



## Seizure-Induced Newborn Neurons Might Not Be So Bad After All

### Impaired Recruitment of Seizure-Generated Neurons into Functional Memory Networks of the Adult Dentate Gyrus Following Long-Term Amygdala Kindling.

Fournier NM, Botterill JJ, Marks WN, Guskjolen AJ, Kalynchuk LE. *Exp Neurol* 2013;244:9–104.

Epileptic seizures increase the birth of new neurons in the adult hippocampus. Although the consequences of aberrant neurogenesis on behavior are not fully understood, one hypothesis is that seizure-generated neurons might form faulty circuits that disrupt hippocampal functions, such as learning and memory. In the present study, we employed long-term amygdala kindling (i.e., rats receive 99-electrical stimulations) to examine the effect of repeated seizures on hippocampal neurogenesis and behavior. We labeled seizure-generated cells with the proliferation marker BrdU after 30-stimulations and continued kindling for an additional 4 weeks to allow newborn neurons to mature under conditions of repeated seizures. After kindling was complete, rats were tested in a trace fear conditioning task and sacrificed 2 h later to examine if 4-week-old newborn cells were recruited into circuits involved in the retrieval of emotional memory. Compared to non-kindled controls, long-term kindled rats showed significant impairments in fear memory reflected in a decrease in conditioned freezing to both tone and contextual cues during testing. Moreover, long-term kindling also prevented the activation of 4-week-old newborn cells in response to fear memory retrieval. These results indicate that the presence of seizure activity during cell maturation impedes the ability of new neurons to integrate properly into circuits important in memory formation. Together, our findings suggest that aberrant seizure-induced neurogenesis might contribute to the development of learning impairments in chronic epilepsy and raise the possibility that targeting the reduced activation of adult born neurons could represent a beneficial strategy to reverse cognitive deficits in some epileptic patients.

### Commentary

Despite early evidence of neurogenesis in adult rodents (1), not until the 1990s was the notion fully acknowledged that new neurons could be generated in the adult brain. Newborn cells were found in humans (2), and their number was found to increase in rodents after exercise, exposure to enriched environment or learning, and decrease after stress (3). In rodent models of temporal lobe epilepsy (TLE), it was also found that status epilepticus (SE) increases neurogenesis in the dentate gyrus (4). However, instead of being a restorative process, dentate epilepsy-induced neurogenesis appeared to be detrimental: SE-newborn cells emerging from the subgranular zone were found to migrate aberrantly to the hilar/CA3 border, where they display abnormal dendritic morphology. These newborn cells—also referred to as “ectopic granule cells”—integrate functionally to the existing network and are hyperexcitable compared to normal granule cells, firing in bursts. They also contribute to mossy fiber sprouting, another hallmark of temporal lobe epilepsy (5, 6). As a consequence, it is believed

that excessive excitatory inputs from cortex reach CA3, violating the excitation–inhibition balance of the so-called “dentate gate,” potentially causing seizures.

In this article, Fournier and colleagues ask whether epilepsy-induced newborn cells could also be involved in cognitive impairments, a common comorbidity of TLE. This question is all the more relevant since the dentate gyrus plays a critical role in certain forms of memory (7) and newborn cells in epilepsy migrate, differentiate, and integrate abnormally in the dentate gyrus. In addition, a large number of studies—although there are some discrepancies—report alterations of memory performance after blockade of adult neurogenesis in non-epileptic rodents (3). Taken together, one would therefore expect that epilepsy-induced newborn cells would negatively impact dentate and memory function. While previous attempts have been made to test this hypothesis by blocking adult neurogenesis after SE (8), it is possible that the behavioral improvements observed could be caused directly by the treatment (here, valproate) and not the decreased neurogenesis. In this study, Fournier and colleagues used a different approach: Instead of blocking adult neurogenesis in their epilepsy model, they let newborn neurons develop freely and asked whether these neurons show signs of involvement in a memory task.

Epilepsy Currents, Vol. 13, No. 5 (September/October) 2013 pp. 229–230  
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To increase the normal rate of adult neurogenesis, the authors used the amygdala kindling model of TLE, where electrical stimulations (99 stimulation sessions at a regimen of 3 per day) were delivered intracranially to the basolateral amygdala. While initial stimulations did not have any behavioral effect, rats started to develop seizures after several days of stimulation. Interestingly, this model rarely produces spontaneous seizures, which allows investigators to control for the number of seizures. Amygdala kindling also increases the rate of adult neurogenesis, which was quantified here with BrdU, a proliferation marker injected during kindling.

At the end of the kindling schedule, rats were subjected to the classical trace fear conditioning task. During the training phase, animals were subjected to a tone followed by a foot shock, following which rats were tested in the presence of the same tone but in another conditioning chamber (tone test). A day later, rats were exposed to the same chamber but without the tone (context test). The ability of rats to remember both the tone–shock association and the context–shock association is traditionally measured by quantifying the amount of time rats spend without moving (freezing) in each task.

Two hours after the end of the experiment, the animals were sacrificed and brain sections were immunostained for BrdU to quantify adult-born cells. Investigators also analyzed the expression of Fos, an immediate early gene that is expressed when neurons are activated. By using this technique, they were able to visualize and quantify the neurons that were activated in the past few hours; that is, as the rats were involved in the context test. Elegantly, the coupling of both BrdU and Fos staining then allowed them to ask whether newborn neurons (tagged with BrdU) were activated during the task (i.e., whether they expressed Fos).

Fournier et al. found that long-term kindling caused a dramatic increase in BrdU-labeled neurons in the dentate gyrus. During both tone and context tests, kindled rats spent significantly less time freezing than did the controls, suggesting that they had memory deficits. In parallel, neuronal activation in the granule cell layer during the task was decreased in kindled rats. Surprisingly, whereas 9% of the control newborn cells expressed Fos after fear conditioning, less than 1% of the newly born cells were activated, and none in the hilus. Since it has been documented that epilepsy-induced newborn neurons—at least the ectopic ones (6, 9)—are hyperexcitable, these results are surprising. Even without any specific learning, one might have expected that more of the kindled newborn cells would have been activated. Instead, kindling-induced newborn cells remained silent.

On one hand, these results could be good news: Although seizure-induced neurogenesis may be involved in epileptogenesis and seizure generation, it does not seem to affect cognition, at least in the amygdala kindling model. Therefore, destruction or inactivation of these cells would have little impact on behavior while limiting and potentially preventing seizures. On the other hand, however, these results seem at odds with previous reports defending the idea that abnormal neurogenesis does contribute to cognitive impairment. The

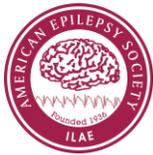
results also raise questions as to whether hyperexcitability of epilepsy-induced newborn cells is a general phenomenon or is limited to specific experimental conditions.

However, a few points should be taken into consideration: First, the authors report a lack of cellular activation in the kindling group while the output behavior (freezing) of this same group was precisely missing. Moreover, the overall granule cell layer Fos staining in this group was significantly lower than in the controls. This general decreased activation of the dentate gyrus in the epileptic group could also prevent potential observation of newborn cell activation. Second, cellular activation was measured after retrieval of only one type of behavior. Other paradigms testing acquisition versus retrieval of trace fear conditioning and other dentate-dependent tasks, such as those depending on pattern separation, could reveal different results. Finally, these results need to be replicated with other epilepsy models, where neurogenesis is more robust. Nevertheless, this report suggests that, in vivo, epilepsy-induced newborn neurons may not be as hyperactive as suggested in previous reports. While more experiments are clearly needed to fully understand the functional role of epilepsy-induced neurogenesis, this type of in vivo approach might prove informative.

by Pierre-Pascal Lenck-Santini, PhD

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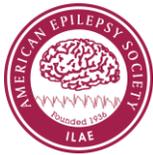
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