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

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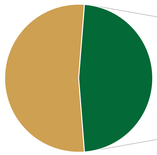
Introduction

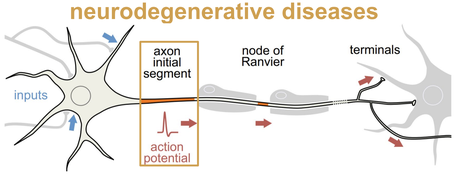
Type 2 diabetes is a risk factor for cognitive impairment and dementia



47.5% of adults in the United States are living with diabetes or prediabetes. Approximately 90% of adults with diabetes have type 2 diabetes[1]

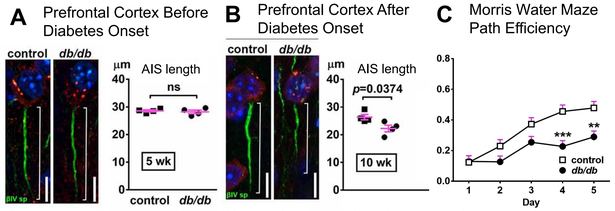
Patients with type 2 diabetes have **1.73x** the risk of developing dementia of those without diabetes. [2]

The AIS is implicated in the pathophysiology of many neurodegenerative diseases



The axon initial segment (AIS), a domain anchored by ankyrinG and βIV spectrin to the neuronal cytoskeleton,[3] regulates action potential initiation via voltage-gated sodium channels and maintains neuronal polarity.[4] Even small AIS shortening decreases neuronal excitability and is associated with cognitive impairment in conditions such as traumatic brain injury,[5] Alzheimer's disease,[6] neuropathic pain,[7] and multiple sclerosis. [8] Adapted from (Leterrier; Current Topics in Membranes, 2016)

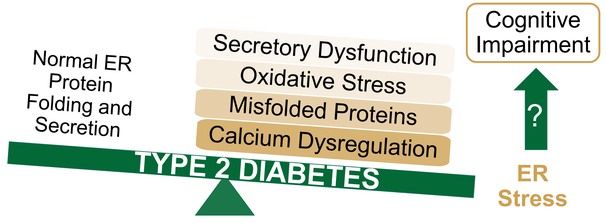
AIS shortening is associated with cognitive impairment in *db/db* mice with type 2 diabetes



Db/db mice **(A)** develop AIS shortening **(B)** after diabetes onset [staining: βIV spectrin (green, AIS), NeuN (red, neuronal soma) and Hoechst (blue, cell nuclei)] associated with impaired cognitive flexibility **(C)** demonstrated by decreased path efficiency during the reversal phase of Morris water maze. Adapted from Yermakov.[9,10]

ER stress mediates diabetes cognitive impairment

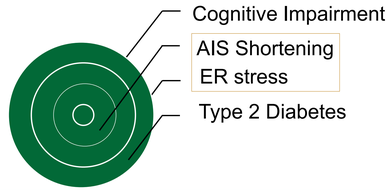
"Endoplasmic reticulum (ER) stress" describes cellular insults that hinder normal functioning of the ER. ER stress plays a role in several complications of diabetes[11] including cognitive impairment.[12]



Hypothesis

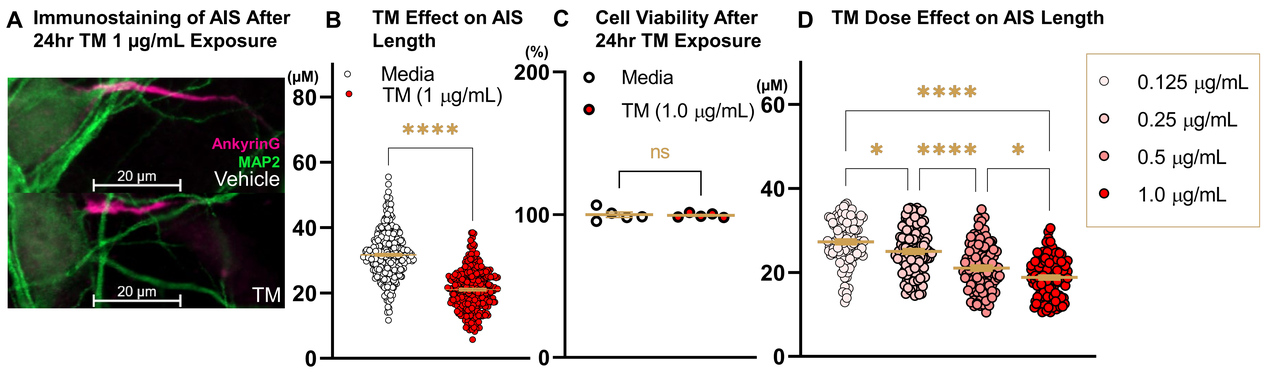
AIS shortening and cognitive impairment may be reversible[13] providing a novel therapeutic target for treatment of a variety of neurodegenerative conditions. Our overall goal is to study the relationship between ER stress, AIS shortening, and cognitive impairment in type 2 diabetes. To examine this, we first hypothesized:

Endoplasmic reticulum stress is sufficient and necessary for axon initial segment shortening *in vitro*



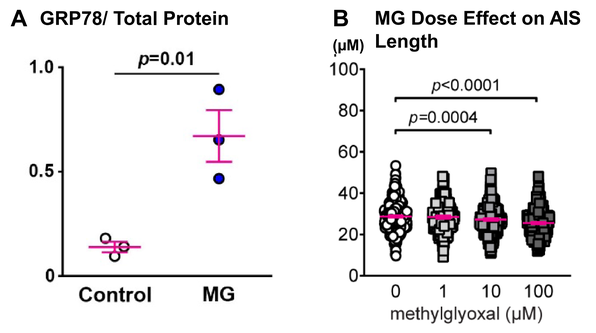
Results

Tunicamycin induces dose dependent shortening of the AIS at non-lethal levels



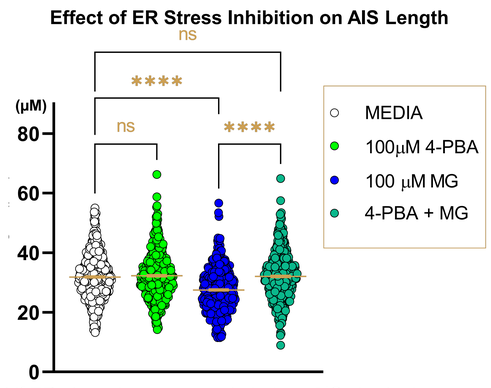
To test if ER Stress is sufficient to cause AIS shortening at non-lethal levels, we utilized the ER stress inducer tunicamycin (TM). **(A,B)** Primary neuronal cortical culture were exposed to TM (1 μg/mL) for 24 hours(staining: magenta: ankyrinG (AIS), green: MAP2 (dendrites)). **(C)** We quantified neuron viability after 24-hour TM 1 μg/mL exposure by comparing the percentage of neurons lacking an AIS, a sign of irreversible neuronal injury. [14] (n=5 coverslips per treatment from 2 preps). TM induced AIS shortening (n= 319-375 AIS per treatment from 2 preps) in a dose dependent manner (n= 96-116 per treatment AIS from 3 coverslips from1 prep).

MG induces ER stress and AIS shortening



Methylglyoxal (MG) is a glucose metabolite elevated in diabetes[15] known to cause ER stress. This is also true in our cell culture model. **(A)** MG exposure (100 μM, 24 hours) causes increased protein expression of the ER stress marker GRP78 on western blot. **(B)** MG also induces AIS shortening in a dose dependent manner in primary cortical neurons.

4-PBA prevents MG induced AIS shortening



To test if ER stress is necessary to induce AIS shortening, we used the ER stress inhibitor sodium phenylbutyrate (4-PBA). We treated mouse primary cortical neurons with media, 4-PBA (100 μM), MG (100 μM), or both for 24 hours. 4-PBA prevents MG induced AIS shortening (n=417-542 AIS per treatment from 3 preps).

Conclusions

01 — Sub-lethal ER Stress is sufficient to cause AIS shortening *in vitro*

02 — ER Stress induces AIS shortening in a dose dependent manner

03 — Inhibition of ER Stress prevents AIS shortening *in vitro*

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