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Enhanced Susceptibility to Chemoconvulsant-Induced Seizures in Ganglioside GM3 Synthase Knockout Mice

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Abstract

Ganglioside GM3 synthase (α-2,3-sialyltransferase, ST3GAL5, GM3S) is a key enzyme involved in the biosynthesis of gangliosides. ST3GAL5 deficiency causes an absence of GM3 and all downstream biosynthetic derivatives. The affected individuals manifest deafness, severe irritability, intractable seizures, and profound intellectual disability. To investigate whether deficiency of GM3 is involved in seizure susceptibility, we induced seizures with different chemoconvulsants in ST3GAL5 knockout mice. We report here that ST3GAL5 knockout mice are hyperactive and more susceptible to seizures induced by chemoconvulsants, including kainate and pilocarpine, compared with normal controls. In the hippocampal dentate gyrus, loss of GM3 aggravates seizure-induced aberrant neurogenesis. These data indicate that GM3 and gangliosides derived from GM3 may serve as important regulators of epilepsy and may play an important role in aberrant neurogenesis associated with seizures.

Keywords

ganglioside GM3, seizure, adult neurogenesis

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Gangliosides are a group of multifunctional molecules found on the surface of virtually all mammalian cells. They are particularly abundant in the central nervous system, where they represent about 10% of the total lipid content (Schnaar et al., 2014).

Four major species (GM1, GD1a, GD1b, and GT1b) comprise > 97% of mature mammalian brain gangliosides, represented roughly equally by members of the a- and b-series gangliosides (Tettamanti et al., 1973). These sialic acid-containing glycosphingolipids are thought to function in the regulation of signaling pathways that impact cell proliferation, survival, cell–cell adhesion, and motility, and they play essential roles in normal neural development and function (Yu et al., 2012). For this reason, mutations in ganglioside biosynthetic enzymes in humans invariably result in intellectual disability and are often accompanied by increased seizure susceptibility (Simpson et al., 2004; Boukhris et al., 2013; Fragaki et al., 2013; Harlalka et al., 2013; Boccuto et al., 2014). The mechanisms by which changes in ganglioside biosynthesis result in learning disabilities and dysregulation of excitatory neurotransmission have not been fully established.

GM3, as the first ganglioside of the synthetic pathway, is a common precursor of all of the downstream derivatives (Ando & Yu, 1979; Yu & Ando, 1980; Sandhoff et al., 2018). Ganglioside GM3 synthase (also called lactosylceramide, α-2,3-sialyltransferase, ST3GAL5, GM3S) is the key enzyme involved in the initial stages of the biosynthesis of the a-, b-, and c-series gangliosides (Kolter et al., 2002; Yu et al., 2011). In 2004, a nonsense variant in ST3GAL5, p.Arg288ter (R288*), was found in

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family members of the Amish community and was reported to be the cause of a severe neurologic syndrome characterized by infantile onset of severe irritability, hearing loss, cortical blindness, profound intellectual disability, and drug-resistant epileptic seizures (Simpson et al., 2004). In patients with ST3GAL5 deficiency, GM3 and all downstream biosynthetic derivatives in the circulation are hardly detectable even with some recently improved analytic assays (Huang et al., 2014). The lack of a-, b-, and c-series gangliosides are replaced by overexpression of asialo- and α-series gangliosides (Seyfried et al., 1994; Huang et al., 2016). The discovery of ST3GAL5 deficiency, a recessive genetic disorder, provided the first evidence for a congenital disorder of glycosylation that affects the biosynthesis of complex glycosphingolipids (Bowser et al., 2019). An additional report from France focused on a family with two members suffering from early onset of epilepsy, deafness, and blindness (Fragaki et al., 2013). Those patients were identified to have homozygous mutations in the ST3GAL5 gene. Yet another report examined ST3GAL5-deficient patients who also showed progressive epilepsy syndrome (Farukhi et al., 2006).

Knockout (KO) mice in which ST3GAL5 is disrupted have been established with total loss of all a-, b-, and c-series gangliosides. Of the two ST3GAL5 KO mouse models generated in the past, the earlier one (Yamashita et al., 2003) revealed a phenotype that is generally normal except for increased insulin sensitivity and a low response to a high-fat diet. In a later study, it was found that ST3GAL5-KO mice exhibit complete hearing loss due to selective degeneration of the organ of Corti (Yoshikawa et al., 2009, 2015), which resembles the deafness found in ST3GAL5-deficient patients. Therefore, ST3GAL5-KO mice may represent a useful model to investigate the pathological processes of neurological disorders in patients with ST3GAL5 deficiency. Interestingly, mice lacking other sialyltransferase are often accompanied by increased seizure susceptibility; enhanced susceptibility to seizures was found in mice lacking B4GALNT1 (Wu et al., 2005), and mice with B4GALNT1 and ST8SIA1double KO are sensitive to sound-induced seizures (Kawai et al., 2001). It will be interesting to examine whether ST3GAL5-KO mice could present an excellent model for investigating the genetic basis of epileptic seizures found in patients.

Hippocampal neurogenesis occurs throughout life in a wide variety of mammalian species, including humans and nonhuman primates (Gage, 2019). After extensive studies based on physiological and pathophysiological stimuli, adult hippocampal neurogenesis appears to be necessary and beneficial (Kang et al., 2016; Baptista & Andrade, 2018). In contrast, epileptic seizures led to aberrant hippocampal neurogenesis, including increased proliferation of neural progenitors, production of ectopic granule cells, and persistence of hilar basal dendrites on adult-generated granule neurons (Parent et al., 1997; Kohman & Rhodes, 2017). On the other hand, hippocampal neurogenesis also plays an important role in the development of chronic seizures (Danzer, 2019). Aberrant hippocampal neurogenesis leads to a reduction in chronic seizure frequency (Cho et al., 2015). Mice lacking ganglioside GD3 have shown impairment of hippocampal neurogenesis (Wang et al., 2014). GM3 is the immediate upstream ganglioside of GD3 (Yu et al., 2012), raising the possibility that GM3 and other "brain-type" gangliosides, may have a function in hippocampal neurogenesis and contribute to the susceptibility to seizures.

In the present study, we showed that ST3GAL5-KO animals were hyperactive and prone to epileptic seizures. In addition, ST3GAL5-KO mice showed an increased susceptibility to convulsants, such as pilocarpine and kainic acid (KA). Loss of GM3 had no effect on hippocampal neurogenesis under physiological conditions but might aggravate seizure-induced aberrant neurogenesis in the hippocampus by promoting neural stem/progenitor cell (NSPC) proliferation. These data shed new light on the functional relevance of gangliosides, including GM3 and other *brain-type* gangliosides, in the etiology and pathogenesis of epilepsy. Recognizing the involvement of gangliosides should facilitate the development of new targets for seizure prevention and treatment, such as genetic engineering and ganglioside replacement.

Methods and Materials

Animals

Mice were cared for according to animal protocols approved by the Institutional Animal Care and Use Committee at our University according to the National Institutes of Health (NIH) guidelines. All mice were housed in standard conditions with food and water provided ad libitum and maintained on a 12 hr dark/light cycle. The generation of ST3GAL5-KO mice was carried out as described previously (Yamashita et al., 2003). The heterozygous animals were kindly provided to us by Dr. Richard Proia, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, and Dr. Ming Dong, Boston Children's Hospital, Harvard Medical School, Cambridge, MA. The following primers were used for genotyping: ST3GAL5 Mut.F: 5′-CAA TAG ATC TGA CCC CTA TGC-3′; ST3GAL5 Mut.R: 5′-TCG CCT TCT TGA CGA GTT CTT CTG AG-3′; ST3GAL5 WT.F: 5′-AGC TCA GAG CTA TGC TCA GGA-3′; ST3GAL5 WT.R: 5′- TAC CAC ATC GAA CTG GTT GAG-3′. Experiments were replicated a minimum of three times with mice derived from independent litters. All phenotypic characterizations of

ST3GAL5-KO and their control mice were in the C57BL/6 background.

Behavioral Analysis

Male 4- to 6-week-old mice were used for this study.

The open-field test was used to examine locomotor function and anxiety, as described previously with minor revisions (Christoffel et al., 2012). A black open box with the dimensions of 56 cm × 56 cm was used. To start the test, a mouse was placed at one corner of the box, facing the wall. During a 5-min period, the number of episodes of rearing and grooming and the travel distance were recorded.

For the pilocarpine model, mice were injected with scopolamine (2 mg kg^{-1}, intraperitoneally [ip], Tocris Bioscience, Minneapolis, USA) 30 min before pilocarpine treatment to block peripheral side effects.Mice were then injected with pilocarpine in normal saline (wt/vol, ip, 200 mg kg^{-1}, Fisher Scientific, USA), followed by injections every 30 min at a dosage of 100 mg kg^{-1}. For the KA model, mice received ip injections of KA (20 mg kg^{-1}; Enzo Life Sciences, NY, USA) dissolved in saline. Seizures were recorded by a video camcorder and scored according to previously described criteria (Curia et al., 2008; Kim & Cho, 2018). We used the following seizure scale: *no response* (0), *staring and reduced locomotion* (1), *activation of extensors and rigidity* (2), *repetitive head and limb movements* (3), *sustained rearing with clonus* (4), *loss of posture* (5), and *status epilepticus and death* (6). Time of seizure initiation, seizure duration, maximum seizure intensity, and seizure scores at various times were analyzed.

The day after KA injection, bromodeoxyuridine (BrdU; 50 mg kg^{-1}; Sigma, St. Louis, USA) dissolved in saline was injected ip into ST3GAL5-KO and wild-type (WT) mice daily for 1 week to monitor cell proliferation, differentiation, and survival. These mice were sacrificed 1 day or 21 days after the last injection of BrdU.

Tissue Processing

Animals were sacrificed using CO_2 suffocation. Mice were transcardially perfused with 100 ml phosphate-buffered saline (PBS; pH 7.4) followed by 100 ml 4% paraformaldehyde (pH 7.4, Roth, Cat# 0335) at a rate of 20 ml/min. Their brains were removed and soaked in the fixative for 2 to 4 hr. After rinsing with PBS, coronal vibratome sections (40 μm in thickness) were prepared (VT1000s, Leica, Buffalo Grove, USA) and used for immunostaining or immunohistochemical staining analyses.

Histology and Counting Procedures

Immunofluorescent staining of free-floating 40-μm sections was performed as follows. For staining with anti-BrdU antibody, brain sections were incubated for 15 min with 2 N HCl. Sections were then rinsed 3 times for 10 min with PBS with 0.1% Tween, rinsed once in a blocking solution (PBS with 3% donkey serum and 0.25% Triton-X) at room temperature for 1 hr, and incubated with primary antibodies in blocking solution for 24 hr at 4°C. After rinsing 3 times for 10 min in PBS with 0.1% Tween, sections were rinsed 30 min in blocking solution and incubated with secondary antibodies in blocking solution at 4°C for 1 hr. After rinsing for 10 min in 4′,6-diamidino-2-phenylindole (Sigma, Cat# D9542, St. Louis, USA), sections were washed 2 times with PBS for 10 min and mounted with Aqua-Poly/Mount (Polysciences, Cat# 18606, Warrington, USA). The following primary antibodies were used: anti-BrdU (1:1,000, Abcam, Cambridge, USA), anti-doublecortin (DCX; 1:500, Santa Cruz, Dallas, USA), anti-NeuN (1:1000, Millipore, St. Louis, USA), and anti-Glial fibrillary acidic protein (GFAP) (1:500, Sigma, St. Louis, USA). All the corresponding conjugated secondary antibodies (1:1000) were purchased from Invitrogen, Thermo Fisher Scientific.

Confocal single plane images and Z stacks were taken by the Nikon A1 confocal microscope with four lasers (405, 488, 559, and 633 nm) and 10× or 63× objective lens. Images were processed using Fiji ImageJ. 3D reconstructions were obtained using Imaris software (Bitplane AG, Zürich, Switzerland).

Cell Counts

BrdU-positive cells within the dentate gyrus (DG) were counted using every 6th section (240 μm apart). The number of counted cells was then multiplied by six to provide an accurate estimation of the number of cells per DG. To calculate the total number of marker-double-positive cells, at least 200 randomly chosen BrdU-positive cells per animal were analyzed. Microscopic analysis yielded a ratio of BrdU-positive cells colabeled with DCX, NeuN, and GFAP. These ratios were multiplied by the total number of BrdU-labeled cells to give estimates of the total number of BrdU-positive immature or mature neurons and BrdU-positive astrocytes. To estimate the ratio of surviving cells after seizure, the total number of BrdU-positive cells at 3 weeks post injection of BrdU was divided by the total number of BrdU-positive cells at Day 1 post injection of BrdU.

Statistics

Although no statistical methods were used to predetermine sample size, our sample sizes are based on our previous experience or publications (Wang et al., 2014; Sun et al., 2018; Tang et al., 2020). All data are presented as mean ± SEM. GraphPad Prism 8 (GraphPad Software) was used for statistical analysis. Data distribution was assumed to be normal, but this was not formally tested. The two-tailed unpaired *t* test was used to evaluate statistical significance of two groups of samples. One-way analysis of variance with a Tukey post hoc test was used to evaluate statistical significance of three or more groups of samples. The *n* numbers can be found in the figure legends. Statistical significance was defined as $p < .05$.

Results

ST3GAL5 KO Mice Weigh Less Than WT Mice and Are Hyperactive

In the initial report, there were no significant differences in weight gain between ST3GAL5-KO and littermate control mice with a C57BL/6-129/svev mixed background (Yamashita et al., 2003). To eliminate the interruption of the weight change due to the mixing of the genetic backgrounds, we first crossed the ST3GAL5 heterozygotes with C57BL/6 WT mice for 10 generations. The mouse colonies were then maintained on a C57BL/6 background through heterozygous mating to generate a littermate control. In our hands, at the first week of birth, there were no differences in body weight between KO and WT mice (4.50 ± 0.14 and 4.87 ± 0.2 g, respectively). However, after postnatal day (P) 28, the body weight of ST3GAL5-KO mice was significantly less than that of the WT littermates (18.10 ± 1.51 and 22.39 ± 0.76 g, respectively, at P42 (Figure 1A).

Juvenile ST3GAL5-KO mice have shown hyperactivity in a motor activity test (Niimi et al., 2011). In accordance with this study, we also observed that exposure to the novel environment triggered a significantly higher level of spontaneous horizontal and vertical activity in KO juvenile mice (1.5 months of age) compared with their WT littermates (Figure 1B). This novelty-induced hyperactivity was also observed in younger animals (data not shown).

Loss of ST3GAL5 Does Not Impair the Macroscopic Histology of the Brain

Progressive microcephaly has been reported in patients with ST3GAL5 deficiency (Simpson et al., 2004; Bowser et al., 2019). To investigate if ST3GAL5 KO mice also showed a similar phenotype, the histology of brain slices was investigated for control and ST3GAL5-KO mice using immunostaining with parvalbumin (PV) and NeuN antibodies (Figure 1C). No apparent differences in gross histology in serial sections throughout the whole mouse brain were detected in age-matched mice of the same sex, suggesting that the ablation of ST3GAL5 did not cause macroscopic dysmorphologies. Notice that the density of the neuronal marker NeuN and the interneuron marker PV are comparable between control and ST3GAL5-KO brain (Figure 1D–F), which suggests that loss of ST3GAL5 may not directly influence early neuronal development.

ST3GAL5-KO Mice Are More Susceptible to Chemoconvulsants Than WT Mice

Although the large majority of electrographic seizures were clinically silent for most of the children with the ST3GAL5 mutation, 72% of the patients had at least one seizure witnessed by a parent (Bowser et al., 2019). Occasional spontaneous seizures were observed in ST3GAL5-KO mice ($n = 3$) and were characterized by abnormal head movements, rearing, and clonus of the forelimbs. No WT littermates showed similar behaviors. We next investigated whether ST3GAL5 deficiency affected seizure susceptibility. Six-week-old ST3GAL5-KO and control mice were first injected with different dosages of pilocarpine to generate status epilepticus. Doses were adjusted from previous reports (Curia et al., 2008; Kim & Cho, 2018), and their behavior was subsequently assessed by an investigator blinded to the genotypes. To exclude the influence of body temperature to seizure responses, immediately after pilocarpine injection, the mice were placed in an incubator (28–30°C) for observation. Most control mice developed status epilepticus after the fifth injection (Figure 2A). In contrast, a majority of ST3GAL5-KO mice developed status epilepticus after the third injection (Figure 2A and B). These data indicate a decreased threshold to seizure in ST3GAL5-KO mice.

To confirm this observation, we injected ip KA (20 mg kg^{-1}), a potent glutamate analogue that triggers neuronal hyperactivation (Yoshihara et al., 2003), to a group of WT ($n = 6$) and littermate KO ($n = 7$) animals. During the first hour following injection, four of the six WT animals became immobile or displayed stereotypic movements (behavioral scores rated 1, 2, or 3), whereas the two others showed convulsions or severe seizures (scores 4, 5, or 6). In contrast, only 1 KO mouse was rated 1 to 3 in this test compared with 6 KO animals showing convulsions or severe seizures (Figure 2C). Furthermore, KO mice were scored significantly higher than WT in the first 15 min following KA injection, as well as throughout the 3 hr monitoring period (Figure 2D). In both groups, several mice died in the week following the KA test ($n = 3$ KO, 1 WT), with 2 KO compared with 0 WT mice

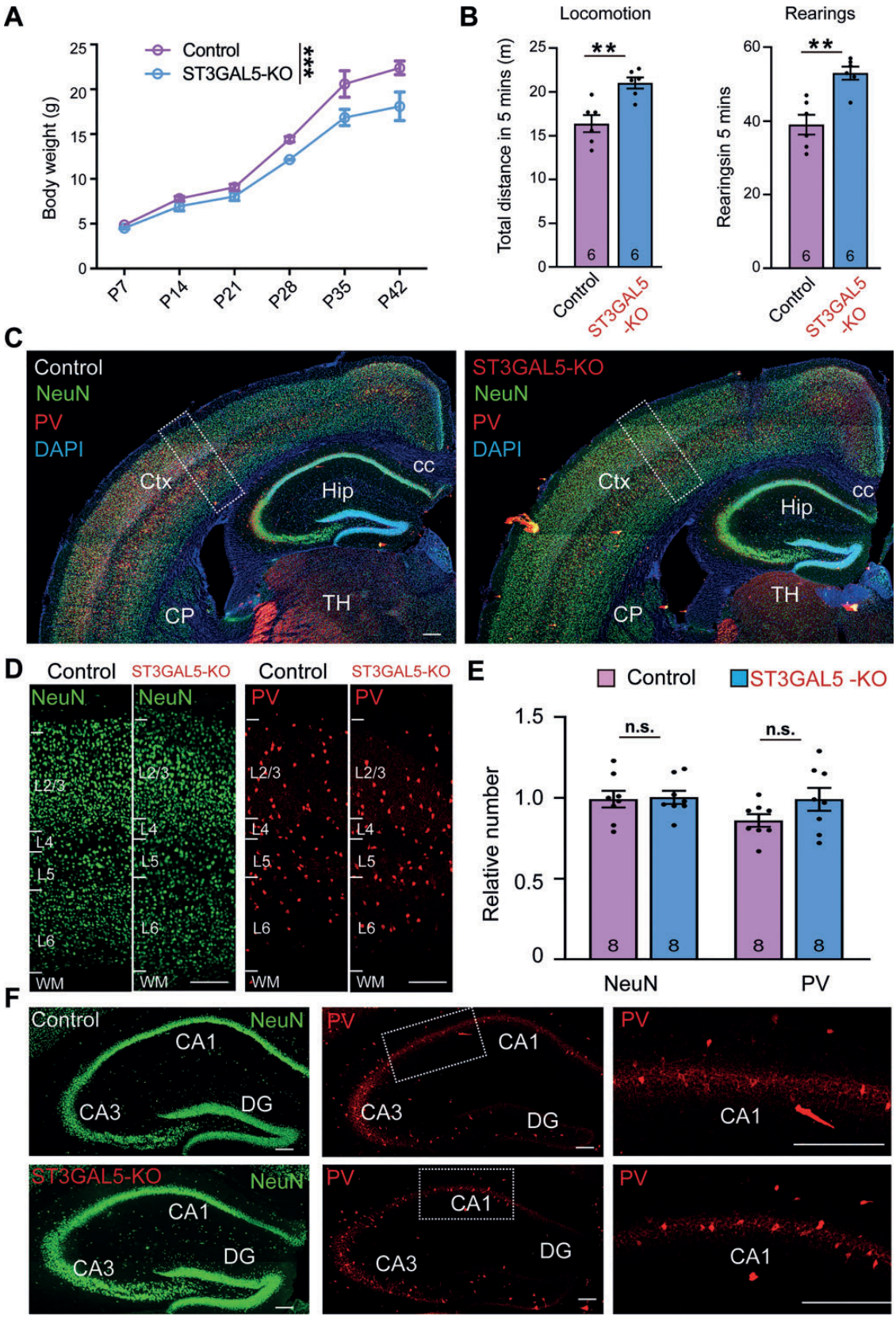

Figure 1. ST3GAL5-KO mice are hyperactive but do not reveal impairment of the macroscopic histology of the brain. (A) Body weight growth curve for ST3GAL5 KO and control mice. Body weights were recorded weekly for each group of mice ($n = 10$ per group).

(continued)

dying in the first 40 min. Together, our findings indicate that ST3GAL5 deficiency strikingly increases susceptibility to chemoconvulsant-induced epileptic seizures due to differences in the property of drugs and analysis of seizure severity.

ST3GAL5 Deficiency Aggravates Seizure-Induced Aberrant Neurogenesis in the Adult Hippocampus

Past reports using pharmacological agents or genetic approaches suggest that ablation of neurogenesis

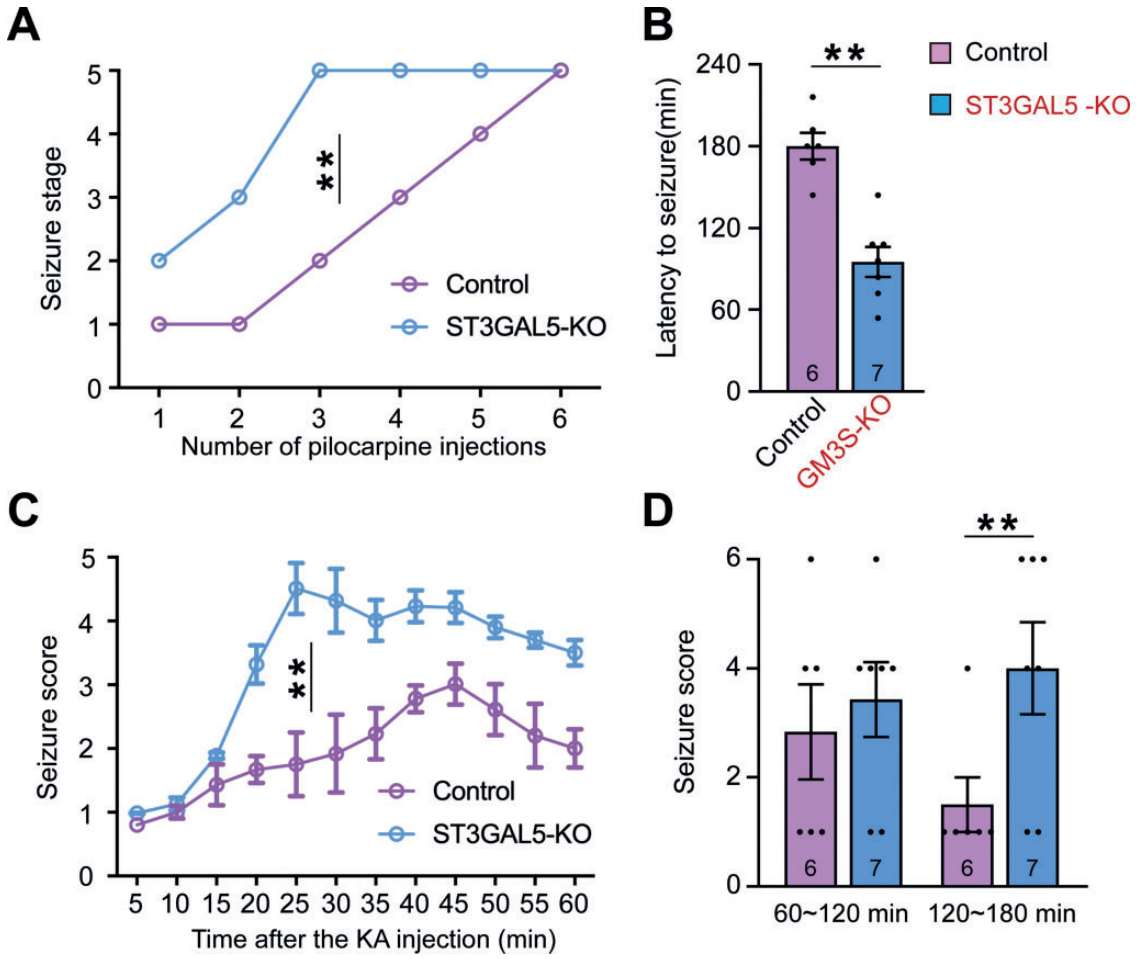

Figure 2. ST3GAL5-KO mice are more susceptible to chemoconvulsant-induced seizures than their WT controls. (A) Representative time course of seizure development by repeated pilocarpine injections. Mice of the two genotypes were subjected to pilocarpine injection every 30 min and scored for seizure stage. (B) Decreased number of pilocarpine injections needed to reach Stage-5 seizure for mutant mice. (C) In the first 60 min after KA injection, a significant increase in the progression of seizure-related behavior was observed in KO mice, compared with WT. (D) Bar histograms show that during the 3-hr monitoring period after KA injection, more KO mice show rearing and falling (Score 4) or progression to severe tonic, clonic seizures (Score 6) compared with WT mice.
KO = knockout; KA = kainic acid.

Figure 1. Continued
(B) Rearing times and travel distances were determined in the open-field test to assess locomotor function of each group of mice ($n = 6$ per group). (C) Representative immunodetection of NeuN and PV in coronal brain sections from 1-month-old mice. Scale bar: 500 μm. (D) Neocortical segment of the primary somatosensory cortex (trunk region/barrel field). Cortical layers are indicated as follows: L1: molecular layer; L2: external granular layer; L3: external pyramidal layer; L4: internal granular layer; L5: internal pyramidal layer; L6: multiform polymorphic layer; and WM: white matter. Scale bar: 200 μm. (E) Quantification of the number of NeuN+ and PV+ cells in cortex. (F) Hippocampus. Scale bar: 50 μm.
KO = knockout; PV = parvalbumin; DAPI = 4′,6-diamidino-2-phenylindole; Ctx = cortex, Hip = hippocampus, CP = caudoputamen, TH = thalamus, cc = corpus callosum.

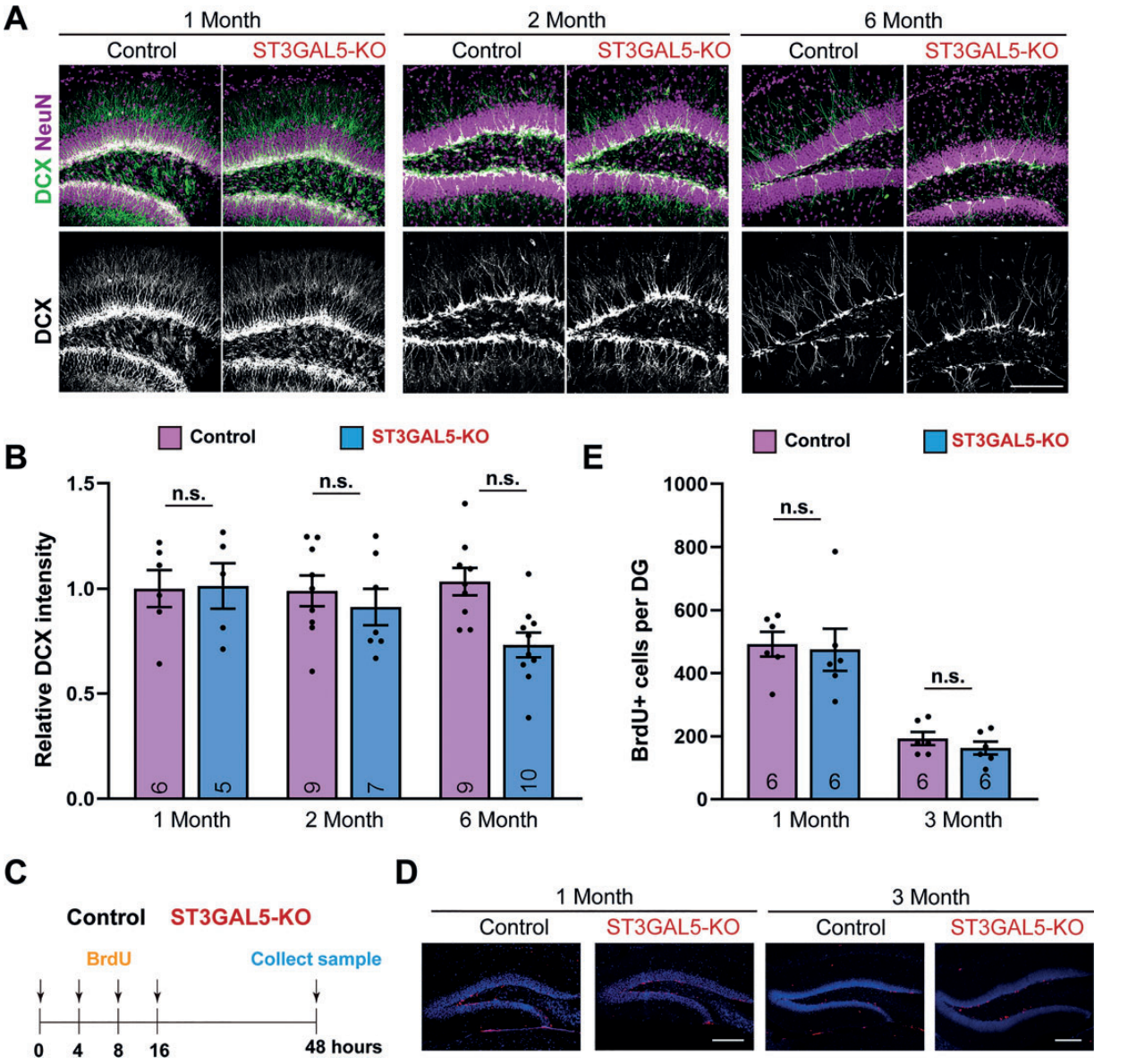

Figure 3. Normal neurogenesis in the DG of ST3GAL5-KO mice. (A, B) DCX immunostaining and quantification of the immature neurons at DG from 1-, 2-, and 6-month-old mice. Note the pronounced decrease in the DCX-expressing cells in the 6-month-old ST3GAL5-KO mice. (C–E) Short-term BrdU lineage tracing of DG neurogenesis. Scale bar: 100 μm in A and D.
KO = knockout; DCX = doublecortin; DG = dentate gyrus; BrdU = bromodeoxyuridine.

influences the susceptibility to seizures (Jung et al., 2004, 2006; Cho et al., 2015). Previously, we showed a progressive loss of neurogenesis in the DG of mice lacking ganglioside GD3 (Wang et al., 2014). Because GM3 is a precursor of GD3, we next analyzed the impact of ST3GAL5 deficiency on adult hippocampal neurogenesis. Unlike the ST8SIA1-KO mice, the number of DCX (an immature neuronal marker)-positive neurons in the DG was comparable between ST3GAL5-KO and control mice at younger ages (1 and 2 months old; Figure 3A and B, left and middle). In 6-month-old mice, the number of DCX-positive cells was only slightly reduced in ST3GAL5-KO mice compared with that in the control (Figure 3B, right). To confirm this observation, we injected mice with BrdU ip 4 times within 1 day to label proliferating NSPCs in the DG. Mice were sacrificed 1 day after the final injection. We observed no significant difference between the ST3GAL5-KO and control mice (Figure 3C–E). These results provided strong evidence that adult neurogenesis was not impaired in the young ST3GAL5-KO mice under physiological conditions.

It is well known that epileptic seizures induce aberrant adult hippocampal neurogenesis, but it remains unclear

what the underlying mechanism(s) is and how animals respond to this pathological condition (Jessberger et al., 2007). It is known that KA-induced acute convulsive seizures trigger aberrant augmentation of neurogenesis in the DG and the migration of newborn neurons to ectopic locations such as the hilus, resulting in impairment of hippocampus-dependent memory (Jiang et al., 2003; Jessberger et al., 2007; Kron et al., 2010). We thus examined the effects of ST3GAL5 deficiency on the response to convulsive seizure induction. Consistent with previous reports, we observed a significant increase in the number of BrdU-positive cells within the subgranular zone (SGZ) in all KA-treated mice (Figure 4A–C). Interestingly, the number of BrdU-positive cells within the SGZ at 1 week after KA administration was higher in ST3GAL5-KO mice than in the WT control (Figure 4A–C). In addition, an increased number of BrdU-retaining and DCX-expressing immature neurons was observed in the DG, including both the SGZ and the hilus, of ST3GAL5-KO mice (Figure 4D–G), suggesting that the loss of ST3GAL5 stimulated KA-induced aberrant neurogenesis.

We further traced the differentiation and survival of newly generated cells in the DG 3 weeks after the final BrdU injection (Figure 5A). ST3GAL5-KO mice showed a higher number of BrdU-retaining cells in the DG than that in WT mice (Figure 5B and D), and the majority of BrdU-retaining cells had become positive for the mature neuronal marker NeuN (Figure 5B–E). However, the loss of ST3GAL5 had no effect on the proportion of NeuN-positive neurons among the BrdU-positive cells. Concordant with the increase in number of BrdU-positive cells, the number of newly generated GFAP-positive astrocytes was also increased in the DG of ST3GAL5-KO compared with WT mice (Figure 5C–E), suggesting that NSPC differentiation was not affected by the loss of ST3GAL5 as shown in Figure 5F for neuronal differentiation. Taken together, these results suggest that ST3GAL5 deficiency aggravates seizure-induced aberrant neurogenesis in the hippocampus by promoting NSPC proliferation. In other words, gangliosides derived from GM3 attenuate seizure-induced abnormal proliferation of NSPCs to maintain homoeostatic neurogenesis in the DG.

Discussion

ST3GAL5 is the key enzyme involved in the initial stages of the biosynthesis of the a-, b-, and c-series gangliosides (Schnaar et al., 2014). Deletion of this enzyme is expected to result in an inability to synthesize not only GM3 but also other complex ganglioside species required at different stages of normal development. In humans, the enzyme deficiency (OMIM 609056) is a rare metabolic disorder inherited as an autosomal recessive trait, initially reported in the Old Order Amish and later found in other ethnic groups as well (Bowser et al., 2019). Although the pathological mechanisms remain obscure, the condition is severe, characterized by infantile onset of severe irritability, failure to thrive, developmental stagnation, cortical blindness, profound intellectual disability, and intractable seizures (Bowser et al., 2019). In the present study, we showed that ST3GAL5-KO mice exhibited increased susceptibility of chemical-induced seizures that were not previously identified in this model. In addition, ST3GAL5 deficiency aggravated seizure-induced aberrant neurogenesis. Taken together, our findings support that GM3 and its downstream brain-type gangliosides play an important role in epileptogenesis, and ST3GAL5-KO mice may be a useful animal model for revealing specific aspects of diseases due to ST3GAL5 deficiency.

Mice lacking ST3GAL5 were first reported in 2003 (Yamashita et al., 2003). Their fetal development was reported to be normal, and the animals appeared healthy at birth and during their postnatal development, except for the development of enhanced insulin sensitivity. In ST3GAL5-deficient mice with the C57BL/6 background, hyperactivity was observed in tests for exploration of novel environments (Figure 1B). We could not ascertain if this phenotype was linked to epilepsy, as spontaneous convulsive seizures with abnormal head movements and clonus of the forelimbs were observed occasionally in the KO mice. To further characterize seizure susceptibility in ST3GAL5-KO mice, we induced epilepsy in these mice by other means. We skipped the audiogenic stimulation approach because it had already been reported that ST3GAL5-KO mice exhibited a progressive hearing impairment phenotype due to the degeneration of cochlear hair cells (Yoshikawa et al., 2015). For this reason, we tested seizure susceptibility by ip injection of chemoconvulsants. We clearly demonstrated that mice lacking ST3GAL5 were more prone to pilocarpine- and KA-induced convulsive seizures compared with WT controls (Figure 2). The seizures induced by KA were significantly more frequent, and with a shorter latency and longer mean duration, than those induced by pilocarpine (Figure 2). At present, the exact mechanisms of the behavioral alterations caused by deletion of ganglioside GM3 and other complex stage- and cell-specific gangliosides in the brain is still not clear. It may be safe to speculate that the nervous system circuitry in these mice is not formed normally due to the lack of complex gangliosides, based on what is known on the dramatic alteration of ganglioside expression during the developmental stages of the normal functioning nervous system.

Although adult neurogenesis remains a controversial topic, a growing body of literature supports the concept of neurogenesis during adult stages (Kang et al., 2016; Gage, 2019). Our data are consistent with the latter

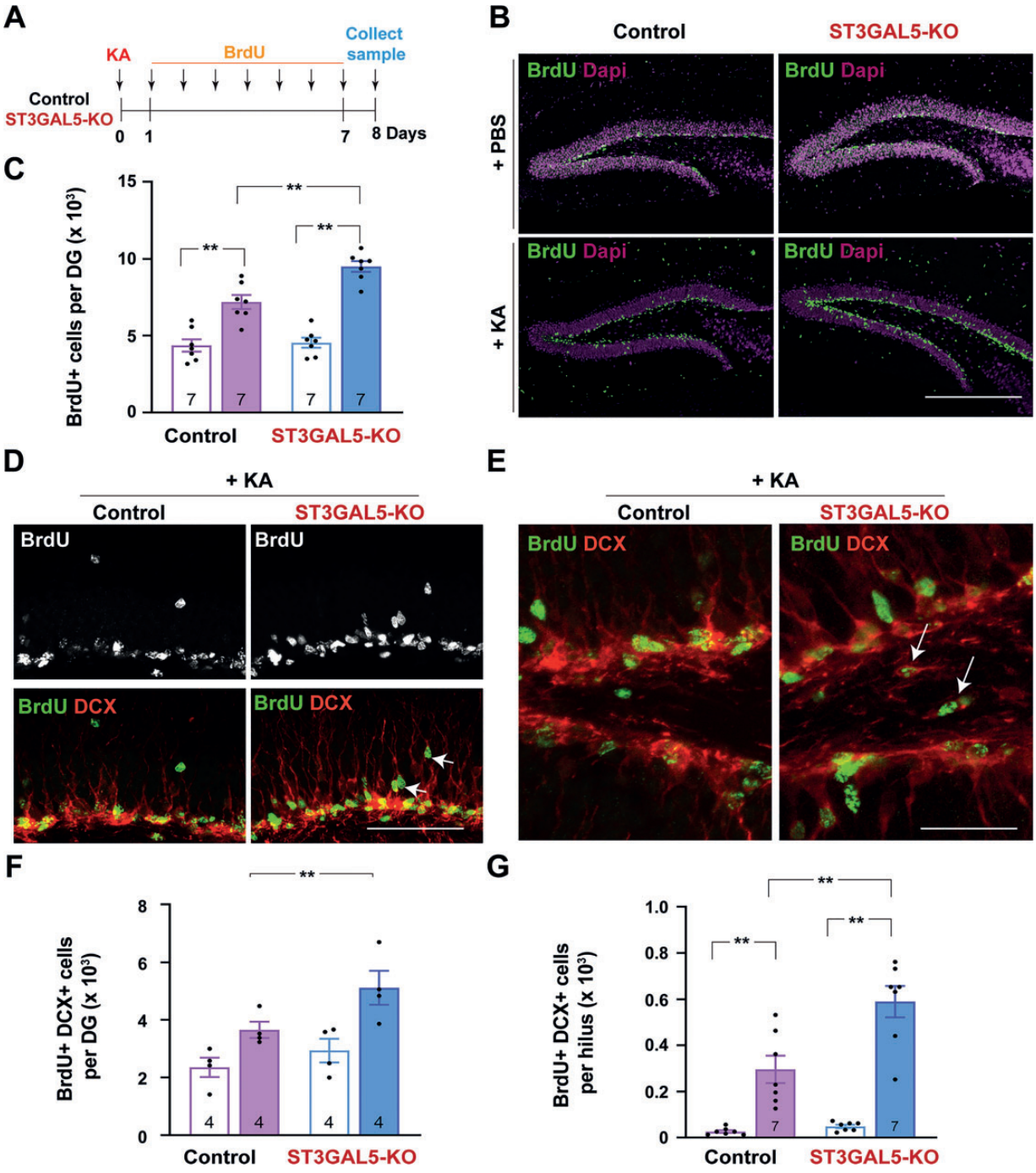

Figure 4. ST3GAL5 deficiency aggravates seizure-induced aberrant neurogenesis. (A) Experimental time line for assessing NSPC proliferation in WT and ST3GAL5-KO mice. (B) Representative images of the DG and immunostaining images with BrdU and DAPI showed that KA-induced proliferation of NSPCs in ST3GAL5-KO mice was more extensive than in WT mice. Scale bar: 50 μm. (C) Quantification of total number of BrdU-positive (BrdU+) cells in the DG with (filled bars) or without (open bars) KA treatment. After seizure, the number of BrdU+ cells was higher in ST3GAL5-KO mice than in WT mice. (D, E) Representative images of BrdU (red) and DCX (green) double-labeled (BrdU+DCX+) newly generated neurons in the SGZ (D) and the hilus (E). Scale bars: 50 μm. White arrowheads indicate new neurons located ectopically in the hilus. (F, G) Quantification of the number of BrdU+DCX+ cells in D and E.
KO = knockout; KA = kainic acid; BrdU = bromodeoxyuridine; DAPI = 4′,6-diamidino-2-phenylindole; DG = dentate gyrus; PBS = phosphate-buffered saline; DCX = doublecortin.

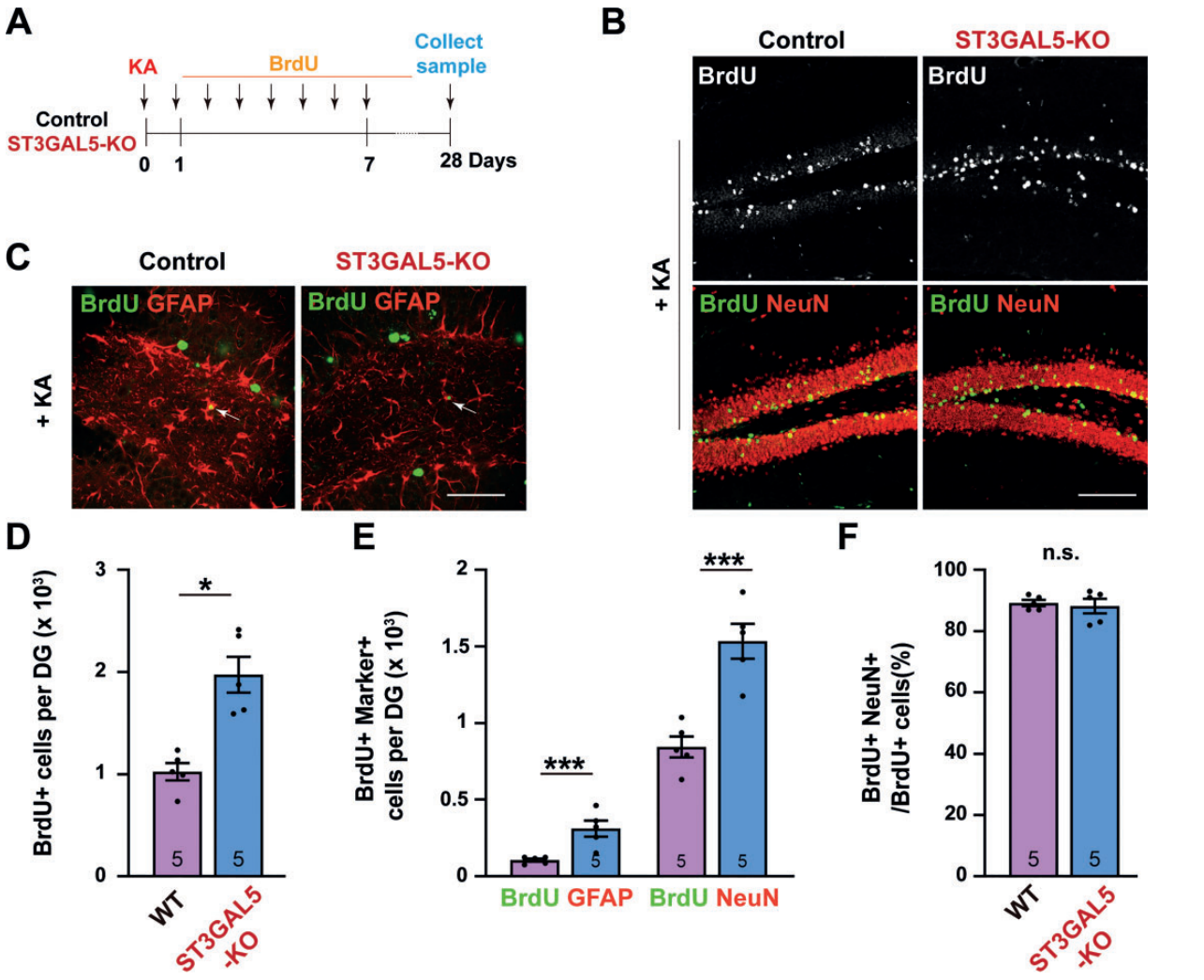

Figure 5. Loss of ST3GAL5 increases newly generated mature neurons after seizure. (A) Experimental scheme for examining the number of newly generated mature neurons. (B) Representative images of staining for NeuN and BrdU in the DG. Scale bar: 50 μm. (C) Representative images of BrdU and GFAP staining in the DG of KA-administered WT and ST3GAL5-KO mice. White arrowheads indicate BrdU and GFAP double-labeled newborn astrocytes. Scale bar: 50 μm. (D, E) The DG of ST3GAL5-KO mice exhibited increased numbers of BrdU+ cells (D) and newly generated BrdU+NeuN+ mature neurons (E). (F) Quantification of BrdU+ cells for assessing differentiation of newly generated cells in the DG.
KO = knockout; KA = kainic acid; BrdU = bromodeoxyuridine; DG = dentate gyrus; WT = wild-type.

observation as seizure activities clearly influence dentate granule cell neurogenesis (Jessberger et al., 2007). Hence, injection of KA could conceivably induce an increased level of neurogenesis in the hippocampus, and the ganglioside abnormalities in ST3GAL5-KO mice could exacerbate the aberrant adult hippocampal neurogenesis (Figures 4 and 5). Because these newly generated neurons were also often disorganized, due to the lack of proper expression of other stage-specific gangliosides during development, it is likely that they contributed to network hyperexcitability and promoted subsequent seizure propagation (Parent et al., 2007). Further studies assessing neurogenesis in ST3GAL5-KO animals and its relationship to epileptic seizures are therefore warranted.

Impaired adult hippocampal neurogenesis has been previously reported in mice lacking GD3 (Wang et al., 2014). Because ST3GAL5-KO mice also lack GD3, it is interesting to note that adult hippocampal neurogenesis was not impaired in the young ST3GAL5-KO mice. It is worth recalling that in the brain of ST3GAL5-KO mice, the missing gangliosides are replaced by asialo- and α-series gangliosides, such as GM1b and GD1α (Seyfried et al., 1994; Yoshihara et al., 2003; Yoshikawa et al., 2009). This situation is analogous to the ceramide galactosyltransferase-KO situation where

the synthesis of galactosylceramide (galactocerebroside) is compensated by an overexpression of ceramide glucosyltransferase, resulting in the formation of the so-called "pseudo-myelin" that expresses glucocerebroside rather than the galactocerebroside in "normal-appearing" myelin (Takamiya et al., 1996). Thus, despite the formation of a myelin-like morphology, pseudo-myelin is physiologically inefficient in insulating axons for conducting saltatory nerve conduction (Sheikh et al., 1999). In the present case, it is remarkable to note that ablation of the ST3GAL5 gene expression, which results in the lack of normal brain gangliosides, is not embryonic lethal and not sufficient to alter the gross brain morphology in agreement with previous findings (Brigande et al., 1998). Suffice to say that some or all of those asialo- and α-series gangliosides could replace some, but not all, of the normal functions of other GM3-derived downstream complex gangliosides. This conclusion is clearly manifested by the enhanced seizure-induced aberrant neurogenesis in ST3GAL5 KO animals.

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