Ethanol withdrawal produces hyperexcitability and lowers seizure threshold in an optogenetic model of seizure.

Klorig, D.C.[1+], Alberto, G.[1+], Masicampo, M.[1], Godwin, D.W.[1]

Chronic ethanol (EtOH) exposure and subsequent withdrawal produces symptoms that include cognitive deficit and seizure. However, the exact mechanisms of neuronal sensitization and its subsequent effect on neural circuitry during withdrawal (WD) are not well understood. We have developed a method for detecting circuit level changes in excitability by using optogenetically mediated synchronous population discharges to probe excitability in the hippocampal and perihippocampal network.

Methods: Mice expressing ChR2 in CA1 pyramidal neurons were implanted with an 8 channel multi-site microwire array, which allowed for chronic simultaneous monitoring of activity in CA1 (bilaterally), subiculum, entorhinal cortex, dentate gyrus, CA3, anterior medial thalamus, and prefrontal cortex. Light-induced synchronous depolarization of CA1 pyramidal neurons in naive, freely moving mice produced interictal spike-like events. Recorded activity was similar in latency and waveform to spontaneously occurring interictal spikes from kindled animals and propagated through the recorded network. Repetitive optical stimulation was sufficient to induce persistent rhythmic afterdischarges and optogenetic kindling at or just above the spike induction threshold resulting in Racine stage 5 or 6 clonic-tonic seizures. Both naïve and kindled mice were then exposed to a chronic intermittent EtOH (CIE) exposure paradigm consisting of 16 hours of ethanol vapor exposure followed by 8 hours of WD. This paradigm was repeated for four days.

Results: Two optogenetic measures of excitability were employed in mice exposed to CIE as a model of WD. First, in non-kindled animals, the response of the larger network to single pulse optogenetic stimulation at different light intensities was tested against pre-EtOH thresholds. Second, in previously kindled mice, seizure thresholds (light intensity), durations, and severity were assessed during WD and compared to the pre-exposed state. Additionally, the number of spontaneously occurring interictal spikes was counted each day during WD. EtOH WD significantly reduced population spike and seizure thresholds, and increased seizure duration and severity. In addition, the rate of spontaneous interictal spiking during baseline periods was increased during WD.

Discussion: Our results indicate that EtOH WD mediated hyperexcitability increases sensitivity to optically induced seizure, thereby validating the CIE model of neuronal sensitization following WD. Additionally; we demonstrate the utility of optogenetic population thresholds as a sensitive measure of excitability and for use in probing changes in excitability due to chronic EtOH exposure. This model not only demonstrates a novel way to investigate population changes following EtOH exposure, but it also sets the stage for investigating the utility of pharmacologic interventions with the hope of finding specific ameliorative and curative therapies for EtOH WD symptoms.

Funding was provided by NIH grants R01AA016852 and F30AA23708-01 and the Tab Williams Family Fund