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Axonal plasticity of age-defined dentate granule cells in a rat model of mesial temporal lobe epilepsy

A.L. Althaus [a,b,1], H. Zhang [b,c,2], J.M. Parent [a,b,c,*,3]

[a] Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI, United States
[b] Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI, United States
[c] VA Ann Arbor Healthcare System, Ann Arbor, MI, United States

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ABSTRACT

Dentate granule cell (DGC) mossy fiber sprouting (MFS) in mesial temporal lobe epilepsy (mTLE) is thought to underlie the creation of aberrant circuitry which promotes the generation or spread of spontaneous seizure activity. Understanding the extent to which populations of DGCs participate in this circuitry could help determine how it develops and potentially identify therapeutic targets for regulating aberrant network activity. In this study, we investigated how DGC birthdate influences participation in MFS and other aspects of axonal plasticity using the rat pilocarpine-induced status epilepticus (SE) model of mTLE. We injected a retrovirus (RV) carrying a synaptophysin-yellow fluorescent protein (syp-YFP) fusion construct to birthdate DGCs and brightly label their axon terminals, and compared DGCs born during the neonatal period with those generated in adulthood. We found that both neonatal and adult-born DGC populations participate, to a similar extent, in SE-induced MFS within the dentate gyrus inner molecular layer (IML). SE did not alter hilar MF bouton density compared to sham-treated controls, but adult-born DGC bouton density was greater in the IML than in the hilus after SE. Interestingly, we also observed MF axonal reorganization in area CA2 in epileptic rats, and these changes arose from DGCs generated both neonatally and in adulthood. These data indicate that both neonatal and adult-generated DGCs contribute to axonal reorganization in the rat pilocarpine mTLE model, and indicate a more complex relationship between DGC age and participation in seizure-related plasticity than was previously thought.

1. Introduction

Dentate granule cell (DGC) axonal reorganization is a common feature of dentate gyrus histopathology in patients with mesial temporal lobe epilepsy (mTLE) that is recapitulated in many animal models of the disease (Sutula and Dudek, 2007). Scheibel and colleagues first discovered the presence of aberrant terminals of DGC axons, known as mossy fibers, in the inner molecular layer (IML) of mTLE patients in 1974 (Scheibel et al., 1974). In rodent models of mTLE, mossy fiber sprouting (MFS) into the IML creates a functional excitatory feedback loop, primarily forming synapses onto DGC apical dendrites, with a minority of sprouted synapses in the IML onto interneurons (Buckmaster et al., 2002, Scharfman et al., 2003). MFS has been linked with recurrent excitation of the DGC network (Scharfman et al., 2003, Tauck and Nadler, 1985, Winokur et al., 2004, Zhang et al., 2012) and positively correlated with the number of spontaneous seizures (Hester and Danzer, 2013, Xu et al., 2004). However, the role of MFS in epileptogenesis remains controversial as some studies find that it is not required for spontaneous seizure activity (Buckmaster, 2014, Buckmaster et al., 2009). Importantly, the focus of most research on DGC axonal reorganization in mTLE has been on IML MFS, but this plasticity also occurs within hippocampal area CA3 and potentially also in the dentate hilus.

The mechanisms that drive axonal reorganization of DGCs in mTLE models are not fully understood. IML sprouting is associated with seizure-induced changes such as cell death, altered extracellular matrix protein expression, and loss of axon guidance cues (Cavazos et al., 1991, Cavazos and Sutula, 1990, Holtmaat et al., 2003, Pollard et al., 1994). Plasticity of MFs in CA3 can occur in response to physiological stimuli such as exercise, in addition to seizures, and has been linked to loss of synaptic contacts in CA3 in a mTLE model (Danzer et al., 2010, McAuliffe et al., 2011, Schwarzer et al., 1995, Toscano-Silva et al., 2010). While nothing is known about seizure-related plasticity at the MF-CA2 synapse, recent data indicate that this synapse can be modulated by exercise and inflammation (Llorens-Martin et al., 2015). In

Abbreviations: mTLE, mesial temporal lobe epilepsy; DGC, dentate granule cell; MFS, mossy fiber sprouting; GCL, granule cell layer; IML, inner molecular layer.

* Corresponding author at: Department of Neurology, University of Michigan Medical Center, 5021 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI 48109, United States.
E-mail address: parent@umich.edu (J.M. Parent).

[1] Collected and analyzed data, developed experimental design, wrote manuscript.
[2] Collected data, contributed to manuscript writing.
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addition to external stimuli, there are cell-autonomous factors, such as mTOR signaling, that influence axonal reorganization within individual DGCs (Buckmaster et al., 2009).

Not every DGC contributes to MFS (Buckmaster and Dudek, 1999), and it is not known what influences the likelihood to contribute. DGC birthdate is one potential factor because of the well-documented role of adult-born DGCs in seizure-related plasticity (Jessberger et al., 2007, Kron et al., 2010, Walter et al., 2007). Previous work suggested that DGCs developing during or after an epileptogenic insult are responsible for most, if not all, MFS in the IML (Kron et al., 2010). However, the retrovirally-delivered green fluorescent protein (RV-GFP) label used in these experiments did not allow for reliable resolution of small MF boutons in the IML and hilus. Moreover, extensive dendritic labeling made it difficult to distinguish GFP-labeled axons in the IML. A potential relationship between DGC birthdate and axonal plasticity within the hilus and hippocampal pyramidal cell regions has not previously been investigated.

In this study, we compared MF structural plasticity of DGCs born in the neonatal period, and thus mature at the time of injury, with those generated in adulthood after injury in the rat pilocarpine model of mTLE. To more specifically label the synaptic boutons of birthdated DGCs, we used a construct carrying the synaptophysin (syp) gene fused to yellow fluorescent protein (YFP) to direct YFP to synaptic terminals (Umemori et al., 2004), and this construct was packaged in a RV vector to restrict infection to actively dividing cells. With this tool, we found that both neonatal and adult-born populations of DGCs participate in MFS in the rat pilocarpine mTLE model. We also showed that the DG-CA2 "alternative trisynaptic circuit" is altered after SE. Our findings suggest that both pre-existing, mature DGCs and those generated after SE contribute to various aspects of seizure-induced plasticity.

2. Methods

2.1. Virus production

We generated the syp-YFP RV using a vesicular stomatitis virus G-protein (VSV-G) pseudotyped Moloney murine leukemia virus backbone in which the expression of syp-YFP is driven by the human synapsin-1 promoter. The syp-YFP fusion gene was amplified from the pCMV-syp-YFP plasmid (Umemori et al., 2004) via PCR using primers 5′-cgagctcaaggatccaattctgcagtcg-3′ and 5′-ctgattatgatcacgcgtcgcggcc-3′. To express the syp-YFP under the control of the human synapsin-1 (syn1) promoter (a generous gift from Ed Callaway), the amplified and isolated syp-YFP fragment was subcloned into the RV vector pCAG-mCherry-WPRE (woodchuck post-transcriptional regulatory element) by replacing the mCherry fragment between pCAG and WPRE (Fig. 1A). The accuracy of the cloned syp-YFP fragment in the construct was verified by DNA sequencing and the expression of syp-YFP was tested by transfecting HEK 293 cells using Lipofectamine™ (Invitrogen, CA) as it is weakly expressed in HEK cells (and strongly in neurons). The cells were cultured at 37 °C for 3 days prior to analysis. We also used a GFP-expressing RV as described previously (Kron et al., 2010).

High-titer, replication incompetent pseudotyped RV was produced as previously described (Kron et al., 2010) by co-transfection of CAG-GFP-WPRE or syn1-syp-YFP-WPRE and VSV-G plasmids into the GP_2-293 packaging cell line (Clontech, CA). Cells were plated in 10 ml DMEM supplemented with 10% FBS the day prior to transfection. The co-transfection was performed using calcium phosphate precipitation.

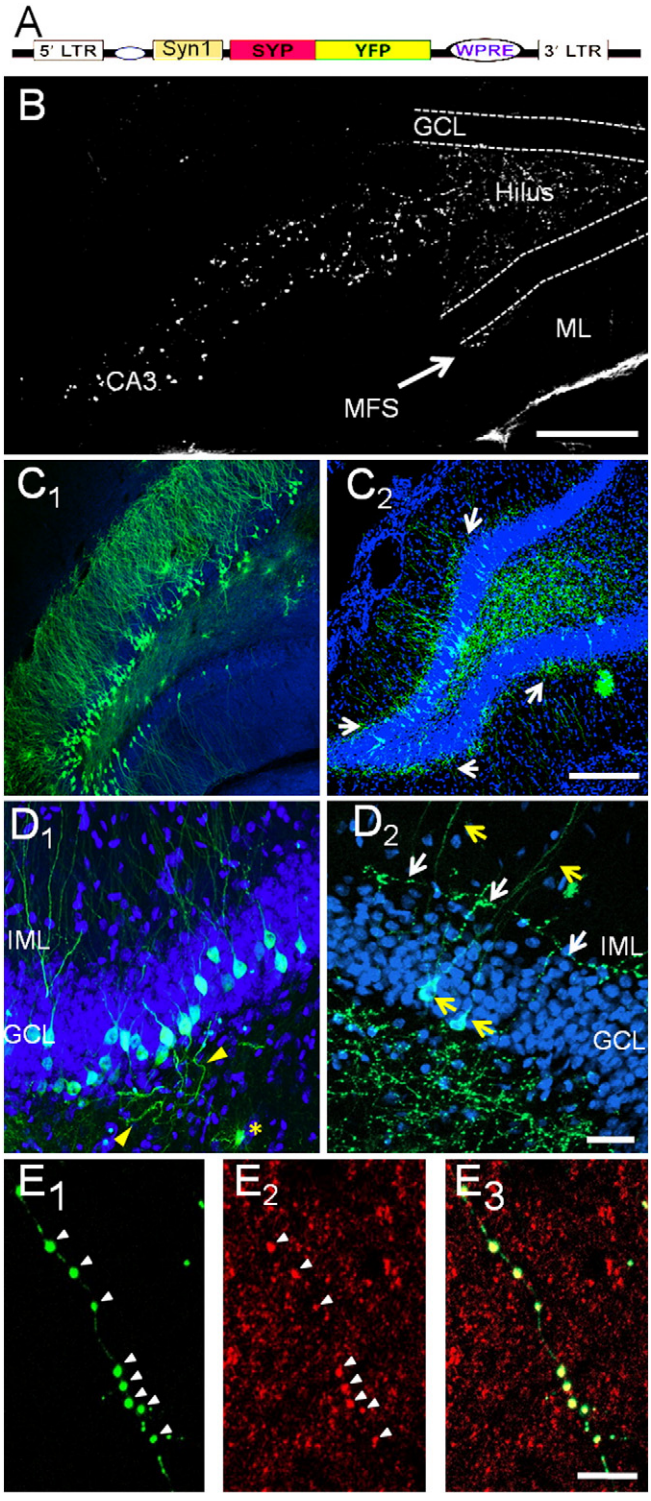

Fig. 1. The syp-YFP RV allows better resolution of small MF boutons in the hilus and IML of the hippocampal dentate gyrus as compared to GFP RV. A. Diagram of the RV-syp-YFP construct with syp-YFP driven by the human synapsin-1 promoter (Syn1). LTR = long terminal repeats of the RV backbone. Dashed lines outline the granule cell layer (GCL). B. Low magnification (5×) confocal image obtained from an animal injected with syp-YFP-RV at P60, 4 days after pilocarpine treatment, and euthanized 8 weeks later. Axon terminals can be seen throughout the hilus, in CA3, and even in the IML (arrow). Scale bar = 200 mm. C, D. Representative lower (C; 10× objective, scale bar = 150 mm) and higher (D; 20× objective with 2× optical zoom, scale bar = 25 mm) magnification confocal images from rats that experienced SE and injections of eGFP RV (C_1, D_1) or syp-YFP RV (C_2, D_2) to label DGC progenitors. Rats were euthanized 8 weeks later. MFS (white arrows in C_2, D_2) and abundant hilar boutons are detected from DGCs that express syp-YFP (C_2, D_2), while the most prominent hilar structures from DGCs that express eGFP (C_1, D_1) are HBDs (yellow arrowheads in D_1) and glia (yellow asterisk in D_1) with minimal axonal labeling. The syp-YFP RV does label some somas and dendrites (yellow arrows in D_2). Blue is Hoescht and green is GFP (C_1, D_1) or YFP (C_2, D_2). E. Confocal z-stack images obtained with a 63× objective and 2× optical zoom of a hilar axon segment from a syp-YFP expressing DGC. E_1) YFP alone (green). E_2) Bassoon (red), a presynaptic terminal marker. E_3) Merged images showing co-localization of YFP and bassoon in the terminals. Scale bar = 10 mm.