Developmentally-Relevant Optogenetic Control of Synaptic Pruning via Light-Cleavable Hevin

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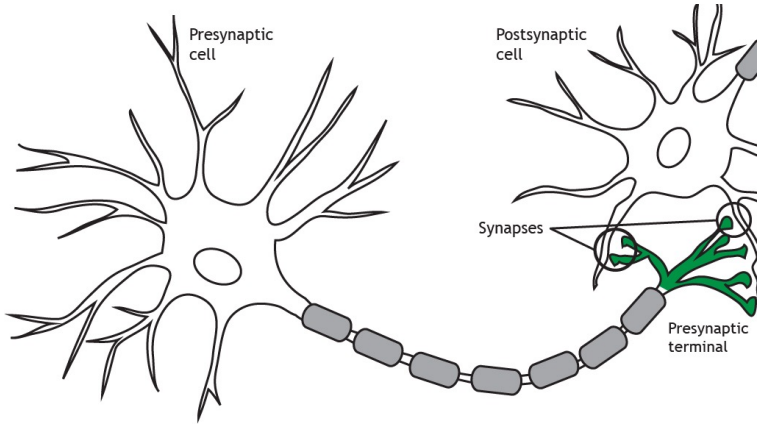
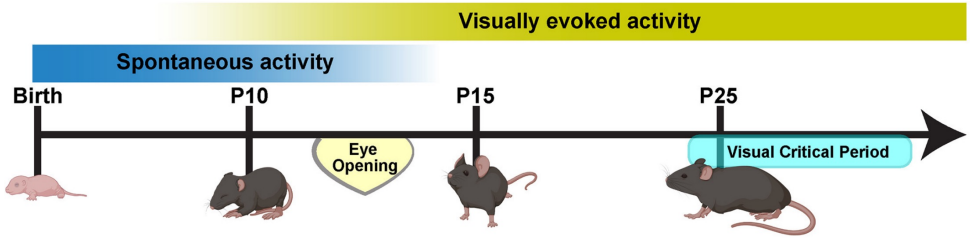
Background & Significance

Understanding how neurons develop early in an organism's life is vital; however, there is no easy toolkit to do so.



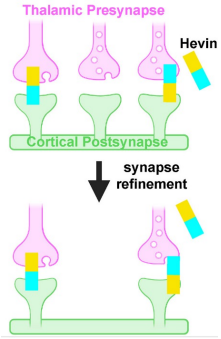
1) *Neurons carry signals in the brain. Synapses pass signal from the pre-synaptic neuron to the post-synaptic neuron. The* ***number of synapses between neurons*** *is quite important for neural signaling. Figure from Singh, 2024.*



2) *Much like in humans, synapses form in mice during their developmental period (above). The brain initially over-creates synaptic connections, then coordinates destruction of the excess. This process of synaptic destruction is linked to* ***autism spectrum disorder*** *and* ***schizophrenia****.*

The cleavage itself is coordinated between two non-neuron cells in the brain – astrocytes and microglia (below). How exactly the astrocytes and microglia coordinated this process was unknown until recently.

Figure above from Ramirez et al., 2026; figure below from Liu et al., 2020.



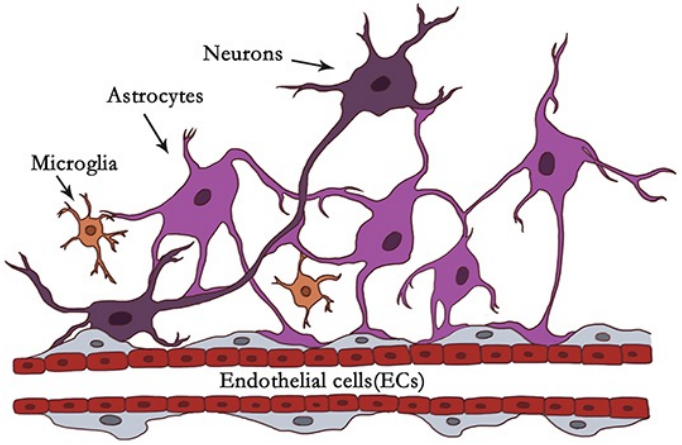
3) *Ramirez et al. show that Hevin, or SPARC-like 1,* ***both helps refine and destroy thalamocortical synapses*** *in its whole and cleaved form, respectively. This is a unique behavior that lends itself to further study.*

The thalamocortex system is the ***primary sensory relay circuit*** *through which sensory information is transmitted to the cerebral cortex.*

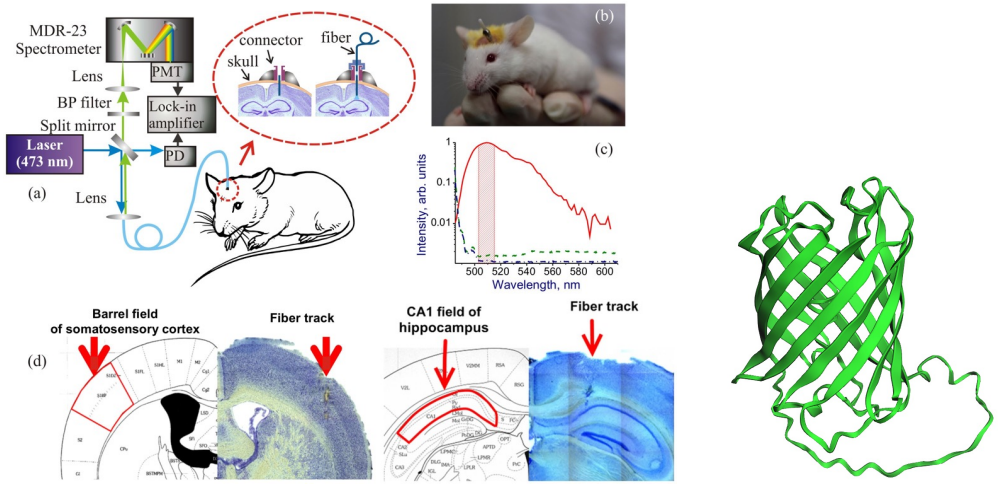
Figure from Ramirez et al., 2026.

Interrogation or utilization of the Hevin synapse assembly/destruction system would advance neurodevelopmental research.

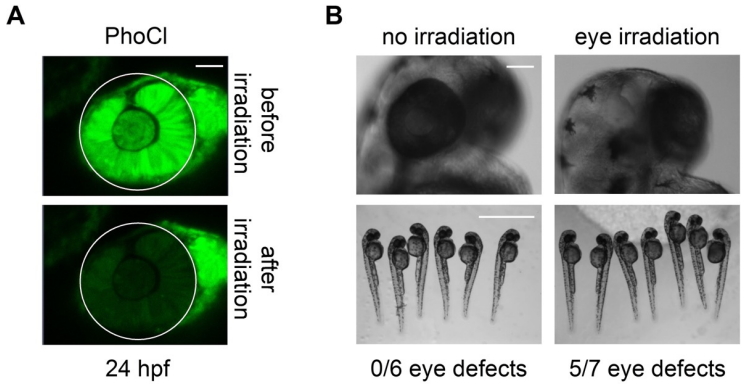
However, we cannot do this using current methods, and at the granular scale we would like.

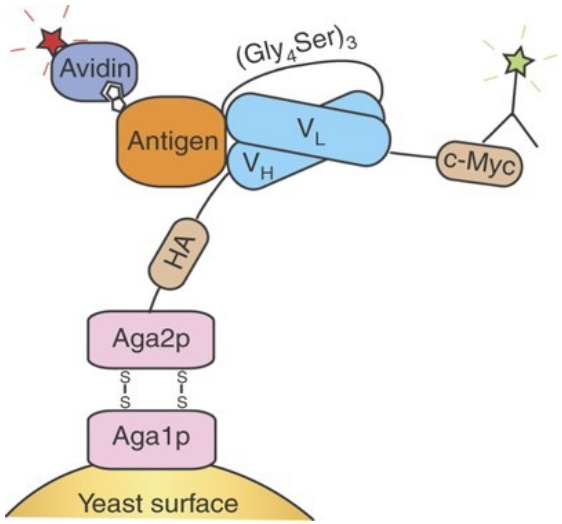


Prior Work



Optogenetics are a viable, widely-used method for in vivo *manipulation. Placing a fiber optic probe into a mouse brain is relatively routine (left; Doronina-Amitonova et al., 2013).*
The PhoCl2f protein (right; sequence from Lu et al., 2021) has been used before in studies of zebrafish development (below; Brown et al., 2022) via rational design.





Yeast surface display is a widely-used method of generating binders, often using fluorescence-activated cell sorting (FACS). Figure from Chao et al., 2006.

Innovation

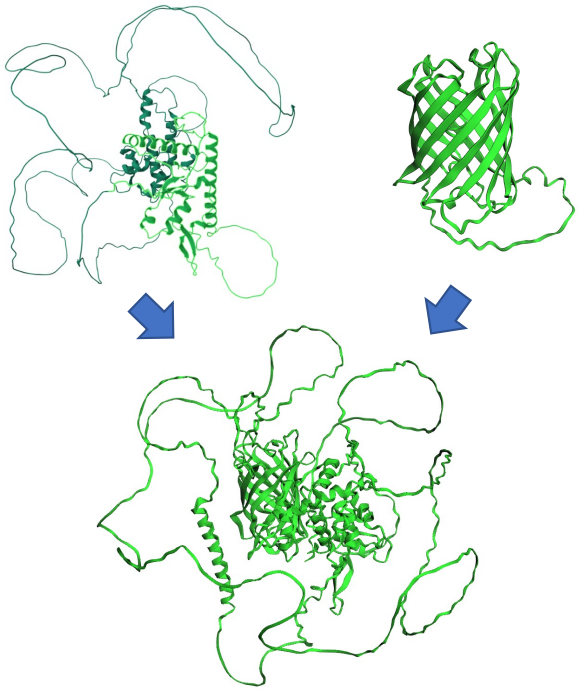
Can we place the Hevin system under optogenetic control?

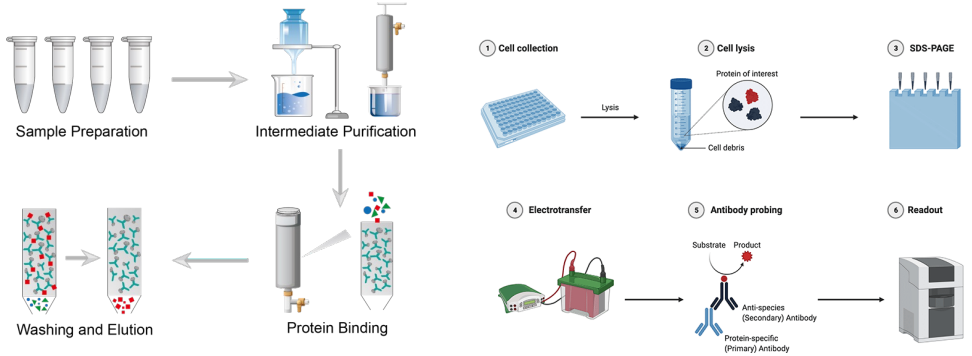
- First optogenetic tool for *in vivo* synaptogenic control via Hevin.
- *Why?* Allows for granular control of where and when synapses cleave, rather than full-thalamocortex synapse cleavage or expression.

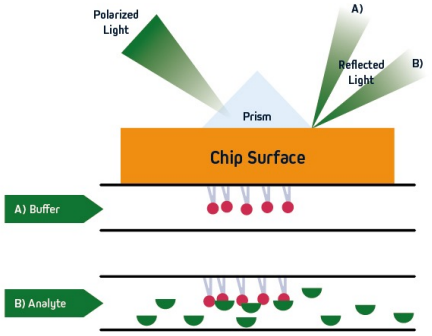
- First engineered variant of Hevin with increased TLR4 binding affinity.
- *Why?* Ensures that more cleaved Hevin reaches the signaling protein required to enable synapse cleavage.

Aim 1: Make Hevin Photocleavable

By making Hevin cleavable optogenetically rather than via a protease, we can control when and where Hevin cleaves, bringing this tool under our control for the first time.







We combine the wild-type Hevin protein (top left; sequence from UniProt) with the PhoCl2f protein (top right, sequence from Lu et al., 2021) which is cleaved optogenetically; this forms the structure seen on the bottom, which is hypothetically photocleavable and retains activity due to its specific location of insertion.

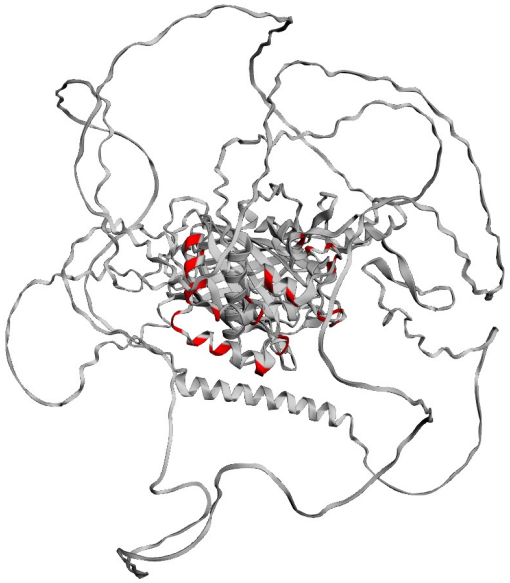
***1)** Protein production will occur in HEK293 cells; purification will be facilitated by an N-terminal FLAG tag (figure from Creative Biolabs). **2)** Western blot will be used to examine production against wild-type Hevin. Western blot will additionally be used to confirm photocleavage in 405nm-exposed construct (Figure from Rockland Immunochemicals). **3)** Surface plasmon resonance will be used to confirm synaptogenic function conservation (figure from Jackson ImmunoResearch).*

Aim 2: Increase Hevin-TLR4 Binding

Increasing Hevin's binding to its signal molecule will help offset the non-100% efficiency of PhoCl2f (88%).

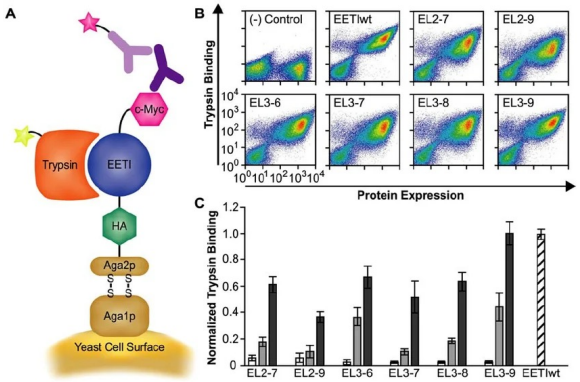
Residues 792-906: Red = Top 25% SASA Exposure

NEKQRNKVKKIYLDEKRLLAGDHPIDLLLRDFKKNYHMYVYPVHWQFSELDQHPMDRVLTHSELAPLRASLVPMEHCITRFFEECDPNKDKHITLKEWGHCFGIKEEDIDENLLF



index	id	name
0	793	GLU
1	794	LYS
2	800	LYS
3	801	LYS
4	804	LEU
5	806	GLU
6	807	LYS
7	810	LEU
8	813	ASP
9	817	ASP
10	821	ARG
11	824	LYS
12	825	LYS
13	828	HIS
14	844	HIS
15	846	MET
16	848	ARG
17	852	HIS
18	865	MET
19	874	GLU
20	878	PRO
21	880	LYS
22	882	LYS
23	887	LYS
24	891	HIS
25	896	LYS
26	898	GLU
27	902	GLU
28	903	ASN

Solvent exposure analysis was used to identify free residues for site-saturation mutagenesis. 29 residues within the non-synaptogenic region were selected.



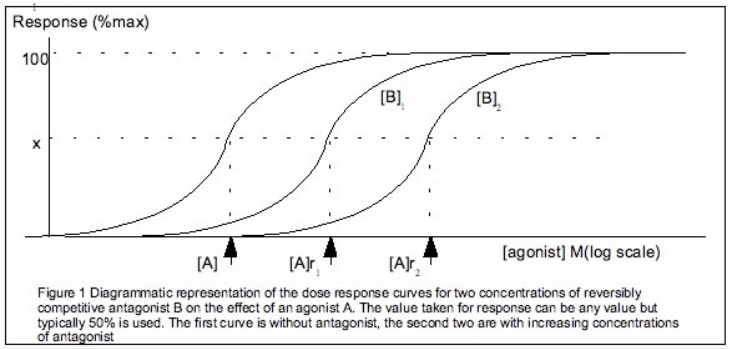
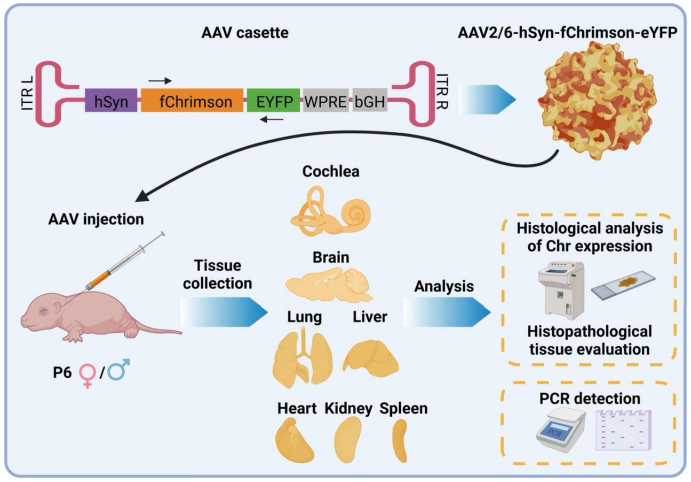


Figure 1 Diagrammatic representation of the dose response curves for two concentrations of reversibly competitive antagonist B on the effect of an agonist A. The value taken for response can be any value but typically 50% is used. The first curve is without antagonist, the second two are with increasing concentrations of antagonist

1) *Yeast display and FACS will be used to screen for variants with higher binding affinity to fluorophore-conjugated TLR4 (Figure from Lahti et al., 2009).* ***2)*** *Serial dilution ELISA will be used to differentiate highest-performing mutants (figure from Wikipedia).* ***3)*** *Protein production, photocleavage, and synaptogenic function will all be tested as described in* Aim 1.

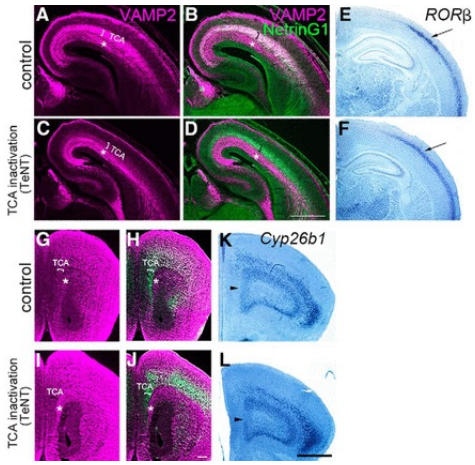
Aim 3: Confirm *In Vivo* Function

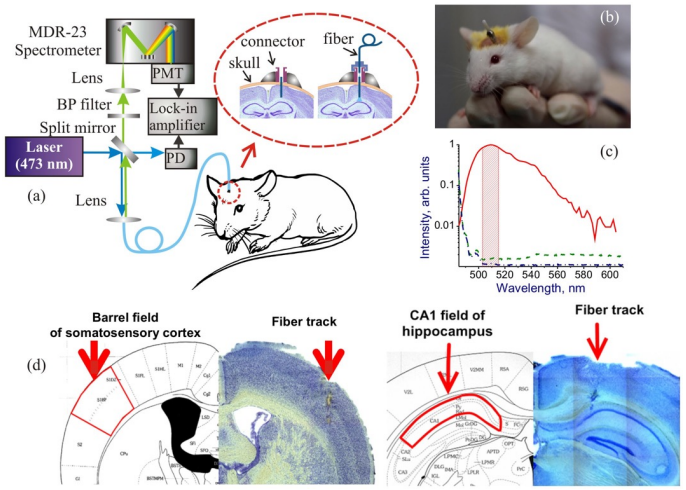
Our system is not perfect if it does not work in vivo.



1) *We deliver the modified construct from* Aim 2 *via an adeno-associated virus (AAV). This is a well-studied delivery method. Figure from Zhou et al., 2022.*

2) *We examine expression of the construct by harvesting mouse brain tissue. Staining and fluorescence microscopy allow for confirmation of expression. Figure from Larsen et al., 2019.*





3) *We examine cleavage* in vivo *by delivering 405nm light to the thalamocortex and comparing the number of thalamocortical synapses to wild-type via microscopy. Figure from Doronina-Amitonova et al., 2013.*

References

Ramirez, J. J., Hardin, E. J., Sakers, K., Kim, J., Colón Ortiz, C., Savage, J. T., Hanamsagar, R., Block, C. L., Singh, S. K., Bilbo, S. D., & Eroglu, C. (2026). Astrocyte-microglia crosstalk through Hevin and Toll-like receptor signaling controls developmental thalamocortical synapse refinement. Neuron, 114(8), 1433–1453.e11. https://doi.org/10.1016/j.neuron.2025.12.028

Brown, W., Albright, S., Tsang, M., & Deiters, A. (2022). Optogenetic protein cleavage in zebrafish embryos. ChemBioChem, 23(23), e202200297.

Chao, G., Lau, W. L., Hackel, B. J., Sazinsky, S. L., Lippow, S. M., & Wittrup, K. D. (2006). Isolating and engineering human antibodies using yeast surface display. Nature Protocols, 1(2), 755–768. https://doi.org/10.1038/nprot.2006.94

Lu, X., Wen, Y., Zhang, S., Zhang, W., Chen, Y., Shen, Y., Lemieux, M. J., & Campbell, R. E. (2021). Photocleavable proteins that undergo fast and efficient dissociation. Chemical Science, 12, 9658–9672. https://doi.org/10.1039/D1SC01059J

UniProt Consortium. (2024). SPARCL1 · Q14515 · SPARCL1_HUMAN. UniProt. Retrieved 5 May 2026, from https://www.uniprot.org/uniprotkb/Q14515/entry

Singh, A. (2024). Synapse structure. In Introduction to Neurobiology. University of Oregon Libraries. https://opentext.uoregon.edu/neurobiology/chapter/synapse-structure/

Liu, L.-R., Liu, J.-C., Bao, J.-S., Bai, Q.-Q., & Wang, G.-Q. (2020). Interaction of microglia and astrocytes in the neurovascular unit. Frontiers in Immunology, 11, 1024. https://doi.org/10.3389/fimmu.2020.01024

Creative BioLabs. (2026). Protein purification protocol & troubleshooting guide. Creative BioLabs. https://www.creativebiolabs.net/protein-purification.htm

Rockland Immunochemicals. (n.d.). Western blotting technique. Rockland. https://www.rockland.com/resources/western-blotting-technique/

Mason, E. (2023, March 29). An introduction to surface plasmon resonance. Jackson ImmunoResearch. https://www.jacksonimmuno.com/secondary-antibody-resource/immuno-techniques/an-introduction-to-surface-plasmon-resonance/

Lahti, J. L., Silverman, A. P., & Cochran, J. R. (2009). Interrogating and predicting tolerated sequence diversity in protein folds: Application to E. elaterium trypsin inhibitor-II cystine-knot miniprotein. PLOS Computational Biology, 5(9), e1000499. https://doi.org/10.1371/journal.pcbi.1000499

EC50. (n.d.). In Wikipedia. https://en.wikipedia.org/wiki/EC50

Zhou, K., Han, J., Wang, Y., Zhang, Y., & Zhu, C. (2022). Routes of administration for adeno-associated viruses carrying gene therapies for brain diseases. Frontiers in Molecular Neuroscience, 15, 988914. https://doi.org/10.3389/fnmol.2022.988914

Larsen, R., Proue, A., Scott, E. P., Christiansen, M., & Nakagawa, Y. (2019). The thalamus regulates retinoic acid signaling and development of parvalbumin interneurons in postnatal mouse prefrontal cortex. eNeuro, 6(1), ENEURO.0018-19.2019. https://doi.org/10.1523/ENEURO.0018-19.2019

Doronina-Amitonova, L. V., Fedotov, I. V., Ivashkina, O. I., Zots, M. A., Fedotov, A. B., Anokhin, K. V., & Zheltikov, A. M. (2013). Implantable fiber-optic interface for parallel multisite long-term optical dynamic brain interrogation in freely moving mice. Scientific Reports, 3, 3265. https://doi.org/10.1038/srep03265