**Endoplasmic Reticulum Stress Induces Axon Initial Segment Shortening in Cortical Neuron Culture.**

The axon initial segment (AIS) is a domain located between the neuronal soma and axon responsible for action-potential initiation. Because even small changes in its length or location alter neuronal excitability and function, the AIS is a key structure for neurodegenerative pathophysiology. AIS shortening is seen in several neurodegenerative conditions such as traumatic brain injury, Alzheimer's disease, neuropathic pain, multiple sclerosis, and diabetic encephalopathy. Diabetic encephalopathy refers to mild cognitive impairment or dementia often experienced in patients with type 2 diabetes. Although patients with type 2 diabetes are 1.73 times more likely to develop dementia, there is currently no course modifying treatment options available. Our lab has previously shown that cognitive impairment in *db/db* mice, a mouse model of type 2 diabetes, is associated with AIS shortening. The cellular process responsible for this AIS shortening is unknown and thus a significant gap in our knowledge. One candidate as a mediator of AIS shortening in diabetic encephalopathy is endoplasmic reticulum (ER) stress. Alterations in protein folding or secretion, oxidative stress, and other cellular disturbances can lead to ER stress. This then leads to a signaling cascade called the unfolded protein response which aims to rectify ER function. Hippocampal UPR protein levels are elevated in *db/db* mice, and ER stress inhibition improves cognitive function in animal models of diabetes. In addition, methylglyoxal, a reactive glucose metabolite increased in type 2 diabetes that induces ER stress, leads to AIS shortening in cortical neuron cultures. These studies led to our hypothesis that endoplasmic reticulum stress induces axon initial segment shortening. To test if ER stress is sufficient to cause AIS shortening, we first exposed primary cortical neuron cultures from wild type mice to the ER-stress inducer tunicamycin (1 µg/mL) for 24 hours. We used immunofluorescence to evaluate AIS morphology by labeling AIS markers and quantifying AIS length. We then evaluated the dose dependent effect of tunicamycin (0.125-1 µg/mL) on AIS length and neuronal viability. Viability was determined by the percentage of neurons with an AIS. To evaluate if ER stress is necessary for AIS shortening, we exposed cortical neuronal cultures to methylglyoxal (100µM) and the ER stress inhibitor sodium phenylbutyrate (100µM) for 24 hours. AIS length and viability measurements for all experiments were completed under observer blinded conditions. Our results show tunicamycin induces significant AIS shortening in a dose dependent manner without significant decrease in viability. These results show induction of ER stress at non-lethal levels is sufficient to induce AIS shortening *in vitro*. Further, sodium phenylbutyrate coadministration prevents methylglyoxal induced AIS shortening. These results suggest ER stress is necessary to induce AIS shortening *in vitro.* Together with previously reported AIS shortening and ER stress in the diabetic brain as discussed above, this data suggests that ER stress induced AIS shortening is a novel mechanism of the pathophysiology of diabetic encephalopathy.