Hippocampal transplants of fetal GABAergic progenitors regulate adult neurogenesis in mice with temporal lobe epilepsy

Muhammad N. Arshad, Simon Oppenheimer, Jaye Jeong, Bilge Buyukdemirtas, Janice R. Naegele

Hall-Atwater Laboratory, Wesleyan University, Department of Biology, Program in Neuroscience and Behavior, Middletown, CT 06459-0170, USA

Abstract

GABAergic interneurons play a role in regulating adult neurogenesis within the dentate gyrus (DG) of the hippocampus. Neurogenesis occurs within a stem cell niche in the subgranular zone (SGZ) of the DG. In this niche, populations of neural progenitors give rise to granule cells that migrate radially into the granule cell layer (GCL) of the DG. Altered neurogenesis in temporal lobe epilepsy (TLE) is linked to a transient increase in the proliferation of new neurons and the abnormal inversion of Type 1 progenitors, resulting in ectopic migration of Type 3 progenitors into the hilus of the DG. These ectopic cells mature into granule cells in the hilus that become hyperexcitable and contribute to the development of spontaneous recurrent seizures. To test whether grafts of GABAergic cells in the DG restore synaptic inhibition, prior work focused on transplanting GABAergic progenitors into the hilus of the DG. This cell-based therapeutic approach was shown to alter the disease phenotype by ameliorating spontaneous recurrent seizures in mice with pilocarpine-induced TLE. Prior optogenetic and immunohistochemical studies demonstrated that the transplanted GABAergic interneurons increased levels of synaptic inhibition by establishing inhibitory synaptic contacts with adult-born granule cells, consistent with the observed suppression of seizures. Whether GABAergic progenitor transplantation into the DG ameliorates underlying abnormalities in adult neurogenesis caused by TLE is not known. As a first step to address this question, we compared the effects of GABAergic progenitor transplantation on Type 1, Type 2, and Type 3 progenitors in the stem cell niche using cell type-specific molecular markers in naïve, non-epileptic mice. The progenitor transplantation increased...
GABAergic interneurons in the DG and led to a significant reduction in Type 2 progenitors and a concomitant increase in Type 3 progenitors. Next, we compared the effects of GABAergic interneuron transplantation in epileptic mice. Transplantation of GABAergic progenitors resulted in reductions in inverted Type 1, Type 2, and hilar ectopic Type 3 cells, concomitant with an increase in the radial migration of Type 3 progenitors into the GCL. Thus, in mice with Pilocarpine induced TLE, hilar transplants of GABA interneurons may reverse abnormal patterns of adult neurogenesis, an outcome that may ameliorate seizures.

Keywords
Granule cell; Dentate; hippocampus; GABA; Pilocarpine; Doublecortin; Neural stem cells; Subgranular zone

1. Introduction

Temporal lobe epilepsy (TLE) is characterized by recurrent spontaneous seizures that originate in the temporal lobes, due to excessive synchronous neuronal firing (Wickham et al., 2019). In severe TLE, characteristic pathological changes in the DG include granule cell dispersion, GABAergic interneuron loss, and astrogliosis; a suite of histopathological changes referred to as hippocampal sclerosis (Mathern et al., 2002; Losi et al., 2012; Asadi-Pooya et al., 2017; Liu et al., 2020; Ammothumkandy et al., 2022). TLE patients often become resistant to anti-convulsant drugs, and may be referred for surgical resection of the hippocampus (Asadi-Pooya et al., 2017) (López-Rivera et al., 2022), a procedure that cannot be performed bilaterally due to the prominent role of the hippocampus in memory (Miller and Hakimian, 2013). Thus, innovative approaches for treatment of drug resistant TLE are needed.

To address this unmet need for new therapeutic approaches for treating TLE, stem cell transplantation is being studied in rodent TLE models that utilize systemic or focal injections of chemo convulsant drugs such as kainic acid or pilocarpine. Injections of systemic pilocarpine have become a common laboratory method for models of TLE in inbred strains of mice, due to the relative ease of inducing status epilepticus (SE) using repeated subthreshold injections of the drug. This method leads to the reliable development of spontaneous, recurrent seizures in mice (Ahmed Juvale and Che Has, 2020; Arshad and Naegle, 2020; Arshad et al., 2021). Pilocarpine-induced SE also results in aberrant neurogenesis (Parent et al., 1997; Parent et al., 2006; Jessberger and Parent, 2015; Danzer, 2018; Sasaki-Takahashi et al., 2020) and loss of GABAergic interneurons (Thind et al., 2010; Buckmaster et al., 2017).

The therapeutic efficacy of stem cell transplantation for treating pilocarpine induced TLE in rodents was shown following transplantation of mouse or human forebrain GABAergic interneuron progenitors (Hunt et al., 2013; Cunningham et al., 2014; Henderson et al., 2014; Anderson et al., 2018; Upadhya et al., 2019; Shrestha et al., 2020). These prior studies suggested that transplantation provides seizure suppression by increasing GABAergic inhibition in the DG. Indeed, patch-clamp electrophysiological recordings in brain slices from TLE mice with transplants showed increased spontaneous inhibitory
postsynaptic currents in granule cells (Henderson et al., 2014). Transplants containing Channel Rhodopsin 2 (ChR2)-expressing GABAergic neurons formed extensive inhibitory synaptic contacts onto adult-born granule cells and, when stimulated with blue light, the transplanted interneurons induced strong inhibitory postsynaptic currents in adult-born granule cells in the hippocampus (Gupta et al., 2019; Arshad et al., 2021). Despite mounting evidence that engraftment of developing GABAergic progenitors enhances inhibitory networks and suppresses seizures in TLE, the underlying mechanisms for this therapeutic effect are not well understood.

Adult neurogenesis is a dynamic process affected by age, physical exercise, hormones, chronic stress, and neurological disorders (Eisch and Petrik, 2012; Lucassen et al., 2015; Vivar et al., 2016; Kuhn et al., 2018; Voss et al., 2019; Gage, 2021). In the rodent brain, neurogenesis occurs in the neurogenic niche of the DG, called the subgranular zone (SGZ). This thin layer of cells between the GCL and the hilus includes a neural stem cell pool and provides an environment suitable for neural stem cell proliferation. Type 1 or radial glia-like (RGL) progenitors are quiescent or proliferative and express nestin, Glial Fibrillary Acidic Protein (GFAP), and SRY-box 2 (SOX2) (Seri et al., 2001; Moss et al., 2016). Type 1 cells extend radial processes through the GCL to the molecular layer (ML) and the hippocampal fissure (Sasaki-Takahashi et al., 2020). These cells also generate Type 2 progenitors, identified by co-expression of SOX2 and TBR2, and exhibit a multipolar morphology (Hutton and Pevny, 2011; Hodge et al., 2012). Type 2 progenitors undergo limited divisions and usually give rise to Type 3 progenitors (neuroblasts). Type 3 progenitors express doublecortin (DCX), differentiate into dentate granule cells (GCs), and integrate into host brain circuits (Martínez-Cerdeño and Noctor, 2018).

Maturation of adult-born GCs is influenced by gamma-aminobutyric acid (GABA) and glutamatergic inputs to the SGZ (Tozuka et al., 2005; Wang et al., 2005; Toni and Schinder, 2015). GABA influences intermediate neural progenitors and immature GCs in the DG by promoting fate selection, proliferation, migration, and dendritic arbor maturation (Ge et al., 2006; Ge et al., 2007; Catavero et al., 2018). Tonic GABA signaling promotes Type 1 progenitor quiescence (Song et al., 2012). Regulation of Type 1 progenitor proliferation occurs via parvalbumin (PV) interneurons, whereby PV interneuron activation represses proliferation and inactivation promotes proliferation (Song et al., 2013). In Type 2 progenitors, GABA signaling reduces proliferation and promotes their differentiation by inducing cell cycle exit (Deisseroth et al., 2004; Deisseroth and Malenka, 2005). Type 3 progenitors require synaptic GABAergic and glutamatergic inputs for their maturation and migration into deeper layers of the GCL (Kozareva et al., 2019).

Adult neurogenesis in the hippocampus is commonly dysregulated in rodent models of TLE (Kang et al., 2016). Prolonged seizure activity triggers a dramatic and transient rise in cell proliferation in the DG (Parent et al., 1997; Gray and Sundstrom, 1998; Jessberger et al., 2005). Seizure activity particularly affects mitotically active progenitors and immature migrating DCX-expressing adult-born GCs (Parent et al., 1999; Hüttmann et al., 2003; Jessberger et al., 2005; Parent et al., 2006; Lugert et al., 2010). Even a single seizure-like discharge induces transient cell proliferation in the DG shortly following the seizure (Bengzon et al., 1997; Römer et al., 2011). The extent of seizure-induced proliferation...
of GCs declines with increased seizure severity (Mohapel et al., 2004). Cell proliferation returns to baseline levels approximately 2 to 4 weeks following the initial episode of SE (Parent et al., 1997; Bonde et al., 2006; Römer et al., 2011), and may even be suppressed below baseline after that period (Hattiangady et al., 2004; Kralic et al., 2005). Such changes may result from depletion of the pool of neural stem cells or altered environment in the SGZ (Jessberger and Parent, 2015). Type 1 or RGL cell polarity is often altered in chronic TLE. Type 1 cells may assume an inverted morphology characterized by one or more radial processes directed toward the hilus of the DG, a change in polarity that directs ectopic migration of newborn GCs and causes some RGL cells to transdifferentiate into astrocytes, reducing the neural stem cell pool that produces new GCs (Sasaki-Takahashi et al., 2020). Additionally, some GCs born after SE migrate to ectopic positions in the hilus and have altered dendritic orientations (Scharfman et al., 2007; Shapiro et al., 2008; Murphy et al., 2012). Hilar ectopic GCs form abnormal synaptic connections with neighboring GCs and CA3 pyramidal cells, forming a hyperexcitable dentate circuit that is more prone to spontaneous recurrent seizures (Scharfman et al., 2000; Scharfman, 2004; Scharfman and Bernstein, 2015; Althaus et al., 2019).

In addition to the GABAergic progenitor transplantation into the hippocampus in TLE mice, ablating adult-born GCs, pre- or post-pilocarpine-induced TLE, reduces seizure burden (Cho et al., 2015; Hosford et al., 2016; Hosford et al., 2017; Varma et al., 2019) but see (Iyengar et al., 2015; Zhu et al., 2017). The mixed results of ablation studies in rodent epilepsy models may be due to the methods of ablation used and their differential effects on Type 1, 2, or 3 progenitors, or the timing of the ablations. However, silencing adult-born GCs using the newer DREADDs approach reduces seizures (Zhou et al., 2019; Lybrand et al., 2021). These findings underscore the role of aberrant adult neurogenesis in TLE (Cossart et al., 2001; Thind et al., 2010; Buckmaster et al., 2017).

Here, we addressed the hypothesis that GABAergic progenitor engraftment into the DG of adult mice alters the neurogenic niche and corrects some of the abnormalities in adult neurogenesis characteristic of the pilocarpine model of human TLE. The MGE (Medial Ganglionic Eminence) progenitors were harvested from embryonic day 13.5 transgenic mouse embryos expressing ChR2-EYFP under control of the vesicular GABA transporter promoter (VGAT-ChR2-EYFP) and transplanted into the hilus of the DG of adult mice. We then compared the effects of the transplants on the composition of the SGZ progenitor populations in naïve vs. TLE mice following engraftment of GABAergic interneuron progenitors. Proliferation of the stem cell populations in the SGZ was analyzed by means of pulse-labeling with bromodeoxyuridine (BrdU), and the composition of different stem cell populations was assessed by immunofluorescent labeling, in combination with high-resolution quantitative confocal microscopy.

2. Materials and methods

All animals and reagents used for these studies are listed in Table 1: Key Resources.
2.1. Animals
Animal use conformed to protocols approved by the Wesleyan University Institutional Animal Care and Use Committee. Experiments were performed using group-housed C57BL/6NHsd mice (Table 1, Envigo) maintained in self-ventilating cages, under a 12 h light–dark cycle with light onset at 7 AM, room temperature of 25 deg. C, humidity of 17%–20%, and light intensity of 193–215 lx at the cage level. The mice were group-housed until they were 4–5 weeks of age. They were handled for 5 min per day for one week prior to the beginning of the experiment. The mice consumed food and water ad libitum. Donor embryos were generated by breeding VGAT-ChR2-eYFP transgenic males (Jackson Labs) with C57BL/6NHsd females (Envigo).

2.2. Timeline for experiments
Week 0: The group of mice designated “TLE” was subjected to pilocarpine-induced status epilepticus, then housed individually due to aggression. The group of mice designated “Naïve” received saline injections but no pilocarpine or midazolam. Naïve mice were housed in groups of 2–3.

Week 1: TLE and Naïve mice received hilus transplants of donor cells from the medial ganglionic eminence (MGE) of VGAT-ChR2-eYFP+ embryos using precision stereotaxis (TLE-TX and Naïve-TX). Controls from each group (TLE-Media and Naïve-Media) were subjected to stereotaxic injections of media, minus MGE cells.

Week 2: All mice were injected with a single dose of BrdU. Approximately half of the mice were euthanized after 2 h and BrdU+ cells were quantified to assess proliferation.

Week 5: Additional cohorts of TLE and Naïve mice were euthanized. Experiments were performed to quantify BrdU+ adult-born cells, Type 1, Type 2, and Type 3 progenitors, and the migration and locations of ectopic and normotopic adult-born granule cells.

2.3. Pilocarpine induced TLE
Two of the established methods to induce TLE in rodents are systemic pilocarpine and intrahippocampal kainic acid injections (Curia et al., 2008; Lévesque and Avoli, 2013; Arshad and Naegele, 2020). For our experimental group, multiple, subthreshold systemic injections of pilocarpine were given to the mice to induce SE (Arshad and Naegele, 2020). Prior studies established that 3 h of SE is sufficient to induce TLE in all animals. Control groups received injections of saline (i.p.). A total of 71 mice were used in this study (45 males and 26 females).

2.4. Naïve group
Thirty mice (Envigo, C57BL/6NHsd strain), weighing 18–22 g, received an injection of methyl scopolamine followed by saline, but did not receive pilocarpine and midazolam (“Naïve” mice). Six naïve mice were used for a control experiment to test whether midazolam had a persistent effect on cell proliferation (3 males; 3 females; see below). Twenty-four “Naïve” (17 males; 7 females) received stereotaxic injections of either fetal
GABAergic progenitor transplants into the DG (12 Naïve-TX; 9 males; 3 females) or media, without cells (12 Naïve-Media; 8 males; 4 females).

2.5. TLE-group

To generate mice with chronic TLE, 41 adult mice (Envigo, C57BL/6NHsd strain; 31 males, 10 females), 5–6 weeks of age, weighing 18–22 g, were injected with 0.07 mL methyl scopolamine (i.p., 0.5 mg/mL diluted in sterile saline; Sigma). After 30 min, we injected pilocarpine-HCl (i.p., 280 mg/kg), as described previously (Arshad and Naegele, 2020). SE was attenuated after 3 h, with a systemic injection of midazolam (i.p., 0.04 mL, 10 mg/kg, Covetrus). Mice that did not develop SE were excluded from further study. Prior work established that 1–3 h of SE reliably leads to abnormal adult neurogenesis and spontaneous recurrent seizures (Parent et al., 1999; Parent et al., 2006; Jessberger and Parent, 2015; Hosford et al., 2016; Hosford et al., 2017; Danzer, 2018; Arshad and Naegele, 2020). Out of the pilocarpine-treated group, 31/41 developed SE (24 males, 7 females).

We defined SE as the stage of continuous generalized seizures in which the mice show immobilization, Stroop tail posture, and prominent motor tics (head bobbing); this stage typically follows 3 or more Racine scale Stage 3, 4, or 5 seizures (Arshad and Naegele, 2020). For the mice that progressed to develop SE, we visually monitored them to ensure that they continued to exhibit SE for 3 h, then the SE was attenuated by injecting midazolam (10 mg/kg). Only mice that experienced 3 h of continuous SE were used in subsequent studies. The mice were subsequently assessed behaviorally for high aggression during handling and jumping behavior, both characteristic of C57Bl/6 N mice with TLE.

For the TLE-TX group, 16 TLE mice (13 males, 3 females), received stereotaxic injections of fetal GABAergic progenitors into the DG (TLE-TX). For the TLE-Media group, 9 TLE mice (5 males, 4 females) received stereotaxic injections of neural transplant media, without cells (TLE-Media). Three TLE-TX mice were excluded from further analysis due to off-target stereotaxic injection sites that passed through the hippocampus.

2.6. Control experiment testing effects of midazolam on proliferation

Three TLE mice and 6 naïve mice were used to establish whether midazolam affected cell proliferation at the time points used in our experiments. The TLE mice and 3 of the naïve mice received an injection of midazolam (10 mg/kg, 0.04 mL, i.p.); the 3 remaining naïve mice received saline (0.04 mL i.p.), instead of midazolam. Two weeks later, all of the mice received a single injection of BrdU (150 mg/kg i.p.) and were euthanized after 2-h. BrdU labeling and detection was assessed as described below.

2.7. Medial Ganglionic Eminence (MGE) dissection, dissociation, and transplantation

Embryonic day 13.5 embryos were obtained by timed breeding of VGAT-ChR2-YFP adult male mice (stock number: 014548, The Jackson Labs) to wild-type females (n = 6; C57Bl/6NHsd, Envigo). VGAT-ChR2-YFP* embryos were identified under fluorescent illumination and the MGEs were isolated, as described (Arshad et al., 2021). Following trypsin digestion and trituration to obtain a single-cell suspension, the cells were re-suspended at a concentration of 1 × 10^5 cells/μL in transplantation media (Leibovitz’s L-15 (Gibco, 11415–
(064), supplemented with caspase inhibitor (Promega G7231), B27 (Gibco, 17504–044), fibroblast growth factor (Sigma, F0291), and epidermal growth factor (Invitrogen, 53,303–018) (Henderson et al., 2014). MGE-derived progenitors were transplanted by stereotaxic injections, bilaterally into four sites in the hilus of the DG (AP −2.5 mm, ML ±2.1 mm, DV −2.2 and −1.8 mm). Control mice in the TLE and Naïve groups received 4 stereotaxic injections of the transplant media, without MGE cells, into four sites in the DG at the same stereotaxic coordinates as the experimental mice with MGE transplants, as a control for any surgical damage to the DG and the neurogenic niche. Mice were returned to the home colony until further use. Naive mice were group-housed (2–3 mice/cage) in the home colony; TLE mice were individually-housed to prevent aggression.

2.8. Immunohistochemistry

Mice were perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), and brains were removed from the skull, postfixed for 15 h at 4 deg. C, and equilibrated in ascending sucrose solutions (10%, 20%, and 30% sucrose in 0.1 M sodium phosphate buffer, pH 7.4). Forty μm cryostat sections in the horizontal plane were collected in 0.1 M sodium phosphate buffer saline (PBS). Immunofluorescent staining to detect transplanted GABAergic interneurons expressing ChR2-EYFP was performed with chicken anti-GFP antibody (GFP #1020, Aves), in combination with neurogenic markers. Type 1 progenitors were identified by immunostaining for GFAP (mouse mAb anti-GFAP; #360, Millipore Sigma) and SOX2 (rabbit polyclonal anti-SOX2 #5603, Millipore Sigma) (Segi-Nishida et al., 2008; Jiruska et al., 2013; Nickell et al., 2017; Micheli et al., 2018). Type 2 progenitors were identified by positive immunoreactivity for SOX2, in the absence of co-expressed GFAP (Suh et al., 2007; Segi-Nishida et al., 2008; Jiruska et al., 2013; Nickell et al., 2017; Micheli et al., 2018). Type 3 progenitors (immature GCs) were identified by immunostaining with guinea pig anti-DCX (#2253, Millipore Sigma). Free-floating sections were blocked in 5% normal goat serum in PBS containing 0.3% Triton-X100 for 1 h, then incubated for 18–22 h at RT, while agitating, in primary antibodies in PBS with 3% normal goat serum and 0.3% Triton-X100. Sections were washed in 0.1 M PBS, then incubated in goat anti-chicken IgY Alexa Fluor 488 (#A11039, Life Technologies) and one or more of the following antibodies: goat anti-rabbit IgG Alexa Fluor 568 (#A11036, Invitrogen), goat antimouse IgG Alexa Fluor 647 (#A21242, Invitrogen), or goat anti-guinea pig IgG Alexa Fluor 647 (#A21450, Invitrogen) for 1 h. The sections were rinsed, incubated in DAPI (#62248, ThermoFisher Scientific), rinsed, and mounted on Superfrost Plus slides (#12-550-15, Fisher Scientific) in Prolong Diamond with DAPI (#P36966, ThermoFisher Scientific).

2.9. BrdU labeling and detection

To examine the effects of transplantation on cell proliferation, mice received a single dose of BrdU (i.p; 150 mg/kg; #B5002, Sigma-Aldrich), after a survival period of 1-week, following stereotaxic transplantation of MGE-derived progenitors or injections of media. Two hours after BrdU was injected, the mice were euthanized by injection of pentobarbital solution (0.1 mL i.p., Fatal Plus, Henry Schein, 035946), and immediately perfused with ice-cold rinse buffer (0.1 M sodium phosphate buffer, pH 7.4, containing 220 units heparin/ml (Fisher BioReagents, BP245), followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). To examine the effects of transplantation on survival of adult-born GCs.
(granule cells) in the DG, BrdU was injected one week following GABAergic progenitor transplantation, and the mice survived for three weeks, before euthanasia and perfusion, cryoprotection, and storage of the brains at −80 deg. C.

Forty μm thick sections were cut in the horizontal plane and collected in 0.1 M sodium phosphate buffer saline (PBS). Sectioning was always done the same day as immunostaining to preserve BrdU antigenicity. Free-floating sections were incubated in 1 N HCl for 20 min at 37 °C to denature the DNA and expose the incorporated BrdU, and the acid was neutralized by immersing sections in Trizma base (pH 8.5) for 2 × 5 min. Additional permeabilization was achieved by incubating sections in PBS with 1% Triton-X for 30 min. Next, sections were incubated in PBS containing anti-BrdU antibodies (Rat anti-BrdU IgG, 1:500; MCA 2060, BioRad) for 18–22 h, rinsed, then incubated in secondary antibodies (goat anti-rat-IgG Alexa Fluor 568, ThermoFisher Scientific). Nuclei were labeled with DAPI (1:2000, 62248, ThermoFisher Scientific), rinsed, and sections were mounted onto Superfrost Plus glass slides (#12550-15, Fisher Scientific) in Prolong Diamond with DAPI (P36966, ThermoFisher Scientific).

2.10. Quantitative confocal microscopy

To quantify immunofluorescent adult-born cells, we obtained confocal images (Leica TCS SP8 confocal microscope) at 1 μm steps through the z-axis, with Leica 20×/0.75 (zoom factor of 0.75) or 63×/1.4 objectives. Counts of labeled neurons were made in 4 sections, spaced 400 μm apart, along the dorso-ventral axis of the hippocampus. The region of interest (ROI) for the Type 1 and Type 2 cell counts included the SGZ region, beneath the GCL, within each section. The subgranular zone (SGZ) was defined as the region within ~10 μm beneath the GCL (Varma et al., 2019). For counts of Type 3 cells, the ROI included the entire GCL and SGZ within each section. For the counts of ectopic Type 3 cells, the ROI was the region of the hilus contained between the two blades of the GCL, excluding the part of CA3 extending into the hilus. Ectopic cells in this region were defined as DCX+ cells located 20 μm or further from the GCL (Singh et al., 2015). Within the ROI, counts were performed in optical sections spaced 1-μm apart (typically 20–22 optical sections along the z-plane of the 40 μm thick tissue section). Counts were not made in guard zones, consisting of 5 μm at the top and bottom of each confocal image.

2.11. Quantitative analyses of granule cell migration

Migration of DCX+ cells into the GCL was assessed in flattened confocal images of brain sections ~ −2.36 mm ventral to the stereotaxic coordinates for bregma. For each treatment group, the ROI was defined as a square box, measuring 100 μm × 100 μm, located in the middle of the lateral blade of the DG. The migration distance for each cell was measured from the center of the soma to the border of the GCL/SGZ, as described previously (Duveau et al., 2011).

2.12. Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM). Shapiro-Wilk test was performed to find normal distribution of the data sets. For all comparisons, one-way ANOVA, Kruskal-Wallis test, or t-tests were performed, followed by post hoc tests,
whenever appropriate. Statistical tests were performed using GraphPad Prism, V7. The confidence interval was 95%. All statistical comparisons are shown in Table 2: Statistical Analyses. Differences were considered statistically significant at *p < 0.05, **p = 0.009, ***p < 0.0009, ****p < 0.0001.

3. Results

Transplantation of GABAergic progenitors into the hippocampus has been shown to suppress seizures in the pilocarpine and kainic acid models of TLE (Hunt et al., 2013; Cunningham et al., 2014; Henderson et al., 2014; Upadhya et al., 2019; Shrestha et al., 2020). Little is known, however, about the impact of transplantation of GABAergic progenitors on the neural stem cell niche of the hippocampus of naïve and epileptic mice. To address this gap, we analyzed the effects of transplanting MGE-derived fetal mouse progenitors on the neural stem cell and progenitor populations in the SGZ. Mice receiving intra-hippocampal stereotaxic injections of media served as controls.

3.1. Does hilar transplantation of embryonic GABAergic neurons in mice with TLE affect the populations of Type 1 and Type 2 progenitors in the SGZ?

Type 1 progenitors are specialized RGL cells that give rise to Type 2 progenitors and self-renew. We first asked whether transplantation of GABAergic neurons alters the numbers of Type 1 and Type 2 progenitors in naïve and epileptic mice (Fig. 1A). Type 1 progenitors are GFAP and SOX2 co-labeled cells while Type 2 progenitors express SOX2 only. Type 1 progenitors are located in the SGZ of the DG and their apical process extend through the granular cell layer toward the pia and the hippocampal fissure. Type 2 progenitors express SOX2 and reside in the SGZ of the DG (Fig. 1B–D). Consistent with the prior studies, we found that Type 1 progenitors were significantly reduced in TLE mice compared to the naïve mice (Fig. 1E–J, K, Naïve-Media vs. TLE-Media **p = 0.002; Naïve-Media vs. TLE-TX ***p = 0.0002) (Singh et al., 2015; Sasaki-Takahashi et al., 2020); GABA progenitor transplantation did not rescue Type 1 progenitors in naïve and TLE mice (Fig. 1E–J, K).

The radial glial processes from Type 1 progenitors normally extend through the GCL into the inner third of the molecular layer, contact blood vessels, provide structural support for radial migration of adult-born neurons, and appear to regulate synapse formation between adult-born GCs and afferent inputs (Xu et al., 2015; Moss et al., 2016). In epilepsy, inversion of the radial glial progenitors may be associated with ectopic migration of adult-born GCs, as the Type 1 progenitors adopt an inverted morphology (Fig. 1I), with processes extending toward the hilus (Sasaki-Takahashi et al., 2020). Given prior literature showing inverted Type 1 progenitors in TLE mice, we next quantified abnormal GFAP*/SOX2* Type 1 progenitors in the SGZ with inverted processes that extended into the hilus. While these cells were significantly increased in TLE mice that received injections of media (Fig. 1L, Naïve-Media vs. TLE-Media ****p < 0.0001; Naïve-Media vs. TLE-TX **p = 0.002), they were reduced in TLE mice with transplants (Fig. 1L, TLE-Media vs. TLE-TX ****p < 0.0001). Consistent with the literature, we found a significant increase in the number of Type 2 progenitors in TLE-Media mice, compared to the Naïve-Media controls (Fig. 1M, ***p =
The effects of GABAergic progenitor transplantation included a significant reduction in the number of Type 2 (GFAP−/SOX2+) progenitors in the SGZ in naïve and TLE mice, compared to the media controls (Fig. 1M, naïve-Media vs. naïve-TX *p = 0.01, TLE-Media vs. TLE-TX ****p < 0.0001).

### 3.2. Does transplantation of GABAergic progenitors into the DG alter the migration or location of Type 3 (DCX+) progenitors in epileptic mice?

Prior work established that recurrent seizures in rodent TLE models are linked to increased numbers of adult-born GCs in the GCL and the hilus of the DG (Scharfman et al., 2000; Scharfman, 2004; Jessberger et al., 2005; Parent et al., 2006; Scharfman et al., 2007; Danzer, 2018; Althaus et al., 2019). DCX is a specific marker for Type 3 progenitors (immature migrating GCs) in the adult hippocampus.

We next investigated whether transplantation altered the population of Type 3 progenitors expressing DCX (Fig. 2A) by comparing DCX+ cells, in the absence of transplants, in epileptic mice (TLE-Media) vs. non-epileptic (naïve-Media) mice. DCX+ cells were significantly increased in the GCL and hilus in TLE-Media mice compared to naïve-Media mice (Fig. 2B, E, H, ****p < 0.0001). Strikingly, in mice with transplanted GABAergic progenitors, we observed a further significant increase in DCX+ cells in the GCL in both naïve and TLE mice (Fig. 2C–D, F–H naïve-Media vs. naïve-TX *p = 0.02, TLE-Media vs. TLE-TX ***p = 0.0003).

These results showing an increase in the number of DCX+ cells in mice with GABA progenitor transplants suggest that transplantation is associated with increased survival of adult-born DCX+ cells. Alternatively, or additionally, GABAergic progenitor transplants may reduce the ectopic migration of DCX+ cells in the DG of TLE mice, and promote radial migration. To distinguish between these alternatives, we compared the number of hilar ectopic DCX+ cells in the four treatment groups of mice. As expected, naïve-Media mice had very few hilar ectopic DCX+ cells compared to the TLE mice (Fig. 2B, E, I; naïve-Media vs. TLE-Media ****p < 0.0001; Fig. 2E). Strikingly however, TLE mice with GABAergic progenitor transplants had significantly reduced numbers of ectopic DCX+ cells (Fig. 2E–G, I; TLE-Media vs. TLE-TX ***p = 0.0009). Taken together, these findings suggest that hilar transplants of GABAergic progenitors likely act to inhibit the ectopic migration of adult-born cells into the hilus and promote their survival and radial migration into the GCL.

To further investigate possible effects of the transplanted cells on migration, we next asked whether the presence of GABAergic cell transplants was associated with greater migration of DCX+ cells into the GCL, and the results are shown in Fig. 2J. When we compared the average migration distance for each group of mice (Duveau et al., 2011), we found that DCX+ cells migrated significantly further into the GCL in the TLE mice with GABAergic cell transplants (naïve-Media vs. naïve-TX *p = 0.03, naïve-Media vs. TLE-Media *p = 0.01, naïve-Media vs. TLE-TX **p = 0.009). Moreover, the number of DCX+ cells that migrated into GCL was positively correlated with the number of transplanted cells (Fig. 2K, naïve-TX r = 0.9, *p = 0.04, TLE-TX r = 0.8 *p = 0.03) suggesting that the number of transplanted GABAergic progenitors may be important for restoring normal patterns.
of radial migration of adult-born granule neurons into the GCL and preventing ectopic migration into the hilus.

The prior literature suggests that the extent of adult neurogenesis differs between the dorsal and ventral hippocampus in an adult mouse (Vivar et al., 2016). To further investigate whether our findings showing that GABA transplants were associated with an increase in DCX<sup>+</sup> cells that had migrated radially into the GCL, we next asked whether this effect was localized or generalized. To address this question, we compared the numbers of DCX<sup>+</sup> cells at different levels of the DG along the dorso-ventral axis of the hippocampus (Fig. 3A). We observed a higher density of DCX<sup>+</sup> cells in the dorsal hippocampus, in the vicinity of the transplants, but no apparent differences in the ventral hippocampus, where we had not transplanted cells (Fig. 3B–F, Naïve-Media vs. Naïve-TX *p-value = 0.03, TLE-Media vs. TLE-TX *p = 0.009, <0.0001). Moreover, we only found a significant reduction in the number of hilar ectopic DCX<sup>+</sup> cells in the dorsal hippocampus in TLE mice with GABAergic progenitor transplants (Fig. 3G *p = 0.02, **p = 0.008). These findings suggest that the observed effects of the GABA progenitor transplants on survival and migration of adult-born GCs may be restricted to the vicinity of the transplants.

3.3. Does transplantation of GABAergic progenitors affect the proliferation or survival of adult-born cells in naïve and epileptic mice?

Our findings support a localized role for GABA progenitor transplants in the survival and migration of adult-born neurons in the DG, consistent with prior work (Ge et al., 2006; Ge et al., 2007; Dieni et al., 2013; Song et al., 2013). We next asked whether the transplanted cells enhanced the proliferation of adult-born neurons, or their survival. To distinguish between these possibilities, we analyzed cell survival at two different time points, by pulse-labeling proliferating cells with BrdU one week after transplanting GABAergic progenitors (Fig. 4A). Short-term survival lasting two hours post-BrdU injection resulted in equivalent labeling of proliferative populations of cells in the SGZ of the DG in naïve and TLE mice with or without transplants (Fig. 4B–F), suggesting that GABAergic transplants do not alter neural progenitor proliferation.

Given these negative results, we next performed an experiment to control for the possible effects of midazolam on cell proliferation by comparing proliferating BrdU<sup>+</sup> cells two weeks after midazolam injections in groups of Naïve-Saline and Naïve midazolam-treated mice. We observed no significant differences in the number of BrdU<sup>+</sup> cells between the two groups of mice, suggesting that any effects of midazolam on cell proliferation are transient and limited to a short period after injection. Comparing the controls with TLE mice, we found a significant increase in the number of BrdU<sup>+</sup> cells in TLE mice treated with midazolam, compared to the naïve mice (Supplementary Fig. 1, (Naïve-Saline vs. TLE-midazolam ***p-value = 0.0003), (Naïve-midazolam vs. TLE-midazolam ***p-value = 0.0003). We next compared survival of BrdU<sup>+</sup> cells at a later post-mitotic time-period in naïve and TLE mice with GABAergic progenitor transplants vs. Media injections. After three-week survival periods (Fig. 5A), the number of BrdU-labeled adult-born cells in the presence of GABA progenitor transplants in naïve and TLE mice was significantly higher, compared to the media controls (Fig. 5B–F Naïve-Media vs. Naïve-TX *p = 0.04, Naïve-Media vs. TLE-
Media ns-\( p = 0.051 \), TLE-Media vs. TLE-TX *\( p = 0.01 \). These findings are consistent with the interpretation that transplants of GABAergic progenitors increase the survival of adult-born GCs.

To determine whether GABA transplants have localized or generalized effects, we compared the survival of BrdU-labeled cells at different dorso-ventral levels of the DG of the hippocampus in naïve and TLE mice with or without transplants. BrdU-labeled adult-born cells were increased only at more dorsal levels of the hippocampus, where the transplanted GABAergic cells were located, suggesting a possible local effect on new neuron survival in mice receiving transplants (Fig. 6 A–C, Naïve-Media vs. Naïve-TX *\( p \)-value = 0.01, TLE-Media vs. TLE-TX *\( p = 0.01 \), ns-\( p = 0.051 \)).

4. Discussion

The major findings of this study are that GABAergic progenitor transplants in the DG of the hippocampus in TLE mice enhance the survival and migration of adult-born GCs. Both TLE and naïve mice receiving GABAergic progenitor transplants had significant increases in surviving numbers of adult-born GCs and these newborn cells exhibited enhanced migration from the SGZ into the GCL. Further, TLE mice with GABAergic progenitor transplants showed a reduction in the number of inverted Type 1 and Type 2 progenitors in the SGZ. These results support the interpretation that intrahippocampal transplants of GABAergic progenitors promote survival of adult-born neurons, through accelerated Type 2 progenitor differentiation into DCX\(^+\) immature neurons, reduced programmed cell death, or both mechanisms.

4.1. Effects of experimental conditions on comparisons of adult neurogenesis

While our findings point to a strong effect of GABAergic interneuron grafts on adult neurogenesis, several experimental factors may have influenced these findings. The experiments in naïve mice showed a consistent and strong effect of the transplants on survival and migration of adult-born neurons. These mice were group housed, a social effect known to increase adult neurogenesis. All mice were group housed, with 2–3 naïve mice per cage, prior to the beginning of the experiments at age 5–6 weeks. Once pilocarpine was injected however, the TLE mice could not be grouped housed due to conspecific aggression (Huang et al., 2012; Zhu et al., 2020). Social isolation in TLE mice increases plasma corticosterone levels which negatively affects neurogenesis. However, our internal controls of singly housed TLE mice were provided, in part to control for the effects of housing. This TLE control group received intracranial injections of media without cells. The observed effects on the different stem cell populations and the survival of adult-born BrdU+ or DCX+ neurons were all significantly reduced compared to TLE mice with GABAergic progenitor transplants, indicating that transplanted GABAergic progenitors, and not social isolation, are likely responsible for the main effects we observed on cell survival, migration into the GCL, and reduction in ectopic adult-born cells.

Another difference between naïve and TLE groups of mice, pertained to the use of a benzodiazepine, midazolam, which was administered to terminate seizures. Naïve mice did not receive pilocarpine or midazolam, but were instead given injections of saline. To address
concerns about the possible suppressive effects of midazolam on cell proliferation, we carried out a pilot experiment in naïve mice to compare BrdU labeling following treatment with midazolam vs. saline. At the time points examined in our study, midazolam does not have an apparent effect on cell proliferation. Additionally, we included within group controls that have identical treatments.

4.2. Transplantation of GABAergic progenitors enhances survival of adult-born granule cells

In rodents, 30–80% of adult-born GCs in the hippocampus undergo programmed cell death (Cameron and McKay, 2001; Dayer et al., 2003; Sun et al., 2004; Kuhn, 2015; Denoth-Lippuner and Jessberger, 2021). Regardless of whether they received transplants, we found that TLE mice exhibited a significant increase in Type 3 progenitors along the entire dorsoventral axis of the hippocampus. Comparisons of TLE mice with transplants vs. TLE controls showed that the survival of Type 3 progenitors and BrdU+ cells was increased in the dorsal hippocampus, in the vicinity of the transplants. These findings suggest that transplanted GABAergic progenitors may have a direct effect on promoting the survival of adult-born neurons. Alternatively, seizure activity reduces adult neurogenesis in chronic rodent models of TLE (Hattiangady et al., 2004; Hattiangady and Shetty, 2008) and transplantation of GABAergic progenitors may suppress the seizure burden (Hunt et al., 2013; Henderson et al., 2014). This alternative interpretation of our findings, showing enhanced survival of adult-born GCs, is that GABAergic transplants exert their main effects by suppressing seizures, and secondarily, this reduction in seizure burden would tend to promote enhanced survival. A counter argument for this interpretation is the finding that transplants in naïve mice were also found to enhance survival of Type 3 progenitors and BrdU+ cells.

The heterogeneity of GABAergic cell types within our transplants provides a possible mechanism for enhanced survival of Type 3 progenitors. Endogenous populations of hippocampal GABAergic interneurons are known to promote survival of GCs (Dieni et al., 2013). Specifically, the PV-expressing subset of hippocampal GABAergic interneurons were found to increase the survival of adult-born GCs (Song et al., 2013). MGE-derived transplants consist of approximately 37% PV+ GABAergic interneurons and many of these cells migrate extensively throughout the molecular and GCL layers, where upon maturation, they form synaptic contacts with the dendrites of adult-born GCs (Hunt et al., 2013; Henderson et al., 2014; Gupta et al., 2019). One possibility worth exploring in future studies is the specific role of the PV+ synaptic contacts formed by the transplanted interneurons on Type 3 progenitor survival and migration.

Additionally, the cAMP response element-binding protein (CREB) is a downstream effector of GABA signaling and its activated form, phospho-CREB, is expressed by a high percentage of adult-born GCs (Nakagawa et al., 2002; Redmond et al., 2002). Phosphorylation of CREB coincides with a developmental phase when adult-born neurons depolarize in response to GABA-signaling (Ge et al., 2007; Jagasia et al., 2009), which is essential for maturation of adult-born GCs (Wang et al., 2003; Ge et al., 2007; Ben-Ari et al., 2012). The depolarizing responses of adult-born GCs to GABA depends upon expression of
NKCC1, as knockdown of NKCC1 in adult-born GCs decreases pCREB, impairs outgrowth of dendrites, and decreases GC survival (Ge et al., 2007; Jagasia et al., 2009). These prior studies suggest that in Type 3 progenitors, an increase in GABA-induced depolarization, due to high expression of NKCC1 and downstream activation of CREB-signaling, may contribute to the increased survival.

4.3. Enhanced radial migration of adult-born granule cells into the GCL due to GABAergic progenitor transplants

We further observed increased migration of Type 3 GCs into the GCL of the DG following transplantation of GABAergic progenitors. Consistent with the literature, we found in Naïve-Media mice, that the majority of DCX⁺ adult-born GCs were located in the SGZ and very few migrated into the inner and middle layers in the GCL. However, in the presence of the transplanted cells, we observed increased radial migration of DCX⁺ cells into the deeper layers of GCL. These observed effects on migration could be due to secretion of Reelin by the transplanted interneurons. Reelin is a large extracellular matrix glycoprotein expressed by GABAergic basket cells in the DG of the hippocampus (Curran and D’Arcangelo, 1998; Pesold et al., 1998), that acts as a repellant signal during the migration of adult-born dentate GCs. Reelin exerts its effect by acting on a migration scaffold formed by Type 1 progenitors (Frotscher et al., 2003), directing the migration of adult-born GCs into the GCL (Wang et al., 2018). Loss of Reelin⁺ cells in the hilus and the ML after epileptic seizures was linked to ectopic migration of adult-born GCs (Parent et al., 2006; Gong et al., 2007), leading to the suggestion that Reelin deficiency in TLE causes a disruption in the normal pattern of radial migration of newborn neurons (Orcinha et al., 2016; Orcinha et al., 2021). Therefore, significant reductions in hilar ectopic DCX⁺ cells in TLE-TX might be due to an increase in the number of Reelin expressing cells after transplantation of GABAergic progenitors. While additional studies are necessary to determine the precise molecular and cellular mechanisms for increasing survival and normalizing the direction of migration of adult-born GCs in TLE mice with GABAergic transplants, our findings suggest that the transplanted exogenous GABAergic cells may provide the signals for these phenomena.

4.4. The role of GABAergic transplants in regulating Type 1 progenitors

Prior studies showed that mice with TLE had reduced numbers of normal Type 1 progenitors with radial processes into the GCL (Sierra et al., 2015; Singh et al., 2015; Sasaki-Takahashi et al., 2020) but increased numbers of inverted Type 1 progenitors. In contrast, we found reduced numbers of inverted Type 1 progenitors in TLE mice that received transplants, while there was no change in prevalence of Type 1 progenitors with radial processes. While we have not examined transplant-induced changes in the morphology of Type 1 cells in TLE mice, another possibility is that the transplants alter the disease-associated pathological properties of Type 1 progenitors, by reducing the length or orientation of hilus-projecting processes, which would reduce ectopic migration of adult-born GCs. This in turn, would attenuate the formation of hyperexcitable circuits formed by ectopic GCs in TLE. Inversion of Type 1 progenitor processes can occur due to reorientation of the centrosome away from the GCL toward the hilus, and centrosomal reorientation can be induced by seizures (Sasaki-Takahashi et al., 2020). This change in orientation of Type 1 progenitors might be regulated by Reelin, which modulates neuronal migration (Zhao et al., 2004). In the absence
of Reelin signaling. Reelin treatment in hippocampal slice culture experiments was shown to rescue the correct radial orientation of Type 1 progenitors (Jossin, 2020). Thus, deficient reelin signaling in TLE may result in the development of inverted Type 1 progenitors and contribute to ectopic migration of adult-born DCX⁺ cells in the hilus. Our findings suggest that transplants help to maintain normal radial fiber orientation and prevent ectopic migration of new GCs.

4.5. The role of GABAergic transplants in regulating Type 2 progenitors

Seizures increase the proliferation of Type 2 progenitors (Indulekha et al., 2010; Jiruska et al., 2013; Jessberger and Parent, 2015; Fu et al., 2019). Accordingly, our experiments showed that TLE mice that received media injections also had significantly increased Type 2 progenitors. Our findings extend prior work by showing that Type 2 progenitors in TLE mice were reduced following GABAergic progenitor transplantation. The reduced number of SOX2-expressing Type 2 progenitors could be due to increased GABA signaling, causing more rapid differentiation into DCX-expressing GCs. In support of this interpretation, prior work showed that GABA receptor agonist treatment induces rapid differentiation of Type 2 progenitors due to downregulation of anti-neuronal genes and upregulation of pro-neural gene neuroD, required for differentiation of Type 2 progenitors into post-mitotic cells (Deisseroth et al., 2004; Deisseroth and Malenka, 2005; Tozuka et al., 2005).

4.6. Relevance for therapeutic treatments in patient populations

Whether adult neurogenesis occurs in humans after childhood is a controversial subject; one study reported undetectable levels after early childhood, (Sorrells et al., 2018), but others provide evidence of persistent adult neurogenesis in humans (Boldrini et al., 2018; Moreno-Jiménez et al., 2019; Tobin et al., 2019). In human TLE, resected hippocampal tissue from patients with more severe seizures had higher levels of cell proliferation compared to patients with milder forms of TLE (Crespel et al., 2005) and tissue from younger patients exhibited higher numbers of neural progenitors, compared to adults (Blümcke et al., 2001). Methodological differences might account for some of these discrepancies. In considering cell replacement therapies for TLE patients, our findings suggest that GABAergic progenitor transplantation would be more likely to correct some of the abnormalities in adult neurogenesis in younger patients, including reductions in formation of hyperexcitable circuits by ectopic GCs.

In conclusion, our current study builds on our prior work establishing that GABAergic progenitor transplants reduce seizure burden in TLE mice. Here, we provide new insights on possible mechanisms of transplant-mediated seizure suppression. Our findings suggest that GABAergic progenitor transplants enhance the survival of adult-born neurons in naïve and epileptic mice. GABAergic progenitor transplants in TLE mice may prevent hyperexcitability in the dentate circuit by reducing the number of ectopic adult-born GCs that migrate abnormally into the hilus. We also showed that GABA transplants are linked to increased survival and migration of adult-born neurons into the GCL. Whether these additional adult-born neurons survive long-term, and wire up with the host brain, is not yet known. Finally, in TLE mice with GABAergic progenitor transplants, we found a significant decrease in inverted Type 1 cells, suggesting disease-modifying effects of the GABAergic...
grafts. Taken together, our findings suggest that GABAergic progenitor transplantation into the dentate gyrus in mice with TLE may partially restore normal radial orientation of Type 1 progenitors and increase radial migration and survival of adult-born granule cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank the following Naegele lab members who assisted with data collection for this study: Nicolas Cimino, Alina Widmann, Fizzroy Wickham, Marion Humphreys, Mirzi Adler-Wachter, Spencer Fox, Sophia Marra, Calista Stevens, Saira Mehra, and Bette Gobeze. We thank Kathleen Sagarin for helpful proof-reading of the manuscript. We thank Jeff Gilarde for technical assistance with confocal microscopy and Sera Brown for animal husbandry.

Funding

This work was supported by the National Institute of Neurological Disorders and Stroke Grant R15NS072879-01A1, a Connecticut Stem Cell Established Investigator Grant, a Challenge Award from Citizens United for Research in Epilepsy (J.R.N), and grants in support of scholarship from Wesleyan University.

Data availability statement

The quantitative data from this study are available by request. These data will also be shared on Figshare.

Data availability

Data will be made available on request.

Abbreviations:

- **GCL**: Granule cell layer
- **DG**: Dentate gyrus
- **SGZ**: Subgranular zone
- **TLE**: Temporal lobe epilepsy
- **BrdU**: Bromodeoxyuridine
- **GABA**: Gamma aminobutyric acid
- **PV**: Parvalbumin
- **SE**: Status epilepticus
- **RGL**: Radial glia-like

References

Ahmed Juvale II, Che Has AT, 2020. The evolution of the pilocarpine animal model of status epilepticus. Heliyon 6, e04557. [PubMed: 32775726]
Althaus AL, Moore SJ, Zhang H, Du X, Murphy GG, Parent JM. 2019. Altered synaptic drive onto birthdated dentate granule cells in experimental temporal lobe epilepsy. J. Neurosci. 39, 7604–7614. [PubMed: 31270158]

Ammothumkandy A, et al. 2022. Altered adult neurogenesis and gliogenesis in patients with mesial temporal lobe epilepsy. Nat. Neurosci. 25, 493–503. [PubMed: 35383330]

Anderson NC, Van Zandt MA, Shrestha S, Lawrence DB, Gupta J, Chen CY, Harrsch FA, Boyi T, Dundes CE, Aaron G, Naegle JR, Grabel L. 2018. Pluripotent stem cell-derived interneuron progenitors mature and restore memory deficits but do not suppress seizures in the epileptic mouse brain. Stem Cell Res. 33, 83–94. [PubMed: 30340090]

Arshad MN, Naegle JR. 2020. Induction of temporal lobe epilepsy in mice with pilocarpine. Bio Protoc. 10, e3533.

Arshad MN, Aaron GB, Naegle JR. 2021. Optogenetic interrogation of ChR2-expressing GABAergic interneurons after transplantation into the mouse brain. Methods Mol. Biol. 2191, 235–259. [PubMed: 32865749]

Asadi-Pooya AA, Stewart GR, Abrams DJ, Sharan A. 2017. Prevalence and incidence of drug-resistant mesial temporal lobe epilepsy in the United States. World Neurosurg. 99, 662–666. [PubMed: 28034810]

Ben-Ari Y, Woodin M, Sernagor E, Cancedda L, Vinay L, Rivera C, Legendre P, Luhmann H, Bordey A, Wenner P, Fukuda A, van den Pol A, Gaiarsa J-L, Cherubini E. 2012. Refuting the challenges of the developmental shift of polarity of GABA actions: GABA more exciting than ever! Front. Cell. Neurosci. 6.

Bengzon J, Kokaia Z, Elmér E, Nanobashvili A, Kokaia M, Lindvall O. 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc. Natl. Acad. Sci. U. S. A. 94, 10432–10437. [PubMed: 9294228]

Blümcke I, Schewe J-C, Normann S, Brüstle O, Schramm J, Elger CE, Wiestler OD. 2001. Increase of nestin-immunoreactive neural precursor cells in the dentate gyrus of pediatric patients with early-onset temporal lobe epilepsy. Hippocampus 11, 311–321. [PubMed: 11769312]

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ. 2018. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 22 (589–599), e585.

Bonde S, Ek Dahl CT, Lindvall O. 2006. Long-term neuronal replacement in adult rat hippocampus after status epilepticus despite chronic inflammation. Eur. J. Neurosci. 23, 965–974. [PubMed: 16519661]

Buckmaster PS, Abrams E, Wen X. 2017. Seizure frequency correlates with loss of dentate gyrus GABAergic neurons in a mouse model of temporal lobe epilepsy. J. Comp. Neurol. 525, 2592–2610. [PubMed: 28425097]

Cameron HA, McKay RD. 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J. Comp. Neurol. 435, 406–417. [PubMed: 11406822]

Catalvero C, Bao H, Song J. 2018. Neural mechanisms underlying GABAergic regulation of adult hippocampal neurogenesis. Cell Tissue Res. 371, 33–46. [PubMed: 28948349]

Cho KO, Lybrand ZR, Ito N, Brulet R, Tafacory F, Zhang L, Good L, Ure K, Kernie SG, Birnbaum SG, Scharfman HE, Eisch AJ, Hsieh J. 2015. Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline. Nat. Commun. 6, 6606. [PubMed: 25808087]

Cossart R, Dinocourt C, Hirsch JC, Merchant-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C. 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat. Neurosci. 4, 52–62. [PubMed: 11135645]

Crespel A, Rigu V, Coubes P, Rouset MC, de Bock F, Okano H, Baldy-Mouliner M, Bockaert J, Lerner-Natoli M. 2005. Increased number of neural progenitors in human temporal lobe epilepsy. Neurobiol. Dis. 19, 436–450. [PubMed: 16023586]

Cunningham M, Cho JH, Leung A, Savvidis G, Ahn S, Moon M, Lee PK, Han JJ, Azimi N, Kim KS, Bolschakov VY, Chung S. 2014. hPSC-derived maturing GABAergic interneurons ameliorate seizures and abnormal behavior in epileptic mice. Cell Stem Cell 15, 559–573. [PubMed: 25517465]
Curia G, Longo D, Biagini G, Jones RSG, Avoli M. 2008. The pilocarpine model of temporal lobe epilepsy. J. Neurosci. Methods 172, 143–157. [PubMed: 18550176]
Curran T, D’Arcangelo G. 1998. Role of reelin in the control of brain development. Brain Res. Brain Res. Rev. 26, 285–294. [PubMed: 9651544]
Danzer SC. 2018. Contributions of adult-generated granule cells to hippocampal pathology in temporal lobe epilepsy: a neuronal bestiary. Brain Plasticity 3, 169–181. [PubMed: 30151341]
Dayer AG, Ford AA, Cleaver KM, Yassae M, Cameron HA. 2003. Short-term and long-term survival of new neurons in the rat dentate gyrus. J. Comp. Neurol. 460, 563–572. [PubMed: 12717714]
Deisseroth K, Malenka RC. 2005. GABA excitation in the adult brain: a mechanism for excitation-neurogenesis coupling. Neuron 47, 775–777. [PubMed: 16157270]
Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC. 2004. Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron 42, 535–552. [PubMed: 15157417]
Denoth-Lippuner A, Jessberger S. 2021. Formation and integration of new neurons in the adult hippocampus. Nat. Rev. Neurosci. 22, 223–236. [PubMed: 33633402]
Dieni C, Chancey J, Overstreet-Wadiche L. 2013. Dynamic functions of GABA signaling during granule cell maturation. Front. Neural Circuits 6.
Duveau V, Laustela S, Barth L, Gianolini F, Vogt KE, Keist R, Chandra D, Homanics GE, Rudolph U, Fritschy JM. 2011. Spatiotemporal specificity of GABA receptor-mediated regulation of adult hippocampal neurogenesis. Eur. J. Neurosci. 34, 362–373. [PubMed: 21722213]
Eisch AJ, Petrik D. 2012. Depression and hippocampal neurogenesis: a road to remission? Science 338, 72–75. [PubMed: 23042885]
Frotscher M, Haas CA, Förster E. 2003. Reelin controls granule cell migration in the dentate gyrus by acting on the radial glial scaffold. Cereb. Cortex 13, 634–640. [PubMed: 12764039]
Fu C-H, Iascone DM, Petrof I, Hazra A, Zhang X, Pyfer MS, Tosi U, Corbett BF, Cai J, Lee J, Park J, Iacovitti L, Scharfman HE, Enikolopov G, Chin J. 2019. Early seizure activity accelerates depletion of hippocampal neural stem cells and impairs spatial discrimination in an Alzheimer’s disease model. Cell Rep. 27, 3741–3751.e3744. [PubMed: 31242408]
Gage FH. 2021. Adult neurogenesis in neurological diseases. Science 374, 1049–1050. [PubMed: 34822282]
Ge S, Goh ELK, Sailor KA, Kitabatake Y, Ming G. I., Song H. 2006. GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439, 589–593. [PubMed: 16341203]
Ge S, Pradhan DA, Ming GL, Song H. 2007. GABA sets the tempo for activity-dependent adult neurogenesis. Trends Neurosci. 30, 1–8. [PubMed: 17116335]
Gong C, Wang T-W, Huang HS, Parent JM. 2007. Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus. J. Neurosci. 27, 1803–1811. [PubMed: 17314278]
Gray WP, Sundstrom LE. 1998. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. Brain Res. 790, 52–59. [PubMed: 9593820]
Gupta J, Bromwich M, Radell J, Arshad MN, Gonzalez S, Luikart BW, Aaron GB, Naegle JR. 2019. Restrained dendritic growth of adult-born granule cells innervated by transplanted fetal GABAergic interneurons in mice with temporal lobe epilepsy. eNeuro 6.
Hattiangady B, Shetty AK. 2008. Implications of decreased hippocampal neurogenesis in chronic temporal lobe epilepsy. Epilepsia 49 (Suppl. 5), 26–41.
Hattiangady B, Rao MS, Shetty AK. 2004. Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. Neurobiol. Dis. 17, 473–490. [PubMed: 15571983]
Henderson KW, Gupta J, Tagliatela S, Litvina E, Zheng X, Van Zandt MA, Woods N, Grund E, Lin D, Royston S, Yanagawa Y, Aaron GB, Naegle JR. 2014. Long-term seizure suppression and optogenetic analyses of synaptic connectivity in epileptic mice with hippocampal grafts of GABAergic interneurons. J. Neurosci. 34, 13492–13504. [PubMed: 25274826]
Hodge RD, Nelson BR, Kahoud RJ, Yang R, Mussar KE, Reiner SL, Hevner RF. 2012. Tbr2 is essential for hippocampal lineage progression from neural stem cells to intermediate progenitors and neurons. J. Neurosci. 32, 6275. [PubMed: 22553033]
Hosford BE, Liska JP, Danzer SC, 2016. Ablation of newly generated hippocampal granule cells has disease-modifying effects in epilepsy. J. Neurosci. 36, 11013–11023. [PubMed: 27798182]

Hosford BE, Rowley S, Liska JP, Danzer SC. 2017. Ablation of peri-insult generated granule cells after epilepsy onset halts disease progression. Sci. Rep. 7, 18015. [PubMed: 29269775]

Huang X, McMahon J, Huang Y, 2012. Rapamycin attenuates aggressive behavior in a rat model of pilocarpine-induced epilepsy. Neuroscience 215, 90–97. [PubMed: 22522471]

Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC, 2013. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. Nat. Neurosci. 16, 692–697. [PubMed: 23644485]

Hüttmann K, Sadgrove M, Wallraff A, Hinterkeuser S, Kirchhoff F, Steinhäuser C, Gray WP, 2003. Seizures preferentially stimulate proliferation of radial glia-like astrocytes in the adult dentate gyrus: functional and immunocytochemical analysis. Eur. J. Neurosci. 18, 2769–2778. [PubMed: 14656326]

Hutton SR, Pevny LH, 2011. SOX2 expression levels distinguish between neural progenitor populations of the developing dorsal telencephalon. Dev. Biol. 352, 40–47. [PubMed: 21256837]

Indulekha CL, Sanalkumar R, Thekkuveettil A, James J, 2010. Seizure induces activation of multiple subtypes of neural progenitors and growth factors in hippocampus with neuronal maturation confined to dentate gyrus. Biochem. Biophys. Res. Commun. 393, 864–871. [PubMed: 20171185]

Iyengar SS, LaFrancois JJ, Friedman D, Drew LJ, Denny CA, Burghardt NS, Wu MV, Hsieh J, Hen R, Scharfman HE, 2015. Suppression of adult neurogenesis increases the acute effects of kainic acid. Exp. Neurol. 264, 135–149. [PubMed: 25476494]

Jagasia R, Steib K, Englberger E, Herold S, Faus-Kessler T, Saxe M, Gage FH, Song H, Lie DC, 2009. GABA-cAMP response element-binding protein signaling regulates maturation and survival of newly generated neurons in the adult Hippocampus. J. Neurosci. 29, 7966–7977. [PubMed: 19553437]

Jessenberger S, Parent JM, 2015. Epilepsy and adult neurogenesis. Cold Spring Harb. Perspect. Biol. 7.

Jessenberger S, Römer B, Babu H, Kempermann G, 2005. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. Exp. Neurol. 196, 342–351. [PubMed: 16168988]

Jiruska P, Shtaya ABY, Bodansky DMS, Chang W-C, Gray WP, Jefferys JGR, 2013. Dentate gyrus progenitor cell proliferation after the onset of spontaneous seizures in the tetanus toxin model of temporal lobe epilepsy. Neurobiol. Dis. 54, 492–498. [PubMed: 23439313]

Jossin Y, 2020. Reelin functions, mechanisms of action and signaling pathways during brain development and maturation. Biomolecules 10, 964. [PubMed: 32604886]

Kang E, Wen Z, Song H, Christian KM, Ming GL, 2016. Adult neurogenesis and psychiatric disorders. Cold Spring Harb. Perspect. Biol. 8.

Kozareva DA, Cryan JF, Nolan YM, 2019. Born this way: hippocampal neurogenesis across the lifespan. Aging Cell 18, e13007. [PubMed: 31298475]

Kralic JE, Ledergerber DA, Fritschy JM, 2005. Disruption of the neurogenic potential of the dentate gyrus in a mouse model of temporal lobe epilepsy with focal seizures. Eur. J. Neurosci. 22, 1916–1927. [PubMed: 16262631]

Kuhn HG, 2015. Control of cell survival in adult mammalian neurogenesis. Cold Spring Harb. Perspect. Biol. 7, a018895. [PubMed: 26511628]

Kuhn HG, Toda T, Gage FH, 2018. Adult hippocampal neurogenesis: a coming-of-age story. J. Neurosci. 38, 10401–10410. [PubMed: 30381404]

Lévesque M, Avoli M, 2013. The kainic acid model of temporal lobe epilepsy. Neurosci. Biobehav. Rev. 37, 2887–2899. [PubMed: 24184743]

Liu JYW, Dzurova N, Al-Kaaby B, Mills K, Sisodiya SM, Thom M, 2020. Granule cell dispersion in human temporal lobe epilepsy: proteomics investigation of neurodevelopmental migratory pathways. Front. Cell. Neurosci. 14.

López-Rivera JA, Smuk V, Leu C, Nasr G, Vegh D, Stefanski A, Pérez-Palma E, Busch R, Jehi L, Najm I, Blümcke I, Lal D, 2022. Incidence and prevalence of major epilepsy-associated brain lesions. Epilepsy Behav. Rep. 18, 100527. [PubMed: 35243289]
Losi G, Cammarota M, Carmignoto G, 2012. The role of Astroglia in the epileptic brain. Front. Pharmacol. 3.

Lucassen PJ, Oomen CA, Naninck EFG, Fitzsimons CP, van Dam A-M, Czeh B, Korosi A, 2015. Regulation of adult neurogenesis and plasticity by (early) stress, glucocorticoids, and inflammation. Cold Spring Harb. Perspect. Biol. 7, a021303. [PubMed: 26330520]

Lugert S, Basak O, Knuckles P, Haussler U, Fabel K, Götz M, Haas CA, Kempermann G, Taylor V, Giachino C, 2010. Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell 6, 445–456. [PubMed: 20452319]

Lybrand ZR, Goswami S, Zhu J, Jarzabek V, Merlock N, Aktar M, Smith C, Zhang L, Varma P, Cho K-O, Ge S, Hsieh J, 2021. A critical period of neuronal activity results in aberrant neurogenesis rewiring hippocampal circuitry in a mouse model of epilepsy. Nat. Commun. 12, 1423. [PubMed: 33658509]

Martínez-Cerdeño V, Noctor SC, 2018. Neural progenitor cell terminology. Front. Neuroanat. 12.

Mathern GW, Adelson PD, Cahan LD, Leite JP, 2002. Hippocampal neuron damage in human epilepsy: Meyer’s hypothesis revisited. Prog. Brain Res. 135, 237–251. [PubMed: 12143344]

Micheli L, Ceccarelli M, D’Andrea G, Costanzi M, Giacovazzo G, Cocchelrollo R, Caruso C, Tirone F, 2018. Fluoxetine or Sox2 reactivate proliferation-defective stem and progenitor cells of the adult and aged dentate gyrus. Neuropharmacology 141, 316–330. [PubMed: 30142401]

Miller JW, Hakimian S, 2013. Surgical treatment of epilepsy. Continuum (Minneap Minn) 19, 730–742. [PubMed: 23739107]

Mohapel P, Ekdahl CT, Lindvall O, 2004. Status epilepticus severity influences the long-term outcome of neurogenesis in the adult dentate gyrus. Neurobiol. Dis. 15, 196–205. [PubMed: 15006689]

Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Ll ores-Martín M, 2019. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer’s disease. Nat. Med. 25, 554–560. [PubMed: 30911133]

Moss J, Gebara E, Bushong EA, Sánchez-Pascual I, O’Laor R, El M’Ghari I, Kocher-Braissant J, Ellisman MH, Toni N, 2016. Fine processes of nestin-GFP–positive radial glia-like stem cells in the adult dentate gyrus ensheath the local synapses and vasculature. Proc. Natl. Acad. Sci. 113, E2536–E2545. [PubMed: 27091993]

Murphy BL, Hofacer RD, Gaulker CN, Loepke AW, Danzer SC, 2012. Abnormalities of granule cell dendritic structure are a prominent feature of the intrahippocampal kainic acid model of epilepsy despite reduced postinjury neurogenesis. Epilepsia 53, 908–921. [PubMed: 22533643]

Nakagawa S, Kim JE, Lee R, Chen J, Fujioka T, Malberg J, Tsuji S, Duman RS, 2002. Localization of phosphorylated cAMP response element-binding protein in immature neurons of adult hippocampus. J. Neurosci. 22, 9868–9876. [PubMed: 12427843]

Nickell CRG, Peng H, Hayes DM, Chen KY, McClain JA, Nixon K, 2017. Type 2 neural progenitor cell activation drives reactive neurogenesis after binge-like alcohol exposure in adolescent male rats. Front. Psychiatry 8, 283. [PubMed: 29326611]

Orcinha C, Münnzer G, Gerlach J, Kiliias A, Folio M, Egert U, Haas CA, 2016. Seizure-induced motility of differentiated dentate granule cells is prevented by the central reelin fragment. Front. Cell. Neurosci. 10.

Orcinha C, Kiliias A, Paschen E, Folio M, Haas CA, 2021. Reelin is required for maintenance of granule cell lamination in the healthy and epileptic Hippocampus. Front. Mol. Neurosci. 14.

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. [PubMed: 9133993]

Parent JM, Tada E, Fike JR, Lowenstein DH, 1999. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. J. Neurosci. 19, 4508–4519. [PubMed: 10341251]

Parent JM, Elliott RC, Pleasure SJ, Barbaro NM, Lowenstein DH, 2006. Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. Ann. Neurol. 59, 81–91. [PubMed: 16261566]
Paxinos G, Franklin KB, 2019. Paxinos and Franklin’s the Mouse Brain in Stereotaxic Coordinates. Academic press.

Pesold C, Impagnatiello F, Pisu MG, Uzunov DP, Costa E, Guidotti A, Caruncho HJ, 1998. Reelin is preferentially expressed in neurons synthesizing γ-aminobutyric acid in cortex and hippocampus of adult rats. Proc. Natl. Acad. Sci. 95, 3221–3226. [PubMed: 9501244]

Redmond L, Kashani AH, Ghosh A, 2002. Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. Neuron 34, 999–1010. [PubMed: 12086646]

Römer B, Krebs J, Overall RW, Fabel K, Babu H, Overstreet-Wadiche L, Brandt MD, Williams RW, Jessberger S, Kempermann G, 2011. Adult hippocampal neurogenesis and plasticity in the infrapyramidal bundle of the mossy fiber projection: I. Co-regulation by activity. Front. Neurosci. 5, 107. [PubMed: 21991243]

Sasaki-Takahashi N, Shinohara H, Shioda S, Seki T, 2020. The polarity and properties of radial glia-like neural stem cells are altered by seizures with status epilepticus: study using an improved mouse pilocarpine model of epilepsy. Hippocampus 30, 250–262. [PubMed: 32101365]

Scharfman HE, 2004. Functional implications of seizure-induced neurogenesis. Adv. Exp. Med. Biol. 548, 192–212. [PubMed: 15250595]

Scharfman HE, Bernstein HL, 2015. Potential implications of a monosynaptic pathway from mossy cells to adult-born granule cells of the dentate gyrus. Front. Syst. Neurosci. 9.

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. [PubMed: 10934264]

Scharfman H, Goodman J, McCloskey D, 2007. Ectopic granule cells of the rat dentate gyrus. Dev. Neurosci. 29, 14–27. [PubMed: 17148946]

Segi-Nishida E, Warner-Schmidt JL, Duman RS, 2008. Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. Proc. Natl. Acad. Sci. 105, 11352–11357. [PubMed: 18682560]

Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A, 2001. Astrocytes give rise to new neurons in the adult mammalian Hippocampus. J. Neurosci. 21, 7153. [PubMed: 11549726]

Shapiro LA, Ribak CE, Jessberger S, 2008. Structural changes for adult-born dentate granule cells after status epilepticus. Epilepsia 49 (Suppl. 5), 13–18.

Shrestha S, Anderson NC, Grabel LB, Naegle JR, Aaron GB, 2020. Development of electrophysiological and morphological properties of human embryonic stem cell-derived GABAergic interneurons at different times after transplantation into the mouse hippocampus. PLoS One 15, e0237426. [PubMed: 32813731]

Sierra A, Martín-Suárez S, Valcácel-Martín R, Pascual-Brazo J, Aelvoet SA, Abiego O, Deudero JJ, Brewster AL, Bernales I, Anderson AE, Baekelandt V, Maletić-Savatić M, Encinas JM, 2015. Neuronal hyperactivity accelerates depletion of neural stem cells and impairs hippocampal neurogenesis. Cell Stem Cell 16, 488–503. [PubMed: 25957904]

Singh SP, LaSarge CL, An A, McAuliffe JJ, Danzer SC, 2015. Clonal Analysis of Newborn Hippocampal Dentate Granule Cell Proliferation and Development in Temporal Lobe Epilepsy eNeuro 2:E986–0015.2015.

Song J, Zhong C, Bonaguidi MA, Sun GJ, Hsu D, Gu Y, Meletis K, Huang ZJ, Ge S, Enikolopov G, Deisseroth K, Luscher B, Christian KM, Ming GL, Song H, 2012. Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature 489, 150–154. [PubMed: 22842902]

Song J, Sun J, Moss J, Wen Z, Sun GJ, Hsu D, Zhong C, Davoudi H, Christian KM, Toni N, Ming G. I., Song H, 2013. Parvalbumin interneurons mediate neuronal circuitry–neurogenesis coupling in the adult hippocampus. Nat. Neurosci. 16, 1728–1730. [PubMed: 24212671]

Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, James D, Mayer S, Chang J, Auguste KI, Chang EF, Gutierrez AJ, Kriegstein AR, Mathern GW, Oldham MC, Huang EJ, Garcia-Verdugo JM, Yang Z, Alvarez-Buylla A, 2018. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555, 377–381. [PubMed: 29513649]
Suh H, Consiglio A, Ray J, Sawai T, D’Amour KA, Gage FH, 2007. In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell 1, 515–528. [PubMed: 18371391]

Sun W, Winseck A, Vinsant S, Park OH, Kim H, Oppenheim RW, 2004. Programmed cell death of adult-generated hippocampal neurons is mediated by the proapoptotic gene Bax. J. Neurosci. 24, 11205–11213. [PubMed: 15590937]

Thind KK, Yamawaki R, Phanwar I, Zhang G, Wen X, Buckmaster PS, 2010. Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. J. Comp. Neurol. 518, 647–667. [PubMed: 20034063]

Tobi MK, Musaraca K, Disouky A, Shetti A, Bheri A, Honer WG, Kim N, Dawe RJ, Bennett DA, Arfanakis K, 2019. Human hippocampal neurogenesis persists in aged adults and Alzheimer’s disease patients. Cell Stem Cell 24 (974–982), e973.

Toni N, Schinder AF, 2015. Maturation and functional integration of new granule cells into the adult Hippocampus. Cold Spring Harb. Perspect. Biol. 8, a018903. [PubMed: 26637288]

Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T, 2005. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron 47, 803–815. [PubMed: 16157276]

Upadhyya D, Hattiangady B, Castro OW, Shuai B, Kodali M, Attaluri S, Bates A, Dong Y, Zhang S-C, Prockop DJ, Shetty AK, 2019. Human induced pluripotent stem cell-derived MGE cell grafting after status epilepticus attenuates chronic epilepsy and comorbidities via synaptic integration. Proc. Natl. Acad. Sci. 116, 287–296. [PubMed: 30559206]

Varma P, Brulet R, Zhang L, Hsieh J, 2019. Targeting seizure-induced neurogenesis in a clinically relevant time period leads to transient but not persistent seizure reduction. J. Neurosci. 39, 7019. [PubMed: 31308098]

Vivar C, Peterson BD, van Praag H, 2016. Running rewires the neuronal network of adult-born dentate granule cells. Neuroimage 131, 29–41. [PubMed: 26589333]

Voss MW, Soto C, Yoo S, Sodoma M, Vivar C, van Praag H, 2019. Exercise and hippocampal memory systems. Trends Cogn. Sci. 23, 318–333. [PubMed: 30777641]

Wang DD, Krueger DD, Bordey A, 2003. GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAA receptor activation. J. Physiol. 550, 785–800. [PubMed: 12807990]

Wang LP, Kempermann G, Kettenmann H, 2005. A subpopulation of precursor cells in the mouse dentate gyrus receives synaptic GABAergic input. Mol. Cell. Neurosci. 29, 181–189. [PubMed: 15911343]

Wang S, Brunne B, Zhao S, Chai X, Li J, Lau J, Failla AV, Zobiak B, Sibbe M, Westbrook GL, Lutz D, Frotscher M, 2018. Trajectory analysis unveils Reelin’s role in the directed migration of granule cells in the dentate gyrus. J. Neurosci. 38, 137–148. [PubMed: 29138282]

Wickham J, Ledri M, Bengzon J, Jespersen B, Pinborg LH, Englund E, Woldbye DPD, Andersson M, Kokaia M, 2019. Inhibition of epileptiform activity by neuropeptide Y in brain tissue from drug-resistant temporal lobe epilepsy patients. Sci. Rep. 9, 19393. [PubMed: 31852985]

Xu L, Tang X, Wang Y, Xu H, Fan X, 2015. Radial glia, the keystone of the development of the hippocampal dentate gyrus. Mol. Neurobiol. 51, 131–141. [PubMed: 24719081]

Zhao S, Chai X, Förster E, Frotscher M, 2004. Reelin is a positional signal for the lamination of dentate granule cells. Development 131, 5117–5125. [PubMed: 15459104]

Zhou Q-G, Nemes AD, Lee D, Ro EJ, Zhang J, Nowacki AS, Dymecki SM, Najm IM, Suh H, 2019. Chemogenetic silencing of hippocampal neurons suppresses epileptic neural circuits. J. Clin. Invest. 129, 310–323. [PubMed: 30507615]

Zhu K, Yuan B, Hu M, Feng GF, Liu Y, Liu JX, 2017. Reduced abnormal integration of adult-generated granule cells does not attenuate spontaneous recurrent seizures in mice. Epilepsy Res. 133, 58–66. [PubMed: 28431266]

Zhu X, Yao Y, Yang J, Ge Q, Niu D, Liu X, Zhang C, Gan G, Zhang A, Yao H, 2020. Seizure-induced neuroinflammation contributes to ectopic neurogenesis and aggressive behavior in pilocarpine-induced status epilepticus mice. Neuropharmacology 170, 108044. [PubMed: 32179291]
Fig. 1. Type 2 and inverted type 1 progenitors are reduced following the transplantation of GABAergic progenitors. A, Experimental timeline (see methods). B, Confocal images of Type 1 (GFAP+/SOX2+) and Type 2 (SOX2+/GFAP−) progenitors in the SGZ of the DG in Naïve-Media mice. The white arrow is pointing toward a Type 1 progenitor. Type 2 progenitors only express SOX2. DAPI is used as a counterstain. C, High-resolution zoomed image of a Type 1 progenitor showing z-axis projection and triple labeling. D, High-resolution zoomed-in image of a Type 2 progenitor, showing z-axis projection and...
double labeling. E, Confocal image of eYFP expressing transplanted cells in the DG of the hippocampus from a Naïve-TX mouse. F, Confocal images of Type 1 and Type 2 progenitors in the SGZ of the DG in Naïve-Media mouse; G, Type 1 and Type 2 progenitors in a Naïve-TX mouse DG., H, Confocal image of eYFP+ transplanted cells in the DG of the hippocampus from a TLE-TX mouse. I-J, Confocal images of Type 1 and Type 2 progenitors in the SGZ of the DG in TLE-Media and TLE-TX mice, respectively. Inverted type 1 progenitors are shown by an inverted arrow in panel I. K, Graph shows a significant reduction in the number of Type 1 progenitors in the SGZ in TLE mice with or without GABAergic transplants compared to naïve mice. L, Quantification shows fewer inverted Type 1 progenitors in TLE mice following transplantation of the GABAergic progenitors vs. TLE-Media mice. M, Graph shows a significant reduction in the number of Type 2 progenitors in the SGZ of the DG in mice with GABA transplants. [One-way ANOVA, Multiple comparisons. *p-value<0.05; **p-value = 0.002; ***p-value <0.0002; ****p-value < 0.0001]. Scale bar = 100 μm (E, H) & 10 μm (C–D, F-G, I-J).
DCX\(^+\) adult-born granule cells in the DG of the hippocampus increase in naïve and TLE mice following transplantation of GABAergic cells. (A) Experimental timeline. Five to six-week-old mice were either subjected to pilocarpine-induced TLE or received a saline injection. One week later both groups of mice received either a GABAergic progenitor transplant or a media transplant. Four weeks following transplantation, all four groups of mice were perfused to perform immunohistochemistry to identify Type 3 progenitors. Naïve and TLE mice that received injections of media (Media; no cells transplanted) are
shown (B, E) for comparison with Naïve and TLE mice with GABA progenitor transplants (C–D, F–G). B, Confocal image shows DCX⁺ Type 3 cells in the DG of a Naïve-Media mouse. E, Confocal images of DCX⁺ cells in a TLE-Media mouse. SE was associated with increased numbers of DCX⁺ cells and ectopic migration into the hilus of the DG (E). C–D; F–G, Confocal images comparing DCX⁺ cells (red) in representative animals from the Naïve-TX and TLE-TX groups. Transplanted VGAT-ChR2-eYFP⁺ GABAergic progenitors formed more extensive processes onto DCX⁺ cells. DAPI is a counterstain. H, Quantification showed increased DCX⁺ cells in naïve mice with TX compared to naïve-Media mice and significantly more DCX⁺ cells in TLE-Media operates compared to naïve-TX mice. TLE-TX mice had significantly higher numbers of DCX⁺ cells compared to other groups. I, Quantification of the effects of GABAergic progenitor transplants on ectopic migration, showing that transplantation in TLE mice resulted in significantly fewer ectopic DCX⁺ cells in the hilus of the DG. J, Graph shows the cumulative distributions (%) of DCX⁺ cells in the four treatment groups based on the distance of radial migration (microns) from the SGZ. Type 3 progenitors traveled farthest in TLE-TX mice. K, Graph shows a positive correlation between the numbers of surviving transplanted eYFP⁺ cells and DCX⁺ cells in naïve and TLE mice. [H–I, One-way ANOVA, followed by Tukey’s multiple comparisons. *p-value < 0.05; ** < 0.009; ***p-value < 0.0009; ****p-value < 0.0001; J, Kolmogorov-Smirnov test; *p-value = 0.03; *p-value = 0.04; **p-value = 0.009; K, Pearson correlation. *p-value<0.05]. Scale bar = 100 μm.
Fig. 3.
DCX⁺ adult-born neurons are increased in the dorsal hippocampus following transplantation of GABAergic interneurons. A, Modified atlas (Paxinos and Franklin, 2019) images showing an overview of the horizontal sections (distance from the bregma). Numbers in the left corner correspond to different dorsal-ventral levels from the bregma. The insets correspond to the confocal images below. B, Confocal images show DCX⁺ cells in the DG at different dorsal-ventral levels of a Naïve-Media-control mouse. C, Confocal images show DCX⁺ cells in the DG at different dorsal-ventral levels of a Naïve-TX control mouse.
Transplanted VGAT-ChR2-eYFP+ GABAergic progenitors formed more extensive processes onto DCX$^+$ cells in more dorsal levels of the DG. D, Confocal images of DCX$^+$ cells in a TLE-Media mouse. TLE was associated with increased numbers of DCX$^+$ cells in the GCL and ectopic migration into the hilus of the DG at different dorsal-ventral levels. E, Confocal images show DCX$^+$ cells in representative animals from the TLE-TX groups. Transplanted VGAT-ChR2-eYFP+ GABAergic progenitors formed more extensive processes onto DCX$^+$ cells in more dorsal levels of the DG. F-G, GABAergic interneuron transplantation significantly increased the number of DCX$^+$ cells in the dorsal hippocampus but not in the ventral hippocampus in naive and TLE mice compared to the Media controls. H) Graph comparing the dorso-ventral distributions of DCX$^+$ cells in the DG shows the most significant reductions in ectopic adult-born GCs were in the more dorsal levels of the hippocampus. [ANOVA, Multiple comparisons. \( \cdot p \text{-value} < 0.05; \quad \cdot \cdot \cdot \cdot \text{-value} < 0.009, \quad \cdot \cdot \cdot \cdot \cdot \text{-value} = 0.0003, \quad \cdot \cdot \cdot \cdot \cdot \cdot \text{-value} < 0.0001, \quad t\text{-test, multiple comparisons.} \cdot \cdot \cdot \cdot \text{-value} < 0.05; \quad \cdot \cdot \cdot \cdot \cdot \cdot \text{-value} < 0.008]. \) Scale bar = 100 μm.
Fig. 4.
Transplantation of GABAergic progenitors does not increase cell proliferation in naïve and TLE mice. A, Experimental timeline. To study the effect of GABAergic transplant on cell proliferation, animals received a single dose of BrdU and were euthanized after 2 h. B, Confocal image shows proliferating BrdU-labeled adult-born cells (red) in the SGZ of the DG from a Naïve-Media mouse. C, Confocal image shows proliferating BrdU-labeled adult-born cells in the SGZ of the DG from a Naïve-TX mouse. D, Confocal image shows proliferating BrdU-labeled adult-born cells in the SGZ of the DG from a TLE-Media mouse. E, Confocal image shows proliferating BrdU-labeled adult-born cells in the SGZ of the DG from a TLE-TX mouse.

Neurobiol Dis. Author manuscript; available in PMC 2022 December 06.
E, Confocal image shows proliferating BrdU-labeled adult-born cells in the SGZ of the DG from a TLE-TX mouse. F, Quantification shows no effect of GABA transplants on the proliferation of BrdU-labeled adult-born cells in naïve and TLE mice. [One-way ANOVA, followed by Tukey’s multiple comparisons; ****p-value < 0.0001]. Scale bar = 25 μm.
Fig. 5.
Transplanted GABAergic progenitors increase the survival of adult-born neurons in TLE mice. A, Experimental timeline. To study the effect of GABAergic transplant on survival of adult-born neurons, animals received a single dose of BrdU and were euthanized after 3 weeks. B, Confocal image shows proliferating BrdU-labeled adult-born neurons in the GCL of the DG from a Naïve-Media mouse. C, Confocal image shows proliferating BrdU-labeled adult-born cells in the GCL of the DG from a Naïve-TX mouse. D, Confocal image shows proliferating BrdU-labeled adult-born cells in the GCL of the DG from a TLE-Media mouse.
E, Confocal image shows proliferating BrdU-labeled adult-born cells in the GCL of the DG from a TLE-TX mouse. G, Quantification shows a significant increase in the number of surviving 3-week-old BrdU-labeled adult-born cells in naïve and TLE mice compared to the media controls. [Kruskal-Wallis, Multiple comparisons. *p-value = 0.04; ***p-value = 0.0001; ns-p-value = 0.051]. Scale bar = 25 μm.
Fig. 6.
BrdU+ adult-born cells are increased in the dorsal hippocampus following transplantation of GABAergic interneurons. A, Modified brain atlas images (Paxinos and Franklin, 2019) showing dorsoventral levels through the DG. Numbers in the left corner correspond to different dorsal-ventral levels from the bregma. B, A significant increase in the number of BrdU+ cells was found at more dorsal levels of the DG in the naïve transplant group compared to the naïve-Media group, corresponding to the levels of the DG that contained the most transplant-derived GABAergic interneurons. C, A significant increase in the number of BrdU+ cells was found at more dorsal levels of the DG in TLE-TX group vs. TLE-Media group. [Kruskal-Wallis. Multiple comparisons. *p-value <0.05; **p-value = 0.006; ****p-value<0.0001].
### Table 1

**Key resources.**

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Antibodies** | | |
| Chicken anti-GFP (1:1000; 10 μg/mL) | Aves Labs | GFP 1020; RRID:AB_10000240 |
| Mouse anti-GFAP (1:1000) | Millipore Sigma | MAB360, RRID:AB_11212597 |
| Rabbit anti-SOX2 (1:500; 2 μg/mL) | Millipore Sigma | AB5603, RRID: AB_2286686 |
| Guinea pig anti-DCX (1:1000) | Millipore Sigma | AB2253, RRID: AB_1586992 |
| Rat anti-BrdU (1:500; 2 μg/mL) | BioRad | MCA2060GA, RRID: AB_10545551 |
| Goat anti-chicken IgY Alexa Fluor 488 (1:1000; 2 μg/mL) | Molecular Probes | A-11039, RRID: AB_142924 |
| Goat anti-rabbit IgG Alexa Fluor 568 (1:1000; 2 μg/mL) | Molecular Probes | A-11036, RRID: AB_10563566 |
| Goat anti-rat IgG Alexa Fluor 650 (1:1000; 2 μg/mL) | Molecular Probes | A-11077, RRID: AB_141874 |
| Goat anti-mouse IgG Alexa Fluor 647 (1:1000; 2 μg/mL) | Thermo Fisher Scientific | A-21242, RRID: AB_2535811 |
| goat anti-guinea pig IgG Alexa Fluor 647 (1:1000; 2 μg/mL) | Thermo Fisher Scientific | A-21450, RRID: AB_2735091 |
| DAPI (1:2000) | ThermoFisher Scientific | 62248 |
| Prolong Diamond with DAPI | ThermoFisher Scientific | P36966 |
| **Chemicals and Instruments** | | |
| L-15 Medium | Gibco | 11415-064 |
| Fibroblast growth factor | Sigma | F0291 |
| Epidermal growth factor | Invitrogen | 53303-018 |
| Caspase Inhibitor | Promega | G7231 |
| B27 | Gibco | 17504-044 |
| HBSS powder | Sigma | H4891 |
| 2.5% Trypsin (10x) | Gibco | 15090-046 |
| Trypsin Inhibitor | Gibco | 007-100 |
| Scopolamine methyl bromide | Sigma | S8502-1G |
| Pilocarpine | Sigma | P6503-5G |
| Paraformaldehyde | Electron Microscopy Sciences | 15174-S |
| Heparin Sodium | Fisher BioReagents | BP245 1 g |
| Midazolam HCL | Covetrus | 23155060142 |
| Fatal Plus | Covetrus | VPL9373 |
| Isothesia | Henry Schein | SKU 029405 |
| Glass syringe (5 μL, removable needle) | Hamilton | 7634-01 |
| Needle 26GA, 0.5" 30° | Hamilton | 7804-03 |
| Automated stereotaxic injection system | Stoeling Quintessential | 53311 |
| Charcoal filter, VetEquip | Harvard Apparatus | 340415 |
| Stereotaxic Apparatus | KOPF | 940 Small |
| **Experimental Model: Strains** | | |
| C57BL/6N Hsd | Envigo | 044 |
| B6.Cg-Tg(Slcl32a1-COP4*H134R/EYFP)y8Gfng/J (VGAT-ChR2-EYFP line 8) | The Jackson Laboratory | 014548 |
| **Software** | | |

*Neurobiol Dis. Author manuscript; available in PMC 2022 December 06.*
| Reagent or Resource | Source | Identifier |
|--------------------|--------|------------|
| Graphpad Prism 7   |        |            |
Table 2

| Fig. 1: Type 1 progenitors | Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE - Media vs. TLE-TX |
|---------------------------|---------------------------|---------------------------|---------------------------|------------------------|------------------------|
| ANOVA; p value            | 0.31                      | 0.002                     | 0.0002                    | 0.31                   |
| Mean (cells)              | Naive-Media: 235          | Naive-Media: 235          | Naive-Media: 235          | TLE - Media: 138       |
|                           | Naive-TX: 265             | TLE-Media: 138            | TLE-TX: 107               | TLE-TX: 107            |
| Difference                | −30                       | 97                        | 128                       | 31                     |
| N (animals)               | Naive-Media: 4            | Naive-Media: 4            | Naive-Media: 4            | TLE - Media: 4         |
|                           | Naive-TX: 4               | TLE-Media: 4              | TLE-TX: 6                 | TLE-TX: 6              |

| Fig. 1: Inverted Type 1 progenitors | Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE - Media vs. TLE-TX |
|---------------------------|---------------------------|---------------------------|---------------------------|------------------------|------------------------|
| ANOVA; p value            | 0.99                      | <0.0001                    | 0.002                     | <0.0001                 |
| Mean (cells)              | Naive-Media: 14           | Naive-Media: 14           | Naive-Media: 14           | TLE - Media: 156       |
|                           | Naive-TX: 18              | TLE-Media: 156            | TLE-TX: 70                | TLE-TX: 70             |
| Difference                | −4                        | −142                      | −56                       | 86                     |
| N (animals)               | Naive-Media: 4            | Naive-Media: 4            | Naive-Media: 4            | TLE - Media: 4         |
|                           | Naive-TX: 4               | TLE-Media: 4              | TLE-TX: 6                 | TLE-TX: 6              |

| Fig. 1: Type 2 progenitors | Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE - Media vs. TLE-TX |
|---------------------------|---------------------------|---------------------------|---------------------------|------------------------|------------------------|
| ANOVA; p value            | 0.04                      | 0.0008                     | 0.01                       | <0.0001                 |
| Mean (cells)              | Naive-Media: 1256         | Naive-Media: 1256         | Naive-Media: 1256         | TLE - Media: 1926      |
|                           | Naive-TX: 871             | TLE-Media: 1926           | TLE-TX: 851               | TLE-TX: 851            |
| Difference                | 385                       | −670                      | 405                       | 1075                   |
| N (animals)               | Naive-Media: 4            | Naive-Media: 4            | Naive-Media: 4            | TLE - Media: 4         |
|                           | Naive-TX: 4               | TLE-Media: 4              | TLE-TX: 6                 | TLE-TX: 6              |

Fig. 2: DCX⁺ cells in GCL
### Quantitative measurements

|                      | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE- Media | Naïve-Media vs. TLE- TX | TLE-Media vs. TLE-TX |
|----------------------|---------------------------|---------------------------|------------------------|----------------------|
| **ANOVA: p value**   | 0.02                      | <0.0001                   | <0.0001                | 0.0003               |
| **Mean (cells)**     | Naïve-Media: 2299         | Naïve-Media: 2299         | Naïve-Media: 2299      | TLE-Media: 3667      |
|                      | Naïve- TX: 2840           | TLE- Media: 3667          | TLE- TX: 4516          | TLE-TX: 4516         |
| **Difference**       | -541                      | -1368                     | -2217                  | -849                 |
| **N (animals)**      | Naïve-Media: 5            | Naïve-Media:               | Naïve-Media:            | TLE-Media:           |
|                      | Naïve- TX: 4              | TLE- Media:               | TLE- TX:               | TLE-TX:              |

**Fig. 2: DCX+ cells in Hilus**

|                      | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE- Media | Naïve-Media vs. TLE- TX | TLE-Media vs. TLE-TX |
|----------------------|---------------------------|---------------------------|------------------------|----------------------|
| **ANOVA: p value**   | 0.8                       | <0.0001                   | <0.0001                | 0.0009               |
| **Mean (cells)**     | Naïve-Media: 22           | Naïve-Media: 22           | Naïve-Media: 22        | TLE-Media: 550       |
|                      | Naïve- TX: 30             | TLE- Media: 550           | TLE- TX: 335           | TLE-TX: 335          |
| **Difference**       | -8                        | -528                      | -313                   | 215                 |
| **N (animals)**      | Naïve-Media: 5            | Naïve-Media: 5            | Naïve-Media: 5         | TLE-Media: 6         |
|                      | Naïve- TX: 4              | TLE- Media: 5             | TLE- TX: 6             | TLE-TX: 6            |

**Fig. 2: Correlation between TX and DCX+ cells**

|                      | Naïve-TX                   | TLE-TX                    |
|----------------------|----------------------------|----------------------------|
| **Pearson correlation: R** | 0.95                      | 0.83                       |
| **P value**          | 0.04                       | 0.03                       |
| **R squared**        | 0.91                       | 0.695                      |
| **N (animals)**      | Naïve-TX: 4                | TLE-TX: 6                  |

**Fig. 2: Migration distance analysis**

|                      | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE-Media | Naïve-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|----------------------|---------------------------|---------------------------|------------------------|----------------------|
| **Kolmogorov- Smirnov test: p value** | 0.03                      | 0.01                      | 0.009                  | 0.3                  |
| **Mean (μm)**        | Naïve-Media: 9.06         | Naïve-Media: 9.06         | Naïve-Media: 9.06      | TLE-Media: 16.37     |
|                      | Naïve- TX: 16.86          | TLE- Media: 16.37         | TLE- TX: 24.5          | TLE-TX: 24.5         |
| **Difference**       | -7.8                      | -7.31                     | -15.44                 | -8.13                |
| N (cells) | Naive-Media: 38 | Naive-Media: 38 | Naive-Media: 38 | TLE-Media: 90 |
|----------|----------------|----------------|----------------|--------------|
| Naive-TX: 91 | TLE-Media: 90 | TLE-TX: 141 | TLE-TX: 141 |
| N (Animals) | Naive-Media: 5 | Naive-Media: 5 | Naive-Media: 5 | TLE-Media: 5 |
| Naive-TX: 4 | TLE-Media: 5 | TLE-TX: 6 | TLE-TX: 6 |

**Fig. 3:** Distribution of DCX⁺ cells (GCL), Distance from Bregma: −2.04 mm

| Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|---------------------------|--------------------------|---------------------------|------------------------|----------------------|
| ANOVA: p value            | 0.03                     | 0.34                      | 0.0003                 | 0.009                |
| Mean (cells)              | Naive-Media: 1828        | Naive-Media: 2048         | TLE-Media: 2497        | TLE-TX: 2497         |
| Naive-TX: 2251            | TLE-Media: 2048          | TLE-Media: 2497           | TLE-TX: 2497           | TLE-TX: 2497         |
| Difference                | −422.6                   | −220                      | −669                   | −449                 |
| N (animals)               | Naive-Media: 5           | Naive-Media: 5            | Naive-Media: 5         | TLE-Media: 5         |
| Naive-TX: 4               | TLE-Media: 5             | TLE-TX: 6                 | TLE-TX: 6              |

**Fig. 3:** Distribution of DCX⁺ cells (GCL), Distance from Bregma: −2.36 mm

| Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|---------------------------|--------------------------|---------------------------|------------------------|----------------------|
| ANOVA: p value            | 0.57                     | <0.0001                   | <0.0001                | <0.0001              |
| Mean (cells)              | Naive-Media: 208         | Naive-Media: 208          | TLE-Media: 834         | TLE-TX: 834          |
| Naive-TX: 274             | TLE-Media: 736           | TLE-TX: 834               | TLE-TX: 1042           | TLE-TX: 1042         |
| Difference                | −66                      | −528                      | −834                   | −306                 |
| N (animals)               | Naive-Media: 5           | Naive-Media: 5            | Naive-Media: 5         | TLE-Media: 5         |
| Naive-TX: 4               | TLE-Media: 5             | TLE-TX: 6                 | TLE-TX: 6              |

**Fig. 3:** Distribution of DCX⁺ cells (GCL), Distance from Bregma: −2.96 mm

| Quantitative measurements | Naive-Media vs. Naive-TX | Naive-Media vs. TLE-Media | Naive-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|---------------------------|--------------------------|---------------------------|------------------------|----------------------|
| ANOVA: p value            | 0.99                     | 0.001                     | 0.002                  | 0.87                 |
| Mean (cells)              | Naive-Media: 149         | Naive-Media: 149          | TLE-Media: 490         | TLE-TX: 490          |
| Naive-TX: 162             | TLE-Media: 490           | TLE-TX: 542               | TLE-TX: 542            | TLE-TX: 542          |
| Difference                | −13                      | −341                      | −393                   | −52                  |
| N (animals)               | Naive-Media: 5           | Naive-Media: 5            | Naive-Media: 5         | TLE-Media: 5         |
| Naive-TX: 4               | TLE-Media: 5             | TLE-TX: 6                 | TLE-TX: 6              |
### Quantitative measurements

#### Fig. 3: Distribution of DCX+ cells (GCL), Distance from Bregma: −3.28 mm

| ANOVA: p value | Naïve-Media vs. Naïve-TX | Naïve-Media vs. TLE-Media | Naïve-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|----------------|-------------------------|----------------------------|------------------------|----------------------|
| 0.99           | 0.001                   | 0.0002                    | 0.87                   |
| Mean (cells)   | Naïve-Media: 113        | Naïve-Media: 113          | Naïve-Media: 113       | TLE-Media: 392       |
|                | Naïve-TX: 152           | TLE-Media: 392           | TLE-TX: 417            | TLE-TX: 417          |
| Difference     | −39                     | −279                      | −304                   | −25                  |
| N (animals)    | Naïve-Media: 5          | Naïve-Media: 5            | Naïve-Media: 5         | TLE-Media: 5         |
|                | Naïve-TX: 4             | TLE-Media: 5              | TLE-TX: 6              | TLE-TX: 6            |

#### Multiple t-test: p value

| −2.04 mm       | −2.36 mm                   | −2.96 mm                   | −3.28 mm               |
|----------------|-----------------------------|-----------------------------|-------------------------|
| 0.02           | 0.008                       | 0.54                        | 0.86                    |
| Mean (cells)   | TLE-Media: 335              | TLE-Media: 114              | TLE-Media: 68           |
|                | TLE-TX: 114                 | TLE-TX: 57                 | TLE-TX: 47              |
| Difference     | 221                         | 57                         | 21                      |
| N (animals)    | TLE-Media: 5                | TLE-Media: 5               | TLE-Media: 5            |
|                | TLE-TX: 6                   | TLE-TX: 6                  | TLE-TX: 6               |

#### Fig. 4: Cell proliferation (BrdU 2-h)

| ANOVA: p value | Naïve-Media vs. Naïve-TX | Naïve-Media vs. TLE-Media | Naïve-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|----------------|-------------------------|----------------------------|------------------------|----------------------|
| 0.86           | <0.0001                  | <0.0001                    | >0.999                 |
| Mean (cells)   | Naïve-Media: 285         | Naïve-Media: 285           | Naïve-Media: 285       | TLE-Media: 478       |
|                | Naïve-TX: 300            | TLE-Media: 478             | TLE-TX: 479            | TLE-TX: 479          |
| Difference     | −15                      | −193                       | −194                   |
| N (animals)    | Naïve-Media: 5           | Naïve-Media: 5             | Naïve-Media: 5         | TLE-Media: 4         |
|                | Naïve-TX: 4              | TLE-Media: 4               | TLE-TX: 4              | TLE-TX: 4            |

#### Fig. 5: Survival (BrdU 3-weeks)

| Quantitative measurements | Naïve-Media vs. Naïve-TX | Naïve-Media vs. TLE-Media | Naïve-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|---------------------------|--------------------------|---------------------------|------------------------|----------------------|
| ANOVA: p value            | 0.99                     | 0.001                     | 0.0002                 | 0.87                 |
| Mean (cells)              | Naïve-Media: 113         | Naïve-Media: 113          | Naïve-Media: 113       | TLE-Media: 392       |
|                           | Naïve-TX: 152            | TLE-Media: 392           | TLE-TX: 417            | TLE-TX: 417          |
| Difference                | −39                      | −279                      | −304                   |
| N (animals)               | Naïve-Media: 5           | Naïve-Media: 5            | Naïve-Media: 5         | TLE-Media: 5         |
|                           | Naïve-TX: 4              | TLE-Media: 5              | TLE-TX: 6              | TLE-TX: 6            |
### Kruskal-Wallis: p value

|                | Naïve-Media: 3 | Naïve-Media: 3 | Naïve-Media: 3 | TLE- Media: 9.8 |
|----------------|----------------|----------------|----------------|-----------------|
| Mean (rank)    | Naïve-Media: 3 | Naïve-Media: 3 | Naïve-Media: 3 | TLE- Media: 9.8 |
| Difference     | −7.5           | −6.8           | −13.8          | −7              |
| N (animals)    | Naïve-Media: 5 | Naïve-Media: 5 | Naïve-Media: 5 | TLE- Media: 5   |

**Fig. 6:** Distribution of BrdU+ cells (GCL), Distance from Bregma: −2.04 mm

### Quantitative measurements

|                | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE- Media | Naïve-Media vs. TLE- TX | TLE- Media vs. TLE- TX |
|----------------|---------------------------|----------------------------|-------------------------|-------------------------|
| Kruskal-Wallis: P value | 0.01                      | 0.16                      | <0.0001                 | 0.01                    |
| Mean (rank)    | Naïve-Media: 3            | Naïve-Media: 3            | Naïve-Media: 3          | TLE- Media: 8           |
| Difference     | −13                       | −5                        | −14                     | −9                      |
| N (animals)    | Naïve-Media: 5            | Naïve-Media: 5            | Naïve-Media: 5          | TLE- Media: 5           |

**Fig. 6:** Distribution of BrdU+ cells (GCL), Distance from Bregma: −2.36 mm

### Quantitative measurements

|                | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE- Media | Naïve-Media vs. TLE- TX | TLE- Media vs. TLE- TX |
|----------------|---------------------------|----------------------------|-------------------------|-------------------------|
| Kruskal-Wallis: P value | 0.01                      | 0.16                      | 0.0002                  | 0.051                   |
| Mean (rank)    | Naïve-Media: 3            | Naïve-Media: 3            | Naïve-Media: 3          | TLE- Media: 9.3         |
| Difference     | −8.8                      | −6.3                      | −13.2                   | −6.9                    |
| N (animals)    | Naïve-Media: 5            | Naïve-Media: 5            | Naïve-Media: 5          | TLE- Media: 5           |

**Fig. 6:** Distribution of BrdU+ cells (GCL), Distance from Bregma: −2.96 mm

### Quantitative measurements

|                | Naïve-Media vs. Naïve- TX | Naïve-Media vs. TLE- Media | Naïve-Media vs. TLE- TX | TLE- Media vs. TLE- TX |
|----------------|---------------------------|----------------------------|-------------------------|-------------------------|
| Kruskal-Wallis: P value | 0.055                     | 0.01                       | 0.13                    | 0.33                    |
| Mean (rank)    | Naïve-Media: 4.8          | Naïve-Media: 4.8           | Naïve-Media: 4.8        | TLE- Media: 13.5        |
| Difference     | −7.2                      | −8.7                      | −5.3                    | 3.4                     |
| N (animals)    | Naïve-Media: 5            | Naïve-Media: 5            | Naïve-Media: 5          | TLE- Media: 5           |

**Fig. 6:** Distribution of BrdU+ cells (GCL), Distance from Bregma: −3.06 mm
| N (animals)  | Naïve-Media: 5 | Naïve-Media: 5 | Naïve-Media: 5 | TLE-Media: 5 | TLE-TX: 5 | TLE-TX: 5 |
|-------------|----------------|----------------|----------------|--------------|------------|-----------|
| Naïve-TX: 4 | TLE-Media: 5   | TLE-Media: 5   | TLE-TX: 5      | TLE-TX: 5    |

**Fig. 6: Distribution of BrdU+ cells (GCL), Distance from Bregma: −3.28 mm**

| Quantitative measurements | Naïve-Media vs. Naïve-TX | Naïve-Media vs. TLE-Media | Naïve-Media vs. TLE-TX | TLE-Media vs. TLE-TX |
|---------------------------|--------------------------|----------------------------|------------------------|----------------------|
| Kruskal-Wallis: p value   | 0.053                    | 0.03                       | 0.006                  | 0.91                 |
| Mean (rank)               | Naïve-Media: 3.2         | Naïve-Media: 3.2           | Naïve-Media: 3.2       | TLE-Media: 13.8      |
|                           | Naïve-TX: 12             | TLE-Media: 13.5            | TLE-TX: 10.1           | TLE-TX: 13.4         |
| Difference                | −7.2                     | −10.6                      | −10.2                  | 0.4                  |
| N (animals)               | Naïve-Media: 5           | Naïve-Media: 5             | Naïve-Media: 5         | TLE-Media: 5         |
|                           | Naïve-TX: 4              | TLE-Media: 5               | TLE-TX: 5              | TLE-TX: 5            |

**Supplementary Fig. 1: Effect of midazolam on cell proliferation**

| Quantitative measurements | Naïve-Saline vs. Naïve-Midazolam | Naïve-Saline vs. TLE-Midazolam | Naïve-Midazolam vs. TLE-Midazolam |
|---------------------------|----------------------------------|--------------------------------|----------------------------------|
| ANOVA: p value            | 0.997                            | 0.003                          | 0.006                            |
| Mean (cells)              | Naïve-Saline: 235                | Naïve-Saline: 235              | Naïve-Midazolam: 236             |
|                           | Naïve-Midazolam: 236             | TLE-Midazolam: 578             | TLE-Midazolam: 578               |
| Difference                | −1                               | −343                           | −342                             |
| N (animals)               | Naïve-Saline: 3                  | Naïve-Saline: 3                | Naïve-Saline: 3                  |
|                           | Naïve-Midazolam: 3               | TLE-Midazolam: 3               | TLE-Midazolam: 3                 |