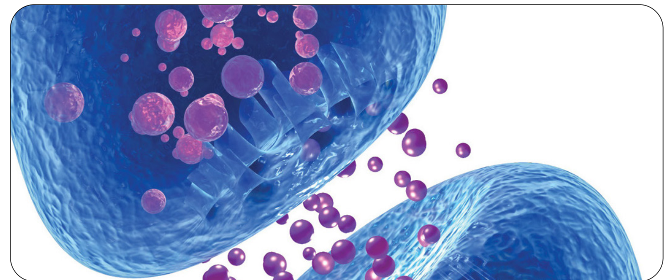
WHITE PAPER

Eyes on synaptotagmin 7 (SYT7) in synaptic function and neuroplastic health for cognitive decline

Introduction

The synaptic vesicle cycle is a highly regulated and reproducibly repeated process that occurs in neurons of the human brain. Numerous proteins are involved in regulating and maintaining the pathways that directly translate to synaptic function, including the family of synaptotagmin (SYT) proteins. Various SYT isoforms function as calcium sensors involved in membrane trafficking and fusion events. These events have been implicated in roles such as neuronal plasticity, synapse remodeling, and glial activation in neurodegenerative disorders.[1] Some studies have suggested that the presence of SYTs in exosomes or cerebral spinal fluid (CSF) could be used as biomarkers for neurodegenerative diseases such as Alzheimer's disease (AD), which may help with risk prediction prior to cognitive impairments.[2]

We spoke to Dr. Jason Vevea, from the laboratory of HHMI Investigator Dr. Edwin Chapman at the University of Wisconsin-Madison, who recently published work describing the role of synaptotagmin 7 (SYT7) in the synaptic vesicle cycle (Figure 1).[3] Using optical imaging tools and novel techniques, he found that SYT7 supports asynchronous release, short-term synaptic plasticity, and synaptic vesicle replenishment from its location on the axonal plasma membrane, at hippocampal synapses. He also discovered that the axonal plasma membrane localization, trafficking,



and stability of SYT7 is dependent on processing by γ-secretase. Interestingly, γ-secretase has a complex history as a high-profile drug target in the fight against AD; however, there have been many failed clinical trials due to a lack of initial biological understanding. Despite this, novel therapeutic strategies involving γ-secretase modulators are still being explored.[4] Here we explore the experimental steps that Dr. Vevea took to make these findings and learn about the implications of his work in neuroscience research.

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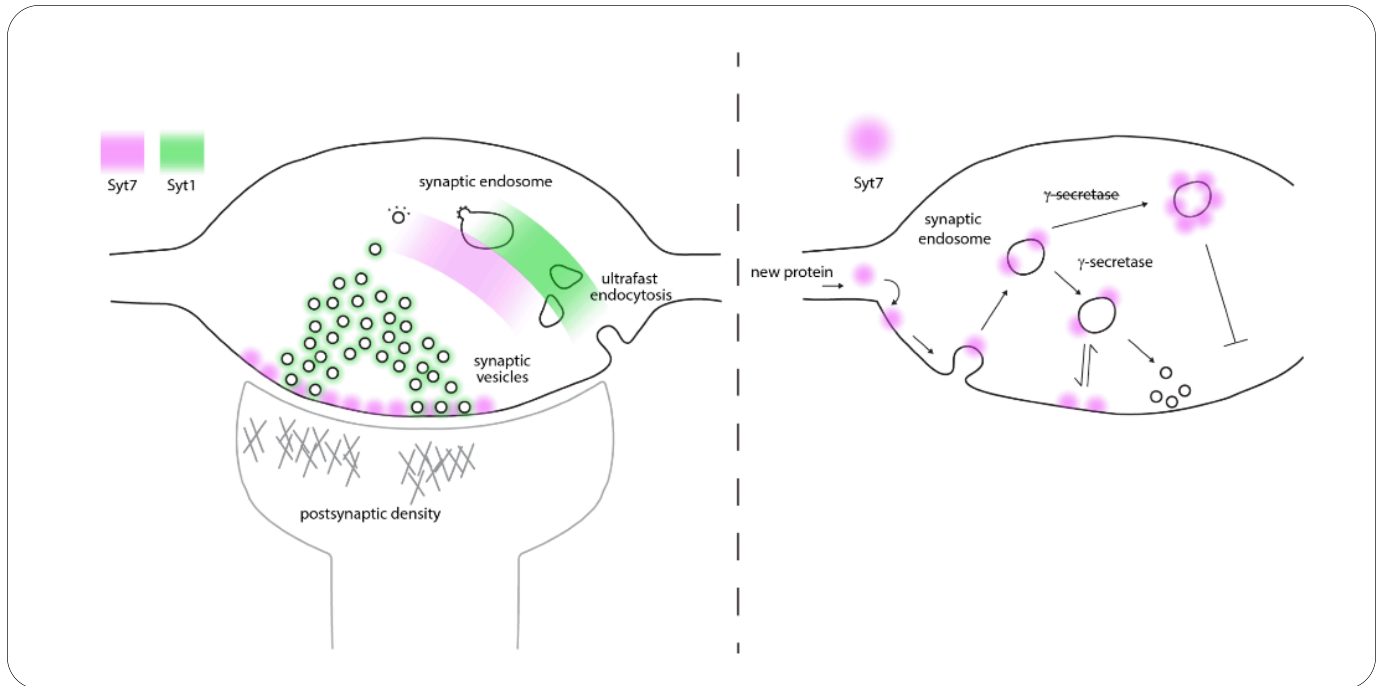

Figure 1: **Graphical Abstract**. Left: A model synapse with roles and locations for SYT1 and SYT7 indicated by green and magenta shading in a presynaptic nerve terminal. Historical research on SYT1 has localized SYT1 to the synaptic vesicle (SV) membrane and has supported a role for SYT1 in fast synchronous SV release as well as a role for SYT1 in endocytosis. The new work by Vevea et al. (2021) has shown that SYT7 physically and functionally localizes to the plasma membrane of the axon, plays a role in supporting release during short-term synaptic activity, as well as reforming SVs. Right: The location of SYT7 (magenta) at the axonal membrane is dependent on γ-secretase processing and palmitoylation near the trans-membrane domain. If palmitoylation is absent (cys –> ala mutations), SYT7 is rapidly degraded. If γ-secretase processing is inhibited, SYT7 mislocalizes to punctae that may be endo-lysosomal intermediate structures. Figure taken from Figure 8;[3] additional figure legend available at https://doi.org/10.7554/eLife.67261.

The synaptic vesicle cycle

Synaptic vesicles are located in the presynaptic terminal of neurons and are essential for propagation of nerve impulses. The vesicles are filled with neurotransmitter and undergo repeated rounds of fusion and regeneration, a process which is known as the synaptic vesicle cycle. “The synaptic vesicle cycle is the foundation for neuronal communication,” affirmed Dr. Vevea. “Some aspects of the underlying biological processes happen so quickly – at the time scale of milliseconds – and reproducibly, it’s a phenomenal feat.”

There are three primary modes by which neurotransmitter is released: synchronous release occurs with a short delay following a stimulus, asynchronous release is characterized by a longer, variable delay following a stimulus, and spontaneous release occurs in absence of electrical activity. Neurotransmitter release is known to depend on changes in intracellular calcium levels, and various calcium-sensing proteins have been shown to govern the synaptic vesicle cycle.

One family of calcium-sensing proteins that has gained considerable attention are the synaptotagmins (SYT), which are characterized by the presence of tandem C2 domains that – in many isoforms – mediate binding to calcium and phospholipid bilayers. The most extensively studied isoform is SYT1, which is the principle regulator of rapid synchronous synaptic vesicle exocytosis. The SYT7 isoform, which is expressed throughout the body and highly expressed in the brain, was originally thought to play no significant role in the synaptic vesicle cycle. However, recent work has suggested that SYT7 is involved in multiple modes of synaptic vesicle release.[5-8] Considering the debate around the function of SYT7 in neurons, Dr. Vevea set out to re-examine the exact role and localization of SYT7 in hippocampal synapses.[3] “The goal for us was to identify exactly how SYT7 influences the synaptic vesicle cycle and the mechanisms behind it,” he said.

For the first stage of his experiment, Dr. Vevea examined synaptic vesicle exocytosis in wild-type and SYT7 knockout (SYT7KO) hippocampal synapses in dissociated cultures using an optical biosensor for glutamate (iGluSnFR).[9] "The optical detection of glutamate is a game changer," he enthused. "To be able to read out, with a normal fluorescence microscope, the fast transient of synaptic glutamate following exocytosis, it is truly awe-inspiring." Following a single stimulus, he observed a tiny change in asynchronous release between wild-type and SYT7KO synapses, but otherwise, there was no apparent change in the amplitude of release. However, when he examined the role of SYT7 in paired-pulse facilitation (PPF), which is a phenomenon where release is transiently increased following an initial stimulation, he detected facilitation (~110%) using iGluSnFR at 50 ms interstimulus intervals, a mild decline at 100 ms, and loss of PPF at 200 and 500 ms. In SYT7KO neurons, PPF was absent at all interstimulus intervals. Additionally, during high frequency train stimulation, SYT7KO synapses also depressed faster and had less asynchronous release, throughout the train. This is the first time that all these deficits have been described at a single dissociated hippocampal preparation. Along with a report from the Jonas lab,[7] this observation helps to simplify the potential mechanism of action of SYT7. SYT7 may not support vastly different modes of release at different synapses, but rather can support PPF, resistance to depression, and asynchronous release wherever it is expressed.

Having demonstrated that release is universally reduced in dissociated SYT7KO neurons after an initial stimulus (e.g. more than one stimulus was needed to uncover the function of SYT7), Dr. Vevea sought help to investigate how this manifested in the synaptic vesicle cycle using 'zap and freeze' electron microscopy with the Shigeki Watanabe lab at Johns Hopkins University.[10] "This is a great technique where you stimulate neurons and immediately freeze them – within milliseconds of simulation. This means you can see how synaptic vesicles are fusing and docking," explained Dr. Vevea. "We tested a whole train of stimuli and froze the neurons at various time points after that train, and we saw this 'dance' of synaptic vesicles. However, this was completely broken in SYT7 knockouts – they just didn't dance." He proposes that the reduction of synaptic vesicle docking during activity observed in the 'zap and freeze' experiments with SYT7KOs could explain the phenotypes observed in the previous experiments (defects in asynchronous release, a complete disruption of PPF, and decreased rates of synaptic vesicle replenishment).

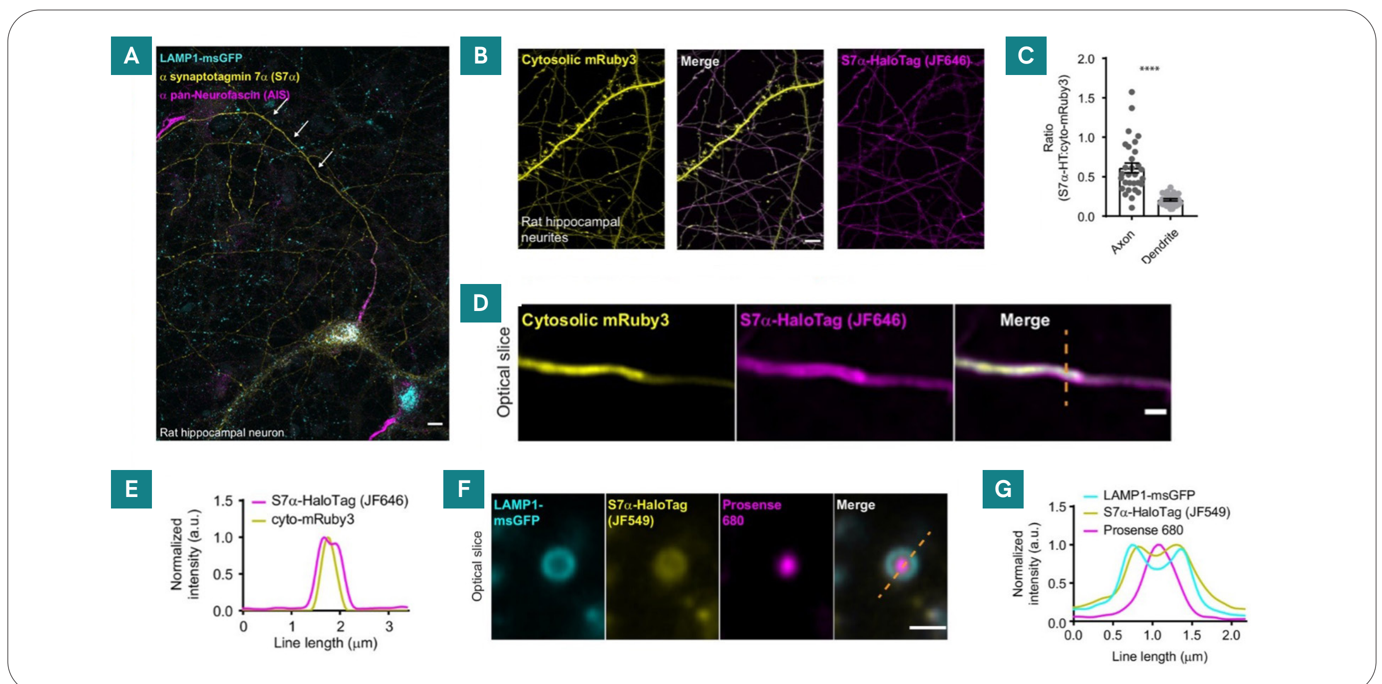

Figure 2: SYT7 was visualized in rat hippocampal neurons at 16 days *in vitro* and found to be localized to the axonal plasma membrane (a-e) and LAMP+ organelles (f-g), including active lysosomes. Figure taken from Figure 4;[3] additional method details can be found in the method and figure legend of https://doi.org/10.7554/eLife.67261.

The role of γ-secretase

A detailed analysis of SYT7 localization in mature neurons has been lacking. Dr. Vevea found that SYT7 was localized to both the axonal plasma membrane and LAMP1+ organelles; the latter included active lysosomes (Figure 2), as shown using a self-quenching and membrane impermeant fluorescent label, via super-resolution optical slices of somatic lysosomes in hippocampal neurons. SYT7 has previously been shown to localize to several distinct subcellular compartments in a variety of cell types; however, its exact localization in mature neurons remained unclear. Dr. Vevea explained that one reason for this is that current antibodies for SYT7 are relatively low affinity and underperform for a lot of applications. "Normally, we would rely on antibodies to detect the protein," he explained. "Something that's held the SYT7 field back is that there aren't great antibodies against this protein."

For this experiment, Dr. Vevea tagged the protein at different ends with molecular tags that reacted with Janelia Fluor® ligands developed in the lab of Dr. Luke Lavis, which led him to a surprising discovery. "When I tagged it at the 'front end' of the protein, I found that the protein was cleaved, but with the same tag at the 'back end' of the protein, it wasn't cleaved. This was really confusing at the time," he said. Further analysis revealed that the amino terminus of SYT7 was cleaved by the Alzheimer's disease relevant γ-secretase complex, and that the stability and localization of SYT7 was dependent on this proteolytic processing step and concurrent palmitoylation. "So, if you don't have γ-secretase activity, this protein doesn't stay in the axon, it's mislocalized and degraded. This may have implications for Alzheimer's disease because familial Alzheimer's disease presenilin mutations are dominant loss of function, and so in theory, if you can't cleave SYT7, you may have defects in short-term synaptic plasticity, which is directly related to memory formation and cognitive function."

Finally, Dr. Vevea used retargeting experiments to investigate how the location of SYT7 influences the synaptic vesicle cycle. Specifically, he restricted SYT7α to the plasma membrane, endolysosomal LAMP1+ membranes, or synaptic vesicles. The results confirmed that the location of SYT7α is paramount to its function – only the plasma membrane-associated SYT7α rescued all investigated phenotypes. "These retargeting experiments were crucial for appreciating that SYT7 has to be in the plasma membrane – it can't just be anywhere in the synapse," he explained. "This backed up something that people have always got right about SYT7, that it's likely located on the plasma membrane and not on a vesicle."

Future plans

Dr. Vevea noted that the nuanced phenotypes associated with SYT7 have previously been difficult for scientists to understand. "The tools available now are so good and so precise that we can start to get more of an understanding of these phenotypes and explore how these proteins give rise to them. These tools may also make the identification of the endosomal intermediates that are influenced by SYT7 possible."

His next steps will be to explore the mechanistic functions of SYT7. "We now know that SYT7 influences synaptic vesicle docking, but we don't know what part of the protein touches the vesicle, for example. We have no idea about the basic mechanisms of how SYT7 physically interacts with effectors, or even what the relevant effectors are, so this is a ripe avenue of research that we will be exploring," he said. "It's a busy future, which means it's a bright future, and potentially implicating SYT7 in a degenerative disease really gives us a potential mechanism for why people might have cognitive decline."

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Information about the chapman lab

Edwin Chapman is an HHMI Investigator and the Ricardo Miledi Professor of Neuroscience at the University of Wisconsin-Madison, where he also serves as the Director of the Quantitative Membrane Biophysics Program. His laboratory studies membrane trafficking in neurons, with an emphasis on the biophysics of Ca2+-triggered exocytosis, synaptic transmission, and synaptic plasticity, using approaches and models that range from reconstituted systems to neuronal circuits.

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