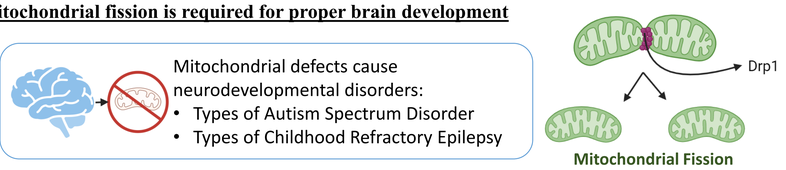
Drp1-induced Mitochondrial Fission Controls Cortical Astrocyte Organization

Sprihaa Kolanukuduru[1,3], Pia Rodriguez[2,3], Cagla Eroglu[3,4,5]

1. Department of Psychology and Neuroscience, UNC-Chapel Hill; 2. Cell and Molecular Biology Program, Duke University; 3. Department of Cell Biology, Duke University; 4. Department of Neurobiology, Duke University; 5. Howard Hughes Medical Institute

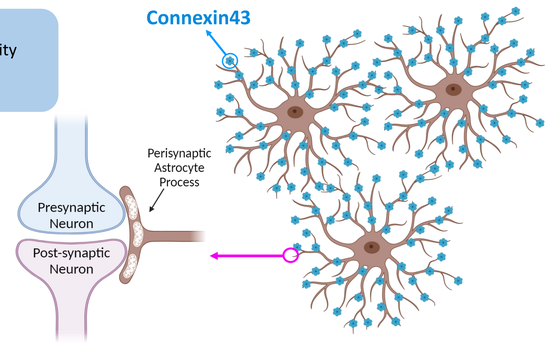
Background

Mitochondrial fission is required for proper brain development



What is the role of mitochondrial fission in astrocyte development in the mouse cortex?

Astrocytic Tiling:
- Established in maturity
- Astrocyte Network
- Lost in disease



How does Drp1-induced mitochondrial fission regulate astrocyte organization?

Drp1 KD causes astrocyte clustering in the adult mouse cortex

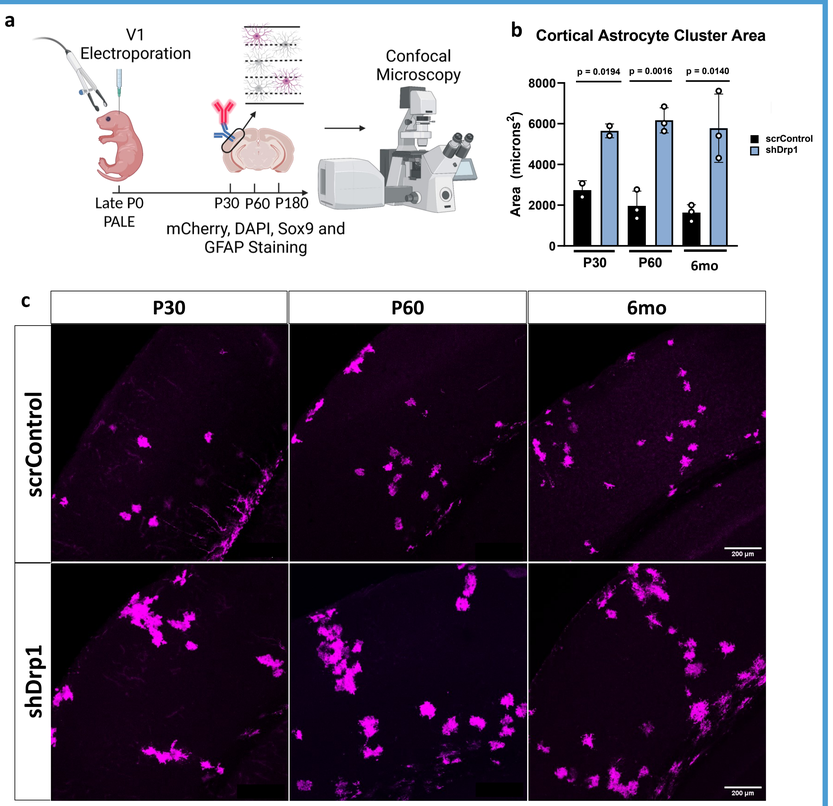


Figure 1. Knockdown of Drp1-induced mitochondrial fission causes astrocyte clustering into adulthood. a. PALE was performed on P0 mice pups injected with either scrControl or shDrp1 constructs and collected at P21, P30, P60, and P180. b. Quantification of cluster area in control and shDrp1 astrocytes, n=3 animals per condition, unpaired t-test. Bars are Mean ± SEM c. Representative images of control (top) and shDrp1 (bottom) P30, P60, and P180 visual cortex PALE brains.

Drp1 KD disrupts cortical astrocyte organization

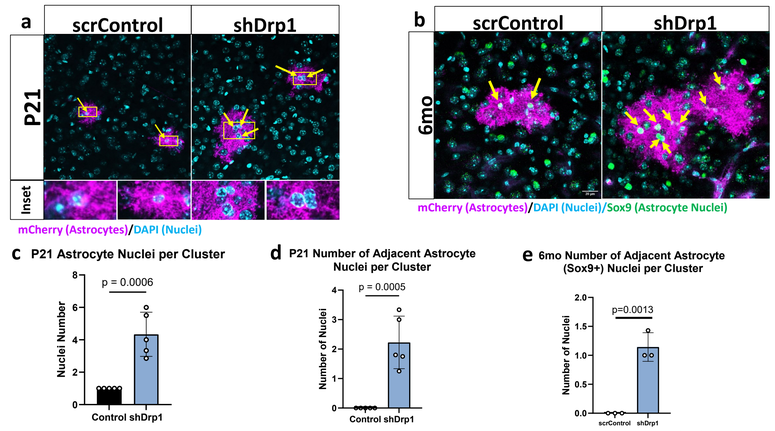


Figure 2. Sparse knockdown of Drp1-mediated mitochondrial fission disrupts astrocyte organization. a. Representative images of control (left) and shDrp1 (right) astrocyte clusters with zoomed in images of nuclei (bottom) in P21 visual cortex PALE brains. b. Representative images of Sox9 (green) and DAPI (cyan) in P180 control (left) and shDrp1 (right) astrocyte clusters. c. Quantification of number of astrocyte nuclei per cluster in P21 control and shDrp1 astrocytes, n=5 animals per condition, unpaired t-test. Bars are Mean ± SEM. d. Quantification of number of adjacent astrocyte nuclei per cluster in P21 control and shDrp1 astrocytes, n=5 animals per condition, unpaired t-test. Bars are Mean ± SEM. e. Quantification of number of adjacent astrocyte nuclei per cluster in P180 control and shDrp1 astrocytes, n=3 animals per condition, unpaired t-test. Bars are Mean ± SEM.

Characterization of an Astrocytic-conditional knockout of Drp1

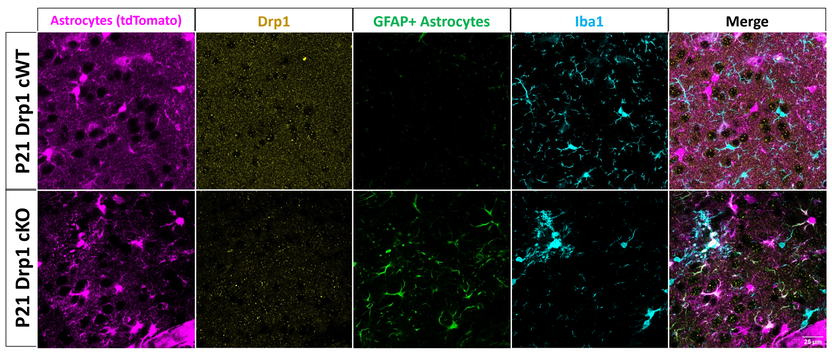


Figure 3. Characterization of Astrocytic-conditional Drp1 Knockout model at P21. a. Representative images of P21 Aldh1l1-Cre ER^{T2}, Drp1 wild-type (Drp1 cWT, top) and Drp1 floxed/floxed (Drp1 cKO, bottom) astrocytes (magenta), stained with Drp1 (yellow), GFAP (green), and Iba1 (cyan).

Drp1 KD and cKO dysregulate Connexin43 expression

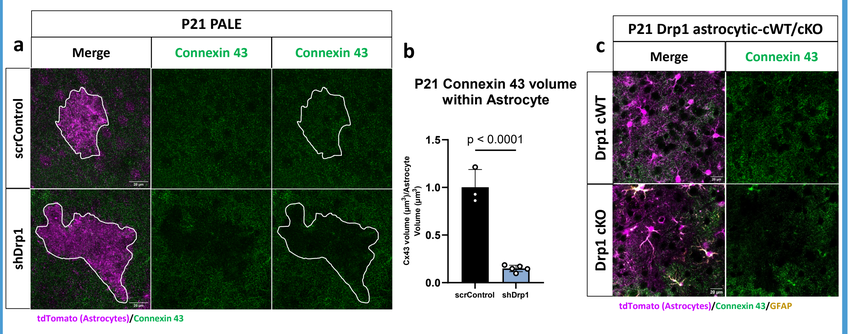
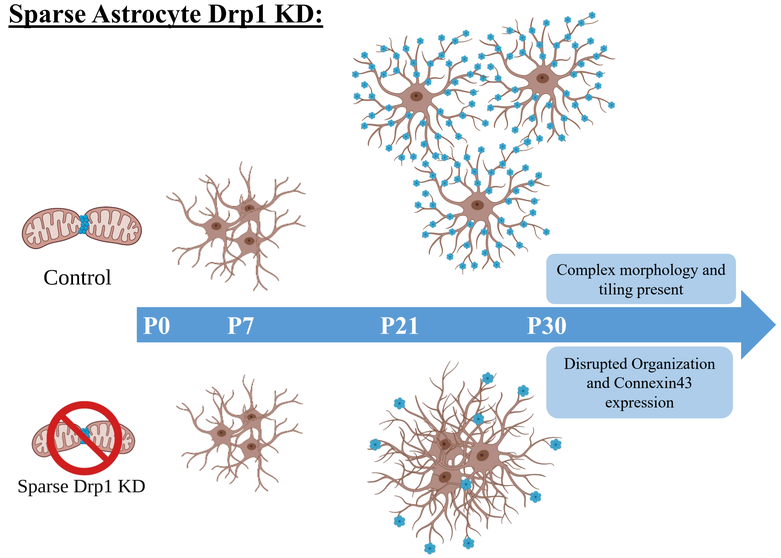


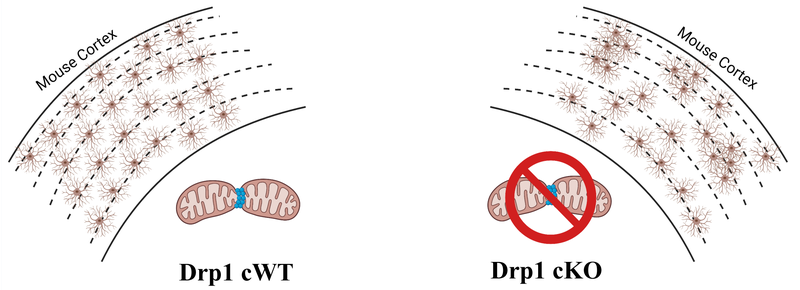
Figure 4. Drp1 knockdown and astrocytic-conditional knockout dysregulate Connexin43 expression. a. Representative images of P21 control (top) and shDrp1 (bottom) visual cortex PALE brains stained with Connexin43 (green). b. Quantification of Connexin43 volume within astrocyte clusters, n=3-5 animals per condition, unpaired t-test. Bars are Mean ± SEM. c. Representative images of P21 visual cortex Aldh1l1-Cre ER^{T2}, Drp1 wild-type (Drp1 cWT, top) and Drp1 floxed/floxed (Drp1 cKO, bottom) astrocytes (magenta), stained with Connexin43 (green) and GFAP (yellow).

Key Findings

Sparse Astrocyte Drp1 KD:



Astrocytic Drp1 cKO:



- Even coverage of astrocytes across the cortex
- Little to no cortical GFAP expression

- Decreased astrocyte coverage
- Increased GFAP expression, disrupted Connexin43 expression

Future Directions

- Does astrocytic cKO of Drp1 impact astrocyte organization in the cortex?
- Do Drp1-deficient astrocytes have decreased synaptogenic ability compared to the wild-type?
- Does the decrease in Connexin 43 in Drp1 KD in astrocytes disrupt astrocyte network communication?

Acknowledgements

- Lindquist Grant Recipient: Fall 2022, UNC-CH Department of Psychology and Neuroscience, OUR Travel Award: UNC-CH Office of Undergraduate Research
- Pia Rodriguez, Mentor
- Dr. Shveta Parekh, Faculty Sponsor/Mentor
- Dr. Cagla Eroglu, Principal Investigator
- Figures: BioRender.com