EXPLORING NEUROANATOMICAL AND GENETIC INFLUENCES ON EPILEPTOGENESIS UTILIZING THE REPEATED FLUROTHYL MODEL IN MICE

by

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ABSTRACT

This thesis explored the genetics and neuroanatomy of epilepsy and epileptogenesis. The change in a normal brain that causes it to become hyperexcitable, which results in epilepsy, is known as epileptogenesis and is still relatively unexplored. Here, we utilized the repeated flurothyl model, a non-invasive animal model of observing epileptogenesis in mice, to examine both seizure susceptibility and epileptogenesis in mice. In this model, mice are given 8 daily flurothyl-induced seizures, 1 seizure per day (induction phase), where their myoclonic jerk threshold (MJT), generalized seizure threshold (GST) and behavioral seizure phenotype are recorded. Then, mice are left in their home cages for 28 days where no flurothyl-induced seizures are administered (incubation phase). Lastly, animals are given 1 flurothyl-induced seizure trial at the conclusion of the incubation phase (retest). The first two studies in this thesis examined differences in seizure behavior between inbred strains of mice examined in the repeated flurothyl model. They highlight 2 strains, C57BL/6J (B6) and DBA/2J (D2) mice as having dissociable seizure traits. B6 mice in the repeated flurothyl model have a high initial MJT and GST that decreases over the 8 trial induction phase. Conversely, D2 mice initially have a low MJT and GST that does not change over the 8 trial induction phase. During the initial 8-trial induction phase, B6 animals display strictly forebrain seizures (characterized by a loss of posture followed by clonus of the face and/or forelimbs). When retested, mice have a change in behavioral seizure phenotype from a forebrain seizure to a forebrain seizure that rapidly progresses into a brainstem seizure (characterized by forebrain seizure behavior followed by wild running, hopping, and/or tonic extension of the limbs; referred to as a forebrain→brainstem seizure). However, D2 mice display strictly forebrain seizures both during the induction phase and at flurothyl retest. The permanent decrease in GST over multiple flurothyl trials and the change in seizure phenotype upon retest are indicative of epileptogenic processes that occur in B6 mice, most likely as a result of changes in the brain due to previous flurothyl-induced seizures. The differences between these 2 inbred strains were exploited for further analysis in the remainder of the thesis. Baseline differences in Fos, a protein used as a marker of neuronal activation, between B6 and D2 mice, and changes in

neuronal activation between these 2 strains throughout the repeated flurothyl model were examined. Specifically, activity in the amygdala increased with multiple flurothyl-induced seizures in B6 mice, however, remained consistently high in D2 mice. Activity in the hippocampus was very high in B6 mice after 1 flurothyl-induced seizure, however, decreased over multiple flurothyl-induced seizure trials. In D2 mice, Fos expression was scattered in the hippocampus after 1 flurothyl-induced seizure, and increased to a moderate level with subsequent flurothyl-induced seizures. These results implicate the hippocampus and the amygdala as two areas being differentially activated during the process of epileptogenesis. Also, the activation of the ventromedial nucleus of the hypothalamus (VMH) seen in B6 mice upon flurothyl retest (as detected by bilateral Fos expression in the VMH) was not seen in D2 mice, further supporting the VMH as a vital area in the propagation of forebrain seizures into the brainstem seizure circuitry responsible for the expression of forebrain→brainstem seizures. Lastly, the inherent genetic differences between B6 and D2 mice were further dissected by observing BXD recombinant inbred strains of mice in the repeated flurothyl model. We found a significant QTL for initial MJT on chromosome 13, a significant QTL for the change in seizure phenotype on chromosome 7, and suggestive QTLs for baseline GST on chromosome 10. Moreover, a QTL for the decrease in GST was observed only when baseline GST was used as an additive covariate on chromosome 5. These results illustrate that seizure traits in the repeated flurothyl model can be mapped to specific areas of the genome, as well as highlight specific areas that are likely controlling these traits.