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 (Herd 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 (Herd et al., Zeitschrift Für Epileptologie 2015).

Methods

Brain slices are selected from the well tray and placed onto positively charged slides. Brain slices are selected from the well tray and placed onto positively charged slides. Brain slices are selected from the well tray and placed onto positively charged slides. Brain slices are selected from the well tray and placed onto positively charged slides. Brain slices are selected from the well tray and placed onto positively charged slides.

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?

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.

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 (Byrne, 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.

Nissl Staining: Graph
- The sectioned brain slices are preserved in a well tray containing PBS. The sectioned brain slices are preserved in a well tray containing PBS. The sectioned brain slices are preserved in a well tray containing PBS. The sectioned brain slices are preserved in a well tray containing PBS. The sectioned brain slices are preserved in a well tray containing PBS.

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.

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. http://precs.ipb.illinois.edu/.
Financial support for this project in the Christian Lab was provided by NIH grant R01 NS105825.