AN ORGANOTYPIC MODEL OF TEMPORAL LOBE EPILEPSY TO INVESTIGATE THE ROLE OF GABAergic TRANSMISSION ON DENDRITIC ARBOR GROWTH IN THE MOUSE HIPPOCAMPUS

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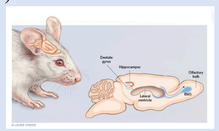
INTRODUCTION/BACKGROUND

Seizures are described as sudden, uncontrollable electrical disturbances in the brain (Mayo Clinic, 2020) brought on by high-frequency synchronous neural activity. In temporal lobe epilepsy (TLE), many patients experience focal seizures in the temporal lobes of the brain, involving the hippocampus or entorhinal cortex and subiculum (Figure 1).

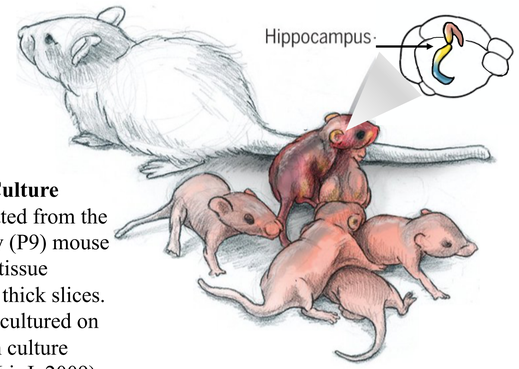


These structures play key roles in learning, memory, and mood. One-third of TLE patients become resistant to anti-convulsant medications and/or show severe side effects of the drugs. In these patients, surgical removal of the temporal lobes is effective for seizure control, but if seizures generalize or spread to both hemispheres, surgical removal may not be possible due to the central role of the temporal lobes in memory. Therefore, novel therapies for patients with intractable TLE are vital. Considerable evidence points to cortical GABAergic interneuron/progenitor transplantation as a potential therapy for intractable epilepsy (Zhu et al. 2018).

Research in the Naegele lab is focused on developing stem cell therapies for TLE using the pilocarpine model of severe TLE (Arshad and Naegele 2020). Prior work in mice with pilocarpine-induced TLE established that hippocampal transplants of fetal GABAergic progenitors significantly reduced seizure frequency (Henderson et al 2014). Optogenetically activating Channelrhodopsin-2 (ChR2)-expressing GABAergic interneurons in the transplants demonstrated that they formed functional inhibitory synapses with adult-born dentate gyrus granule cells (Gupta et al. 2020; Arshad et al. 2020). These transplant-derived synaptic inputs were linked to structural changes in adult-born granule cells, including smaller dendritic arbors (Gupta et al. 2020).

These results suggest that transplanted GABAergic progenitors may suppress seizures by altering the growth of dendrites in adult-born granule cells. We hypothesize that these cellular changes are mediated by synaptic release of the neurotransmitter GABA from the transplanted cells, which would bind to $GABA_A$ receptors, and cause activity-dependent changes in intracellular signaling cascades mediating dendritic arbor growth. To test this hypothesis, we are using a brain slice model of TLE and transplanting GABAergic progenitors that are defective for the release of GABA. Comparisons of the dendritic arbor growth of adult-born granule cells innervated by GABA transporter deficient (VGAT-/-) vs. wildtype transplants (VGAT +/+) will allow us to test whether synaptic release of GABA is required for constraining dendritic arbor growth.

METHODOLOGY



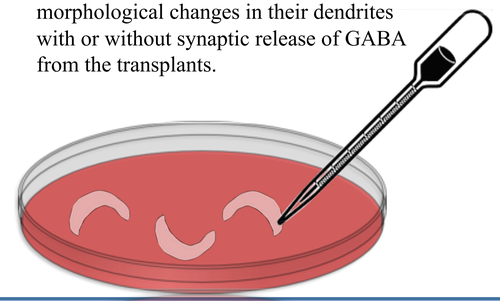
Organotypic Slice Culture

Hippocampi are isolated from the brain of postnatal day (P9) mouse pups and sliced with tissue chopper into 350 µm thick slices. The tissue slices are cultured on porous membranes in culture media (Koito, H., & Li, J. 2009). Morphological and functional integrity of slices is confirmed with Hoechst staining and Mini-Ruby.

MGE Transplants

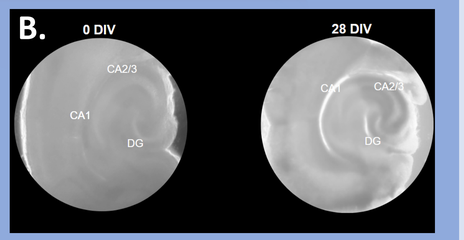
MGE progenitor cells from VGAT deficient and wildtype mouse embryos will be transplanted onto hippocampal slices.

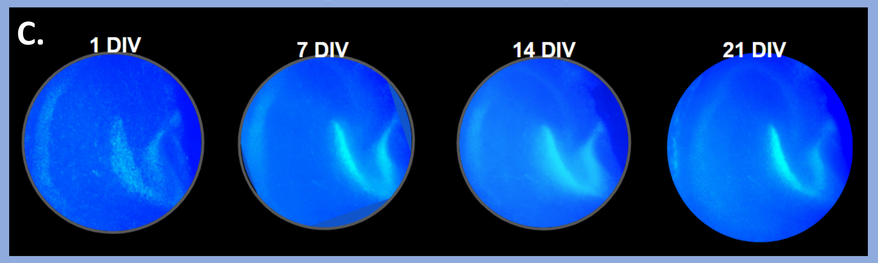
Newborn granule cells in the hippocampi will be labeled with pRubi, a retrovirus that expresses red fluorescent protein throughout the cells. Retrovirally-labeled granule cells will then be analyzed for morphological changes in their dendrites with or without synaptic release of GABA from the transplants.



RESULTS

A. Dissected hippocampal slices from P9 mouse pups as seen under the microscope. The entorhinal cortex is still intact.
B. Hippocampal slices from mouse pups shown at 0 and 28 days in vitro. The hippocampal architecture is retained after 4 weeks of being cultured.
C. Slices fixed and stained with Hoechst showing neurons remain viable after being cultured in vitro for 21 days.





FUTURE DIRECTIONS

Over the summer, we have successfully isolated the hippocampi from mouse pups and kept the slice cultures alive for more than four weeks. The process proved to be a challenging one, as even a small amount of contamination resulted in the death of the specimen. Currently, we are staining the hippocampal slices with the DNA dye Hoechst and using anterograde tracing with rhodamine-conjugate dextran (Mini-ruby) in order to visualize neuronal architecture.

Once we confirm the preservation of the neuronal circuitry and its proper functioning long term in the organotypic model, we will study neurogenesis in the slices to visualize the newborn granule cells.

Finally, we will transplant MGE progenitors from embryonic day 13.5 (E13.5) mouse embryos into the hippocampal slice culture and monitor the cellular mechanism by which they suppress seizure activity.

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