Epilepsy Currents
2022, Vol. 22(5) 315-317

DOI: 10.1177_15357597221113704
journals.sagepub.com/home/epi
SAGE

A Star is Born: Newborn Astroglia in Epilepsy

Louis T. Dang, MD, PhD[1]

Altered Adult Neurogenesis and Gliogenesis in Patients With Mesial Temporal Lobe Epilepsy

Ammothumkandy A, Ravina K, Wolseley V, et al. *Nat Neurosci.* 2022;25:493-503. doi:10.1038/s41593-022-01044-2.

The hippocampus is the most common seizure focus in people. In the hippocampus, aberrant neurogenesis plays a critical role in the initiation and progression of epilepsy in rodent models, but it is unknown whether this also holds true in humans. To address this question, we used immunofluorescence on control healthy hippocampus and surgical resections from mesial temporal lobe epilepsy (MTLE), plus neural stem-cell cultures and multi-electrode recordings of ex vivo hippocampal slices. We found that a longer duration of epilepsy is associated with a sharp decline in neuronal production and persistent numbers in astrogenesis. Further, immature neurons in MTLE are mostly inactive, and are not observed in cases with local epileptiform-like activity. However, immature astroglia are present in every MTLE case and their location and activity are dependent on epileptiform-like activity. Immature astroglia, rather than newborn neurons, therefore represent a potential target to continually modulate adult human neuronal hyperactivity.

Commentary

The long-standing dogma that the adult brain is unable to regenerate or produce new neurons was transformed by evidence for persistent adult neurogenesis in the hippocampus of rodents and humans,[1,2] although persistent neurogenesis in the adult hippocampus has come under question recently[3] and remains controversial. In healthy rodents, these new neurons have a positive effect on learning and memory.[4] In rodent models of epilepsy, pilocarpine-induced status epilepticus results in a dramatic increase in hippocampal neurogenesis.[5] These newborn neurons integrate aberrantly and can contribute to temporal lobe epileptogenesis (reviewed by Danzer).[6] The presence and effect of altered hippocampal neurogenesis in mesial temporal lobe epilepsy (MTLE) is less clear. A prior study examined tissue from individuals undergoing temporal lobe resection for refractory MTLE and found increased doublecortin-positive (Dcx+) cells compared to autopsy control specimens.[7] Dcx is expressed in migrating neuroblasts, and these findings suggested increased hippocampal neurogenesis in the epileptic human brain.

A recent study by Ammothumkandy *et al.* asked whether newborn neurons contribute to epileptogenesis.[8] Surgically resected hippocampal tissue from individuals with refractory MTLE was examined using immunohistochemistry, neurosphere cultures, and multi-electrode array recordings. Postmortem tissue from age-matched individuals served as histological controls. In half of the MTLE cases and almost all the age-matched controls, there were cells immunoreactive for both Dcx and Prox1, consistent with newborn dentate granule neurons. The number of newborn neurons in MTLE dropped precipitously with longer disease. These findings provided additional evidence of human hippocampal neurogenesis in healthy adults and revealed that MTLE is associated with a substantial decline in hippocampal neurogenesis over time.

Surprisingly, a large majority of the Dcx+ cells in MTLE were immunoreactive for glial fibrillary acidic protein (GFAP) and S100β, glial markers. Combined with Dcx expression these cells were deemed to be immature astroglia, and these cells were not found in controls. To determine if immature neurons and astroglia can be newly generated from the hippocampus, the authors isolated primary cells from MTLE specimens, grew them into neurospheres (clusters of neural cells that arise from proliferating cells *in vitro*), and then plated cells for neural differentiation. After 6 weeks, cultures from specimens with a longer disease duration generated a higher percentage of immature astroglia (Tuj1+/GFAP+) than immature granule neurons (Tuj1+/Prox1+), and the converse was true for cultures from specimens with a lower disease duration. This suggested

[1] University of Michigan, Michigan Medicine, Ann Arbor, MI, USA

that ongoing astrogenesis was present but neurogenesis declined during the course of disease.

The authors next asked whether the newborn neurons and immature astroglia contribute to epileptiform activity in MTLE. Acute hippocampal slices from MTLE specimens were recorded using a multi-electrode array, to measure and localize interictal-like activity (IIA). These slices were then used for histology. Specimens with no IIA in the dentate gyrus (DG) had Dcx+/Prox1+ newborn neurons, while those with IIA had none. The cells were labeled for immediate early genes (IEGs) c-fos and Arc, which are expressed in active neurons. There was only minimal IEG expression in newborn neurons in the granule cell layer, or in ectopic newborn neurons in the hilus and molecular layer of the DG. These data suggest that newborn neurons do not contribute to IIA in chronic MTLE and do not exhibit abnormally increased activity. Conversely, immature astroglia were present in MTLE specimens irrespective of whether there was IIA in the DG. In subregions of MTLE specimens with IIA in the DG, there were more immature astroglia in the hilus and fewer in the granule cell layer compared to subregions that did not have IIA. This may be explained by increased migration of newborn astroglia from the granule cell layer to the hilus. Immature astroglia were stained for c-fos, which marks activity in astrocytes, and they were positive in slices that lacked IIA and negative in slices that had IIA.

One strength of the study is that the integration of clinical data, histology, MEA recording, and *in vitro* culture allowed correlatation of various findings with disease duration. The MTLE samples ranged in disease duration from 3 to 53 years, allowing inferences about the chronology of histological findings in MTLE. How these findings relate to histological changes found in rodents during epileptogenesis remains uncertain, considering most rodent models are studied early in the course of disease.

This study raises a number of intriguing questions about the potential role of adult neurogenesis and immature astroglia in epilepsy. Are newborn neurons important to epileptogenesis in human MTLE? Prior rodent studies have demonstrated that newborn neurons mediate various mechanisms of epileptogenesis, and the present study suggests that newborn neurons are not necessary for IIA in chronic epilepsy. A key difference is that findings in this study reflect chronic changes observed with refractory epilepsy rather than initial stages of epileptogenesis. One possible explanation is that newborn neurons are involved in early epileptogenesis and subsequently form mature neurons that contribute to hyperexcitable epileptic networks. With chronic seizures, the stem cell pool becomes depleted and newborn neurons are no longer generated, yet seizures continue. Findings that the newborn neurons did not express IEGs and were absent in slices with IIA do not preclude the possibility that the mature neurons that arise from newborn neurons are involved in generating IIA.

Do the immature astroglia originate from newborn astrocytes, a dedifferentiated mature astrocyte, or something else? How do they relate to reactive astrogliosis seen in chronic epilepsy? These "immature astroglia" expressed Dcx, GFAP, and S100β. Reliance on immunoreactivity and morphology to infer cell identity has its shortfalls, especially in a disease context where the cells in question are not found in healthy controls. While Dcx is often used as a marker of immature neurons, one study identified Dcx expression in mature astrocytes in post-mortem tissue from healthy individuals,[9] and another study on human MTLE tissue noted that Dcx cells did not express GFAP.[7] Conflicting results could be attributed to variations in tissue processing, antibodies, and other technical aspects. If the immature astroglia in the current study were from newborn glia rather than dedifferentiated mature glia, it would be strange that they all lack expression of the cell proliferation marker Ki67. The authors studied potential for proliferation and differentiation of primary cells from MTLE specimens using neurosphere cultures, but this only provided indirect evidence of what occurs *in vivo.* Additional analysis of single cell RNA sequencing data from non-neuronal cells in human MTLE specimens[10] or gene expression analysis after laser capture microdissection may help elucidate the identity and developmental origin of these cells.

Are the immature astroglia involved in epileptogenesis? Possibly. When IIA was present, immature astroglia did not express c-fos, and vice versa. It is possible that IIA is a result of the inability for the immature astroglia to activate inhibitory interneurons, buffer potassium and glutamate, and maintain inhibitory/excitatory balance. There was a subtle difference in the location of immature astroglia between DG regions with higher IIA compared to lower IIA, raising the possibility that misplaced immature astroglia may cause the IIA, but also it is possible that the IIA resulted in misplaced immature astroglia.

This study reaffirms the presence of adult neurogenesis in MTLE and healthy subjects and shows that neurogenesis declines with longer disease duration. Surprisingly, in MTLE there were abnormal cells that may be immature astroglia, possibly from newborn astroglia. The activity and location of these cells varied with the presence of IIA, raising the possibility that they modulate seizure activity and are involved in epileptogenesis. However, a primary weakness of this human tissue study is the findings are correlative only, and the cause-and-effect relationship between the astroglia and the IIA is unclear. Additional mechanistic studies that perturb the function or presence of immature astroglia will better elucidate the role of these cells in epilepsy. If the immature astroglia aberrantly modulate neuronal activity, then targeting these cells could be a promising therapeutic strategy for epilepsy.

ORCID iD

Louis T. Dang https://orcid.org/0000-0003-4853-4952

References

1. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology* 1965;124:319-325.

2. Eriksson PS, Perfilieva E, Björk-Eriksson T, et al. Neurogenesis in the adult human hippocampus. *Nat Med* 1998;4:1313-1317.
3. Sorrells SF, Paredes MF, Cebrian-Silla A, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature* 2018;555:377-381.
4. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and indexing. *Nat Neurosci* 2019;22:1565-1575.
5. Parent JM, Yu TW, Leibowitz RT, et al. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* 1997;17:3727-3738.
6. Danzer SC. Adult neurogenesis in the development of epilepsy. *Epilepsy Curr* 2019;19:316-320.
7. Liu YWJ, Curtis MA, Gibbons HM, et al. Doublecortin expression in the normal and epileptic adult human brain. *European Journal of Neuroscience* 2008;28:2254-2265.
8. Ammothumkandy A, Ravina K, Wolseley V, et al. Altered adult neurogenesis and gliogenesis in patients with mesial temporal lobe epilepsy. *Nat Neurosci* 2022;25:493-503.
9. Verwer RWH, Sluiter AA, Balesar RA, et al. Mature astrocytes in the adult human neocortex express the early neuronal marker doublecortin. *Brain* 2007;130:3321-3335.
10. Pfisterer U, Petukhov V, Demharter S, et al. Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis. *Nature Communications* 2020;11:5038.