Verification of Successful Kainic Acid Injections in a Temporal Lobe Epilepsy Mouse Model

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Introduction: Temporal Lobe Epilepsy

- Epilepsy is a neurological disorder that is characterized by repeated seizures. It affects ~ 2.9 million Americans (Karasin B, Karasin M., AORN 2017).
- Temporal lobe epilepsy (TLE) is the most prominent form of partial epilepsy in adults (Herzog et al., Arch Neurol 1986).
- Reproductive endocrine comorbidities appear in patients with TLE at significantly higher rates than in the general population. These comorbidities include polycystic ovary syndrome, hypothalamic amenorrhea, hyperandrogenism, irregular menstrual cycle, lower testosterone levels, hypogonadism, erectile dysfunction, and decreased semen motility (Herzog, Zeitschrift Für Epileptologie 2015).

Background

- It is believed that gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus play an important role in the development of the aforementioned comorbidities.
- The kainic acid (KA) mouse model is used to study epilepsy-associated changes in GnRH neurophysiology.
- Our lab recently observed that the activity of GnRH neurons is altered in KA-injected mice (Li et al., eNeuro 2018).
- The brain is extracted 2 months after injection. The hypothalamus is used for patch clamp experiments, and the hippocampus is fixed for later use.
- To ensure validity of the data, we must verify the success of all KA injections as indicated by the presence of sclerosis and/or gliosis in the hippocampus.
- Nissl (Cresyl violet) staining is used to visually verify the presence of sclerosis.

Questions

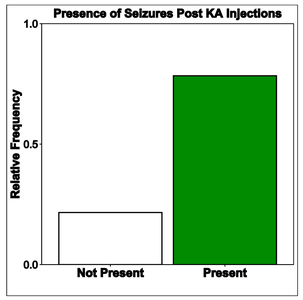
- Do all KA injections effectively trigger seizures and later development of hippocampal damage?
- How effective are the different methods of verification?

Methods

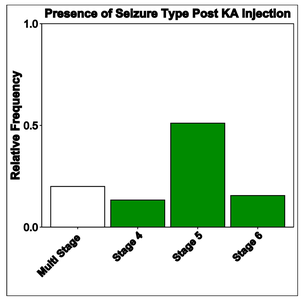
- Post-injection video recordings were used to identify all seizures identifiable by behavioral changes.
- Nissl staining was used to screen for sclerosis in preserved hippocampal sections.

Acute Behavioral Seizure Screening

- Following KA injections, the mice are recorded in an observation chamber for 4-5 hours.
- The recordings are viewed at a later time and screened for acute seizures to verify the KA injection.
- Acute seizures are divided into stages:
  - Stage 1-3: Not recorded
  - Stage 4: Repetitive head and jaw movement
  - Stage 5: Repetitive movement of front paws ("clawing") and thrashing of tail
  - Stage 6: Violent jumping and dashing around the cage
- The times and stages of all seizures are recorded.



Proportion of mice that had seizures



Prevalence of seizure types observed during the video recordings

Nissl Staining: Background

- This staining method stains for intracellular granules called Nissl bodies—rosette-like formations of polysomes that are scattered within the rows of the rough endoplasmic reticulum (Bryne, Academic Press 2014).
- Dispersion of the granule cell layer is an indicator of TLE (Figure 2, arrows).
- Granule cell layer dispersion is confirmed by visual inspection.
- The dispersion is caused by the demyelination of neurons (sclerosis).
- If dispersion is not present in any KA-injected mice, then gliosis is confirmed with combined GFAP/DAPI staining.
- Sclerosis and gliosis—the death of glial cells—are both strong indicators of epilepsy.

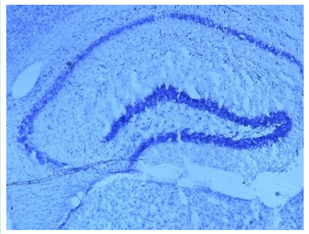


***FIG 1**: Stained dorsal hippocampus at 40x magnification. Dispersion is not present.*

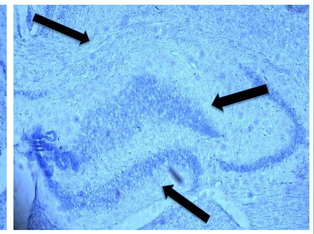
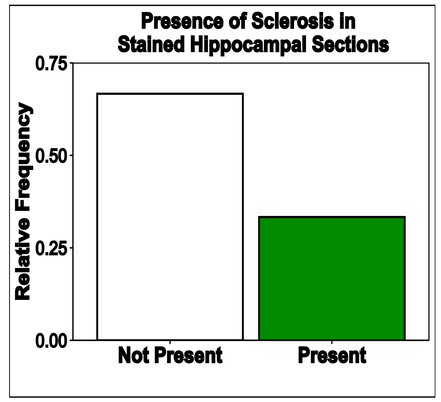


***FIG 2**:Stained dorsal hippocampus at 40x magnification. Dispersion is present.*

Nissl Staining: Graph



Percentage of slices that showed dispersion of neurons.

Conclusions

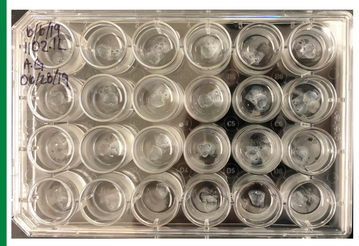
- Post KA injections, mice do not always have acute seizures; however, most do display signs of acute seizures.
- Nissl staining does not always allow one to determine whether a mouse has TLE.
- Additional methods must be utilized to provide sufficient evidence that the KA injections were successful.

Future Work

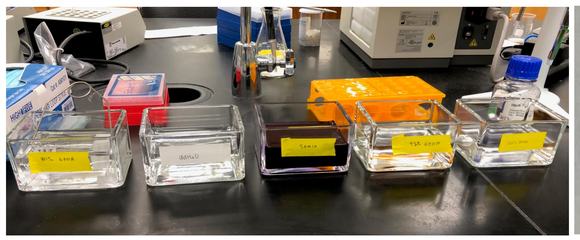
- GFAP/DAPI staining will be used to determine gliosis.
- Recording of seizure activity by electroencephalogram (EEG) will verify seizures and development of TLE.

Nissl Staining: Procedure

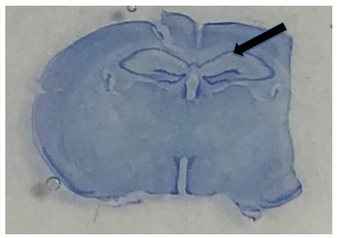
1. The mouse is killed, and the brain is quickly extracted.
2. After collection of hypothalamic slices for recording, the remaining brain containing the hippocampus is fixed in paraformaldehyde.
3. Fixed brain tissue is sectioned into 40-micrometer thick slices and preserved in phosphate buffered saline (PBS).
4. Brain slices are selected from the well tray and placed onto positively charged slides.
5. The slides are put into PBS, EtOH, and Cresyl violet to stain for Nissl bodies.
6. A coverslip is placed over the slices and secured with Permount.
7. Slices are analyzed under a microscope for sclerosis.



The sectioned brain slices are preserved in a well tray containing PBS.



EtOH, PBS, and Cresyl violet stain are the 3 main chemicals used.



These are brain slices from a mouse after Nissl staining. The arrow points at the dorsal hippocampus.

Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 1559908/1559929, as part of the Phenotypic Plasticity Research Experience for Community College Students, through the University of Illinois at Urbana-Champaign Institute for Genomic Biology and Parkland College.
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Financial support for this project in the Christian Lab was provided by NIH grant R01 NS105825.

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