

## ENGINEERING

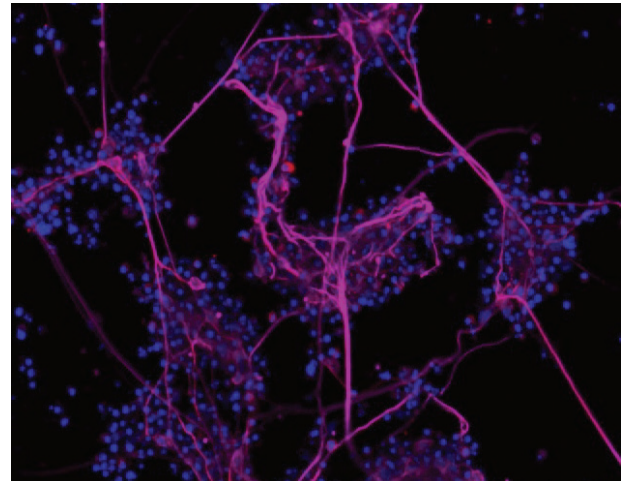
### Characterization of Neuronal Differentiation and Activity in Human-Induced Pluripotent Neural Stem Cells

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Traumatic brain injury (TBI) is one of the leading causes of death and disability in the United States, with more than 1.7 million people seeking medical care for TBI annually. Although there are currently no effective clinical treatments for TBI, pre-clinical treatments using neural stem cells (NSCs) have shown promising results to promote tissue neuroprotection and recovery post-injury. This study aims to analyze how NSCs differentiate into neural networks and seeks to characterize their corresponding activity level to help improve pre-clinical TBI treatments. Based upon past studies, we hypothesized that NSCs would differentiate into mature neurons within two weeks, but would have a delayed functional activity response of 1–2 months.

Human-induced pluripotent (hIP) NSCs were differentiated into neuronal networks over a 14-day period using fibroblast growth factor (FGF-2) withdrawal. Subsequently, immunocytochemistry and MATLAB-based imaging analysis were performed to quantify the percentage of immature and mature neurons on day 1, 7, and 14 of the assay. Conversely, neuronal activity was investigated by seeding hIP-NSCs and primary rat cortical neurons concurrently on microelectrode plates to measure neuronal action potentials. To visualize neuronal activity, a red fluorescent calcium sensor protein was introduced into differentiated hIP-NSCs via viral, chemical, and electrical transfection methods and imaged via fluorescent imaging techniques.

After image analysis, we found hIP-NSCs successfully differentiated into mature neurons within two weeks; however, there was no correlated increase in the hIP-NSC activity level with neuronal differentiation, as hIP-NSCs showed diminutive



Representative staining of hIP-NSC differentiation at day 14 using MAP2 (mature neurons; red), beta-III Tubulin (immature neurons; magenta), and the counter stain with DAPI (cellular nucleus; blue). As indicated by the long, thin axonal connections, a neuronal network has formed.

activity during the 14-day period. As a result, the preliminary transduction results indicate the susceptibility of primary neurons to express fluorescent calcium sensors more readily than differentiated hIP-NSCs, signifying the presence of desired activity levels. Future works will focus on optimizing the differentiation of hIP-NSCs into functional neurons and understanding their application in in vitro and in vivo models of TBI.

*Research mentor Charles Latchoumane writes: "Characterization of hIP-NSC differentiation and neuronal activity is essential for the development of in vitro and in vivo models of TBI, and TBI-associated treatments. During her REU summer program in Dr. Karumbaiah's lab (UGA), Allison's research provided a more complete characterization of our NSC differentiation protocols, and established several techniques for electrical and image-based measurements of neuronal activity. These assessments are now facilitating the development of novel in vitro devices and approaches to study the role of NSCs post-brain injury."*