Quantification of Synapse Number for Identification of Molecules Influencing Cholinergic Synapse Formation in *Drosophila*

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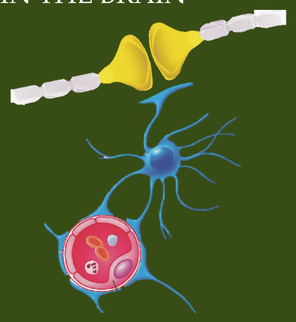
ABSTRACT

Synapses are chemical junctions between neurons that allow signals to be transmitted from one neuron to another. Although disruptions to synapse structure and function contribute to symptoms of most neurological disorders, not much is known about the molecular mechanisms that are responsible for synapse formation and maintenance. Glial cells are a group of non-neuronal cells in the nervous system known for protecting neurons and mediating neuronal function. Astrocytes are glial cells that secrete synaptogenic compounds required for synapse formation. Here, we combine the reverse genetic technique of RNAi and light microscopy to identify novel secreted and cell surface molecules from astrocytes that influence understudied cholinergic synapses in *Drosophila melanogaster*. I studied two established techniques for labeling the active-zone protein Bruchpilot (Brp) in cholinergic dorsal bipolar dendritic (Dbd) neurons to quantify synapses: 1) Brp-short and 2) Synaptic tagging with recombination (STaR). I used light microscopy to quantify Dbd-synapse number at three larval (L) stages: L1, L2, and L3. We will use this information to identify novel regulators of synapse development by performing an astrocyte-specific RNAi screen choosing genes that are predicted as cell surface or secreted, are highly conserved in humans, and are highly expressed by astrocytes. This screen will allow us to identify new genes that instruct synapse formation and maintenance that could ultimately contribute to the establishment of therapies for neurological disorders.

INTRODUCTION

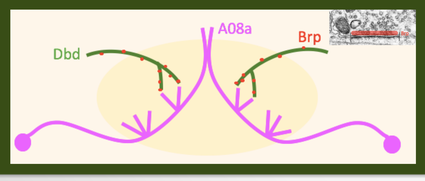
NEURON GLIA INTERACTIONS IN THE BRAIN

- Neurological disorders are prevalent, and are associated with disruptions to synapse formation.[1,2]
- Glial cells are non-neuronal cells in the nervous system that help to mediate neuronal function.
- Astrocytes are glial cells that regulate secretion of synaptogenic compounds.[1]



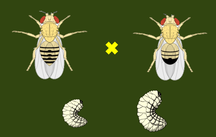
MOLECULAR MECHANISMS REGULATING SYNAPSE DEVELOPMENT ARE POORLY DEFINED

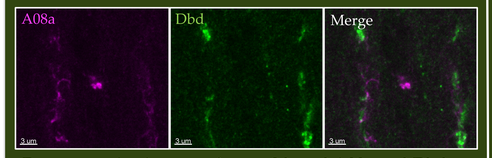
- Bruchpilot (Brp) is a pre-synaptic active-zone protein present on all Drosophila neurons that ensures synapse formation.[3]
- The dorsal bipolar dendritic (Dbd) sensory neuron forms cholinergic synapses onto the A08a interneuron.
- Brp-short and synaptic tagging with recombination (STaR) are two alternative techniques to transmission electron microscopy (TEM) used to label pre-synaptic sites.[3,4]



METHODS

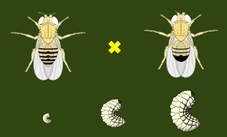
Brp-short

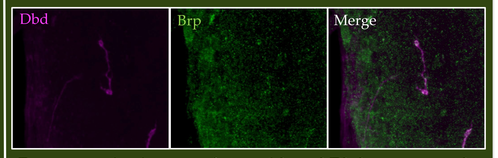




Representative images of stage 3 larval A08a and Dbd neurons taken on a Zeiss LSM 800 confocal microscope.

STaR

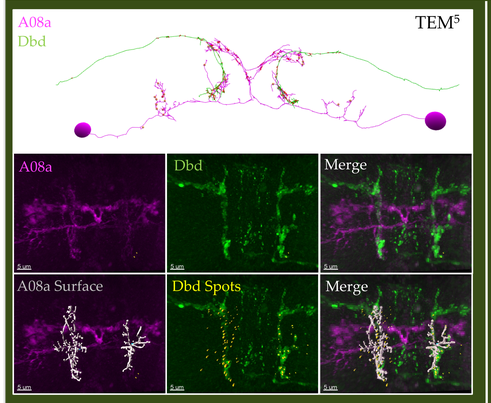




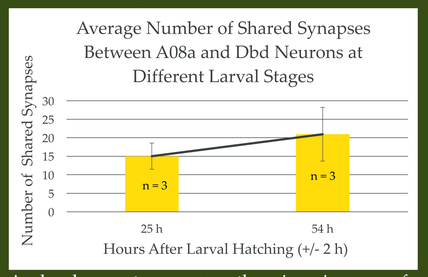
Representative images of stage 1 larval Dbd neurons and Brp puncta taken on a Zeiss LSM 700 confocal microscope.

RESULTS

Brp-short

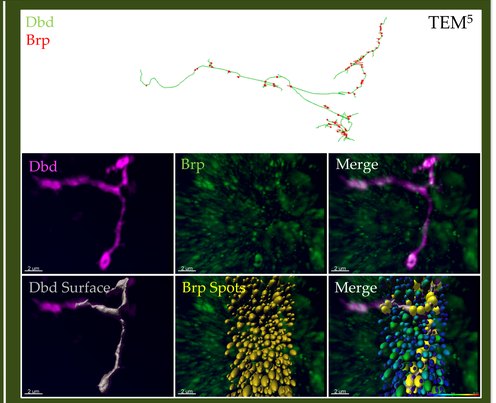


Imaris software was used to construct a surface on the A08a neuron and spots on the Dbd neuron to locate and quantify shared synapses. Spots <90 nanometers from the surface denote synapses.

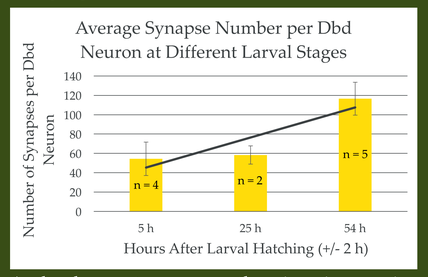


As development progresses, there is an increase of shared synapses between A08a and Dbd neurons.

STaR



Imaris software was used to construct a surface on the Dbd neuron and spots on Brp puncta to locate and quantify synapses. In the merged image, yellow spots denote synapses (<90 nanometers from the surface).



As development progresses, there is an increase in synapse number per Dbd neuron.

DISCUSSION

- As development in *Drosophila* progresses, the number of synapses increases as well.
- Imaris pipeline allows for quantification of synapses across several methods.
- Better staining techniques will allow for more optimization of the Imaris pipeline.
- At the L1 stage, synapses per Dbd using STaR was comparable to that found in TEM.
- Need more TEM data for a greater number of samples and different time points for comparison.

FUTURE DIRECTIONS

- Genetic screen to identify astrocyte-derived molecules influencing cholinergic synapse formation.
- Use of different imaging software to conduct a cross-analyses of accuracy of quantification of synapse number.
- Use of different antibody to reduce background.
- Analyses of a larger sample of animals per time-point.

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