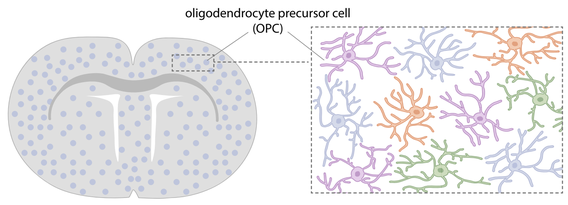
Oligodendrocyte precursor cells use an activity dependent feedback mechanism to stabilise developing retinal ganglion cells

Emma Dumble, Denis Yuan, Yan Xiao and Tim Czopka
Centre for Clinical Brain Sciences, University of Edinburgh

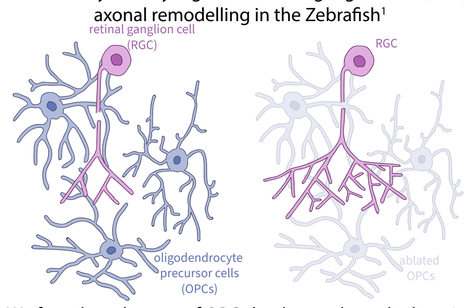
Background

Oligodendrocyte precursor cells (OPCs) tile the CNS with their elaborate process networks, and persist throughout life



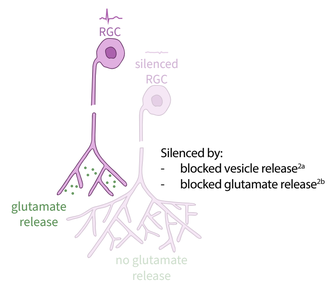
Making us wonder what they do beyond forming myelinating oligodendrocytes...

Our recent research showed that OPCs play a role in sculpting the visual system by regulated retinal ganglion cell (RGC) axonal remodelling in the Zebrafish[1]



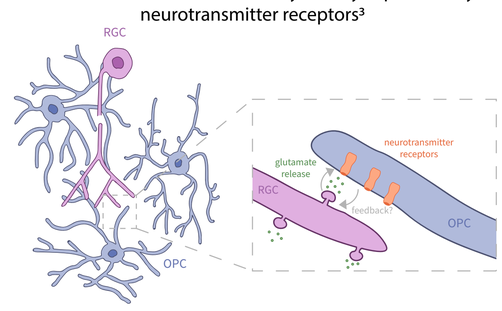
We found an absence of OPCs leads to enlarged arbor size and degraded visual processing!

RGC arbor development is an activity dependent process, where silencing a single RGC leads to it being enlarged![2]



Is this a neuron autonomous mechanism, or are OPCs involved?

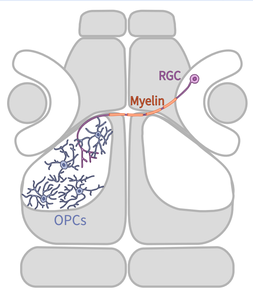
OPCs can sense neuronal activity as they express many neurotransmitter receptors[3]



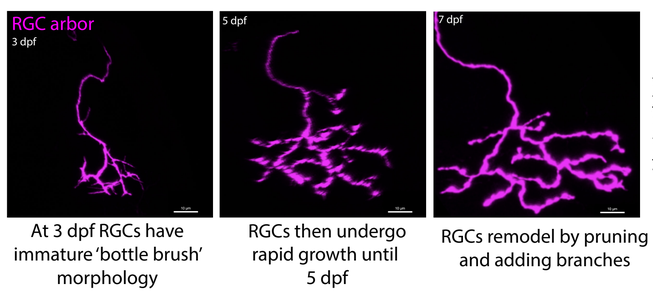
Do OPCs receive activity-dependent glutamatergic signals from RGCs? And do OPCs, in turn, modulate RGC development?

Studying and manipulating OPCs in zebrafish visual system

Zebrafish visual circuitry



RGC arbor development

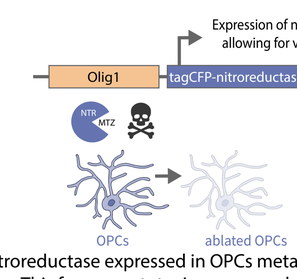


At 3 dpf RGCs have immature 'bottle brush' morphology

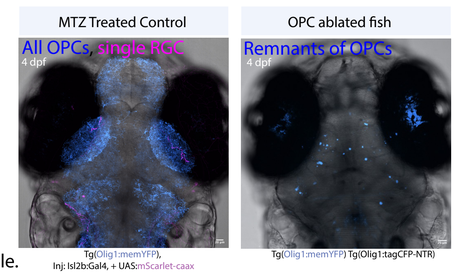
RGCs then undergo rapid growth until 5 dpf

RGCs remodel by pruning and adding branches

NTR-MTZ system robustly ablates OPCs



Nitroreductase expressed in OPCs metabolises metranidozole. This forms a cytotoxic compound causing cell death

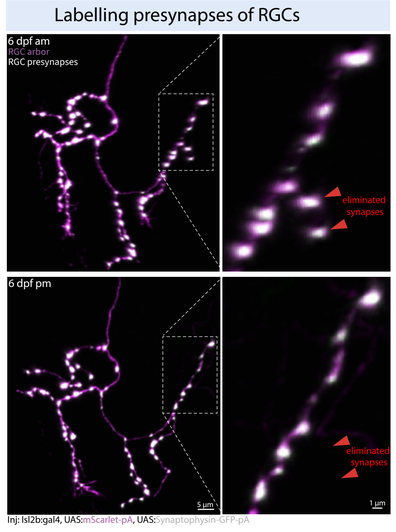


Tg(Olig1:memYFP), Inj: Isl2b:Gal4, + UAS:mScarlet-caax

Tg(Olig1:memYFP) Tg(Olig1:tagCFP-NTR)

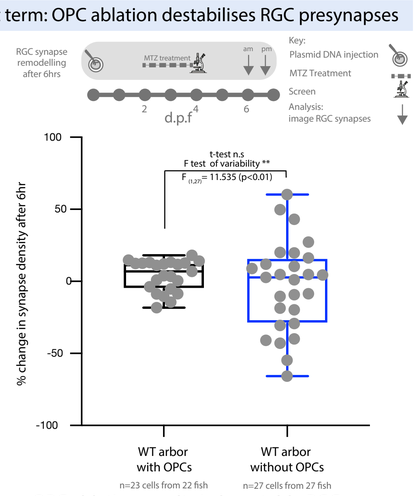
OPCs stablises RGC presynapses

Labelling presynapses of RGCs



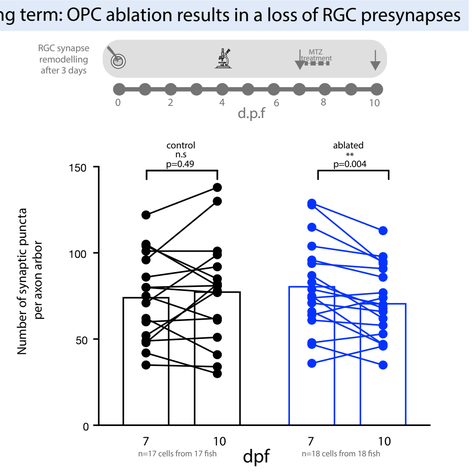
Inj: Isl2b:gal4, UAS:mScarlet-pA, UAS:Synaptophysin-GFP-pA

Short term: OPC ablation destabilises RGC presynapses



OPC ablation results in less stable RGC synapses

Long term: OPC ablation results in a loss of RGC presynapses

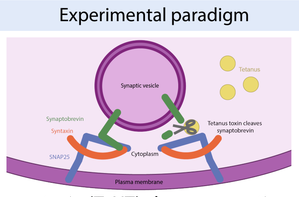


Is the loss of RGC presynapses after OPC ablation due to a loss of axon:OPC synapses?

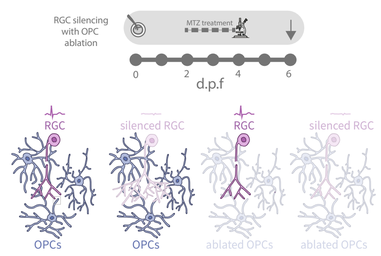
How are OPCs feeding back this synapse stabilising signal?

OPC ablation rescues enlarged silenced RGCs

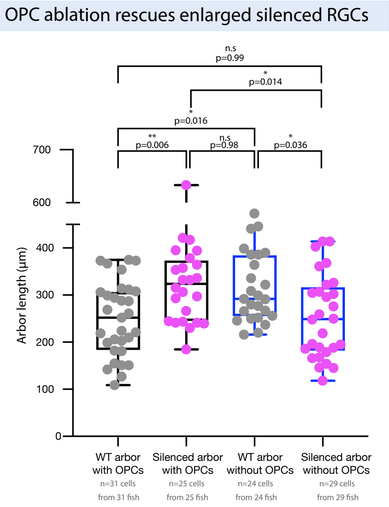
Experimental paradigm



Tetanus toxin (TeNT) cleaves synaptic vesicle membrane proteins, inhibiting neurosecretion.



OPC ablation rescues enlarged silenced RGCs

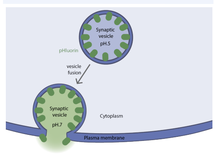


Does this activity dependent feedback by OPCs affect RGC presynapses? How do OPCs feedback to RGCs?

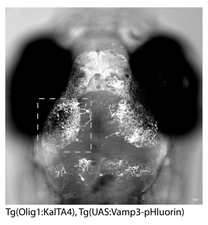
OPC exocytosis is dependent on neural activity

Visualising OPC exocytosis using pHluorins

pHfluorins labelling

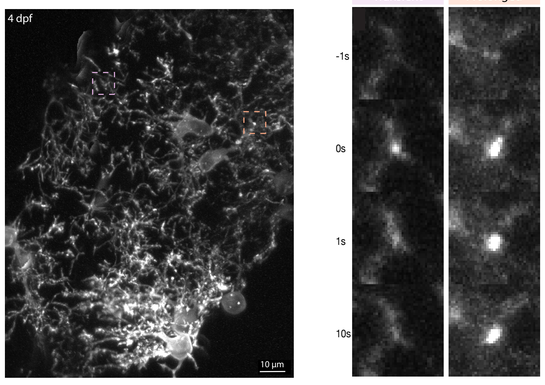


VAMP fused with pH sensitive pHluorins enables imaging of exocytosis in real-time.



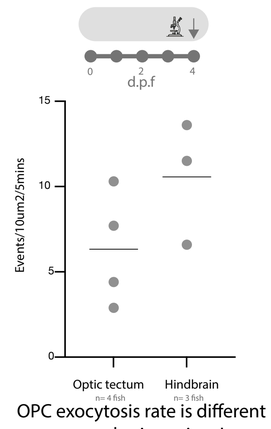
Tg(Olig1:KalTA4), Tg(UAS:Vamp3-pHluorin)

Detecting exocytosis events in OPCs



What triggers OPC exocytosis?

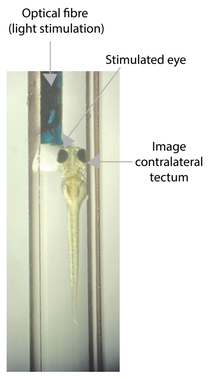
OPC exocytosis across the brain



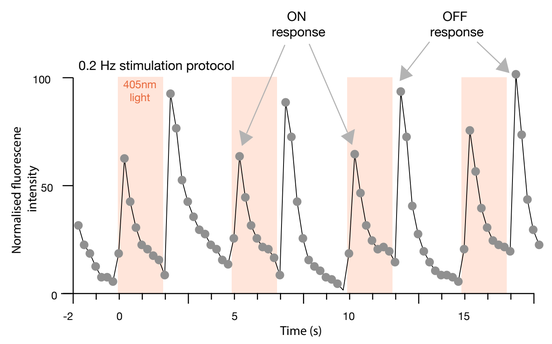
OPC exocytosis rate is different across brain regions!

OPC exocytosis is regulated by RGC activity

Light stimulation setup

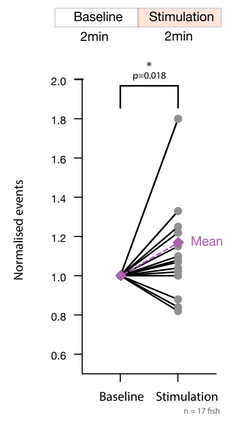


Visual circuitry Ca^{2+} response to light stimulation



0.2Hz light stimulation reliably invokes on/off Ca^{2+} responses in RGCs

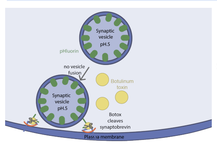
OPC exocytosis increases with visual stimulation



Is OPC acitivty dependent exocytosis how OPCs feedback and modulate RGC development?

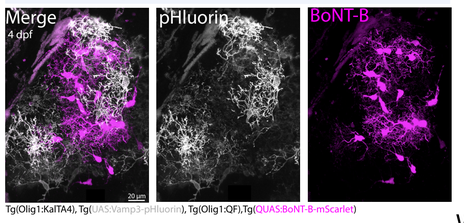
OPCs feedback to RGC via exocytosis a synapse stabilising signal

OPC BoNT-B expression eliminates exocytosis

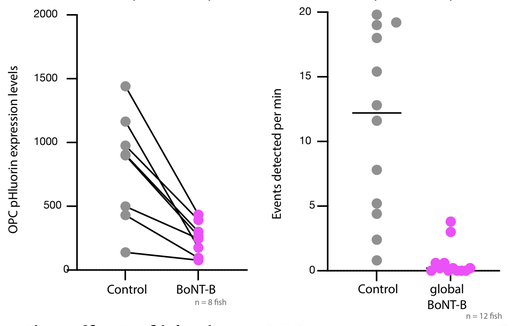


Botulinum toxin (BoNT-B) cleaved synaptic vesicle proteins, inhibiting exocytosis!

Transgenic line with OPCs expressing BoNT-B

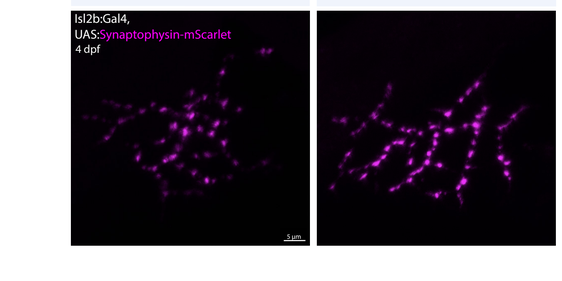


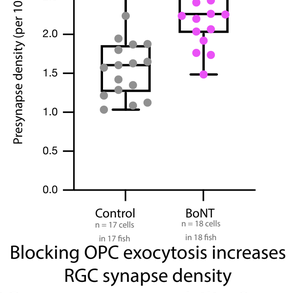
Tg(Olig1:KalTA4), Tg(UAS:Vamp3-pHluorin), Tg(Olig1:QF),Tg(QUAS:BoNT-B-mScarlet)



What is the effect of blocking OPC exocytosis on RGC synapses?

OPC exocytosis regulates RGC presynapse stability



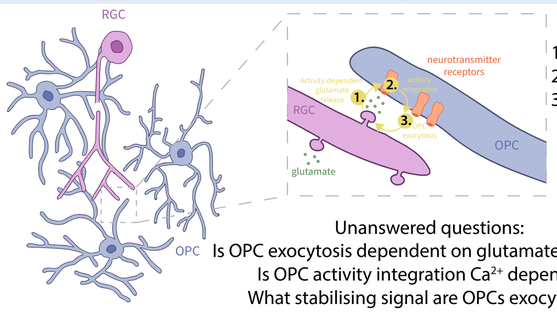


Blocking OPC exocytosis increases RGC synapse density

What do OPCs exocytose that modulates RGC presynapse stability?

Conclusion/Future directions

Working Model

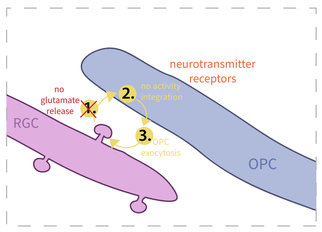


1. Active RGCs release glutamate
2. OPCs sense glutamate
3. This triggers OPCs to exocytose a synapse stabilising signal

Unanswered questions:
Is OPC exocytosis dependent on glutamate signalling?
Is OPC activity integration Ca^{2+} dependent?
What stabilising signal are OPCs exocytosing?

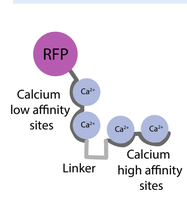
Is OPC stabilisation of RGC presynapses dependent on glutamate release?

Perform CRISPR/Cas9 mediated KD of VGlut1

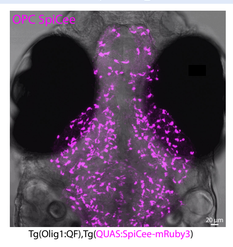


Does a KO of VGlut1 (necessary component for glutamatergic signalling) also rescue RGC arbor length in OPC ablation?

Is OPC activity integration Ca^{2+} dependent?

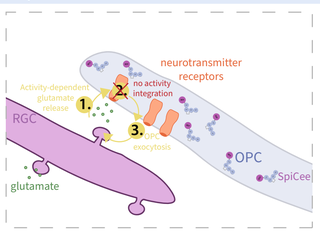


SpiCee (a calcium chelator) dampens Ca^{2+} activity



Tg(Olig1:QF),Tg(QUAS:SpiCee-mRuby3)

Transgenic line with OPCs expressing SpiCee



Does dampening OPC Ca^{2+} modulate RGC presynpase stability?

References/Acknowledgements

1.Xiao, Y., Petrucco, L., Hoodless, L.J., Portugues, R., Czopka, T., 2022. Oligodendrocyte precursor cells sculpt the visual system by regulating axonal remodeling. Nat Neurosci 25, 280–284
2.a. Fredj, N. B., Hammond, S., Otsuna, H., Chien, C.-B., Burrone, J., & Meyer, M. P. (2010). Synaptic Activity and Activity-Dependent Competition Regulates Axon Arbor Maturation, Growth Arrest, and Territory in the Retinotectal Projection. Journal of Neuroscience, 30(32), 10939–10951.
2b. Smear, M. C., Tao, H. W., Staub, W., Orger, M. B., Gosse, N. J., Liu, Y., Takahashi, K., Poo, M., & Baier, H. (2007). Vesicular Glutamate Transport at a Central Synapse Limits the Acuity of Visual Perception in Zebrafish. Neuron, 53(1), 65–77.
3. Bergles, D. E., Roberts, J. D., Somogyi, P., & Jahr, C. E. (2000). Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature, 405(6783), 187–191.

Thanks to the Aquatics team, Bioresearch and Veterinary Services, University of Edinburgh!