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Adult Neurogenesis in the Development of Epilepsy

Steve C. Danzer, PhD[1,2,3,4]*

[1] Department of Anesthesia, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[2] Department of Anesthesia, University of Cincinnati, Cincinnati, OH, USA
[3] Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[4] Center for Pediatric Neuroscience, Cincinnati Children's Hospital, Cincinnati, OH, USA
*Correspondence: Steve C. Danzer, Department of Anesthesia, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, ML 2001, Cincinnati, OH 45229-3039, USA; e-mail: steve.danzer@cchmc.org

Abstract
Compelling evidence indicates that hippocampal dentate granule cells are generated throughout human life and into old age. While animal studies demonstrate that these new neurons are important for memory function, animal research also implicates these cells in the pathogenesis of temporal lobe epilepsy. Several recent preclinical studies in rodents now suggest that targeting these new neurons can have disease-modifying effects in epilepsy.

Keywords
dentate granule cell, dentate gyrus, epileptogenesis, epilepsy therapy, hippocampus, cell ablation, mossy fiber sprouting, ectopic cells, basal dendrite

Introduction

The hippocampal dentate gyrus has long been implicated in the development of temporal lobe epilepsy. Functional studies of the dentate in rodents demonstrate that this brain region is important for regulating the flow of excitatory input into the hippocampus. Physiological studies in animals establish that this function is impaired in epilepsy.[1,2] Both physiological and anatomical studies reveal extensive restructuring of the dentate circuitry in animals and humans with epilepsy; restructuring that is predictive of an increase in excitability.[3]

Granule cells are unusual—they are generated throughout life in animals and are the only neuronal type with evidence for significant adult neurogenesis in humans.[4,5] Over the past 2 decades, it has become clear from animal studies that these newborn granule cells are particularly vulnerable to disruption in epilepsy and are responsible for many of the abnormalities observed in the epileptic dentate.[6-12] The vulnerability of these new neurons to disruption, their role in regulating hippocampal excitability, and their protracted production throughout life has led to the hypothesis that disrupted proliferation and integration of adult-generated granule cells mediates the development of temporal lobe epilepsy (Parent and Kron, 2012).[13] Consistent with this hypothesis, genetic deletion of the mechanistic target of rapamycin (mTOR) pathway inhibitor phosphatase and tensin homolog (PTEN) from newborn granule cells produces epilepsy in rodents, demonstrating that disruption of this neuronal population is capable of causing epilepsy.[14]

Evidence That Adult-Generated Granule Cells Contribute to Epileptogenesis

Initial studies focused on determining whether adult-generated granule cells are required for epileptogenesis. These studies took advantage of existing antimitotic drugs to kill proliferating granule cells. For example, Jung and colleagues[15] found that the chemotherapy agent cytosine-b-D-arabinofuranoside reduced the number of abnormal granule cells following pilocarpine status epilepticus in rats, leading to a reduction in

seizure frequency. A later study produced similar results in pilocarpine-treated rats.[16] More recently, Neuberger and colleagues[17] suppressed neurogenesis after lateral fluid percussion injury using a vascular endothelial growth factor receptor 2 antagonist and demonstrated that treated animals took significantly more time to develop seizures following chemoconvulsant challenge with kainic acid.

Advances in the development of transgenic mouse model systems have provided new opportunities to test the role of adult neurogenesis in epilepsy. These approaches provide superior cellular and temporal specificity, overcoming some of the limitations of pharmacological methods. Cho and colleagues[18] used a transgenic mouse model to block neurogenesis beginning 1 month before pilocarpine-induced status epilepticus. Blocking neurogenesis significantly reduced seizure frequency in the animals and improved cognitive function. Hosford and colleagues[19,20] found a similar effect when they induced expression of the diphtheria toxin receptor in newborn granule cells 5 weeks prior to pilocarpine-induced status epilepticus, and then ablated the newborn cells expressing the receptor 3 days or 3 to 4 months after status epilepticus. Newborn cell ablation reduced seizure frequency by about 50%. Notably, treatment was still effective when applied months after pilocarpine treatment—after the onset of spontaneous seizures—suggesting that manipulations targeting newborn cells could be beneficial in the treatment of chronic epilepsy.

Despite promising results in some studies, reducing neurogenesis has not always been found to mitigate epilepsy development. Pekcec and colleagues[21] used a pharmacological approach to reduce neurogenesis in the self-sustained status epilepticus model in rats and found no effect of treatment on seizure frequency. Zhu and colleagues[22] used methylazoxymethanol acetate in a variation of the pilocarpine model in mice, and also found no effect. Brulet and colleagues[23] used a transgenic mouse model approach to reduce neurogenesis by deleting the transcription factor NeuroD1 from granule cell progenitors. This produced a partial reduction in neurogenesis, but seizure frequency was similar between control and knockout pilocarpine-treated mice. Negative results could be attributed to off-target drug effects, potential toxic effects of systemic antimitotic drugs, and/or insufficient reductions in neurogenesis. However, it is also possible that newborn granule cells are not *required* for the development of epilepsy in all cases. This wouldn't be a huge surprise, as many epilepsies exhibit little hippocampal involvement (eg, focal cortical dysplasia). Even among temporal lobe epilepsy models, however, the dentate—and, correspondingly, adult neurogenesis—may only play a role under certain conditions. Classic work, for example, shows that ablation of the dentate gyrus with colchicine delays but does not prevent or reverse electrical kindling. This work demonstrates that early stages of epileptogenesis can proceed and be maintained without the dentate gyrus.[24] Similarly, studies indicating that neurogenesis ceases after intrahippocampal kainic acid injection argue against a role for new cells in this rodent model of temporal lobe epilepsy,[25] although a role for cells born shortly before the insult cannot be excluded.[26] Neurogenesis also shows complex temporal dynamics, increasing in the weeks after an insult, but decreasing chronically.[27] Based on these considerations, the efficacy of blocking neurogenesis on seizure development may depend on both the model used and the time-point targeted.

Evidence for Protective Effects of Adult-Generated Granule Cells

Further complexity arises from studies examining the role of adult-generated granule cells in normal animals. Physiological studies of newborn granule cells in rodents demonstrate that these new neurons go through a developmental period during which they preferentially activate inhibitory interneurons in the dentate.[28] Correspondingly, blocking neurogenesis in rodents can enhance hippocampal excitability[29] and increase the severity of kainic acid and pilocarpine-induced status epilepticus.[30,31] These apparently conflicting findings can be explained by postulating that newborn granule cells play different roles in healthy and epileptic brains. Under healthy conditions, granule cells innervate excitatory CA3 pyramidal cells, but also innervate large numbers of hilar interneurons and mossy cells.[32] Both hilar interneurons and mossy cells mediate feedback inhibition of the dentate. In temporal lobe epilepsy, by contrast, many hilar neurons are lost, and newborn granule cells form recurrent connections with neighboring granule cells via sprouted mossy fiber axons and newly formed basal dendrites.[7,12,33,34] The altered network structure of adult-generated granule cells in the epileptic brain, therefore, could account for their contrasting effects on hippocampal excitability.

It also appears that a subset of adult-generated granule cells in the epileptic brain may retain their protective properties. Morphological studies of newborn cells in epilepsy reveal a broad diversity in integration patterns.[35] Some migrate to occupy appropriate positions in the granule cell body layer, while others migrate to ectopic locations in the hilus or molecular layer. Some newborn cells develop relatively normal axonal and dendritic projections, while others form de novo connections with neighboring granule cells via axonal sprouting or formation of aberrant basal dendrites. This morphological diversity is paralleled by broad physiological differences. Ectopic cells exhibit hyperexcitable features,[36-38] while cells correctly located in the cell body layer are comparatively normal.[39,40] This diversity highlights a key limitation of current experiments to manipulate newborn granule cells in epilepsy. Specifically, existing approaches cannot discriminate between morphologically abnormal granule cells that are predicted to be pathological, and morphologically normal granule cells that may be beneficial. Studies showing beneficial effects of granule cell ablation, therefore, may have hit on fortuitous circumstances where the net effect of the newborn cells is harmful. It is conceivable that approaches targeting only abnormal cells will be more effective, and more broadly applicable.

Targeting Epileptogenesis to Treat Epilepsy

Studies support the conclusion that aberrant granule cells promote the development of temporal lobe epileptogenesis in rodents. It is therefore worth considering if neurogenesis could be targeted as a treatment for epilepsy in humans.

The first question that has to be resolved is whether adult neurogenesis occurs in humans. Recent studies provide evidence both for[41,42] and against[43] the occurrence of adult neurogenesis. The topic has been covered in depth elsewhere,[44,45] and will ultimately require further studies to fully resolve. It is abundantly clear, however, that abnormal granule cells are present in patients with temporal lobe epilepsy.[46] These abnormal neurons could be generated in adulthood after an epileptogenic brain injury, as occurs in animal models. Alternatively, abnormal granule cells in human temporal lobe epilepsy could follow a different pattern, arising from mature neurons generated in early development. A third possibility is that abnormal granule cells observed in patients with adult-onset epilepsy are generated and develop abnormal features early in development, but remain clinically "dormant" until adulthood.

Regardless of when aberrant granule cells are generated in patients with temporal lobe epilepsy, therapeutic strategies aimed at mitigating the hyperexcitable effects of these neurons are likely to be similar, because in the majority of clinical scenarios, these abnormal cells will already be present by the time most patients are identified as having epilepsy. While antimitotics could be used to block neurogenesis after an epileptogenic brain injury, as has been done in epilepsy models,[15,22] the low incidence of epilepsy development after most injury types—and the lack of biomarkers for epileptogenesis—make this approach impractical. While blocking neurogenesis in epileptic rodents improves cognitive performance,[18] blocking neurogenesis in healthy rodents consistently impairs performance,[47] and the same can be predicted for humans.

Approaches that could target aberrant granule cells after the development of clinical epilepsy would have the broadest applicability. While transgenic mouse approaches used to eliminate abnormal granule cells after disease onset are obviously not translatable to humans, evidence for the efficacy of delayed treatment is encouraging,[20] and suggests that the therapeutic window extends beyond the first clinical seizure. Advances in clinically approved viral delivery vehicles, somatic genetic manipulations, and epigenetic approaches all hold promise for new ways to target granule cells. Such approaches also offer opportunity for the selective targeting of pathological granule cells—if key molecular differences between these cells and healthy granule cells can be identified and exploited. While the exact form of such therapies remains uncertain, one could imagine that approaches to selectively ablate, silence, or modify aberrant granule cells could change the course of epilepsy.

Conclusion

Identification of disease-modifying treatments for epilepsy is a critical focus of epilepsy research. Many laboratories are exploring a variety of promising approaches to disrupt epileptogenesis (targeting mTOR, neurotrophins, inflammation, stem cell therapies, transcriptional regulators etc). Given this diversity of mechanisms linked to epileptogenesis, and the efficacy of approaches targeting distinct mechanisms in preclinical studies, it seems unlikely that any single "silver bullet" will prevent epilepsy in all at-risk patients. Studies targeting disrupted granule cell neurogenesis seem to follow this pattern, showing promising effects in some animal models, but not producing complete seizure remission. Nonetheless, promising results support continued research toward treatments that may ultimately look more like current clinical treatments for cancer, in which multiple pathways are often targeted simultaneously to improve outcomes. Such treatments take advantage of an in-depth knowledge of cancer mechanisms to target multiple cell signaling pathways simultaneously, including advances in understanding mechanisms of cancer development, progression, compensatory pathways, tumor microenvironment, immune interactions, and disease evolution over time.[48] Our understanding of epileptogenesis is still in its early stages relative to cancer biology. As our knowledge of epilepsy expands, however, such a multipronged, adaptive-treatment approach may serve as a model for antiepileptogenesis.

Highlights

- Adult-generated granule cells exert anticonvulsive effects in healthy brains
- During epilepsy development, adult-generated granule cells develop abnormal morphological and physiological properties
- Animal studies implicate adult-generated granule cells in the development of epilepsy

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ORCID iD

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

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