Supporting Information

Adult neurogenesis in the mouse dentate gyrus protects the hippocampus from neuronal injury following severe seizures

Swati Jain, John J. LaFrancois, Justin J. Botterill, David Alcantara-Gonzalez, Helen E. Scharfman

Supporting Information Table S1

DNA	Primers	Seq 5' to 3'	Barcode ID
TK	TKup	GCAGCAAGAAGCCACGGAAGTCC	1018927138
	TKdown	GCGAATCGCAGCCAGCATAGCC	1018923467
Cre	CreUP	AATGCTTCTGTCCGTTTGC	1022833414
	CreDown	TAGCGCCGTAAATCAATCG	1022833415

Table S1: Primers used for genotyping

The genotypes of mice were determined by polymerase chain reaction (PCR) of DNA extracted from a tail sample. The primers for individual DNA (TK or Cre, Eurofins Genomics, Louisville, KY) are listed.

Supporting Information Table S2

Type of control	Male			Female
	FJ	SE analysis	Power analysis	
1) Tamoxifen-treated NestinCre-	7/10	6/9	5/7	10/13
2) Vehicle-treated NestinCre+	3/10	0	0	3/13
3) Vehicle-treated NestinCre-	0	3/9	2/7	0

Table S2: Numbers of control NestinCreER^{T2}*Bax*$^{f/f}$ mice

The number of male mice in each type of control are listed for 1) Tamoxifen-treated NestinCre-, 2) Vehicle-treated NestinCre+, and 3) Vehicle-treated NestinCre- mice used for FJ staining and EEG. The number of control female mice that had pilocarpine injection are listed for each type of control.

Supporting Information Fig. S1

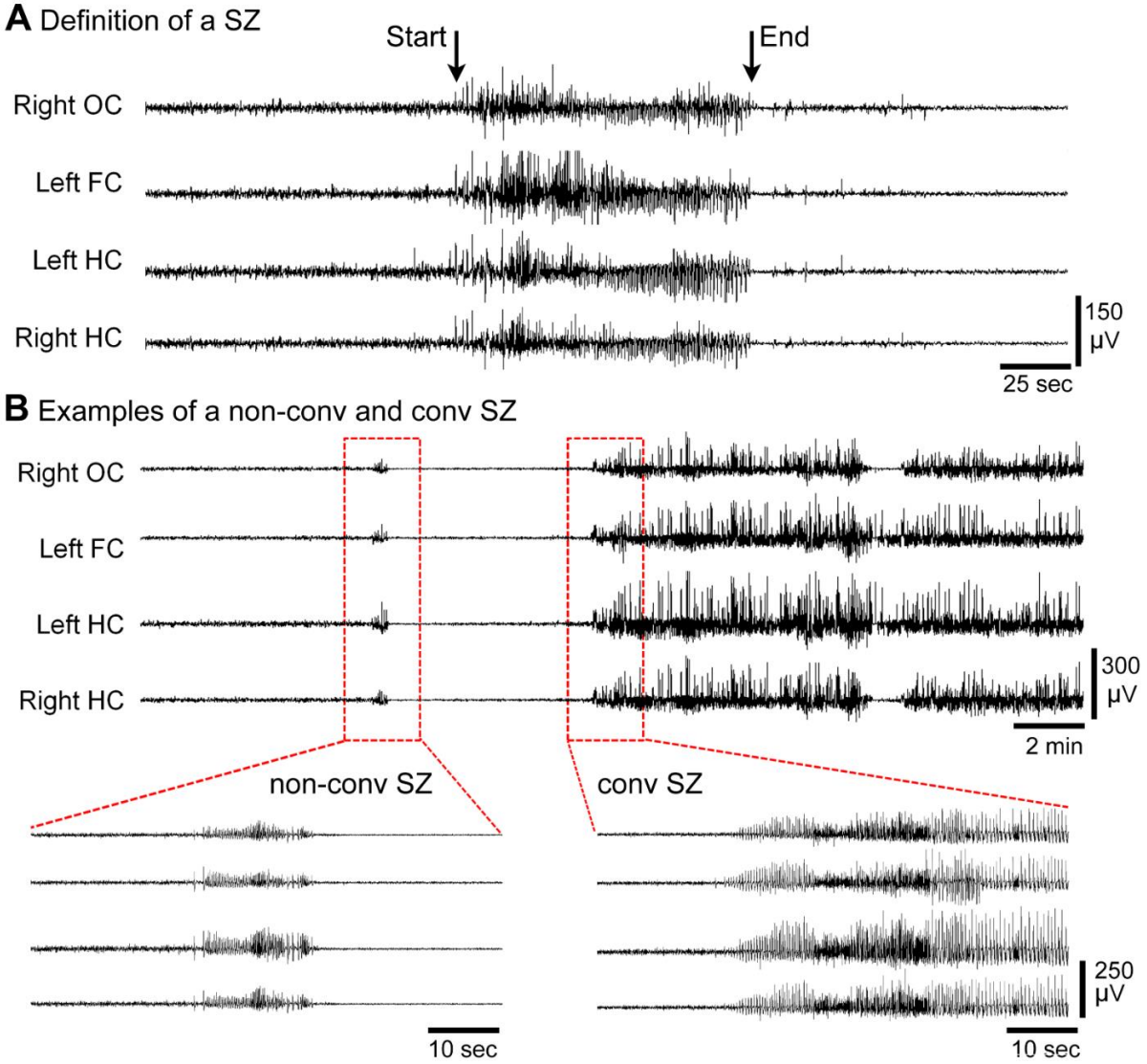


Fig. S1: Definition and examples of a seizure

A. A seizure was defined as a period of rhythmic (>3 Hz) deflections that were >2x the standard deviation of baseline mean noise and lasted at least 10 secs (Fisher et al., 2014; Cho et al., 2015; Iyengar et al., 2015; Trinka et al., 2015; Hosford et al., 2016).

B. Representative traces are shown for a non-convulsive seizure (non-conv SZ, defined as an electrographic seizure without stage 3-5 behavioral convulsions) and a convulsive seizure (conv SZ, an electrographic seizure accompanied by stage 3-5 behavioral convulsions; Racine, 1972).

Supporting Information Fig. S2

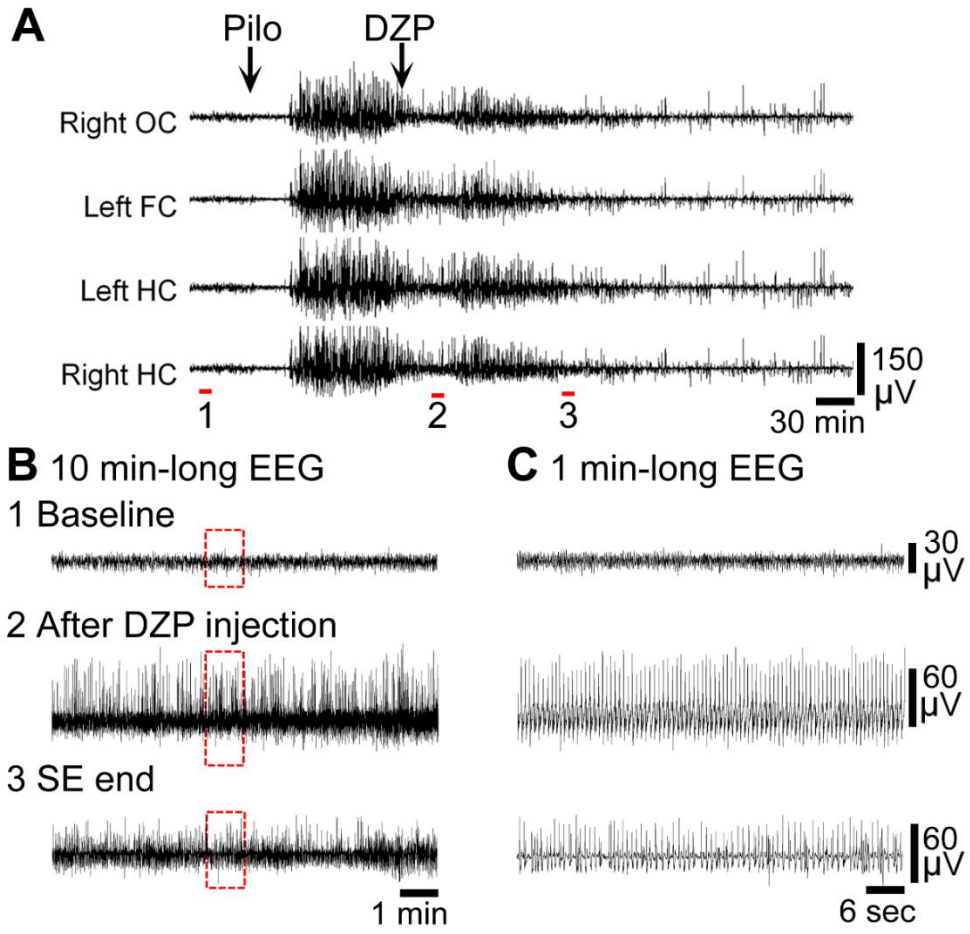


Fig. S2: Definition of the end of SE

A. Representative example of an 8 h-long EEG which includes the period used as a baseline and also shows SE. Criteria to define the end of SE for the purposes of this study were 1) when EEG amplitude was reduced to approximately 2 times the amplitude of the baseline peak-to-peak noise, 2) the decline occurred in at least 3 channels, and 3) the decline persisted for at least 10 min. The last criterion was adopted because when the decline lasted 10 min it did not return to a large amplitude.

B. The EEG indicated by red bars in A are expanded to show the differences between the baseline period (no seizure activity), period of SE after DZP had been injected (high amplitude seizure activity) and when the amplitude of the EEG substantially decreased (the end of SE, as defined in A).

C. The areas surrounded by the red boxes in B are expanded.

Supporting Information Fig. S3

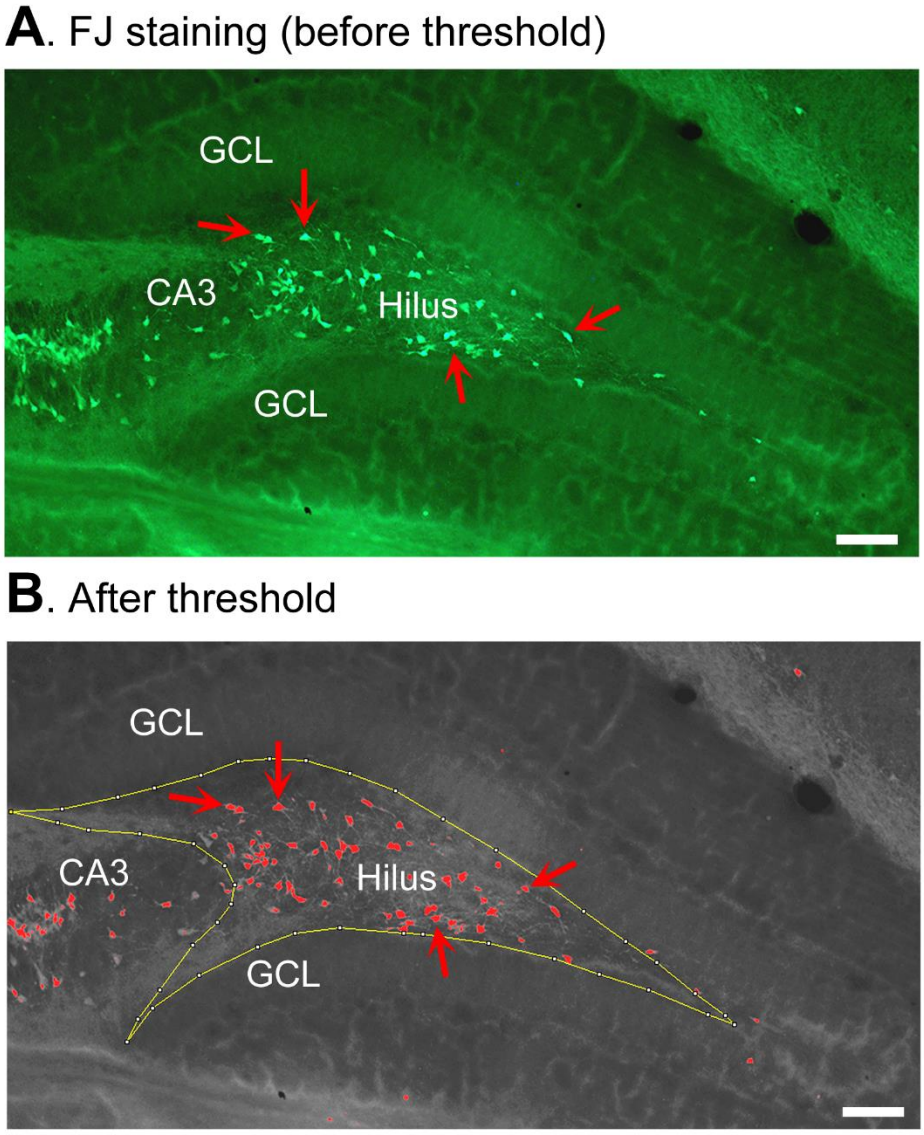


Fig. S3: Analysis of hilar FJ+ cells

A. An example of FJ staining in the DG is shown. Calibration, 100 µm. Red arrows indicate FJ+ cells.

B. Thresholding of hilar FJ+ cells (red arrow) is shown. The surrounded area demarcates the hilus. Calibration, 100 µm.

Supporting Information Fig. S4

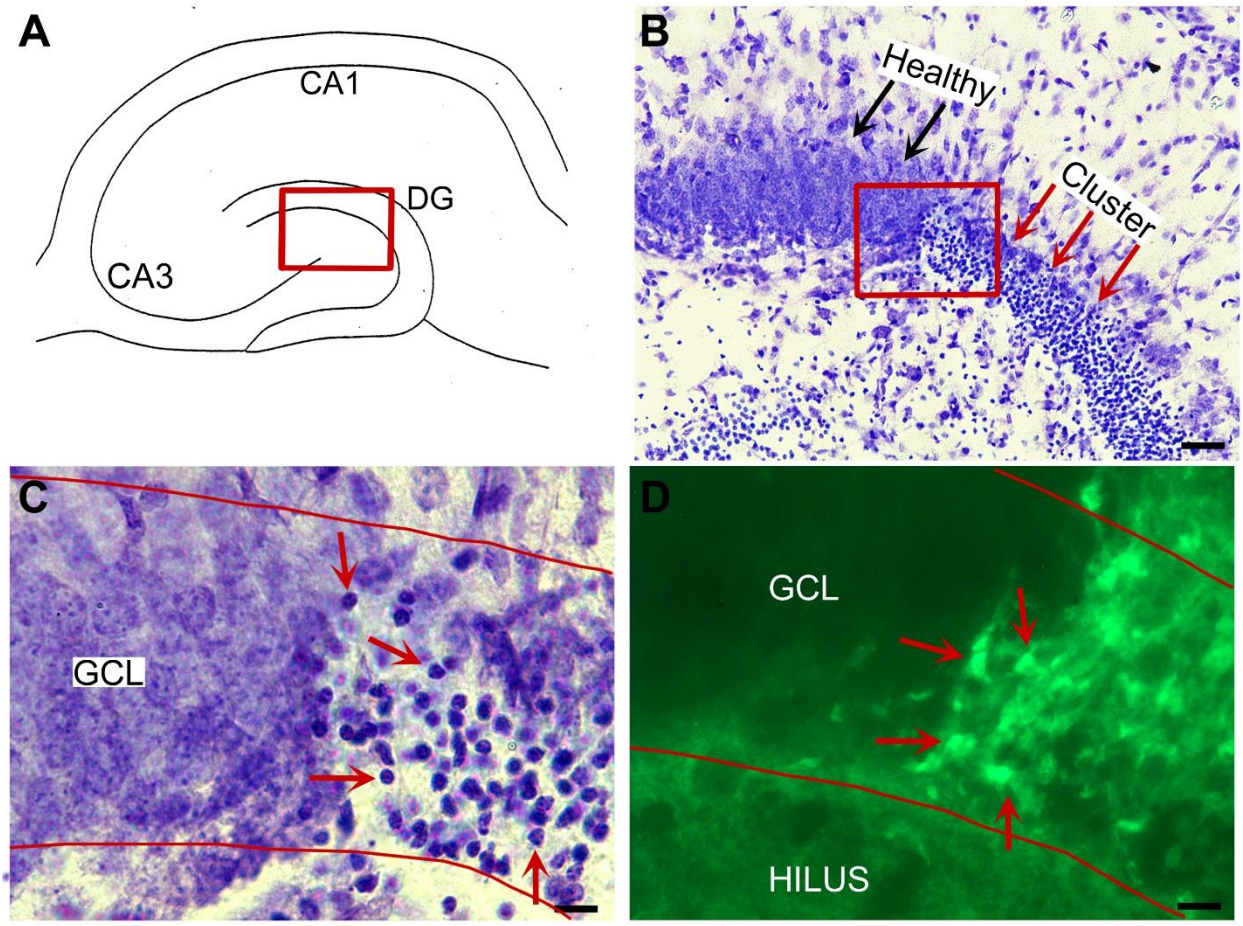


Fig. S4: GCL staining of damaged cells following SE with cresyl violet and FJ

A. A schematic illustration of the hippocampus.

B. Cresyl violet staining of the area surrounded by the box in A in mice with reduced neurogenesis showing healthy cells (black arrows) and a large area of clustered cells which often look abnormal because they are shrunken, dark and unlike the healthy cells. This 'cluster' is indicated by the red arrows. Calibration, 30 µm.

C. The area surrounded by box in B is shown at higher power. Red arrows point to abnormal cells. The red lines are the approximate locations of the borders of the GCL. Calibration, 10 µm.

D. In a section adjacent to the one used in C, FJ staining shows that many cells are FJ+. The FJ staining corresponds to the cells in the cresyl violet-stained cluster, rather than the cells in the area that appeared to be healthy based on cresyl violet staining. However, from section to section the exact location of the abnormal cells

was slightly different so there is not a perfect correspondence between abnormal cells in C and FJ+ cells in D. The red arrows in D point to some of the FJ+ cells. Calibration, 10 μm.

Supporting Information Fig. S5

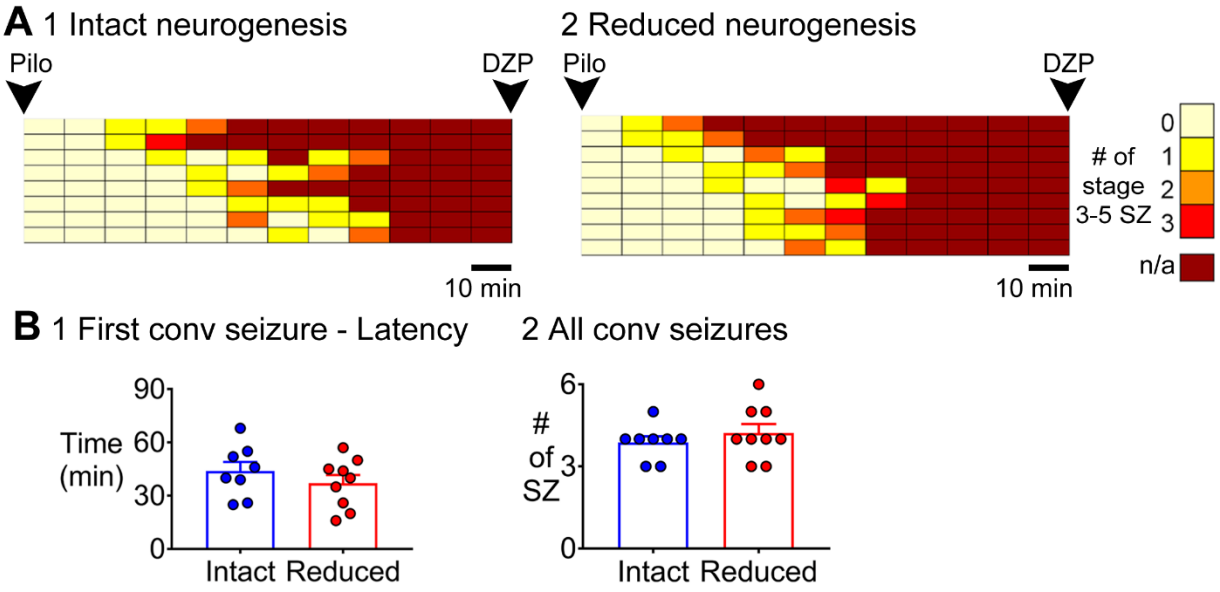


Fig. S5: Similarities of SE in mice without electrode implantation with intact and reduced adult neurogenesis

A. Data from mice with intact and suppressed neurogenesis are shown. These mice did not have electrodes implanted. The 2 h between pilocarpine and diazepam injection were divided into 10 min-long bins as shown in Fig. 3. Each row reflects data from a different mouse. GFAP-TK-: intact neurogenesis (n=8); GFAP-TK+: reduced neurogenesis (n=9).

B. 1. The latency to the onset of first convulsive seizure after pilocarpine injection was similar in mice with intact and reduced neurogenesis [Student's t-test, $t(15) = 1.0$; $p = 0.336$]. 2. The total number of convulsive seizure following pilocarpine injection was similar also [Student's t-test, $t(15) = 0.8$; $p = 0.405$]. Animals used for B1-2 are the same as those used in A.

Supporting Information Fig. S6

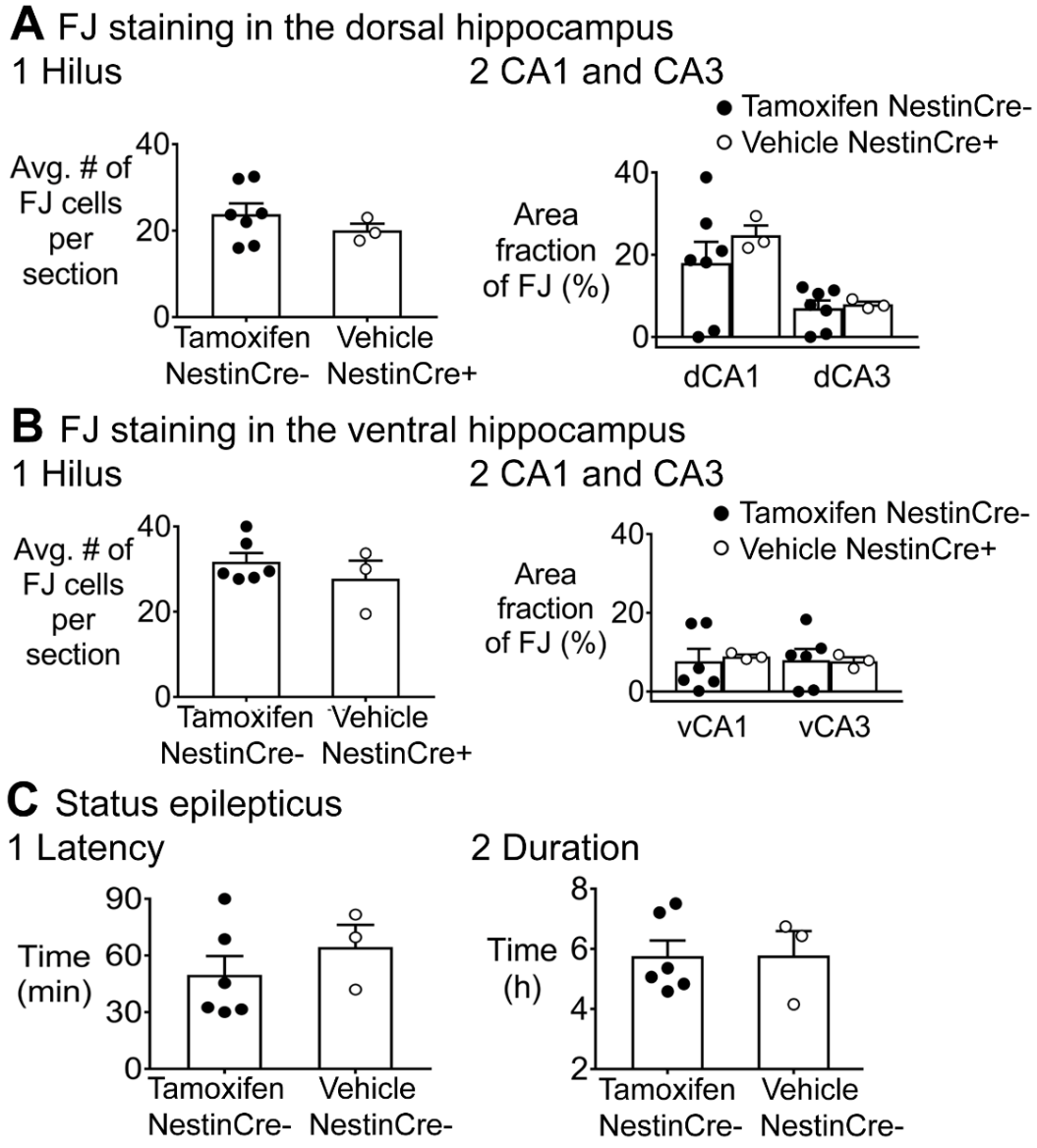


Fig. S6: Similarities in different types of NestinCreER^{T2}*Bax*$^{f/f}$ control mice

A. There were 3 types of controls (See Supplementary Information Table S2). 1. The number of FJ+ cells in dorsal hilus were similar in tamoxifen-treated NestinCre- and vehicle-treated NestinCre+ mice [Student's t-test, t(8) = 0.9; p = 0.381]. 2. There was no effect of treatment on FJ staining in dorsal CA1 and CA3 (Kruskal-Wallis test, H = 7.4, p = 0.058).

B. 1. The number of FJ+ cells in ventral hilus were similar in tamoxifen-treated NestinCre- and vehicle-treated NestinCre+ mice [Student's t-test, t(7) = 0.9; p =

0.368]. 2. There was no effect of treatment on FJ staining in ventral CA1 and CA3 (Kruskal-Wallis test, H = 0.5 p = 0.927).

C. 1. The latency to the onset of SE was similar in tamoxifen- or vehicle-treated, NestinCre- mice [Student's t-test, t(7) = 0.9; p = 0.403]. 2. The duration of SE was also similar [Student's t-test, t(7) = 0.02 t; p = 0.985].

Supporting Information Fig. S7

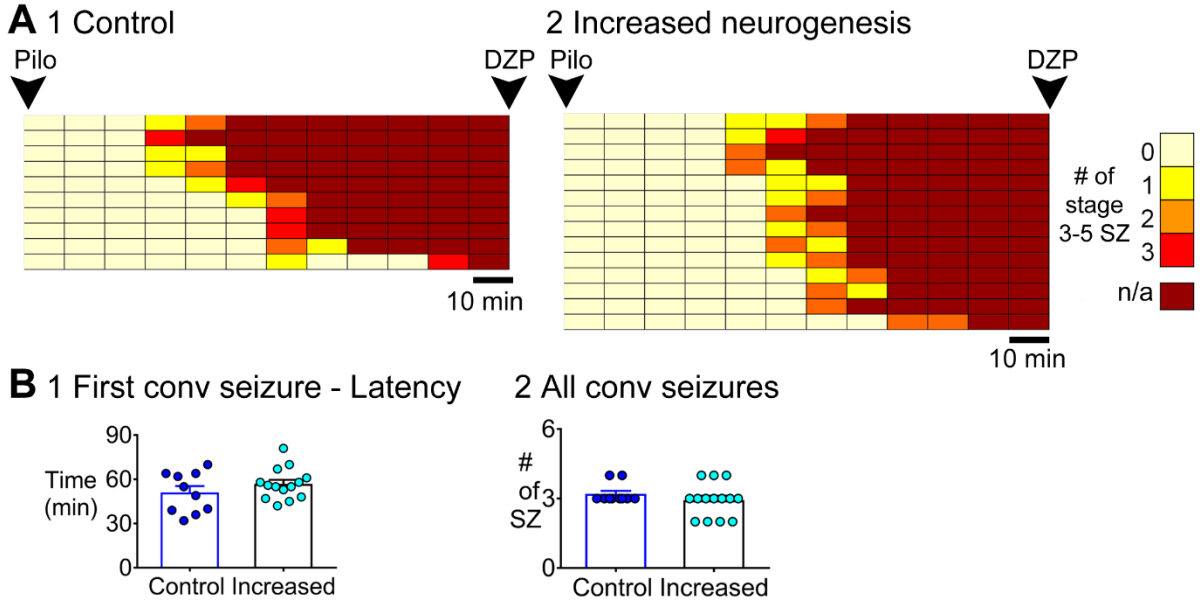


Fig. S7: Similarities of SE in mice without electrode implantation with increased adult neurogenesis and control mice

A. The 2 h between pilocarpine and diazepam injection was divided into 10 min-long bins as shown in Fig. 3. Each row is for a different mouse. Controls: tamoxifen-treated NestinCre- (n=7) and vehicle-treated NestinCre+ (n=3); Increased neurogenesis: tamoxifen-treated NestinCre+, n=14.

B. 1. The latency to the onset of first seizure was similar [Student's t-test, $t(22) = 0.2$; $p = 0.256$]. 2. Total number of convulsive seizure per mouse were similar between control and mice with increased neurogenesis [Mann-Whitney's U test, $U = 55$; $p = 0.339$]. Animals in A and B are the same.

Supporting Information Fig. S8

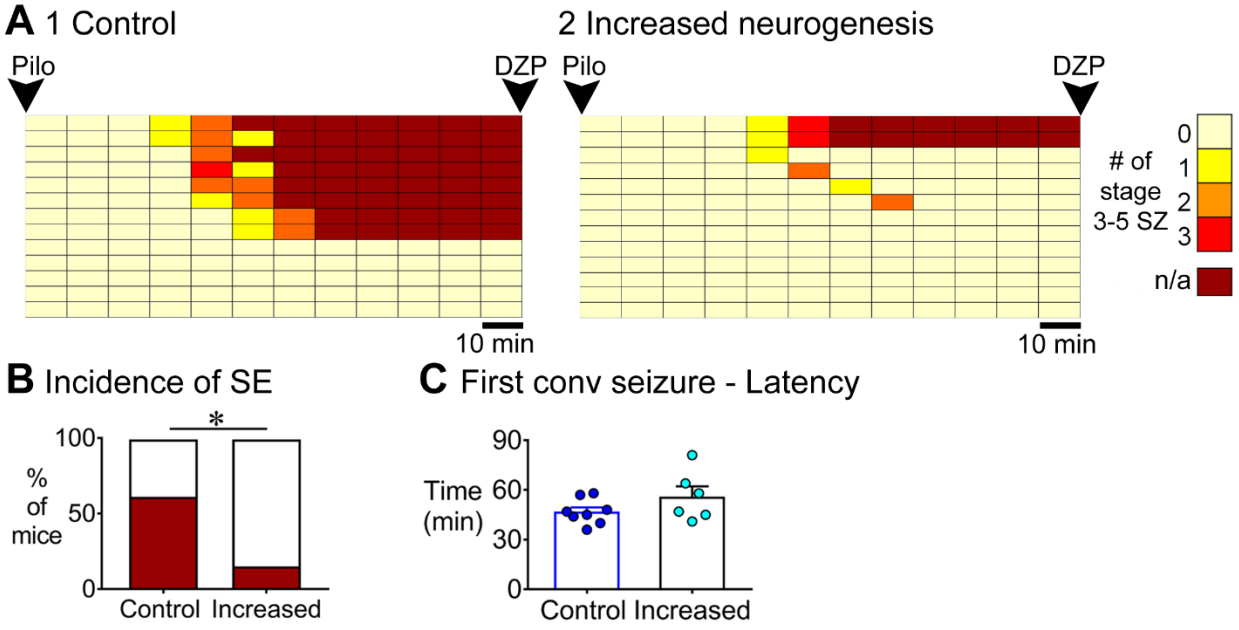


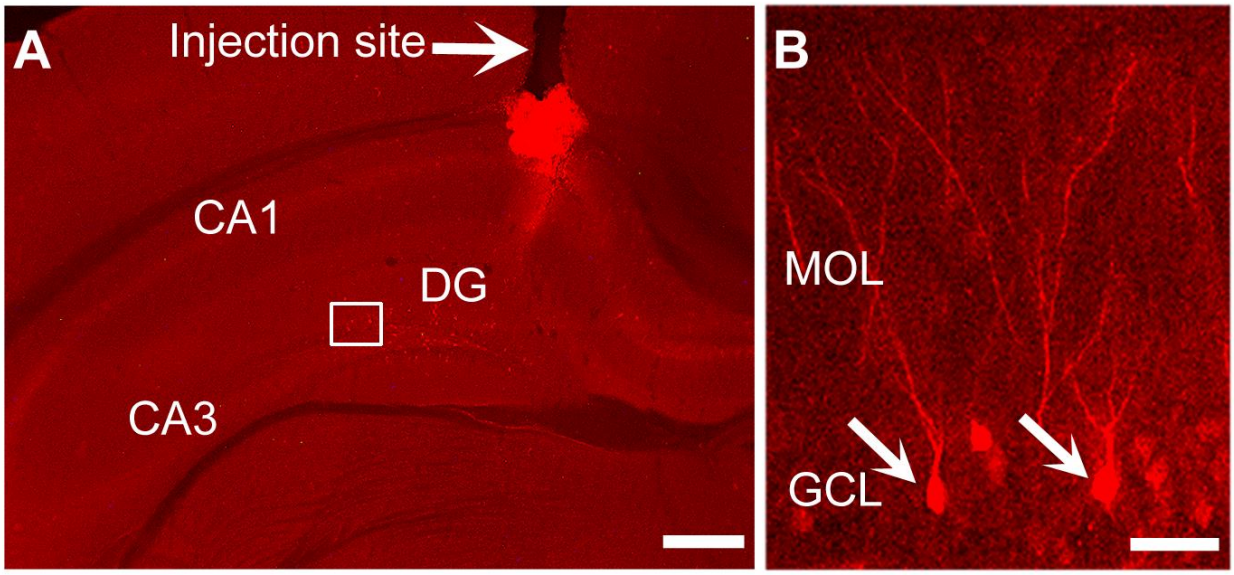
Fig. S8: Lower incidence of SE in females with increased adult neurogenesis compared to controls

A. The 2 h between pilocarpine and diazepam injection was divided into 10 min-long bins as described in Fig. 3. The data show that there were few mice with increased neurogenesis with SE. Each row is for a different mouse.

B. The incidence of SE was significantly reduced in mice with increased neurogenesis than control (control: 8/13, 61.5%; increased: 2/13, 15.28%, Fisher's exact test; $p = 0.041$).

C. The latency to the first convulsive seizure was not significantly different [Student's t-test, $t(12) = 1.5$; $p = 0.160$]. Same animals as B.

Supporting Information Fig. S9



A. A representative example of dorsal hippocampus 3 weeks after viral injection of AAV5-mCherry. Arrow indicates the injection site. Cells expressing mCherry were only in the DG. Calibration, 75 µm.

B. The area surrounded by the box in A is shown at higher power. Cells expressing mCherry (white arrows) had the morphology of granule cells. Calibration, 30 µm.