Glutamate clearance in the glia-deprived *C. elegans* synaptic hub

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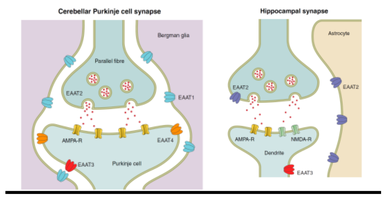
ABSTRACT

As the major excitatory neurotransmitter in the mammalian brain, Glutamate (Glu) is critical for normal neuronal physiology. Normal processing depends on Glu clearance and the ability to separate synapses in order to maintain specificity of neuronal circuits, while failure in ensuring this results in hyper-stimulation of glutamatergic circuits. The canonical model of brain connectivity describes glutamatergic synapses as well insulated and enveloped by glia expressing Glu Transporters (GluTs), which work to clear Glu following synaptic activity. However, only a third of glutamatergic synapses in the hippocampus and other critical brain areas are isolated from other synapses by glia. Poor synaptic isolation may facilitate Glu spillover between adjacent synapses, resulting in loss of circuit specificity. Recent findings also show that brain extracellular space (ECS) is larger than previously believed, suggesting a role for perfusion and bulk flow in robust synaptic clearance and spillover avoidance in critical brain areas. How accurate signal transmission is achieved in such areas remains unclear. The *C. elegans* model is a powerful system to study circuit specificity in absence of glia, with detailed connectome information and animal transparency that allows optogenetic recording of neuronal activity in the intact animal. We suggest a dual mechanism for Glu clearance in absence of glial isolation: 1) A complement of differentially localized GluTs, paired with mechanical agitation of body fluids, facilitating clearance by bulk flow; 2) Robust clearance is achieved by cooperation between GluTs with different physiological properties, which maintain low ambient Glu concentrations. We hypothesize that the combined function of these two mechanisms separate neuronal signals, compensating for lack of glial isolation. We demonstrate a combined role of synaptic location and pharyngeal pulsatility in Glu clearance. We find that distal GluTs exert preferential clearance on some synapses, while proximal GluT preferentially clears other synapses. Synaptic responses in both cases are inhibited when agitation of intracellular fluids ceases. We further propose that active-core differences in amino acid sequence between the different GluTs might be the cause of distinct functional properties, contributing to the ability of these GluTs to work in concert to sufficiently clear Glu, and provide functional circuit isolation where anatomical separation is lacking. These studies will provide novel insights to mechanisms of robust Glu clearance in the absence of glia, a feature shared between nematodes and vital areas of the mammalian brain.

BACKGROUND AND METHODS

Canonical model of the brain portrays each Glu synapses as isolated; in reality, only 1/3 of hippocampal synapses are



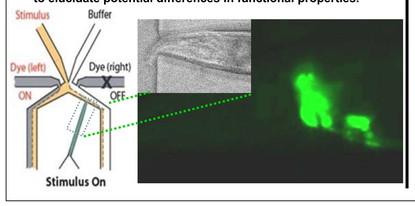
Approach

We study the effect of GluTs on neuronal activity in intact animals:

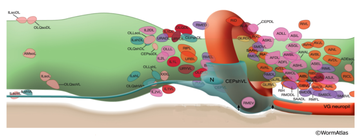
A) Monitor behaviors known to be mediated by specific circuits.

B) Record the effect of GluT on specific circuits: we use a micro-fluidics system and expression of fluorescent extracellular Glu sensor (iGluSnFR) or postsynaptic Ca^{2+} sensor (GCaMP) to follow responses to sensory stimulation in GluT KO strains.

C) Cross-substituting two types of GluTs by promoter swapping, to elucidate potential differences in functional properties.



***C. elegans* Nerve Ring: a highly compact synaptic area lacking glial separation**



Two separate Glutamatergic circuits mediating opposite behaviors are located adjacent to one another in the nerve ring. What prevents spillover during signal transmission?

Expression pattern divides nematode GluTs into Proximal vs. Distal classes

Proximal: GLT-1, GLT4

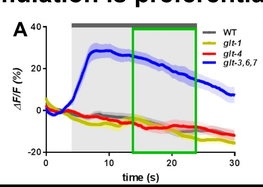
Distal: GLT-3, GLT-6, GLT-7

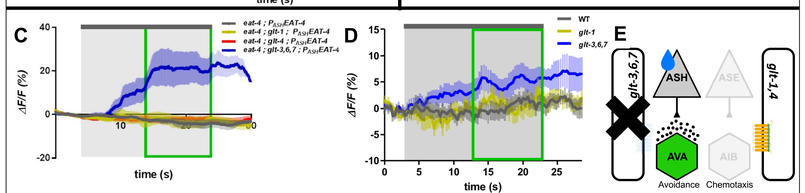
Hypothesis

Can robust clearance by GluTs provide *functional* separation to compensate for the lack of anatomical separation?

RESULTS

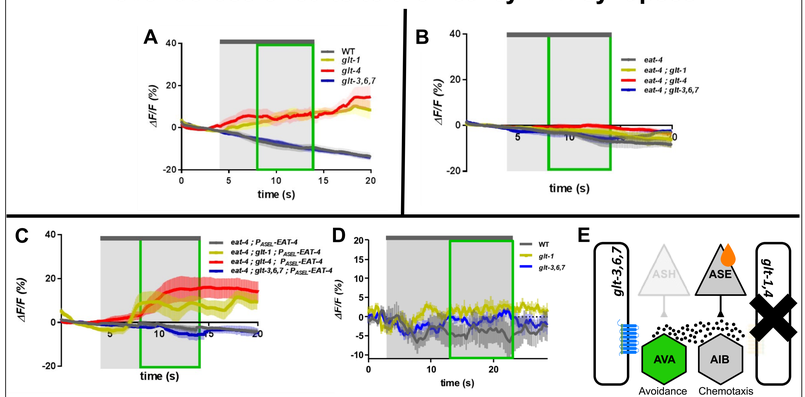
Glu released from ASH onto AVA following nociceptive stimulation is preferentially cleared by distal GluTs





Ca^{2+} responses in **A)** AVA (n = 15 per strain); **B)** AVA in *eat-4* LOF mutants (n = 5-8 per strain); and **C)** AVA in *eat-4* animals with ASH-specific *eat-4* rescue (n = 5 per strain) upon glycerol stimulation. **D)** Glu responses in AVA upon glycerol stimulation (n = 5-6 per strain). **E)** Distal GluTs have a privileged role in ASH responses. In their absence, ASH -> AVA responses are exaggerated.

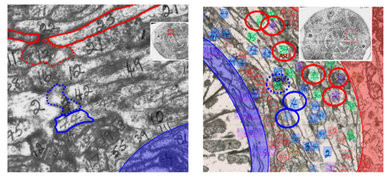
In the absence of proximal GluTs, Glu released from ASE spills over across circuits to the nearby AVA synapses



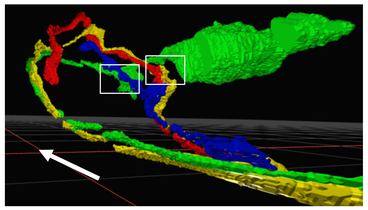
Ca^{2+} responses in **A)** AVA (n = 15-16 per strain); **B)** AVA in *eat-4* LOF mutants (n = 5 per strain); and **C)** AVA in *eat-4* animals with ASE-specific *eat-4* rescue (n = 5-7 per strain) upon 1 mM NaCl stimulation. **D)** Glu responses in AVA upon 1 mM NaCl stimulation (n = 5-7 per strain). **E)** Proximal GluTs have a privileged role in ASE responses. In their absence, Glu seems spill over to AVA.

Location of synapses in the nerve ring may contribute to differential clearance by proximal and distal GluTs

EM Slice of Nerve Ring



3D Reconstruction



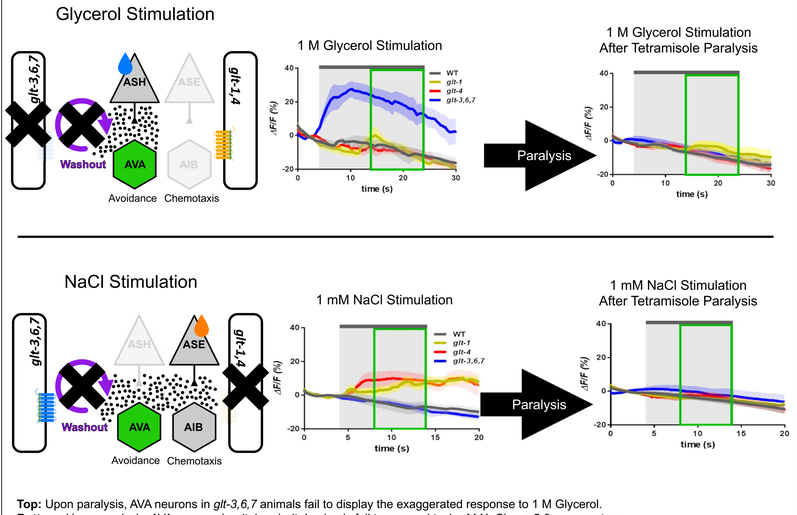
ASH circuit: Blue ; ASE circuit: Red

Pre-synaptic: Dashed ; Post-synaptic: Solid

ASH: Blue AVA: Green ASE: Red AIB: Yellow

Left: Annotated EM images of neurons in the ASH and ASE circuits. Red circles: neurons in the ASE circuit ; Red shaded area: GLT-1 expression territory. Blue circles: ASH circuit neurons. Blue shaded area: a compartment filled with interstitial/body fluids that can be regulated by GLT-3.
Right: 3D reconstruction of ASH and ASE sensory neurons and their interneurons AVA & AIB (respectively). White box indicates the location at which the sensory neurons synapse onto the interneuron. The direction of the white arrow denotes the anterior direction of the worm .
(Modified from WormAtlas.org ; WormWiring.org ; CytoShow.org, wormwiring.org/apps/neuronVolume [3] , Hall Lab, Emmons Lab)

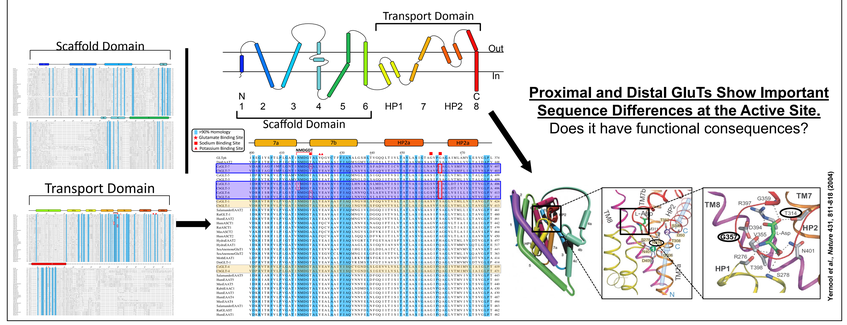
Both normal and spillover responses of the inner-rim synapses of AVA are affected by interstitial fluid agitation.



Top: Upon paralysis, AVA neurons in *glt-3,6,7* animals fail to display the exaggerated response to 1 M Glycerol.
Bottom: Upon paralysis, AVA neurons in *glt-1* and *glt-4* animals fail to respond to 1 mM NaCl. n = 5-9 per genotype.

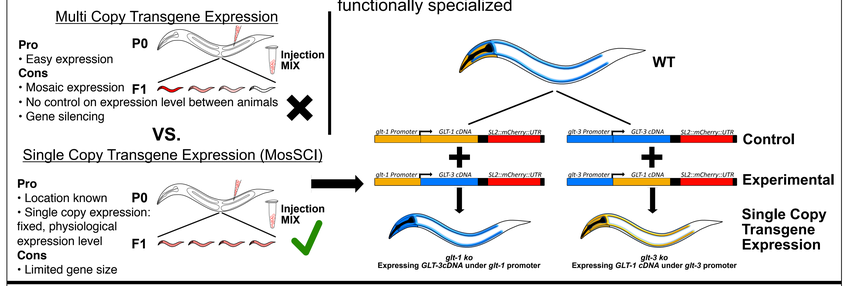
Work in Progress: Structural Determinants of GluTs Physiology

Proximal vs Distal GluTs in the Worm: Transport Domain is Highly Conserved; Important Variations in the Distal GluTs' Active Site



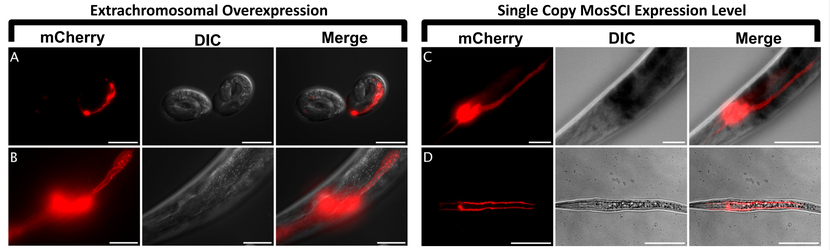
Are Distal Vs Proximal GluTs Interchangeable, or Do They Have Different Physiological Properties?

Swapping transporters and expressing them at physiological levels can show if they are interchangeable or functionally specialized



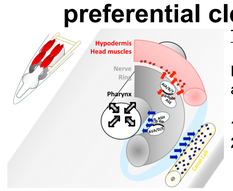
So far, we have: Control glt-3 Promoter GLT-3 cDNA SL2::mCherry::UTR ; Test glt-3 Promoter GLT-1 cDNA SL2::mCherry::UTR

glt-3 ko
Expressing *GLT-1 cDNA* under *glt-3* promoter



Expression of *glt-3p::glt-1cDNA::SL2mCherry::Unc-54 3'UTR* in the *C. elegans* canal cell. **A)** Extrachromosomal expression in eggs (40X oil mag). **B)** Extrachromosomal expression in adult hermaphrodite (40X oil mag). **C)** Single copy MosSCI expression in L4 hermaphrodite (40X oil mag) **D)** Single copy insertion expression throughout entire canal cell in L1 hermaphrodite (20X mag).

Conclusions: Location of ASE vs ASH synapses may explain preferential clearance patterns of proximal vs distal GluTs



Two principles for effective clearance without anatomical glial separation:

Physiological isolation: Robust clearance by a combination of different GluTs can keep ambient Glu concentration low, and compensate for lack of synaptic isolation.

1) **Initial Glu clearance can occur by diffusion** out of the synapse.
2) **Interstitial fluid perfusion facilitated by pharyngeal pumping** may be important for Glu clearance from interstitial fluids by distal GluTs, allowing Glu diffusion out of the synapse to continue, maintaining robust clearance in some synapses.

Funding

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References

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