A Star is Born: Newborn Astroglia in Epilepsy

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Altered Adult Neurogenesis and Gliogenesis in Patients With Mesial Temporal Lobe Epilepsy
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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 recently3 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. Post-mortem 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...
that ongoing astrogensis was present but neurogenesis de-
clined 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 recor-
ded 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 un-
certain, 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 epilepto-
genesis, 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 in-
volved in generating IIA.

Do the immature astroglia originate from newborn astro-
cytes, 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 shortcomings, 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 astro-
cytes 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
specimens10 or gene expression analysis after laser capture
microdissection may help elucidate the identity and develop-
mental origin of these cells.

Are the immature astroglia involved in epileptogenesis? Per-
haps. 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 de-
clines 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 rela-
tionship 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 ther-
apapeutic strategy for epilepsy.

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