Epilepsy Currents
2021, Vol. 21(5) 363–365

DOI: 10.1177/15357597211027012
journals.sagepub.com/home/epi
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What do Newborn Granule Cells Do, and When Do They Do It?

A Critical Period of Neuronal Activity Results in Aberrant Neurogenesis Rewiring Hippocampal Circuitry in a Mouse Model of Epilepsy

Lybrand ZR, Goswami S, Zhu J, et al. *Nat Commun*. 2021;12:1423. doi: 10.1038/s41467-021-21649-8

In the mammalian hippocampus, adult-born granule cells (abGCs) contribute to the function of the dentate gyrus (DG). Disruption of the DG circuitry causes spontaneous recurrent seizures (SRS), which can lead to epilepsy. Although abGCs contribute to local inhibitory feedback circuitry, whether they are involved in epileptogenesis remains elusive. Here, we identify a critical window of activity associated with the aberrant maturation of abGCs characterized by abnormal dendrite morphology, ectopic migration, and SRS. Importantly, in a mouse model of temporal lobe epilepsy, silencing aberrant abGCs during this critical period reduces abnormal dendrite morphology, cell migration, and SRS. Using mono-synaptic tracers, we show silencing aberrant abGCs decreases recurrent CA3 back-projections and restores proper cortical connections to the hippocampus. Furthermore, we show that GABA-mediated amplification of intracellular calcium regulates the early critical period of activity. Our results demonstrate that aberrant neurogenesis rewires hippocampal circuitry aggravating epilepsy in mice.

Commentary

Hippocampal dentate granule cells possess 2 intriguing features that position them to play a unique role in the development of temporal lobe epilepsy. Firstly, granule cells are produced throughout life in most laboratory mammals and likely humans,[1] creating a *tabula rasa* for epileptogenic forces to act on. Secondly, newborn granule cells go through a transient developmental stage during which they modulate hippocampal function (for review see Ref. 2) This second feature has made newborn granule cells particularly challenging to study, as their functional role depends on their maturational state.

Studies in animals have demonstrated that newborn granule cells go through critical periods during which they can develop characteristic pathologies of temporal lobe epilepsy. At the time of an epileptogenic insult, such as status epilepticus, the youngest cells can migrate the wrong direction into the hilus, slightly more mature cells can develop dendritic abnormalities, and both immature and mature cells can contribute to mossy fiber sprouting. Targeted ablation of newborn cells reduces seizure incidence, directly implicating these abnormally integrated cells in epileptogenesis.[3] Utilizing newly available DREADD technology (Designer Receptors Exclusively Activated by Designer Drugs), Lybrand and colleagues further investigated this critical period of cell morphogenesis and migration by selectively activating or silencing immature granule cells at specific developmental ages.[4] Daily activation of newborn granule cells during their first or second week of development was sufficient to promote abnormal hilar migration. Furthermore, this early neuronal activation was associated with spontaneous seizures in the mice when cells were 8 weeks old. The timing of the manipulation was important; activating eight-week-old granule cells had no effect on the cell migration, morphology, or spontaneous seizures. Lybrand and colleagues then induced pilocarpine status epilepticus in a group of mice and subsequently silenced newborn granule cells over a 2-week period following the insult. Mice exposed to status without intervention developed spontaneous seizures accompanied by hilar ectopic granule cells and granule cells with abnormal dendrites. However, 2-week silencing after status decreased the number of spontaneous seizures, reduced the number of granule cells found in the hilus, and prevented dendritic abnormalities when the animals were examined 6 weeks later. Again, a critical period was detected; silencing these same cells 6 weeks post insult did not significantly alter seizure frequency, while seizure duration actually increased. This early critical period matches the stage in granule cell development when calcium transients are largely GABA dependent; at 2-weeks, cells from status exposed mice had an enhanced intracellular calcium baseline that was dependent on $GABA_A$ receptor activation. Notably, using in vitro calcium imaging, the investigators demonstrated that DREADD-mediated silencing decreased intracellular calcium, providing a potential mechanism for the observed effects on epilepsy readouts. Together, the data highlight a critical period for immature adult born granule cells, when increased activation impacts proper migration of the developing neurons, and further

corroborate that abnormal granule cells are sufficient to cause spontaneous seizures. Importantly, these findings suggest a bimodal role for newborn granule cells: promoting epileptogenesis early in their lives and facilitating seizure termination once they reach maturity.

The most surprising finding of the study by Lybrand and colleagues is that DREADD-mediated activation of one- or two-week-old granule cells is sufficient to disrupt their integration and induce spontaneous seizures 6 weeks later. Relative to other manipulations that disrupt new granule cells and induce epilepsy (eg status epilepticus, traumatic brain injury), this insult is quite subtle. The finding indicates that new granule cells are remarkably sensitive to disruption and can exert a powerful impact on brain excitability. A caveat to the observation is that animals also underwent 2 virus injection surgeries and a later surgery for electrode implantation, so DREADD activation of new granule cells occurred in the context of an injured brain. Anesthetics can increase apoptosis among newborn granule cells,[5] while mechanical disruption and inflammatory changes following surgery can alter granule cell development and might "prime" the animal for epileptogenesis.[6] Indeed, some control animals did exhibit seizures with this protocol. Follow up studies using transgenic approaches to express DREADDs in granule cells would eliminate the need for virus injection surgeries, but EEG surgery is unavoidable for obtaining the highest quality data and detecting non-convulsive seizures. Regardless, the finding is a remarkable example of the disruptive potential of new granule cells and raises the question of whether physiological variations in new granule cell activity might alter epilepsy risk after injury.

The second key finding is that silencing newborn granule cells after status epilepticus reduces later seizure incidence, while silencing the same population of cells after maturation does not alter seizure incidence and increases seizure duration. The experiment addresses fundamental questions in the field. Specifically, do new granule cells promote epileptogenesis, or do they promote seizure occurrence in established epilepsy? And, if they perform either (or both) functions, what is the age of the cells relative to the epileptogenic insult? Lybrand and colleagues provided an answer: new granule cells play a transient role in promoting epileptogenesis, but only a limited role (reducing seizure duration) once epilepsy is established. This answer is broadly consistent with prior studies using targeted ablation of cells born in the weeks before or after the insult. Acute ablation reduced later seizure incidence[7] and increased seizure duration,[8] while delayed ablation had no immediate impact on seizure frequency, but prevented further increases in seizure rate.[9] Importantly, transient silencing reduced dendritic abnormalities and migration deficits and, unlike ablation studies, allowed the continued integration of new granule cells that are important for hippocampal function. Reduction of abnormal network connections may decrease both the epileptogenic influence of these granule cells and prevent common comorbidities. As is typical for studies of adult neurogenesis, however, conflicting findings have been observed. In a recent study using a similar DREADD approach, investigators found that silencing a comparable population of newborn cells in chronically epileptic animals reduced seizure frequency, while activating the cells increased seizure frequency.[10] The conflicting results highlight the challenges in studying granule cell neurogenesis. In addition to the usual technical differences, subtle variations in cell age at the time of labeling, cell age at the time of analysis, and the relationship of the two with the time of the epileptogenic insult all have the potential to impact outcomes.

Despite ongoing questions, the work by Lybrand and colleagues further bolsters the case that newborn granule cells play a critical role in temporal lobe epilepsy. Most relevant for clinical translation, the observation that transient silencing of newborn granule cells has a disease-modifying effect is very exciting. Prior studies using cell ablation approaches could be hard to translate. By contrast, introducing a granule-cell targeted DREADD receptor following an epileptogenic injury (eg traumatic brain injury) might be feasible. Importantly, the receptors could be activated transiently, chronically or not at all–facilitating treatment optimization and reducing risk.[11] Further research into the timing, mechanisms and applicability across epilepsy models is still needed, as is the development of epilepsy biomarkers to ascertain when to treat, yet the overall approach is extremely appealing as an anti-epileptogenic therapy.

By Candi L. LaSarge and Steve C. Danzer

Acknowledgments

I would like to thank Keri Kaeding for useful comments on earlier versions of this manuscript.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of Neurological Disorders and Stroke (SCD: R01NS065020, R01NS062806).

ORCID iDs

Steve C. Danzer https://orcid.org/0000-0003-4405-2999

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