According to the Center for Disease Control and Prevention, epilepsy affects 2.2 million Americans and 65 million people worldwide. Epileptic seizures can severely limit school achievements, employment prospects, and participation in other life experiences. Traumatic brain injury is one of the major risk factors causing epileptogenesis; the development of epilepsy.

Insulin-like Growth Factor 1 (IGF-1) is a powerful growth factor involved in several physiological and pathological processes. It is known that the binding of IGF-1 to IGF-1 receptors leads to the activation of MAPK and PI3K signaling cascades, but it is not clear whether IGF-1 activates mTOR, or operates in parallel to the PI3K-Akt-mTOR cascade through activation of MAPK.

**Aim 1:** What are the downstream effectors of IGF-1 in an organotypic hippocampal culture model of posttraumatic epileptogenesis, and is there cross-talk with pro-epileptogenic PI3K-Akt-mTOR signaling? In order to answer these questions, Western Blot is used to measure levels of phosphorylation of Akt, MAPK, and S6 (a marker of mTOR activation), during early post injury period and latent period with and without IGF-1 in the culture medium.

**Aim 2:** Determine the timeline of the signaling cascade. Measuring the presence of phosphorylated Akt, MAPK, and S6 both with and without IGF-1 will lead to conclusions on the effects of IGF-1 on the different steps in the cascade. These conclusions will allow us to determine the sequence in which the pathway operates.

**Background Information**
- Insulin-like growth factor 1 (IGF-1) levels are elevated in the brain following head injury.
- Earlier research suggests that IGF-1 has pro-epileptogenic effects.
- It is known that the binding of IGF-1 to IGF-1 receptors leads to the activation of MAPK and PI3K signaling cascades, but it is not clear whether IGF-1 activates mTOR, or operates in parallel to the PI3K-Akt-mTOR cascade through activation of MAPK.

**Significance**
- According to the Center for Disease Control and Prevention, epilepsy affects 2.2 million Americans and 65 million people worldwide.
- Epileptic seizures can severely limit school achievements, employment prospects, and participation in other life experiences.
- Traumatic brain injury is one of the major risk factors causing epileptogenesis; the development of epilepsy.

**Results**
- Figures 1-4 show the results of Western Blot analysis, where phosphorylated and total protein levels are compared.
- Figures 5 and 6 illustrate graphically the relative ratios of phosphorylated protein to total protein.

**Analysis**
A program called Fiji ImageJ was used to collect the data for this experiment. This program gave values to each band based on band brightness, and these numerical values can be used to interpret the data and make relative ratios of phosphorylated protein to total protein.

**Conclusion**
Over 30 experiments have been done, some being repeat experiments. So far, we have concluded that +IGF gives approximately 50% increase in phosphorylation of Akt and pS6. This is our only consistent conclusion to date, but it is clear that IGF-1 does have a positive effect on protein phosphorylation. MAPK also shows evidence of playing a role in the signaling cascade, but it is not clear how it effects the pathway. The question now is how exactly does IGF-1 respond to injury and what is the timeline of the cascade? This experiment is still in the workings, but once all data is collected and analyzed it will be possible to answer our first aim, make strides toward the second, and to continue on to accomplish the long term aim in the future.

**Acknowledgements**
Special thanks to Dr. Yevgeny Berdichevsky who coordinated this experiment, and to Ms. Alexandra M Dryer, Mr. Yero Saponjian, Mr. Mark Mahoney, Ms. Corrina A Lucini, and Ms. Marija Usevonic; contributors to our project.