The Role of NMDA Receptor Activity in Retinal Ganglion Cell Dendrite Development

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Introduction

Background- *Retinal ganglion cell dendrites undergo dramatic structural changes during development as they grow out into the inner plexiform layer, stratify, and form synapses with bipolar and amacrine cells*

Problem- *Conflicting studies cannot agree whether or not retinal ganglion cell dendritic development is activity dependent*

Hypothesis- *Activity-dependent dendritic development depends on ganglion cell subtype. We predict that the refining RGC subtype, JamB, requires glutamatergic activity for dendrite development*

Methods

- *Model: JamB-CreER:YFP mice label OFF DS ganglion cell*
- *Imaging: confocal microscopy of WT and Grin1$^{-/-}$JamB-CreER:YFP retinal whole-mounts*
- *Experimental treatments: 400 uM AP5 and 40 uM CNQX were injected intraocularly to block spontaneous glutamatergic activity before eye-opening. Dark reared mice were housed in a dark box from P6 to P30.*
- *Analysis: Individual neurons were traced using Neurolucida and analyzed with NeuroExplorer to measure dendrite length, protrusion number, and dendritic field area.*

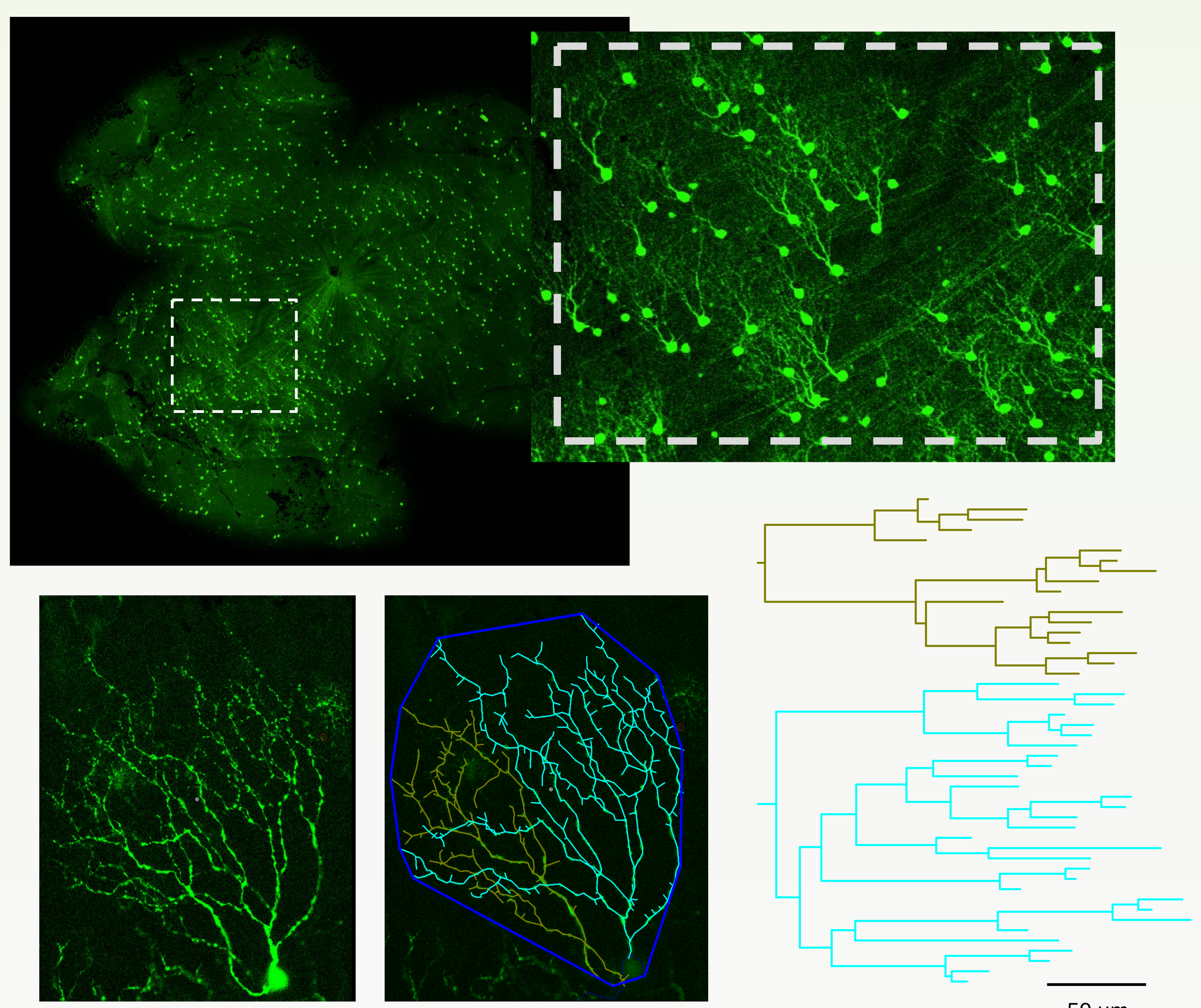

Figure 1. Imaging and dendrite tracing procedure of JamB RGCs. Dendrites are tracing in cyan and olive, dendritic field in blue.

Results

JamB RGC dendritic development occurs in two phases

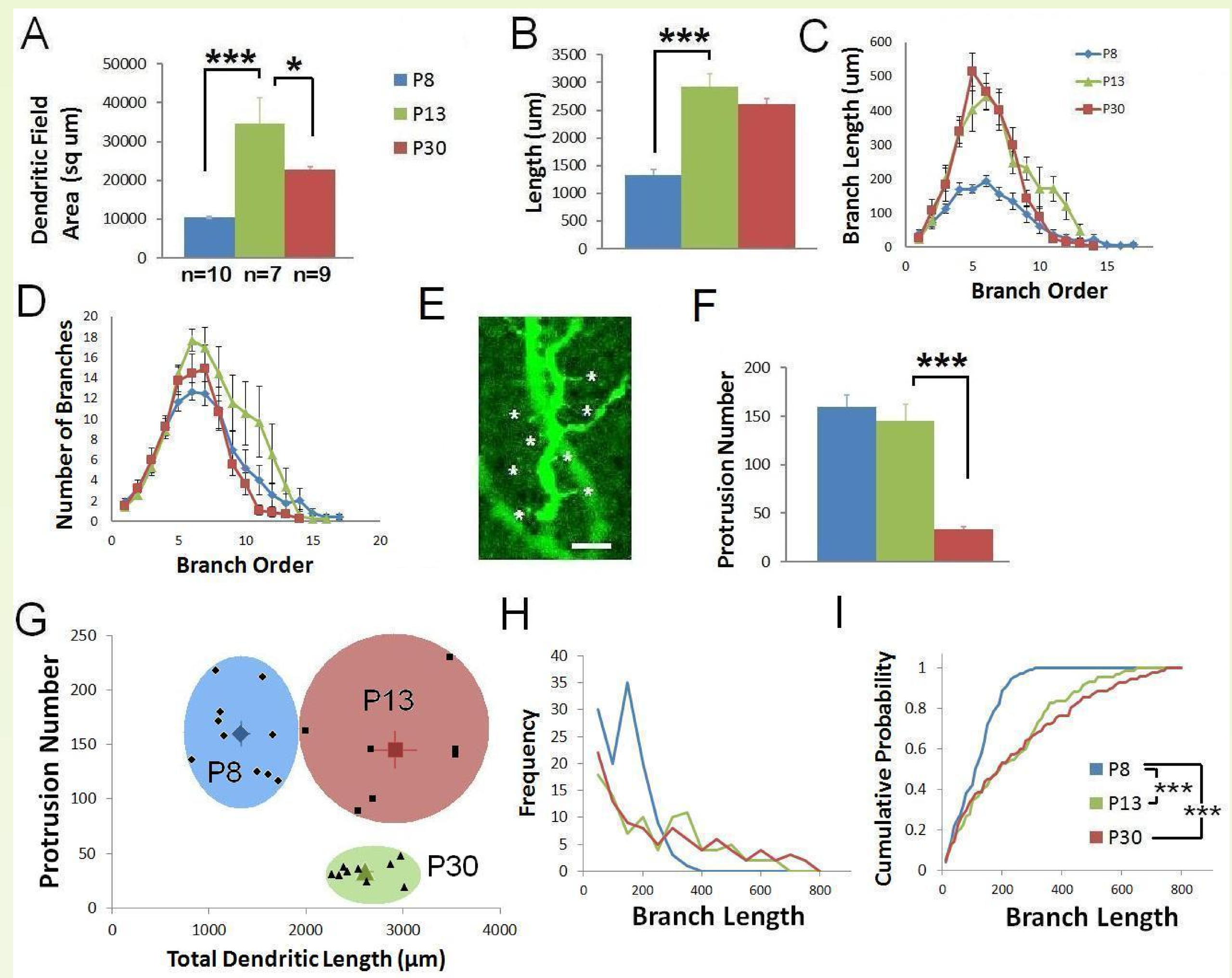

Figure 2. JamB RGC dendrites lengthen and eliminate protrusions during two distinct postnatal time periods. (A) Average dendritic field (DF) area of JamB RGCs at P8, P13, and P30. (B) Average dendritic length of the same three groups of JamB RGCs. (C) Branch length as a function of branch order of the same three groups of JamB RGCs. (D) Number of branches as a function of branch order. (E) Confocal image of a P8 GFP-labeled JamB RGC showing numerous protrusions/filopodia along dendrites. Scale 6.5 um. (F) Number of protrusions per neuron. (G) Scatter plot of protrusion number versus total dendritic length. (H) Histogram of dendritic branch lengths. (I) Branch length cumulative probabilities of the three groups of JamB RGCs. Values are mean ± s.e.m.

Dendritic expansion requires glutamatergic activity

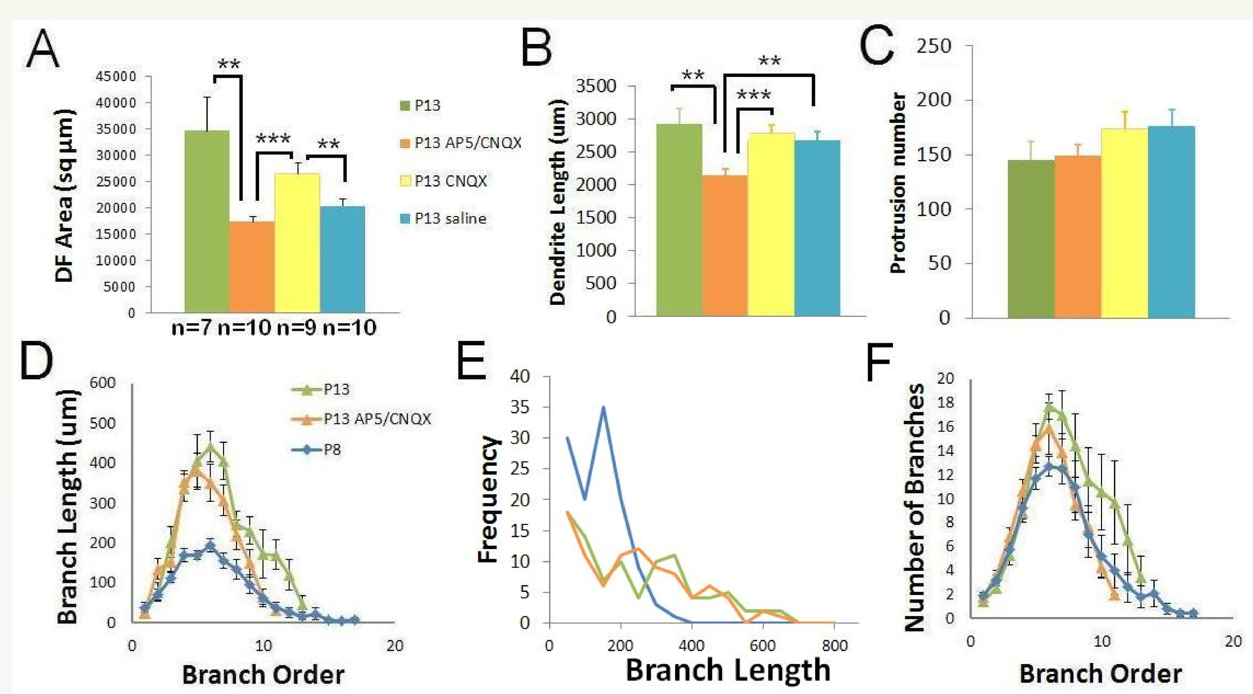

Figure 3. Blockade of synaptic activity mediated by glutamate receptor (GluR) selectively impairs the dendritic elongation of JamB RGCs. (A) Average DF area of P13 JamB RGCs treated and untreated with GluR blockers. (B) Average dendritic length of the same four groups of P13 JamB RGCs. (C) Average number of protrusions per neuron. (D) Branch length as a function of dendrite branch order. (E) Histogram of individual branch lengths. (F) Branch number as a function of branch order.

Results

NMDA receptors drive dendritic expansion

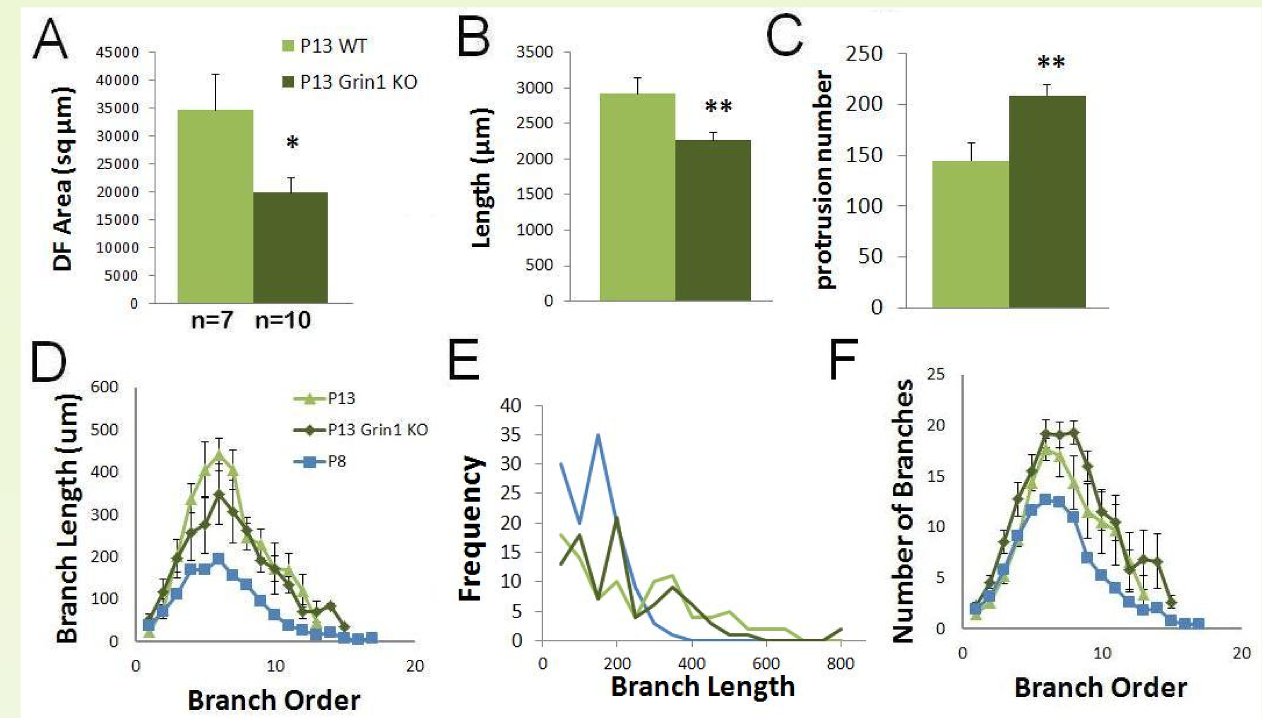

Figure 4. NMDA receptors are required for JamB dendritic expansion. (A) Average DF area of JamB RGCs of wild type (WT) mice and mice with conditional knockout of NR2 expression by JamB RGCs (Grin1 KO) at the age of P13. (B) Average total length of dendrites of WT and Grin1$^{-/-}$ JamB RGCs. (C) Average protrusion number per neuron. (D) Average branch length as a function of branch order. (E) Histogram of individual branch lengths. (F) Branch number as a function of branch order.

Dendritic field consolidation is absent in NMDAR KO JamB RGCs

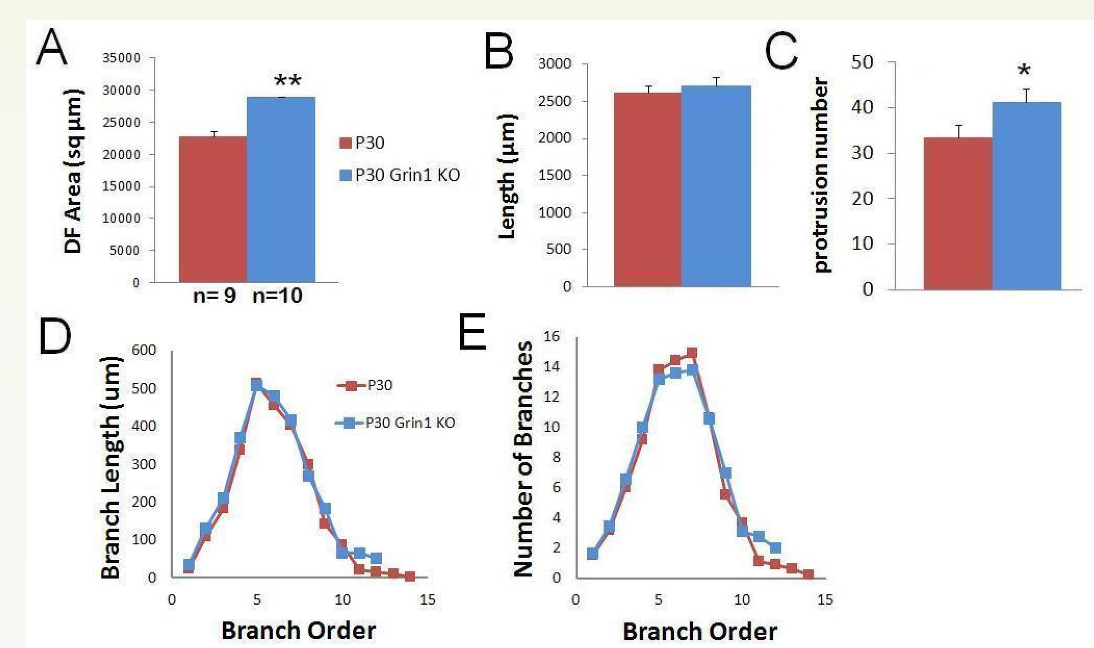

Figure 5. NMDA KO JamB RGCs lack dendritic field consolidation. (A) Average DF area of JamB RGCs of WT and Grin1 KO mice at the age of P30. (B) Average total length of dendrites of the same two groups of JamB RGCs. (C) Average total protrusion number per neuron. (D) Average branch length as a function of branch order. (E) Average number of branches as a function of branch order.

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Visual inputs drive dendritic field consolidation

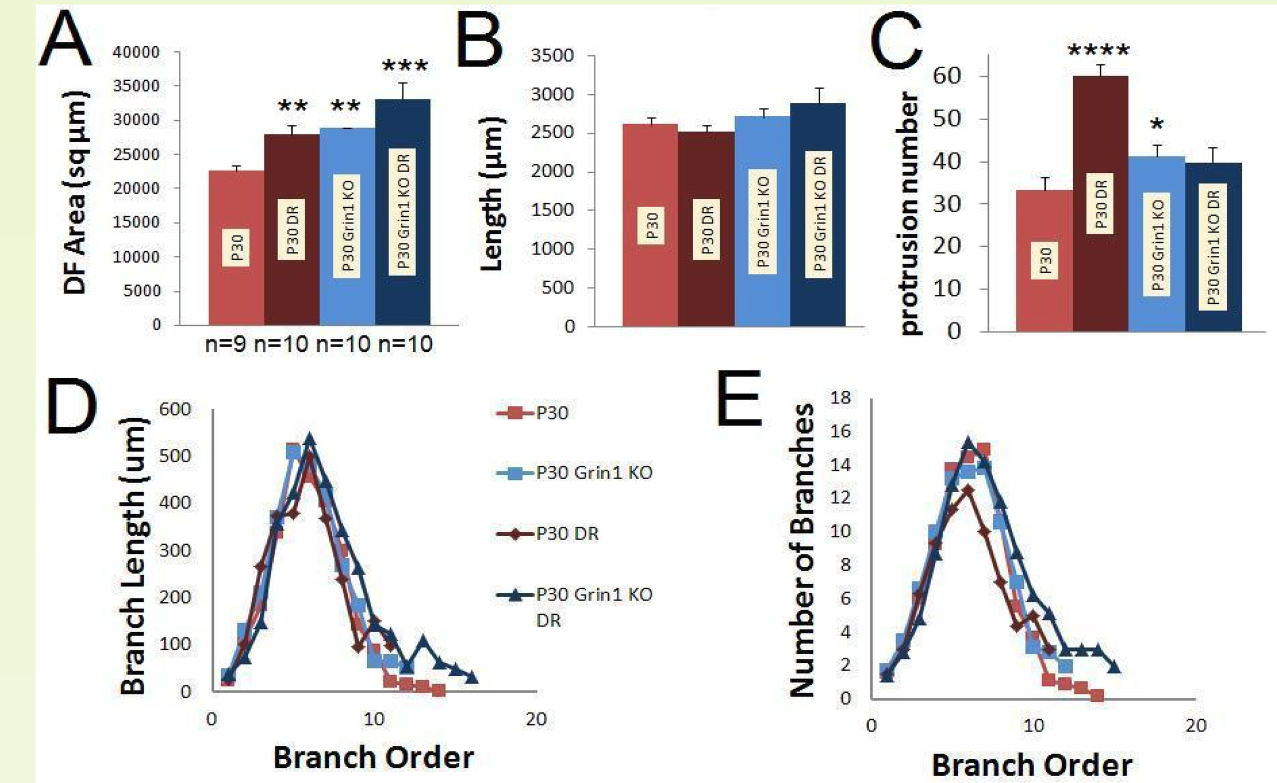

Figure 6. JamB-CreER:YFP and Grin1$^{-/-}$:JamB-CreER:YFP mice were raised in constant darkness from P6 to P30 and the dendritic structure of JamB RGCs of these mice were characterized and compared with the same two strains of mice raised under cyclic light/dark conditions. (A) Average DF area of JamB RGCs of four different groups. (B) Average total dendritic length of the same four groups of RGCs. (C) Number of protrusions per neuron. (D) Branch length as a function of branch order. (E) Number of branches as a function of branch order. Asterisks are comparison to P30 control.

Summary

- *JamB RGC dendritic development occurs in two phases, (1) dendrite elongation and field expansion and (2) elimination of dendritic protrusions and DF consolidation*
- *Dendritic elongation requires glutamatergic activity in the retina*
- *NMDA receptors on JamB RGCs are required for protrusion elimination, dendrite elongation, and DF expansion before eye-opening*
- *NMDA receptors may be required for DF consolidation*
- *Visual input drives DF consolidation*

References

- Kim, I.-J., Zhang, Y., Yamagata, M., Meister, M., & Sanes, J. R. (2008). Molecular identification of a retinal cell type that responds to upward motion. *Nature*, *452*(7186), 478–82
- Wong, R. O. L., Yamawaki, R. M., & Shatz, C. J. (1992). Synaptic Contacts and the Transient Dendritic Spines of Developing Retinal Ganglion Cells. *The European Journal of Neuroscience*, *4*(12), 1387–1397
- Xu, H., Chen, H., Ding, Q., Xie, Z.-H., Chen, L., Diao, L., … Tian, N. (2010). The immune protein CD3zeta is required for normal development of neural circuits in the retina. *Neuron*, *65*(4), 503–15
- Xu, H., Sun, J.H., & Tian, N. (2014) A General Principle Governs Vision-Dependent Dendritic Patterning of Retinal Ganglion Cells. *Journal of Comparative Neurology.* In press.