# Iron Deficiency-Induced Axonal Branching Deficits in Developing Hippocampal Neurons

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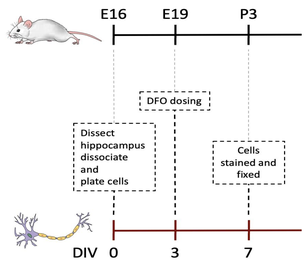
## BACKGROUND

- Iron deficiency (ID) affects 2 billion people worldwide, and it is the most common micronutrient deficiency.[1]
- Iron is particularly important in cell development, and iron needs are greater in developing brains, as in fetal and postnatal developmental periods.[2] Iron is an essential component of proteins involved in cellular metabolism, including the four electron transport chain complexes, cytochrome c oxidase, and some of the enzymes in the citric acid cycle.[2]
- Development is an energy dependent process, and iron is a vital component of cellular metabolism and ATP production. Limited ATP production, due to ID, in periods of high development could therefore limit the ability of cells to grow.[5]
- It is known that ID affects the complexity of dendrite branching in hippocampal neurons. Dendrites grown in ID conditions exhibit decreased arborization, which is observed as decreased branching as a measure of complexity.[6] However, the specific effects of ID on axon growth and branching are unknown.[6]

**Hypothesis:**
Iron deficiency impairs energy dependent processes required for neuron development and axon maturation, reducing axon length and branching and ultimately overall axonal field complexity. This can be corroborated by decreased iron expression genes.

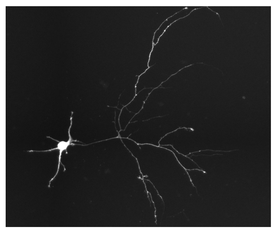
## METHODS

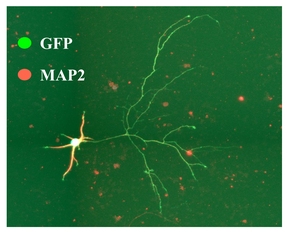
**Modeling Chronic Iron Deficiency in Hippocampal Cultures**
Treatment Groups:
- 0 µM DFO (Control)
- 9 µM DFO (Iron-deficient)




**Measuring Axon Complexity**
- Using Fiji software and Simple Neurite Tracer
- Sholl analysis performed

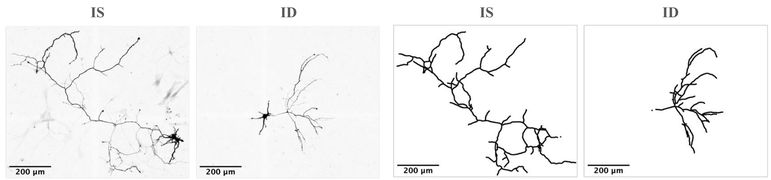
**Measuring Gene Expression**
- qPCR: Tfrc, GDA, GAPDH, RPS18

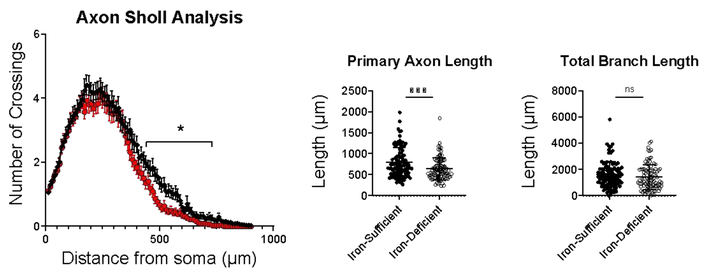


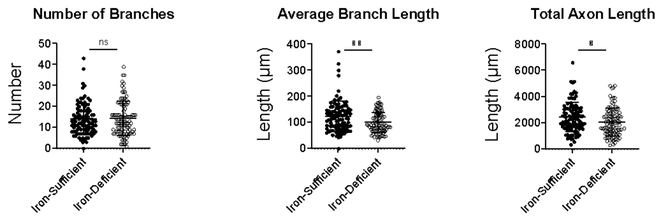


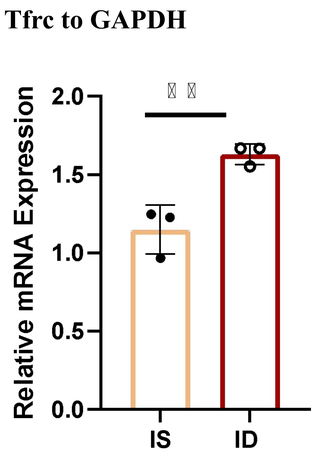

traces

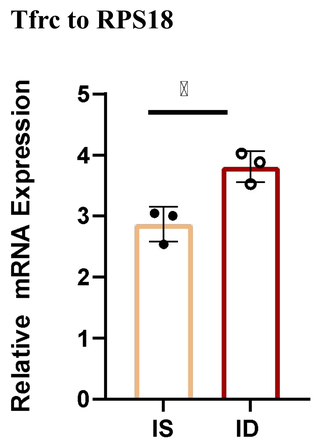
## RESULTS



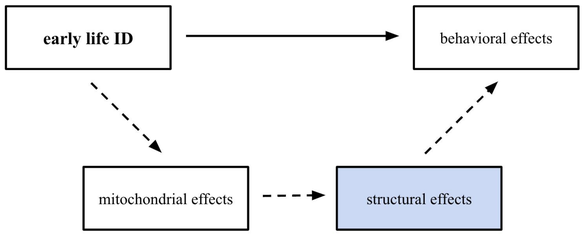

## SUMMARY

### CONCLUSIONS

- ID affects overall axon complexity and growth
  - Observed a significant difference between primary axon length, average branch length, and total axon length
- ID has structural effects on hippocampal axons and dendrites
- Higher expression of Tfrc in ID condition indicates successful iron deficiency

### FUTURE DIRECTIONS

- More analysis of gene expression, including housekeeping genes and genes involved in axonogenesis (cofilin)
- The following questions remain:
  - Why does ID affect neuron structure?
  - Is impaired mitochondrial respiration and ATP production during brain development mechanistically responsible?
- How does neonatal ID result in behavioral and neurological dysfunction?

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