SPECIFIC AIMS - PROJECT 1 - BIOMARKERS OF EPILEPTOGENESIS AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

This preclinical project aims to find single and/or combinatory biomarkers for epileptogenesis after temporal lobe TBI, the most common injury site for post-traumatic epilepsy (PTE) in humans[12, 13]. We will induce temporal lobe TBI in rats using lateral fluid-percussion injury (FPI), taking into account neuroanatomic differences between rodents and humans. Studies show FPI results in the development of PTE in >50% of rats in 1-year follow-up, depending on the injury severity[7, 21, 38]. With protocols emphasizing scientific rigor and reproducibility, we propose a prospective, statistically powered, longitudinal in vivo multi-center 12-months follow-up study in rats with temporal lobe TBI. We will compare the evolution of clinically translatable biomarkers between injured animals which develop or do not develop epilepsy, and validate the results in an independent animal cohort in *Project 2* and in a human cohort in *Project 3*. Results will be communicated to *Public Engagement Core* to obtain consumer input. These biomarkers will be used to develop (AEG) treatments tested in *Project 2.* We will provide EEG and structural MRI datasets for the *Informatics and Analytics Core* to develop automated methodological pipelines to identify biomarkers of epileptogenesis.

Overarching hypothesis: Plasma molecular, electrophysiological, and imaging markers measured at different post-TBI time points, alone or in combination, will diagnose ongoing epileptogenesis after experimental TBI with high sensitivity and specificity, independent of the severity of brain damage. The identified pre-clinical biomarkers can be applied to (a) preclinical AEG studies to predict treatment outcome (*Project 2)* and (b) clinical studies to identify patients undergoing epileptogenesis following temporal lobe TBI (comparison with *Project 3* data) to inform the targeting of epileptogenic interventions, and to monitor their effectiveness.

Specific Aim 1: (1) To investigate (a) the occurrence and progression of abnormalities in cortical and hippocampal wideband EEG recordings acquired immediately after temporal lobe TBI for up to 12 months post-injury. (2) To compare the severity and progression of changes between animals that will or will not develop epilepsy. (3) To provide a set of candidate biomarkers to be validated in an independent animal cohort in *Project* 2 and human cohort with temporal lobe TBI in *Project 3*. (4) To assess whether a combination of imaging and plasma markers increases the sensitivity and specificity of electrophysiological markers.

Hypothesis 1: Temporal lobe TBI in the rat will result in *electrophysiological abnormalities* in the perilesional cortex and hippocampus. Some will be biomarkers for epileptogenesis both in animals and humans. Presence of concomitant molecular and structural abnormalities will increase the sensitivity/specificity of electrophysiological biomarkers.

Specific Aim 2: (1) To investigate the evolution of structural pathology in the cortex, hippocampus, and thalamus and in their connectivity after temporal lobe TBI using repeated high-resolution structural MRI. (2) To compare findings between animals which develop or do not develop epilepsy. (3) To provide a set of candidate biomarkers to be validated in independent animal and human cohorts in *Project 2* and in *Project 3.* (4) To assess whether a combination of electrophysiological and plasma markers increases the sensitivity and specificity of imaging markers.

Hypothesis 2: Temporal lobe TBI in the rat will result in *structural pathology* in hippocampal and thalamocortical networks. Evolution of pathologies will be most prominent in hippocampal networks, and some of them will be biomarkers for epileptogenesis. The presence of extratemporal pathologies, evolution of abnormalities in electrophysiological and/or plasma markers will increase the sensitivity and specificity of imaging biomarkers.

***Specific Aim* 3**: (1) To identify the evolution of abnormalities in plasma proteins and circulating microRNAs which alone or in combination with electrophysiological and imaging markers predict epileptogenesis after TBI. (2) To provide a set of candidate biomarkers to be validated in an independent animal cohort in *Project 2* and in human cohort with temporal lobe TBI in *Project 3.*

Hypothesis 3: TBI will induce *changes in plasma proteins and/or microRNAs*, which signal about neuronal degeneration, axonal and dendritic injury, neuroinflammation, and metabolic changes. Abnormalities in plasma markers will correlate with the degree of hippocampal and thalamocortical damage in MRI and with some electrophysiological markers. Some plasma markers will be specific for TBI-induced epilepsy rather than TBI alone both in animals and humans.

Deliverables: Validated (1) Plasma molecular, (2) Electrophysiological, (3) Imaging and (4) Combinatory biomarker panels to predict epileptogenesis and treatment response in preclinical and clinical AEG studies.