Distinctive Effects of α-Klotho Isoforms on Dendritic Morphology in Hippocampal Neurons

RUTGERS Aresty Research Center for Undergraduates

Allen Y. Fu, Marina M. Cararo-Lopes, & Dr. Bonnie L. Firestein

Department of Cell Biology and Neuroscience, Rutgers, The State University of New Jersey, 604 Allison Road, Piscataway NJ 08854

Firestein Laboratory

Background

- α-Klotho (α-KL) is a neuroprotective protein that enhances cognitive function, learning, and memory [1,2]
- There is a lack of knowledge regarding how the two isoforms of α-KL, a membrane (m-KL) and a secreted form (s-KL), mediate neuroprotection and cognitive enhancement [2].
- α-Klotho may alter the dendrite, a branch-like structure in neurons responsible for receiving information.
- Alterations in dendritic branching are implicated in numerous disorders involving changes in cognition and memory, including neurodegenerative disorders, ASD, and schizophrenia [3]

Aim

To analyze the effects of m-KL and s-KL overexpression on dendritic morphology of cultured neurons via semi-automated Sholl analysis.

Methods

Neuronal Culture and α-KL overexpression

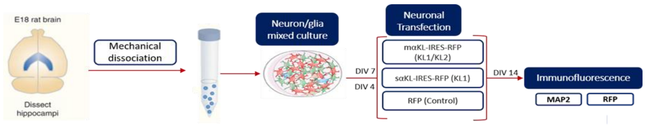

Figure 1. The hippocampi of embryonic day 18 rats were extracted and mechanically dissociated to produce neuronal cultures. These cultures were subsequently transfected with m-KL/s-KL/control plasmids. Immunofluorescence was used to visualize cells on day *in vitro* 14.

Neuronal tracing

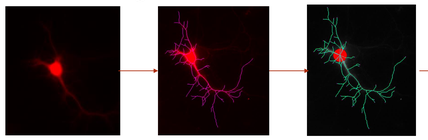

Figure 2. RFP images (example shown above) were loaded and traced by condition-blinded experimenters using ImageJ, finalized in NeuronStudio, and used to generate data in .mat files that could be exported to Excel and analyzed in GraphPad Prism 8.4.3.

Sholl Analysis and dendrite categorization

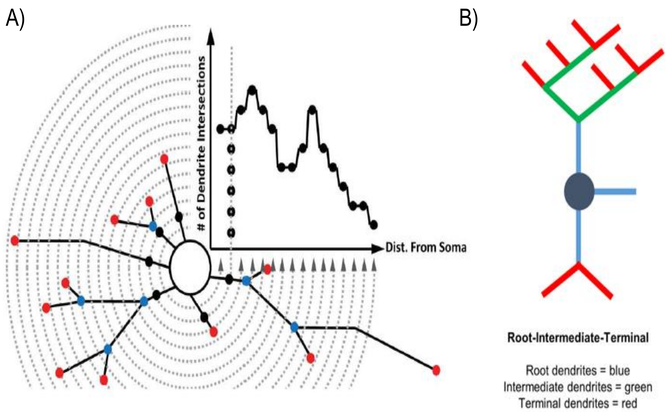

Figure 3. A) Schematics of Sholl analysis, figure from [5]. B) Root-Intermediate-Terminal labeling scheme, figure from [4].

Results

α-KL isoforms differentially affect proximal and distal dendrite branching

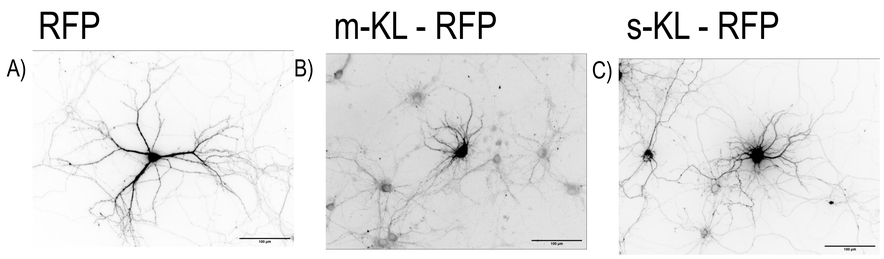

Figure 4. Representative images of cultured hippocampal neurons in the overexpressing RFP(A), m-KL - RFP(B), and s-KL - RFP(C) conditions. 100 µm scale bars shown in the bottom right.

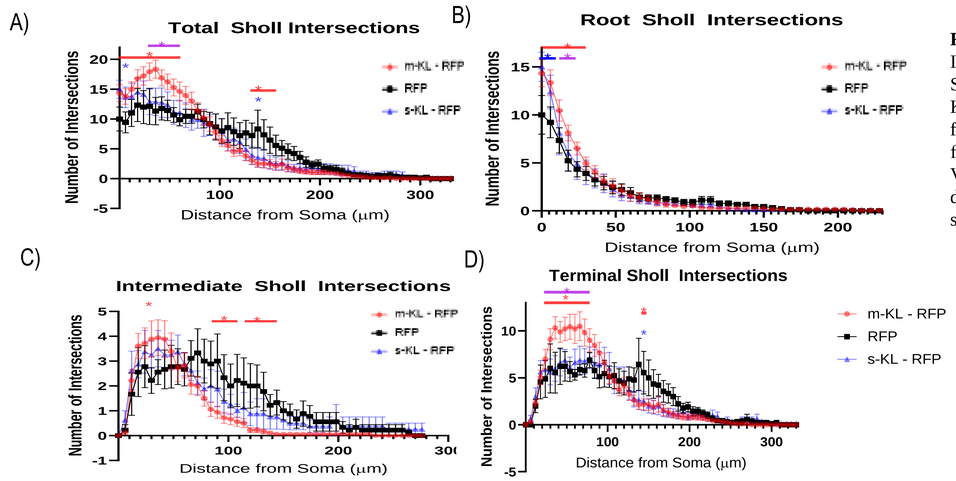

Figure 5. Total(A), Root (B), Intermediate (C) and Terminal (D) Sholl Intersections for m-KL, s-KL, and control groups. * - p<.05 found from two-way ANOVA followed by Tukey's post-test. Violet lines indicate significant differences between the m-KL and s-KL groups. (n=8-18)

α-KL isoforms' effects are independent of dendrite number

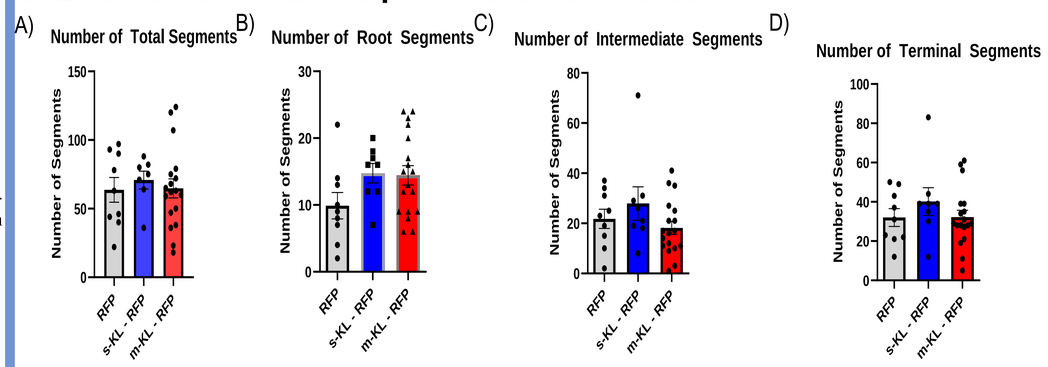

Figure 6. Total(A), Root (B), Intermediate (C) and Terminal (D) segments for all groups. Statistics: one-way ANOVA followed by Tukey's post-test.; n=8-18. Outliers identified and removed using ROUT, Q=1%.

Membrane and secreted klotho have distinct effects on dendritic length

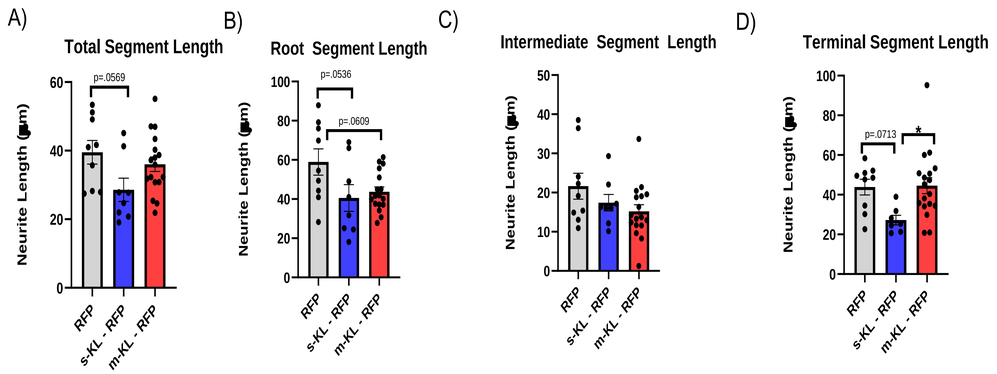

Figure 7. Total, Root, Intermediate, and Terminal Segment Length for all groups. *p<.05 from one-way ANOVA followed by Tukey's post-test. Differences with p<.07 were noted. (n=8-18). Outliers identified and removed using ROUT, Q=1%.

Conclusions

- Overexpression of m-KL and s-KL increasse proximal root Sholl intersections (Figure 5b)
- Dendrites branch off into terminal and intermediate branches earlier in cells overexpressing m-KL (Figures 5c, 5d)
- s-KL overexpression group had significantly shorter terminal segment length and no increase in terminal intersections closer to the soma, unlike m-KL (Figures 5d, 7d)
- **Differences between the m-KL and s-KL overexpression groups suggest they may have distinct mechanisms of controlling neuronal plasticity**
- **α-KL may mediate neuroprotection and the enhancement of cognitive function through modifying the dynamics of dendrite formation.**

Future Directions

- Replicate findings in additional independent experiments
  - Have already been replicated once (DIV 7 neurons)
- Investigate molecular mechanism of changes
- Address the functional implications on animal behavior

Acknowledgements

I would like to sincerely thank Dr. Firestein and Ms. Cararo-Lopes for providing me with the opportunity to perform this research and the guidance I needed to create this presentation.

I'd also like to thank the Aresty Research Center for their generous financial support of our projects, as well as support through our Peer Instructors and community.

These figures made it possible for this project to be presented today.

References

[1] Arking, D.E., Krebsova, A., Macek, M., Sr., Macek, M., Jr., Arking, A., Mian, I.S., Fried, L., Hamosh, A., Dey, S., McIntosh, I., & Dietz, H.C. (2002). Association of human aging with a functional variant of klotho. *Proceedings of the National Academy of Sciences of the United States of America, 99(2)*, 856-861. doi: 10.1073/pnas.022484299

[2] Cararo-Lopes, M.M., Mazucanti, C.H.Y., Scavone, C., Kawamoto, E.M., & Berwick, D.C. (2017). The relevance of α-KLOTHO to the central nervous system: Some key questions. *Ageing Research Reviews, 36*, 137-148. doi: 10.1016/j.arr.2017.03.003.

[3] Kulkarni, V.A., & Firestein, B.L. The dendritic tree and brain disorders. *Molecular and Cellular Neuroscience, 50*(1), 10-20. doi: 10.1016/j.mcn.2012.03.005.

[4] O'Neill, K.M., Akum, B.F., Dhawan, S.T., Kwon, M., Langhammer, C.G., & Firestein, B.L. (2015). Assessing effects on dendritic arborization using novel Sholl analyses. *Frontiers in Cellular Neuroscience, 9*. https://doi.org/10.3389/fncel.2015.00285

[5] Langhammer, C. G., Previtera, M. L., Sweet, E. S., Sran, S. S., Chen, M., & Firestein, B. L. (2010). Automated Sholl analysis of digitized neuronal morphology at multiple scales: whole cell Sholl analysis versus Sholl analysis of arbor subregions. Cytometry Part A, 77(12), 1160-1168.