In the United States more than 2.9 million people have active epilepsy or seizure disorder (US Census Bureau 2010). The economic costs of epilepsy exceed 15 billion dollars, and individuals and their family members experience a significant decrease in quality of life. Temporal lobe epilepsy (TLE) remains the most frequent type of epilepsy, and is the most prevalent classification of partial epilepsy and is associated with persistent deficits in cognition. Rendett et al. (2008) showed that electrical stimulation can be used to induce matrix-dependent structural and functional changes in theta-modulated neurons, leading to impaired cognition (McNaughton 2006). The development of antiepileptic drugs (AEDs) has been based on the prevention of the initial occurrence of seizures, but is not sufficient to prevent development of the pathological neuronal activity and to improve cognitive function while also increasing seizure threshold. In the present study we examine the cumulative effects of theta stimulation of the MSN during early epileptogenesis, prior to the development of spontaneous seizures. We hypothesized that long-term effects of theta stimulation of the MSN during early epileptogenesis, prior to the development of spontaneous seizures, could be modeled by injecting the acetylcholine receptor agonist pilocarpine systemically. Similar to humans, pilocarpine-induced seizures can be modeled by systemic administration of pilocarpine in the pilot experiments in rats. Pilocarpine-induced seizures can be sustained for 1-2 hours before reaching a peak. The effects of pilocarpine on the hippocampus can be characterized by changes in EEG, behavioral testing, and measurements of neuronal activity.

### Methods

- **Pilocarpine-Induced Epilepsy (Day 0)**: Animals were intraperitoneally injected with pilocarpine (30 mg/kg, IP) to induce seizures.
- **Electroencephalography (EEG)**: EEG was recorded during pilocarpine-induced seizures.
- **Hippocampal Oscillations**: Oscillations in the theta range (4-8 Hz) were measured in vivo using EEG recordings.
- **Seizure Threshold Assessments (Days 3, 32, 50)**: Seizure thresholds were assessed using flurothyl (infused at a rate of 20 µg/kg/min). The threshold was defined as the concentration of flurothyl required to produce ictal discharges.
- **Novel Object Recognition Task (Days 40-43)**: Animals were placed in a large dark Plexiglas box with 24 spatial cues. A dark escape box was placed in a fixed location under one of the holes. The box was then filled with water, and the animals were allowed to explore the box for 5 minutes. Animals were then placed back into the box 3 hours later with a novel object replacing one of the originally explored objects.
- **Behavioral Testing (Days 32, 50)**: Behavioral testing was performed using the Barnes maze, a behavioral task that assesses spatial learning and memory.
- **EEG as compared between stimulated and non-stimulated animals**: EEG was recorded in freely moving animals. Individual paired t-tests were used to compare the threshold at each of the chronic time points with PID 3.

### Results

- **Fig 1**: Hippocampal oscillations are generated and maintained by multiple inputs, both direct (blue arrow) and indirect (green arrow). SEEG from the hippocampus was normalized to baseline levels, and was significantly reduced in stimulated animals.
- **Fig 2**: Percent time oscillating in theta increased in stimulated rats as compared to baseline levels.
- **Fig 3**: Seizure thresholds were assessed using flurothyl (infused at a rate of 20 µg/kg/min). There was a significant reduction of stage 3 and stage 5 seizure thresholds on both days 32 and 50 in pilocarpine-treated animals as compared to baseline.
- **Fig 4**: Behavioral testing was performed using the Barnes maze, a behavioral task that assesses spatial learning and memory. The Barnes maze spatial learning task and object recognition task.

### Conclusions

- **EED**: The present study demonstrates that electrical stimulation of the MSN during early epileptogenesis, prior to the development of spontaneous seizures, results in an decrease in seizure threshold, and impairment of cognitive function. The effects of pilocarpine on the hippocampus can be characterized by changes in EEG, behavioral testing, and measurements of neuronal activity.
- **Behavioral Testing**: Behavioral testing improves cognitive performance in both tasks to sham levels.

### Future Directions

- **More complex analyses of EEG at individual nodes as well as across the circuit**: More complex analyses of EEG at individual nodes as well as across the circuit. This may include spectral analysis, coherence, and phase relationships.
- **Comparison of multiple stimulation paradigms**: The comparison of multiple stimulation paradigms (e.g., high-frequency oscillations, theta bursts, delta, and duration of stimulation)
- **Testing of novel objects**: Testing of novel objects, such as videos, images, and textures.

### References


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