figure 1. DCX-labeled cells from an aged control rat. **A** shows a low magnification of the SGZ and GL from an aged control rat. These sections were not counterstained with thionin so that the DCX-labeled cells would be more obvious. Note that very low numbers of DCX-labeled cell bodies along the border between the SGZ and GL. **B** shows an enlargement of the DCX-labeled cell indicated by the box in A. Not e that this cell has only a few thin processes (arrows) arising from its fusiform cell body (arrowhead). **C** shows three more examples of DCX-labeled cells with fusiform cell bodies (arrowheads) and thin dendrites (arrows). **D** shows two examples of DCX-labeled cells with multipolar dendrites. One of these cells has a prominent apical dendrite (long arrow) that passes through the GL while the other has a prominent basal dendrite (short arrow) in the SGZ. **E** shows two DCX-labeled cells with short thick proximal dendrites (arrowheads) but neither of them passes through the GL. The DCX-labeled cell in **F** has a prominent apical dendrite (long arrow) that courses through the GL but there are several smaller processes (short arrows) arising from its cell body and oriented in unusual directions. Panel **G** shows a DCX-labeled cell with a prominent basal dendrite (long arrow) and a stunted apical dendrite (arrowhead). Another basal process (short arrow), an axon or a dendrite, arises from the other side of this cell. Scale bars = 100 µm for **A** and 20 µm for **B-G**.

in the rate of neurogenesis [4,8], indicating that at least in some conditions, neurogenesis can be stimulated in aged rodents. However, unlike young adult rodents where seizures were shown to increase progenitor cell and newborn neuron proliferation, this relationship has not been shown following kainate acid-induced seizures in aged Fisher 344 rats [22] and has not been examined in other seizure models using aged rats. Therefore, the current study aimed to determine whether pilocarpine-induced seizures in aged Sprague-Dawley rats increase

neurogenesis in the dentate gyrus. Doublecortin (DCX) was used for this study because it has been shown to be a reliable marker for labeling immature neurons in the dentate gyrus and is found within these neurons from 4 hours up to three weeks after they are generated [23,24].

METHODS

Adult, male, Sprague-Dawley rats that were 22 months of age (680-850g, Charles River Labs, Wilmington, MA, USA) were used at the onset of this study. All experiments were performed in accordance with the Institutional Animal Care and Use Committee at the University of California, Irvine. Thirty minutes prior to seizure induction methyl-scopolamine (1mg/kg) was injected i.p., to reduce peripheral discomfort. To induce seizures, a single pilocarpine (300mg/kg, i.p.) injection was given to the rats. Animals were monitored for two hours to confirm that they demonstrated stage 5 seizures [25]. Animals that did not reach stage 5 seizures were omitted from the analysis. At two hours after seizure-induction, the rats were injected with diazepam (10mg/kg) to stop seizure activity. Control rats received identical treatment except pilocarpine was substituted with an equal quantity of 0.9% saline. During the recovery stage, the animals were placed in individual cages and provided with food and water ad libitum. The rats with stage 5 seizures were allowed to survive for 30 days (n = 5) after the initiation of seizures, as were age matched controls (n = 4).

It should be noted that there was a high rate of mortality (~50%) with the aged animals following pilocarpine-induced seizures. It is possible that aging, increased weight, declining liver function to break down pilocarpine, or some other factors contributed to this observed increased mortality rate.

Both epileptic and control rats, at 23 months of age, were anesthetized with an overdose of euthasol (1ml/kg), and perfused intracardially with 400 ml of saline followed by 400 ml of 4% paraformaldehyde in 0.1M phosphate buffer. The brains were allowed to sit within the skull for 48 h to improve their fixation and then they were extracted and post-fixed in 4% paraformaldehyde for 48 h. Coronal sections were cut at 50 µm with a Vibratome (Pelco 1000 Plus), and serial sections were collected into 12-well culture dishes containing 0.1M PBS.

All immunocytochemical reactions were carried out simultaneously using the same reagents for all animals. Please see Shapiro et al. [11] for details about the DCX-labeling method used. Sections containing DCX-labeled cells and their labeled dendrites were viewed using a Zeiss Axioplan light microscope. Images were captured using a Zeiss Axio-Vision camera. The number of DCX-labeled cells was quantified by using the Stereo Investigator program (MicroBrightfield Inc., Burlington, VT, USA). Briefly, every 12th hippocampal section was analyzed from each animal in the study. Since aged rats demonstrate lower rates of neurogenesis than young rats, we needed a stereological method that would only take random samples of 300 µm by 300 µm in areas of tissue that actually had cells. Thus, the preliminary step of manually selecting the boundaries was necessary to ensure that our cell counts were not artificially deflated. This was performed by using a cursor to draw a boundary around the portion of the granule cell layer immediately subjacent to the subgranular zone and including the underlying subgranular zone of the dentate gyrus of both the infra- and supra-pyramidal blades. These boundaries were drawn for four consecutive images per blade, starting from the apex and moving outward, with no sections overlapping. The program then randomly selected 300 µm by 300 µm square areas from which the number of DCX-labeled cell bodies would be counted. The counts were made by an individual who was blinded to the identity of the animals' treatment.

Descriptive statistics were calculated for the number of DCX-labeled cells to examine the variation in means for the group of control rats (n = 4) and in the pilocarpine-induced seizure group at 30 days post seizure (n = 5). Student's t-test was used to test for an overall difference between group means.

RESULTS

Hippocampal sections immunolabeled for DCX were examined from 23 month-old control animals. The location of DCX-immunolabeled cells in the dentate gyrus was similar to that previously described. Thus, DCX-labeled cell bodies were found at the border of the granule cell layer and the subgranular zone **(**Fig. 1**)**. Apical and basal dendrites were observed to extend from these DCX-labeled cell bodies. It should be noted that the frequency of DCX-labeled cell bodies along the border between the granule cell layer and the hilus was greatly reduced relative to that in young adult rats (cf. Fig. 2E from [26]). Thus, the DCX-labeled cells in the aged rat were sparsely located along the infra- and supra-pyramidal blades of the hippocampus, with slightly more of them located at the apex of the two blades. It should be noted that pairs and triads of DCX-labeled cell bodies were also observed.

The morphology of DCX-labeled cells in the aged rats also differed from that previously observed in young adult rats (cf. Fig. 2E from [26]). First, the shapes of their cell bodies ranged from fusiform to round **(**Fig. 1**)**.

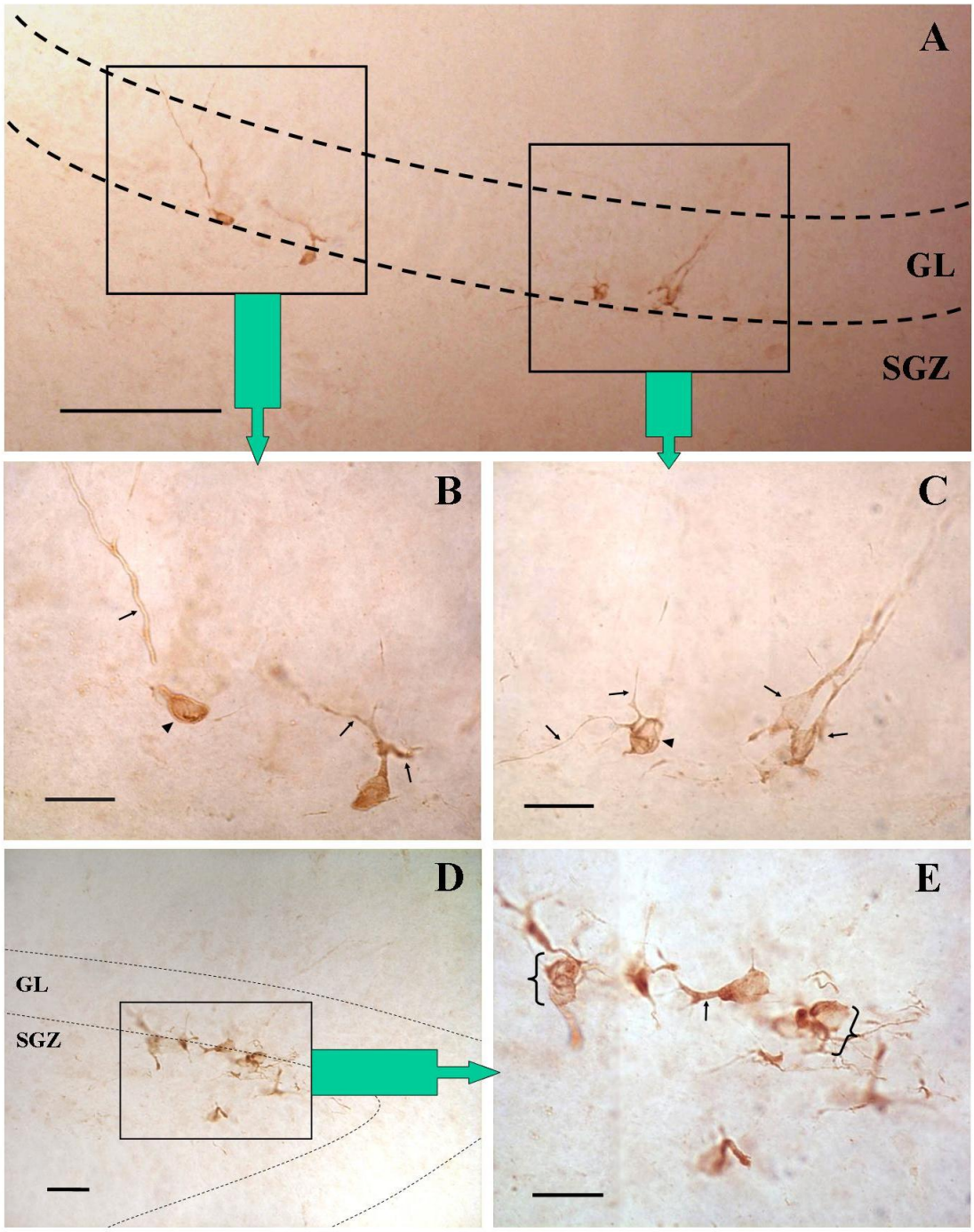

Figure 2. DCX-labeled cells from an aged epileptic rat. A shows a low magnification of the SGZ and GL from an aged epileptic rat. These sections were also not counterstained with thionin so that the details of the DCX-labeled cells would be obvious. **B** and **C** show enlargements of the DCX-labeled cells indicated by the boxes in **A**. Note that the cells in **B** lack apical dendrites that pass orthogonally through the GL. In contrast, two (arrows) of the three DCX-labeled cells in **C** have prominent apical dendrites that pass through the GL. Note that both of these cells with prominent apical dendrites are closely grouped. The third cell (arrowhead) has dendrites (arrows) that are thin and mis-oriented. **D** and **E** are low and high magnification images, respectively, of the same group of DCX-labeled cells (box in **D** is enlarged in **E**). Several of the cells are grouped into pairs and triads (brackets in **E**). None of these DCX-labeled cells display prominent apical dendrites that pass through the GL but instead have basal dendrites (arrow). Scale bars = 100 µm for **A** and 20 µm for **B - E**.

In contrast, most of the DCX-labeled cell bodies found in young adult rats were round [26]. Second, the dendrites arising from the DCX-labeled cell bodies mainly stayed in the subgranular zone running parallel to the granule cell layer. Although some entered the granule cell layer, most of their dendritic processes did not extend through the granule cell layer and into the molecular layer. In contrast, the majority of the dendrites from DCX-labeled cell bodies in young adult rats entered the granule cell layer [24].

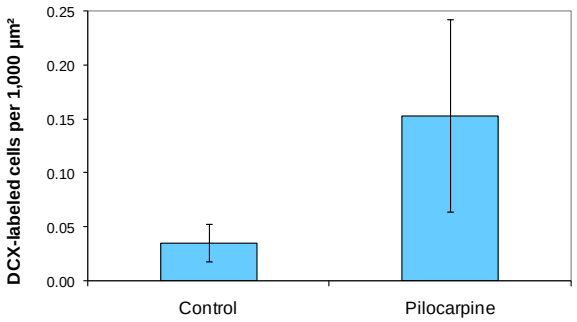

Figure 3. DCX-labeled cells in the dentate gyrus. Quantitative data on the mean number of DCX-labeled cells per unit area (1,000 μm^2) are plotted for both aged control and aged epileptic rats (30 days post seizures). The control animals (n = 4) had a mean of 0.035 newly generated cells (σ = 0.017) per unit area. In contrast, the aged epileptic rats (pilocarpine) (n = 5) had a mean of 0.154 newly generated cells (σ = 0.089) per unit area. This increase was 4.36 times that of the control group. The Student's t-test showed that this increase was statistically significant (t = 2.899 and a P < 0.03). Vertical thin line on each bar indicates the standard deviation ($\pm\sigma$) for the corresponding group.

DCX-labeled cells from the dentate gyrus of pilocarpine-injected, aged rats were found in the same location as described above for control rats (Fig. 2). In addition, the DCX-labeled cells from epileptic aged rats exhibited irregular-shaped cell bodies with flattened and elliptical shapes similar to those in aged-matched control rats. It should be noted that the DCX-labeled cell bodies in epileptic aged rats were also commonly observed in groups of two or three, especially near the apex of the two blades of the granule cell layer (Fig. 2). Similar to the control aged rats, most of the dendrites arising from the DCX-labeled cell bodies from epileptic aged rats lacked apical dendrites that passed through the granule cell layer.

The quantitative data on the mean number of DCX-labeled cells per unit area were based on an average of 24 images that had DCX-immunolabeling and are reported per 1,000 μm^2. The control animals (n = 4) showed 0.024, 0.061, 0.026, and 0.031 DCX-positive cells, for a mean total of 0.035. Animals treated with pilocarpine injections (n = 5) showed 0.165, 0.056, 0.082, 0.185, and 0.280 DCX-positive cells, with the mean number of newly generated cells being 0.154, or 4.36 times that of the control mean (Fig. 3). Statistical analysis using Student's t-test showed that this increase was statistically significant (t= 2.899, P < 0.03). Overall mean coefficient of error of estimating the DCX-labeled cells over a 90,000 μm^2 field for the aged epileptic rats was 9.4% (see Table 1).

DISCUSSION

The main finding of this study was the significant increase in the number of DCX-labeled cells in epileptic, aged rats as compared to control, aged rats. These data show that with relative respect to a seizure-induced increase in neurogenesis at 30 days after pilocarpine-induced seizures, the aged brain responds similarly to the young adult brain, despite the relatively low number of DCX-labeled newly generated cells in the aged rodent brain.

This effect of status epilepticus on the number of newborn neurons has not been previously shown in aged animals. A previous study reported no significant change in the number of DCX-positive cells in aged epileptic rats as compared to aged control rats [22] and this discrepancy could be explained by a few differences in the methods used. First, Rao et al. [22] used a series of graded intraperitoneal injections of kainic acid as a chemoconvulsant, whereas the data in the present study were obtained using a single dose of pilocarpine. Kainic acid and pilocarpine mediate their convulsant effects through two different mechanisms, each affecting a different neurotransmitter receptor. Kainic acid has its effect through the glutamate synapse and pilocarpine through the acetylcholine synapse. Second, Rao et al. [22] used Fisher 344 rats (F344) while the present study employed Sprague-Dawley rats. A strain difference could provide the basis for differences in results. For example, some mice strains with kainic acid-induced epilepsy show no neuronal loss in the hippocampus whereas other strains show robust hippocampal cell loss [27]. Third, Rao et al. [22] examined their Fisher 344 rats 12 days following kainic seizures, and the current study examined neurogenesis 30 days after the pilocarpine-induced seizures. The significance of having a longer survival period is that spontaneous seizures caused by the chemoconvulsant do not usually begin until about a month after the initial pilocarpine-induced status epilepticus and the spontaneous seizures have been previously demonstrated to increase neurogenesis [7].

Table 1. Comparison of DCX-labeled cells in control and aged epileptic rats (30 days post seizure) per 1,000µm². Refer to Larsen [30] for the method used to estimate the coefficient of error (CE) and the overall mean CE of counted cells.

Rat Index	Number of DCX-labeled cells per 1,000µm²		CE of Pilocarpine Group (over 90,000µm² field)
	Control Group	Pilocarpine Group	
1	0.024	0.165	7.6%
2	0.061	0.056	7.9%
3	0.026	0.082	14.3%
4	0.031	0.185	3.2%
5	-	0.280	10.2%
Mean (±σ)	**0.035 ± 0.017**	**0.154 ± 0.089**	**Overall mean CE of epileptic group: 9.4%**

Fourth, Rao et al. [22] used rats 24 months of age, whereas the current study examined 23 month old rats. And fifth, Rao et al. [22] used a stereological counting method similar to that used in young adult rats, whereas the present study used a modified stereological method to take into account the much smaller population of DCX-labeled cells in aged rats.

A second study using the pilocarpine epilepsy model failed to show basal dendrites on newborn neurons at 2 months following seizures that were induced in 20-month old rats [28]. It is important to note that in this latter study, very few newborn granule cells were reported, with the values of newborn neurons appearing to be almost 0 cells per mm^2 (see Fig. 2 [28]). Considering this very low baseline of neurogenesis in their aged-animals, it is not surprising that very few cells with basal dendrites were observed. Furthermore, they used a method of counting that included tracing the entire granule cell layer, whereas the current study only traced the portion of the dentate gyrus where the majority of newborn neurons are located. In the present study, DCX-labeled cells with prominent basal dendrites were clearly observed (Figs 1 and 2). Another difference between the studies is that Avanzi et al. [28] only gave a half-dose (160 mg.kg) of pilocarpine to the aged animals. While it is unclear if a lower dose of pilocarpine has differential effects on neurogenesis, previous studies have demonstrated a relationship between seizure severity and altered neurogenesis. Moreover, Avanzi et al. [28] examined the tissue at 2 months after pilocarpine-induced seizures in 22-month-old rats, whereas analysis for the current study was conducted 1 month after pilocarpine in 23-month-old rats. At later timepoints relative to the 30 days examined in the present study, previous studies have demonstrated that seizure-induced neurogenesis is decreased, as opposed to the initial increases observed at the earlier timepoints [29]. Therefore, there are several possible explanations as to why these studies differed in regard to seizure-induced effects on hippocampal neurogenesis.

Another interesting characteristic of the cells in aged animals was their tendency to appear in groups of 2 or 3. These groupings consisted of cells located in close proximity to one another, with cell bodies typically separated by no more than 5 µm. Both the control and aged epileptic rats showed this grouping phenomenon and there were no significant differences between the two groups (data not shown). The reason for these groups to appear in aged rats is unclear. It is possible that the rate of migration into the granule cell layer is different in aged animals, and this could be the cause for greater numbers of cells in clusters. Another possibility is that there are fewer progenitor cells that produce neurons in aged rats. Thus, seizure-induced stimulation of progenitor cell proliferation might result in increased neuron production by these few progenitor cells. Other possibilities such as altered guidance cues or an alteration to the molecular milieu also cannot be excluded.

In addition to environmental enrichment, this study shows that seizures also have the potential to enhance neurogenesis in the aged rodent brain. Although it is unclear whether seizures result in aberrant circuit formation in the aged rat as it does in younger adult rats, the results from this study support the idea that progenitor cells persist in the aged rat brain. Understanding the mechanisms regulating neurogenesis in the adult and aged brain are important for understanding how these cells might be a therapeutic target.

Acknowledgments

This material is the result of work supported with resources and the use of facilities at the Central Texas Veterans Health Care System, Temple, Texas. Grant funding came from NIH and the Epilepsy Foundation through the generous support of the Patricia L. Nangle fund. Grant numbers: NS038331 and EF-42056, respectively.

References

[1] Kaplan M, Hinds J (1977). Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science, 197:1092-1094.

[2] Bayer SA, Yackel JW, Puri PS (1982). Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science, 216:890-892.

[3] Kuhn HG, Dickinson-Anson H, Gage FH (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci, 16:2027-2033.

[4] van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002). Functional neurogenesis in the adult hippocampus. Nature, 415:1030-1034.

[5] Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH (2007). Synapse formation on neurons born in the adult hippocampus. Nat Neurosci, 10:727-734.

[6] Toni N, Laplagne DA, Zhao C, Lombardi G, Ribak CE, Gage FH, Schinder AF (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. Nat Neurosci, 11:901-907.

[7] Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci, 17:3727-3738.

[8] Kempermann G, Kuhn HG, Gage FH (1998). Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci, 18:3206-3212.

[9] van Praag H, Shubert T, Zhao C, Gage FH (2005.) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci, 25:8680-8685.

[10] Scharfman HE, Goodman JH, Sollas AL (2000). Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. J Neurosci, 20:6144-6158.

[11] Shapiro LA, Korn MJ, Ribak CE (2005). Newly generated dentate granule cells from epileptic rats exhibit elongated hilar basal dendrites that align along GFAP-immunolabeled processes. Neurosci,136:823-831.

[12] Dashtipour K, Tran PH, Okazaki MM, Nadler JV, Ribak CE (2001). Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer. Brain Res, 890:261-271.

[13] Pierce JP, Melton J, Punsoni M, McCloskey DP, Scharfman HE (2005). Mossy fibers are the primary source of afferent input to ectopic granule cells that are born after pilocarpine-induced seizures. Exp Neurol, 196:316-331.

[14] Scharfman HE, Sollas AE, Berger RE, Goodman JH, Pierce JP (2003). Perforant path activation of ectopic granule cells that are born after pilocarpine-induced seizures. Neurosci, 121:1017-1029.

[15] Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV (2000). Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. J Comp Neurol, 428:240-253.

[16] Shapiro LA, Ribak CE (2005). Integration of newly born dentate granule cells into adult brains: hypotheses based on normal and epileptic rodents. Brain Res Rev, 48:43-56.

[17] Shapiro LA, Ribak CE (2006). Newly born dentate granule neurons after pilocarpine-induced epilepsy have hilar basal dendrites with immature synapses. Epilepsy Res, 69:53-66.

[18] Thind KK, Ribak CE, Buckmaster PS (2008). Synaptic input to dentate granule cell basal dendrites in a rat model of temporal lobe epilepsy. J Comp Neurol, 509:190-202.

[19] McDonald HY, Wojtowicz JM. 2005. Dynamics of neurogenesis in the dentate gyrus of adult rats. Neurosci Lett, 385:70-75.

[20] Rao MS, Hattiangady B, Abdel-Rahman A, Stanley DP, Shetty AK (2005). Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. Eur J Neurosci, 21:464-476.

[21] Olariu A, Cleaver KM, Cameron HA (2007). Decreased neurogenesis in aged rats results from loss of granule cell precursors without lengthening of the cell cycle. J Comp Neurol, 501:659-667.

[22] Rao MS, Hattiangady B, Shetty AK (2008). Status epilepticus during old age is not associated with enhanced hippocampal neurogenesis. Hippocampus, 18:931-944.

[23] Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, Kuhn HG (2003). Enriched environment and physical activity stimulate

hippocampal but not olfactory bulb neurogenesis. Eur J Neurosci, 17:2042-2046.

[24] Shapiro LA, Upadhyaya P, Ribak CE (2007). Spatio-temporal profile of dendritic outgrowth from newly born granule cells in the adult rat dentate gyrus. Brain Res, 1149: 30-37.

[25] Racine RJ (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol, 32:281-294.

[26] Ribak CE, Korn MJ, Shan Z, Obenaus A (2004). Dendritic growth cones and recurrent basal dendrites are typical features of newly generated dentate granule cells in the adult hippocampus. Brain Res, 1000:195-199.

[27] Schauwecker PE, Steward O (1997). Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. Proc Natl Acad Sci USA, 94:4103-4108.

[28] Avanzi RDT, Cavarsan CF, Santos Jr JG, Hamani C, Mello LE, Covolan L (2010). Basal dendrites are present in newly born dentate granule cells of young but not aged pilocarpine-treated chronic epileptic rats. Neurosci, 170: 687-691.

[29] Kuruba R, Hattiangady B, Shetty AK (2009). Hippocampal neurogenesis and neural stem cells in temporal lobe epilepsy. Epilepsy and Behav, Supp 1: 65-73.

[30] Larsen JO (1998). Stereology of nerve cross sections. J Neurosci Methods. 85:107-18.