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Research Paper

Mice with conditional NeuroD1 knockout display reduced aberrant hippocampal neurogenesis but no change in epileptic seizures

Rebecca Brulet [a], Jingfei Zhu [a], Mahafuza Aktar [a], Jenny Hsieh [a,*,1], Kyung-Ok Cho [b,*,1]

[a] Department of Molecular Biology and Hamon Center for Regenerative Science and Medicine, UT Southwestern Medical Center, Dallas, TX 75390, USA
[b] Department of Pharmacology, Catholic Neuroscience Institute, School of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

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ABSTRACT

Adult neurogenesis is significantly increased in the hippocampus of rodent models of temporal lobe epilepsy (TLE). These adult-generated neurons have recently been shown to play a contributing role in the development of spontaneous recurrent seizures (SRS). In order to eventually target pro-epileptic adult neurogenesis in the clinical setting, it will be important to identify molecular players involved in the control of aberrant neurogenesis after seizures. Here, we focused on NeuroD1 (ND1), a member of the bHLH family of transcription factors previously shown to play an essential role in the differentiation and maturation of adult-generated neurons in the hippocampus. Wild-type mice treated with pilocarpine to induce status epilepticus (SE) showed a significant upregulation of NeuroD1 + immature neuroblasts located in both the granule cell layer (GCL), and ectopically localized to the hilar region of the hippocampus. As expected, conditional knockout (cKO) of NeuroD1 in Nestin-expressing stem/progenitors and their progeny led to a reduction in the number of NeuroD1 + adult-generated neurons after pilocarpine treatment compared to WT littermates. Surprisingly, there was no change in SRS in NeuroD1 cKO mice, suggesting that NeuroD1 cKO fails to reduce aberrant neurogenesis below the threshold needed to impact SRS. Consistent with this conclusion, the total number of adult-generated neurons in the pilocarpine model, especially the total number of Prox1 + hilar ectopic granule cells were unchanged after NeuroD1 cKO, suggesting strategies to reduce SRS will need to achieve a greater removal of aberrant adult-generated neurons.

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1. Introduction

Epilepsy is a set of neurological disorders characterized by spontaneous recurrent seizures (SRS). Mesial temporal lobe epilepsy (mTLE) is one of the most common adult epilepsies, but is often refractory to currently available therapeutics (Jessberger and Parent, 2015). Animal models of mTLE have demonstrated several important cellular changes in the hippocampal formation and surrounding structures. These include astrogliosis, mossy fiber sprouting, hilar basal dendrites, ectopic migration of newborn neurons, neuronal cell death, granule cell layer dispersion, and an increase in adult neurogenesis (Jessberger et al., 2005; Shapiro et al., 2005; Binder and Steinhauser, 2006; Noebels et al., 2012; Jessberger and Parent, 2015). Neurons born after epileptic seizures have been demonstrated to become aberrant, often mis-migrating and forming inappropriate connections with the surrounding hippocampal network (Parent et al., 1997; Scharfman, 2002). This has led to the hypothesis that these aberrantly integrated adult-generated neurons contribute to the SRS seen in mTLE (Noebels et al., 2012; Bielefeld et al., 2013; Cho et al., 2015). Use of genetic ablation or pharmacological approaches to suppress adult neurogenesis resulted in a 40–70% reduction in SRS frequency in rodent models of epilepsy, supporting this hypothesis (Jung et al., 2004; Jung et al., 2006; Cho et al., 2015). In addition, ablation of neurogenesis normalized many of the epilepsy-associated cognitive deficits indicating that neurogenesis plays a significant contributing role in the development of epilepsy-related comorbidities (Cho et al., 2015). In order to translate these findings to the clinic, it would be ideal to identify molecules that specifically promote aberrant adult-generated neurons as they may represent molecular targets to reduce chronic seizures.

To better understand which molecular regulators of neurogenesis contribute to epilepsy, we have decided to study NeuroD1, a bHLH transcription factor expressed in immature neuroblasts in the hippocampus (Pleasure et al., 2000; Guillemot, 2007; Gao et al., 2009). Previously, we showed NeuroD1 is essential for the survival and maturation of adult-generated neurons in the hippocampus under physiological conditions (Gao et al., 2009). Furthermore, kainic acid (KA)-induced seizures

Abbreviations: SRS, spontaneous recurring seizures; TLE, temporal lobe epilepsy; GCL, granule cell layer; SGZ, subgranular zone; DG, dentate gyrus; YFP, yellow fluorescent protein; KA, kainic acid; Dcx, doublecortin; cKO, conditional knock-out; WT, wildtype; ND1, NeuroD1.

* Corresponding authors.
E-mail addresses: Jenny.Hsieh@utsouthwestern.edu (J. Hsieh), kocho@catholic.ac.kr (K.-O. Cho).
[1] Equal author.

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increased the number of NeuroD1 + immature neuroblasts in the dentate and hilus, while conditional deletion of NeuroD1 in that model reduced the number of YFP + recombined neuroblasts, suggesting it may play a role in chronic seizure development(Cho et al., 2015). Here, we employ the pilocarpine model of epilepsy to investigate the role NeuroD1 plays in aberrant neurogenesis and SRS. Using this approach, deletion of NeuroD1 in mice prior to status epilepticus (SE) led to a significant reduction in the total number of NeuroD1 + neuroblasts, however, this did not affect the overall seizure frequency. These data reinforce the idea that aberrant neurogenesis needs to be reduced below a threshold to impact chronic epilepsy.

2. Materials and methods

2.1. Animals

All the experiments were performed in compliance with the animal care guidelines issued by the National Institutes of Health and by the Institutional Animal Use and Care Committee (IACUC) at University of Texas Southwestern Medical Center. All mice were bred and housed in the animal facility with a 12-h light, 12-h dark cycle with no more than five mice per cage, food (2916 Global irradiated diet, Teklad Labs), and water ad libitum. To generate the mice used in this study, we crossed homozygous Nestin-CreER^{T2}; NeuroD1$^{loxP/+}$ mice with homozygous R26R-YFP; NeuroD1$^{loxP/+}$ mice (Goebbels et al., 2005; Lagace et al., 2007) to generate male and female Nestin-CreER^{T2}/R26R-YFP; NeuroD1$^{+/+}$ (NeuroD1 WT) and Nestin-CreER^{T2}/R26R-YFP; NeuroD1$^{loxP/loxP}$ (NeuroD1 cKO) mice. Mice were genotyped by PCR using genomic DNA and primers for NeuroD1 (5′ GTT TTT GTG AGT TGG GAG TG 3′, 5′ TGA CAG AGC CCA GAT GTA 3′), NestinCreER^{T2} (5′ GGT CGA TGC AAC GAG TGA TGA GG 3′, 5′ GCT AAG TGC CTT CTC TAC ACC TGC G 3′), and R26R-YFP (5′ AAA GTC GCT CTG AGT TGT TAT 3′, 5′ GCG AAG AGT TTG TCC TCA ACC 3′, 5′ GGA GCG GGA GAA ATG GAT ATG 3′). NeuroD1 WT and cKO mice were backcrossed to C57BL/6NHsd mice obtained from Envigo Laboratories (Cat no: 4403F/M) for at least 4–6 generations prior to beginning studies. Male and female NeuroD1 WT and cKO mice at approximately 5.5 weeks of age were administered tamoxifen (TAM) intraperitoneally (i.p.) at 150 mg/kg per day for 5 days prior to SE. TAM was dissolved in 10% EtOH/90% sunflower oil. In Fig. 1 experiments, we used female C57BL/6NHsd (Cat no: 4403F) purchased from Envigo. SNP analysis testing performed for NeuroD1 WT and cKO and C57BL/6NHsd mice showed an average 99.33% genetic similarity between these two lines.

2.2. Chemoconvulsant model of TLE

Male and female NeuroD1 WT and cKO mice at approximately 6 weeks of age were administered scopolamine methyl nitrate (i.p.; 2 mg/kg; Sigma-Aldrich S2250) and terbutaline hemisulfate salt (i.p.; 2 mg/kg; Sigma-Aldrich T2528) to block the peripheral effects of pilocarpine and dilate the respiratory tract, respectively. Thirty minutes later, pilocarpine hydrochloride (i.p.; Sigma-Aldrich P6503) at 220 mg/kg for males and 260 mg/kg for females was injected, and mice were placed in an incubator maintained at 31 °C (ThermoCare). Acute seizures were behaviorally monitored using a modified Racine's scale (Racine, 1972) (stage 1, mouth and facial movement; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing with forelimb clonus; stage 5, rearing and falling with forelimb clonus). Once SE began (defined by continuous tonic-clonic convulsive seizures), mice were placed at room temperature for 3 h and returned to the incubator after seizure activity was reduced using diazepam (10 mg/kg; Sigma-Aldrich D0899). Only mice showing SE for a total of 3 h were included in EEG recording studies. After SE, mice were administered 5% dextrose solution (i.p.; 1 ml) and saline (i.p.; 1 ml) to facilitate their recovery. Mice were weighed each day during the recovery period and if found to have lost ≥2 g from the previous recorded weight were given a single i.p. dose of 1 ml 5% dextrose, and moistened chow. At 3 days after SE, mice were returned to their home cage. Male and female NeuroD1 WT and cKO mice included in the sham seizure group were given (TAM) intraperitoneally (i.p.) at 150 mg/kg per day for 5 days prior to sham treatment. All sham animals were administered scopolamine methyl nitrate (i.p.; 2 mg/kg; Sigma-Aldrich S2250) and terbutaline hemisulfate salt (i.p.; 2 mg/kg; Sigma-Aldrich T2528) 30 min prior to an i.p. vehicle (saline) injection. In some cases, male and female NeuroD1 WT and cKO mice were given access to Metoclopramide (Henry Schein Animal Health, Cat no. 055411) treated water (1 mg/kg) for 5 days prior to SE to help alleviate gastrointestinal symptoms (constipation) associated with use of pilocarpine in this mouse line. EnvigoC57BL/6NHsd female mice were treated the same as above, but did not receive TAM injections, and received a dose of 185 mg/kg pilocarpine to induce SE. For reference we have made available data on the total number of male and female animals used in our experiments, the total number of each that entered

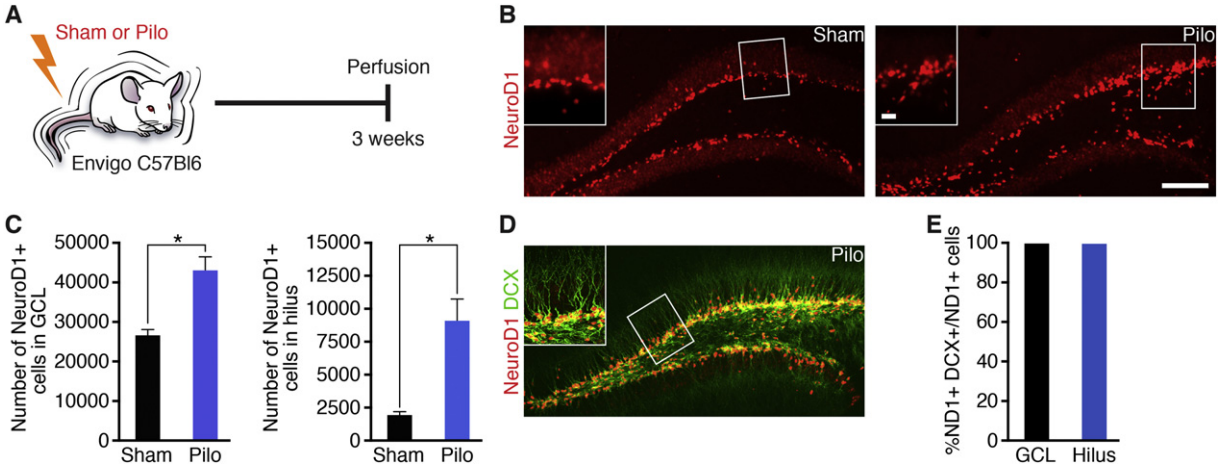

Fig. 1. Acute seizures increase the number of NeuroD1-positive cells in adult dentate gyrus. A) Experimental timeline. Female C57BL/6NHsd Envigo animals were subjected to pilocarpine induced status epilepticus (SE) and then euthanized 3 weeks post pilocarpine. B) Animals were euthanized 3 weeks post pilocarpine treatment and brains were stained with NeuroD1 to determine the total cell number in the granule cell layer (GCL) and hilus (n = 6 sham, n = 6 pilo). C) There was a significant increase in the NeuroD1 + cells in both the GCL ($p = 0.0013$) and hilus ($p = 0.0016$) in pilocarpine treated animals compared to sham treated animals. D, E) The majority of pilocarpine induced NeuroD1 + cells co-localized with the immature neuronal marker Dcx. (n = 8 sham, n = 8 pilo). Scale bar in B: 100 um, inset bar in B: 25 um, both valid for D.