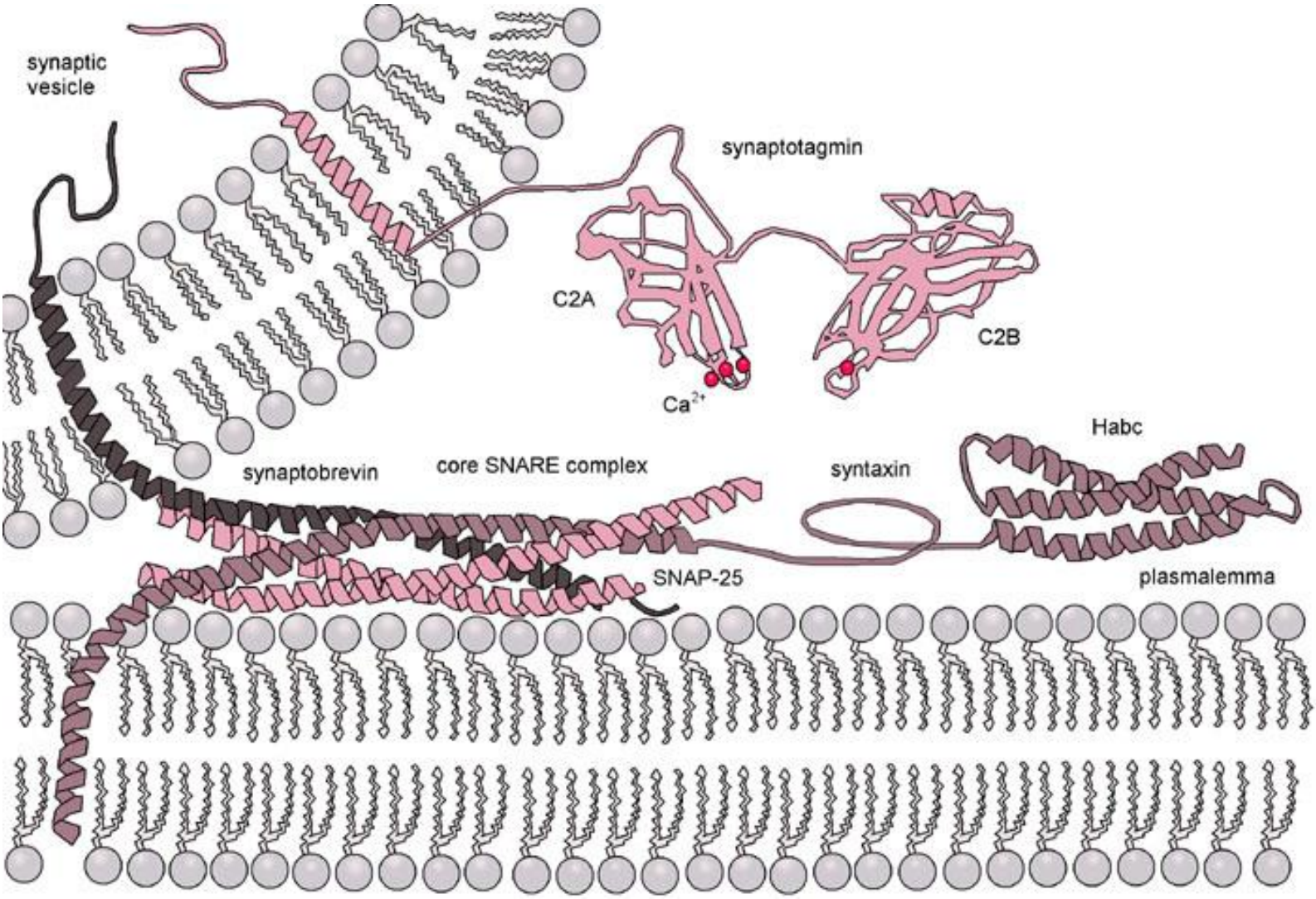

ELUCIDATING THE MECHANISMS OF SYNAPTIC VESICLE RELEASE

The role of synaptotagmin

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April 7th, 2016

ISCI 2A18: EP

Title page image from Georgiev and Glazebrook, 2007.

ABSTRACT

Synaptotagmin is a Ca^{2+}-sensing protein located in the presynaptic terminal essential to calcium-dependent neurotransmission. Sixteen isoforms have been identified, all with slightly different structures and functions. This literature review examines the research that led neuroscientists to discover synaptotagmin 1 (Syt1) and its function as a calcium sensor for synchronous neurotransmitter release, as well as the function of a different isoform, synaptotagmin 7 (Syt7), which is still under deliberation. Finally, a series of experiments is proposed in order to determine if Syt1 is implicated in paired-pulse facilitation at CA3-CA1 hippocampal synapses.

DEVELOPMENT OF OUR UNDERSTANDING OF SYNAPTIC VESICLE EXOCYTOSIS

WHAT IS THE ROLE OF CALCIUM IN NEUROTRANSMITTER RELEASE?

Calcium ions have long been known to be essential to neural signalling. In 1957, Hodgkin and Keynes, in their work on squid giant axons, demonstrated that a large calcium influx occurs when the axon is stimulated (Hodgkin and Keynes 1957). Later that year, working with Frankenhauser, Hodgkin used the voltage clamp technique on squid giant axons in order to measure the effects of varying the extracellular calcium concentration on potassium and sodium conductances inside and outside the axon (Frankenhauser and Hodgkin 1957). Their experiments showed, remarkably, that changing the extracellular calcium concentration had similar effects on potassium and sodium conductances as did changes in membrane potential. Frankenhauser and Hodgkin speculated on two explanations for this phenomenon. The first was that calcium ions may be adsorbed on the outer edge of the membrane of the neuron and create an electric field, which would alter the resting potential inside the membrane. The altered potential inside the membrane would cause a change in the distribution of other charged particles inside the membrane, and thus in the conductances of potassium and sodium.

Nearly ten years later, Miledi and Slater further explored the role of calcium in what was now known to be signal transduction between neurons (Miledi and Slater 1966). They worked with the isolated stellate squid ganglion, and recorded potentials with intracellular and extracellular microelectrodes. A lack of extracellular calcium was found to abolish the postsynaptic response but not the presynaptic action potential, and the application of extracellular calcium restored it. This established that synaptic transmission in the squid stellate ganglion is not electrical in nature, but is a result of some unidentified, calcium-dependent transmitter release. Miledi and Slater went on to suggest that because intracellular injection of calcium ions did not rescue synaptic transmission in a low-Ca^{2+} extracellular environment, that some calcium receptor found on the outside of the presynaptic membrane binds to calcium upon the receipt of an electrical signal, and somehow mediated transmitter release. Though the calcium-mediated mechanism of neurotransmitter is much more complicated, Miledi and Slater were onto something: calcium was required for neurotransmission, and synaptic vesicle release would soon be found to be dependent on the detection of calcium.

Sodium channel blockage by tetrodotoxin proved to be a valuable technique for determining the role and time course of calcium in neurotransmission (Katz and Miledi 1967a). Katz and Miledi determined that depolarization of the presynaptic membrane of squid stellate ganglions increases the permeability of the membrane to calcium (Katz and Miledi 1967a). They later examined the time course of calcium action during neuromuscular transmission (Katz and Miledi 1967b). The results of their work on

paralysed nerve-muscle preparations showed that neurotransmitter release occurred when calcium was applied to the motor nerve ending briefly, and only immediately before the depolarizing pulse. Katz and Miledi thus concluded that calcium is used by the neuromuscular junction in a brief period preceding transmitter release which barely outlasts depolarization. As such, they concluded that the depolarization of the nerve ending must open some kind of gate to calcium, which allows calcium to move inside the axon membrane, and somehow causes an increase in the rate of transmitter release. In later studies, Katz and Miledi further discovered that the relationship between neurotransmitter release and external calcium concentration is non-linear and very steep, suggesting that transmitter release sites bind multiple calcium ions (Katz and Miledi 1970).

Prior to 1973, neuronal stimulation was conducted electrically. However, Miledi showed that injecting squid synapses intracellularly with calcium could induce transmitter release, even with the sodium channel blockage associated with tetrodotoxin application (Miledi 1973). Furthermore, Miledi determined that intracellular injections of calcium caused synaptic potentials that were much smaller than those evoked by an action potential, which was called asynchronous release. Finally, Miledi suggested that the time course and amplitude of neurotransmitter release following intracellular injections of calcium implied that calcium ions act as a transmitter-releasing factor on the inside of the terminal. This provided more evidence towards the hypothesis that calcium binds to transmitter release sites, and disproved the earlier conclusions made by Miledi and Slater that the calcium receptor is found on the outside of the presynaptic membrane.

WHAT IS THE CALCIUM SENSOR FOR SYNAPTIC VESICLE RELEASE?

The identity of this calcium sensor soon because a subject of interest. Work by Matthew, Tsavaler, and Reichardt identified a 65 kDa synaptic vesicle membrane protein, widely distributed in neuronal and neural secretory tissues, and with similar distribution to cAMP and Ca^{2+}-activated protein kinases (Matthew et al. 1981). The role of the protein was not determined in Matthew, Tsavaler, and Reichardt's experiments, but this was the first glimpse of the protein that would be called synaptotagmin, ten years later (M.S.. Perin et al. 1991). This protein (p65) was subsequently shown to bind calmodulin in chromaffin granule membranes in a calcium-dependent manner (Bader et al. 1985). At the time, calmodulin was thought to mediate chromaffin granule release, as calmodulin antagonism inhibits it (Bader et al. 1985). The results of these experiments showed that a 65-kDa protein bound calmodulin only at low calcium concentrations (approximately 10^{-7} M Ca^{2+}) and was therefore likely implicated in the mechanism for chromaffin granule release in a calcium-dependent manner.

Calmodulin, in fact, was at the time considered to be a candidate to be the calcium receptor that triggers synaptic vesicle release (Augustine et al. 1987). However, calmodulin had been shown not to cause fusion between artificial membranes, and thus calmodulin-related proteins and vesicle-associated calcium-binding proteins were determined as the primary candidates for being trigger proteins for calcium-dependent synaptic vesicle release (Augustine et al. 1987). Identification of vesicle-associated proteins and glycoproteins was an important objective in identifying the calcium sensor in synaptic vesicle release, since any proposed mechanism for vesicle release would involve the interaction of the calcium sensor with synaptic vesicles (Augustine et al. 1987). The discovery that p65 contains two repeats of protein kinase C's (PKC) regulatory domain, thought to be responsible for

its Ca^{2+}-binding properties, as well as the capability of p65 to bind phospholipids, suggested that p65 is at the very least implicated in synaptic vesicle exocytosis (Perin et al. 1990; Brose et al. 1992).

P65 was formally named "synaptotagmin" in 1991 (Perin et al. 1991a). By comparing full-length cDNA sequences for synaptotagmin between rats, humans, and *Drosophila*, the protein was shown to be highly conserved evolutionarily, along with its phospholipid binding properties (Perin et al. 1991a). Subsequent studies demonstrated that synaptotagmin contains five protein domains: an intravesicular portion, a single transmembrane region, a section separating the transmembrane region from the repeats homologous to PKC, the repeating PKC-homologous section, and a sequence following the PKC-homologous region (Perin et al. 1991b). Again, the protein appeared to bind negatively-charged phospholipids, and was found to be part of a potential docking or fusion complex in the synaptic vesicle membrane (Perin et al. 1991b).

Finally, in 1992, synaptotagmin was finally shown to bind to phospholipids tightly in a Ca^{2+}-dependent manner, with some phospholipid binding occurring in the absence of Ca^{2+} as well (Brose et al. 1992). This was done using a fluorescence resonance energy transfer assay, where the energy transfer is dependent on the distance between two fluorophores, located on tryptophan residues in synaptotagmin, and the dansyl group in the liposomes (Brose et al. 1992). Ca^{2+}-triggered fluorescence resonance energy transfers occurred in a dose-dependent manner, suggesting that binding Ca^{2+} causes a close association of synaptotagmin with the liposomes (Brose et al. 1992). Furthermore, proteolysis of synaptotagmin abolished its binding capability to phospholipids Ca^{2+}-dependently or Ca^{2+}, implying that an intact tetrameric structure of the protein is required for Ca^{2+} binding (Brose et al. 1992). Brose and colleagues suggested the following, updated model for vesicle exocytosis: while the intracellular calcium concentration remains at baseline, synaptotagmin is bound to some specific acceptor protein in the presynaptic membrane, and upon increased calcium ion concentration inside the nerve terminal, the cytoplasmic arms of synaptotagmin interact with the phospholipids of the plasma membrane, triggering fusion via interaction with other as-of-yet uncharacterized proteins (Brose et al. 1992).

Multiple isoforms of synaptotagmin were soon characterized, and found to be expressed differentially in different parts of the brain (Ullrich et al. 1994). The original synaptotagmin was thus named synaptotagmin 1 (Syt1) (Geppert et al. 1994). Syt1's essential role in Ca^{2+}-dependent vesicle release was further confirmed when Syt1-knockout mice did not display synchronous release of neurotransmitters (Geppert et al. 1994). However, asynchronous release was not affected by Syt1-knockout, showing that some other calcium sensor may be implicated in asynchronous release mechanisms (Geppert et al. 1994). This study also uncovered that Syt1 has a low calcium affinity and high efficiency, thus increasing the probability of vesicle exocytosis (p) manifold when four calcium ions are bound (Geppert et al. 1994). Conversely, the sensor for asynchronous release would display a high affinity for calcium ions, but a low efficiency in increasing p (Geppert et al. 1994). As such, in high Ca^{2+} concentrations, due to the opening of voltage-gated Ca^{2+} channels in the presynaptic membrane, synaptotagmin 1 dominates and causes the release of neurotransmitter quanta (Geppert et al. 1994). At resting calcium levels, the second sensor would produce spontaneous release events, or asynchronous release (Geppert et al. 1994).

WHAT IS THE MOLECULAR MECHANISM FOR CALCIUM-DEPENDENT VESICLE RELEASE?

The identity of this second calcium sensor for asynchronous release remained unknown, as did the exact mechanism for Syt1-mediated Ca^{2+}-dependent synchronous release. However, within the next several years, a more complete mechanism for synchronous release was elucidated (Augustine 2001). Firstly, the C_2 domains (PKC regulatory domain homologs) of Syt1 were characterized, with C_2A being the domain to which Ca^{2+} ions bind, and with the whole C_2 domain ostensibly binding to negatively charged phospholipids via four basic, positively-charged lysine residues (Sutton et al. 1995). The C_2A and C_2B domains form an eight-stranded β-sandwich, with Ca^{2+} binding in cup-shaped depressions at the N- and C-termini of the C_2 domain (Sutton et al. 1995). More proteins involved in the exocytotic machinery were identified, with Ca^{2+} binding to the C_2A domain causing the protein to bind acidic phospholipids, as well as the plasma-membrane proteins SNARE, syntaxin, and SNAP-25 (Augustine 2001). Furthermore, the cytosolic protein complexin was identified as a fusion clamp that prevents continuous synaptic vesicle fusion with the presynaptic membrane, with which Ca^{2+}-activated synaptotagmin 1 must compete in order to bind SNARE complexes (Huntwork and Littleton 2007).

Part of the mechanism of synaptotagmin 1-mediated synchronous synaptic vesicle release requires synaptotagmin 1 to displace the stabilizing complexin and to form a quaternary complex with the presynaptic phospholipid membrane and a SNARE complex, named the SNARE-synaptotagmin-Ca^{2+}-phospholipid (SSCAP) complex (Dai et al. 2007). The SNARE complex consists of synaptobrevin/VAMP, syntaxin, and SNAP-25, forming a tight four-helix bundle (Sollner et al. 1993; Sutton et al. 1998; Poirier et al. 1998). Interestingly, though the two C_2 domains of synaptotagmin 1 are both required for release, mutating C_2B to disrupt Ca^{2+} binding has much more deleterious effects on release than mutating C_2A (Dai et al. 2007). In order to determine whether synaptotagmin 1 binds phospholipids and SNARE complexes simultaneously, Dai and colleagues forced fluorescently marked C_2AB fragments of the synaptotagmin 1 protein to bind samples of phospholipid membranes either containing SNARE complexes or lacking them, deposited in a microfluidic channel (Dai et al. 2007). Furthermore, they tested whether the same marked C_2AB fragments would bind soluble SNARE complexes more frequently than SNARE-free phospholipids (Dai et al. 2007). The C_2AB fragments were found to bind SNARE-containing phospholipids more frequently than SNARE-free phospholipids, and bound phospholipids preferentially over soluble SNARE complexes, indicating that the C_2AB fragment of synaptotagmin 1 almost certainly binds the phospholipid membrane and the SNARE complex simultaneously (Dai et al. 2007). Furthermore, disrupting the C_2A domain's ability to bind Ca^{2+} did not affect its ability to displace complexin, whereas disrupting the C_2B domain almost completely abolished this ability (Dai et al. 2007). As such, the following model was proposed: the C_2B domain of synaptotagmin binds directly to the SNAREs in the SSCAP complex and displaces complexin, whereas the C_2A domain plays an auxiliary role by increasing the affinity of C_2B for the SNARE complex (Dai et al. 2007).

Overall, the mechanism of calcium-dependent, synchronous neurotransmitter release can be summarized as follows (adapted from Pang and Südhof 2010):

1. An action potential floods the presynaptic terminal, opening voltage-gated calcium ion channels.
2. $[Ca^{2+}]$ increases inside the axon terminal.

3. Ca^{2+} binds to the cytosolic C_2A and C_2B domains of the synaptic vesicle membrane-associated protein synaptotagmin 1, which causes Syt1 to bind to plasma membrane-associated SNARE complexes and the phospholipid membrane, displacing complexin.
4. The SNARE-synaptotagmin-Ca^{2+}-phospholipid (SSCAP) complex catalyzes fusion by forcing the synaptic vesicle membrane and the plasma membrane together, forming a fusion pore.
5. The contents of the synaptic vesicle are emptied to diffuse into the synaptic cleft.

As such, synaptotagmin 1's role has been established as an essential mediator of calcium-dependent synchronous neurotransmitter release.

The architecture of the synaptotagmin 1-SNARE machinery for neuronal exocytosis has recently been crystallized, and therefore, a more detailed mechanism has been proposed (Zhou et al. 2015). Syt1 binds to SNARE via its C_2B domain, forming a flat, positively-charged face, containing the basic lysine residues of Syt1, as well as the basic residues of the SNARE complex (Zhou et al. 2015). Ca^{2+} binding to the Syt1 C_2B domain causes the Ca^{2+} binding loops to insert partially into the phospholipid synaptic membrane (Zhou et al. 2015). This results in a membrane deformation, and leads to fusion pore opening (Zhou et al. 2015).

WHAT IS THE FUNCTION OF SYNAPTOTAGMIN 7?

Synaptotagmin has at least sixteen different isoforms, the two most abundant being the closely related Syt1 and Syt2, with Syt2 exhibiting faster kinetics than Syt1 and mediating synchronous transmission at the neuromuscular junction and calyx of Held in mammals (Sugita et al. 2001; Pang et al. 2006; Wen et al. 2010; Cooper and Gillespie 2011). Syt1 and Syt2 are followed in abundancy by synaptotagmin 7 (Sugita et al. 2001).

Synaptotagmin 7, a presynaptic plasma-membrane-associated protein (Sugita et al. 2001), has been shown to function as a high-affinity calcium sensor, triggering synaptic vesicle exocytosis at lower concentrations than Syt1 in PC12 cells (Wang et al. 2005). Its slow disassembly rate has contributed to the hypothesis that Syt7 may mediate asynchronous release, and it may also be implicated in paired-pulse facilitation (Hui et al. 2005). Knockdown of Syt7 at the zebrafish neuromuscular function has been found to significantly reduce asynchronous release, while synchronous release remained intact, supporting the idea that Syt7 may mediate asynchronous release (Wen et al. 2010).

Recent evidence supports this claim. Syt1 knockout in Syt1- and Syt7-expressing hippocampal neurons has been shown to block synchronous release but not asynchronous release, and further knockout of Syt7 in Syt1-KO neurons has been shown to suppress asynchronous release as well (Bacaj et al. 2013). Furthermore, these asynchronous release suppressing effects in Syt1-KO were rescued by expressing wild-type but not mutant Syt7 incapable of Ca^{2+}-binding, suggesting that Syt7 is the calcium sensor for asynchronous release (Bacaj et al. 2013). However, Syt7 KO in wild-type neurons had no effect on either synchronous or asynchronous release (Bacaj et al. 2013). As such, the mechanism for Syt7-mediated asynchronous release is still elusive, and has been found not to be valid in hippocampal neurons (Liu et al. 2014).

Syt7's role as the calcium sensor for asynchronous release has been shown to be associated with the SNARE protein SNAP-23, where the expression of SNAP-23 in knockout SNAP-25 (one SNARE protein considered to form a SSCAP complex with Syt1) neurons has been shown to create strongly

asynchronous excitatory post-synaptic currents (Weber et al. 2014). Such observations imply that different synaptotagmin isoforms may act as specific release mediators for specific sets of SNARE proteins, where Syt7/SNAP-23 forms a calcium sensor with slow kinetics (Weber et al. 2014).

Syt7 has also been associated with short-term plasticity (Liu et al. 2014). High-frequency stimulation of cultured Syt7-KO mouse hippocampal neurons demonstrated that Syt7 is a Ca^{2+} sensor that regulates Ca^{2+}-dependent synaptic vesicle replenishment in association with calmodulin (CaM) (Liu et al. 2014). Defective synaptic vesicle replenishment is associated with paired-pulse depression, and the recovery of the readily-releasable pool of synaptic vesicles was found to be slower in Syt7-KO neurons (Liu et al. 2014).

Most recently, synaptotagmin 7 has been found to be the calcium sensor for synaptic facilitation, another form of short-term synaptic plasticity (Jackman et al. 2016). Some synapses with a low initial release probability (*p*) can release a larger amount of neurotransmitter following closely-spaced presynaptic action potentials (Jackman et al. 2016). Paired-pulse facilitation was eliminated in Syt7-KO CA3-CA1 synapses, and rescued by subsequent expression of Syt7, but not by mutated Syt7 with a Ca^{2+}-insensitive domain (Jackman et al. 2016). However, facilitated release has fast, Syt1-like kinetics, rather than slow kinetics, as would be expected if Syt7 was the only calcium sensor involved in paired-pulse facilitation (Jackman et al. 2016). Therefore, the mechanism for Syt7 mediation of paired-pulse facilitation is unknown, but begs the question: does Syt7 interact with Syt1 (or some other fast synaptotagmin) in a Ca^{2+}-dependent manner to induce paired-pulse facilitation? If so, would deleting Syt1 abolish paired-pulse facilitation in CA3-CA1 synapses?

RESEARCH QUESTION: DOES SYT7 INTERACT WITH SYT1 TO INDUCE PAIRED-PULSE FACILITATION?

Facilitation is exhibited at all neocortical and hippocampal synapses studied in detail to date, and usually at those with a low initial probability of synaptic vesicle release (*p*) (Thomson 2000; Jackman et al. 2016). It is defined to be a phenomenon where repeated, small action potentials flooding the presynaptic axon terminal (paired pulses) subsequently increase *p* (Thomson 2000). Differences in *p* in different synapses can be attributed to differential expression of synaptotagmin, due to the calcium-sensing properties of these proteins (Thomson 2000). As such, variability in expression of synaptotagmin isoforms with different sensitivities to calcium could have a significant effect on the probability of synaptic vesicle release, and thus on facilitation (Thomson 2000).

Synaptotagmin 7 has been identified to be required for facilitation in Schaffer collateral synapses between hippocampal CA3-CA1 pyramidal cells (Jackman et al. 2016). This conclusion was made based on the results of a series of experiments with Syt7-knockout mice, where paired-pulse facilitation was eliminated, compared to wild type animals, where sustained stimulation can produce up to tenfold enhancement (Jackman et al. 2016). Furthermore, facilitation was shown to be Ca^{2+}-dependent, as Syt7(C_2A^*), a mutated Syt7 with a Ca^{2+}-insensitive C_2A domain, was not able to rescue facilitation in knockouts (Jackman et al. 2016). Finally, facilitated release was found to exhibit rapid kinetics, and coupled with previously observed interactions of Syt7 with Syt1 to induce vesicle exocytosis, this suggests that Syt7 somehow interacts with Syt1 to increase *p* (Jackman et al. 2016).

Given the previous research, the following research question is proposed: does Syt7 interact with Syt1 calcium-dependently to induce paired-pulse facilitation? In order to examine this research question, the following experiment is proposed, (adapted from Jackman et al. 2016).

First, Syt1-knockout mice would be generated, and transverse slices of both Syt1-knockout and wild-type mouse brains would be obtained, in order to access CA3-CA1 synapses (Jackman et al. 2016). Separate brain samples would be stained with labeled antibodies for Syt7 and Syt1 in order to determine whether knockout had been successful. To generate paired pulses and stimulate the presynaptic axon, an extracellular stimulus electrode would be used, and whole cell voltage clamp would be used to record postsynaptic currents (Jackman et al. 2016). EPSCs would be recorded following the generation of paired pulses in the presynaptic membrane in both Syt1-knockout and wild-type CA3-CA1 synapses.

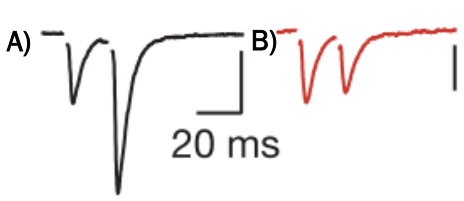

Figure 1. Representative traces of postsynaptic currents in the CA1 pyramidal cell following paired-pulse stimulation, where A) represents typical traces of a synapse with paired-pulse facilitation, and where B) represents a typical trace of a synapse with no paired-pulse facilitation or depression (Jackman et al. 2016).

I hypothesize that if Sy1 is essential to paired-pulse facilitation in CA3-CA1 hippocampal synapses, then no facilitation would be observed in Syt1-KO, that is, following paired pulses, the second postsynaptic response would not be greater than the first (Figure 1B). The null hypothesis would therefore be that if Syt1 is not essential to paired-pulse facilitation in CA3-CA1 hippocampal synapses, then both wild-type and Syt1-KO synapses would display paired-pulse facilitation, and postsynaptic traces would resemble those found in Figure 1A.

If the null hypothesis is rejected, more experiments would need to be conducted in order to determine whether expression of Syt1 would rescue paired-pulse facilitation. I propose using a similar expression system as used in Jackman and colleagues' experiment, where channelrhodopsin and Syt1 were expressed bicistronically using viral vectors in previously Syt7-KO neurons (Jackman et al. 2016). Channelrhodopsin is a light-gated ion channel, and expressing it bicistronically with a protein of interest allows the stimulation of only neurons where the protein of interest was successfully expressed, using laser light (Jackman et al. 2016). Light stimulation of these neurons and recording EPSCs following paired pulses would then allow us to determine whether facilitation occurred.

The results of such this study would allow the determination of whether Syt1 specifically is implicated in the fast kinetics of paired-pulse facilitation. If not, then either Syt2 or Syt9, the two other synaptotagmin isoforms known to trigger fast synchronous release, would need to be examined as candidates for interaction with Syt7 (Südhof 2012). Alternatively, calmodulin could potentially mediate the mechanism for paired-pulse facilitation, as it has already been shown to interact with Syt7 in the Ca^{2+}-dependent replenishment of synaptic vesicles (Liu et al. 2014; Jackman et al. 2016).

WORKS CITED

Augustine GJ. 2001. How does calcium trigger neurotransmitter release? Curr. Opin. Neurobiol. 11:320–326.

Augustine GJ, Charlton MP, Smith SJ. 1987. Calcium action in synaptic transmitter release. Annu. Rev. Neurosci. 10:633–693.

Bacaj T, Wu D, Yang X, Morishita W, Zhou P, Xu W, Malenka RC, Südhof TC. 2013. Synaptotagmin-1 and synaptotagmin-7 trigger synchronous and asynchronous phases of neurotransmitter release. Neuron 80:947–959.

Bader MF, Hikita T, Trifaro JM. 1985. Calcium-dependent calmodulin binding to chromaffin granule membranes: presence of a 65-kilodalton calmodulin-binding protein. J. Neurochem. 44:526–539.

Brose N, Petrenko AG, Südhof TC, Jahn R. 1992. Synaptotagmin: a calcium sensor on the synaptic vesicle surface. Science (80-.). 256:1021–1025.

Cooper AP, Gillespie DC. 2011. Synaptotagmins I and II in the developing rat auditory brainstem: Synaptotagmin I is transiently expressed in glutamate-releasing immature inhibitory terminals. J. Comp. Neurol. 519:2417–2433.

Dai H, Shen N, Araç D, Rizo J. 2007. A quaternary SNARE-synaptotagmin-Ca2+-phospholipid complex in neurotransmitter release. J. Mol. Biol. 367:848–863.

Frankenhauser B, Hodgkin AL. 1957. The action of calcium on the electrical properties of squid axons. J. Physiol. 137:218–244.

Georgiev DD, Glazebrook JF. 2007. Subneuronal processing of information by solitary waves and stochastic processes. In: Edward S, editor. Nano and Molecular Electronics Handbook. CRC Press. p. 17–41.

Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Südhof TC. 1994. Synaptotagmin I: A major Ca2+ sensor for transmitter release at a central synapse. Cell 79:717–727.

Hodgkin AL, Keynes RD. 1957. Movements of labelled calcium in squid giant axons. J. Physiol. 138:253–81.

Hui E, Bai J, Wang P, Sugimori M, Llinas RR, Chapman ER. 2005. Three distinct kinetic groupings of the synaptotagmin family: candidate sensors for rapid and delayed exocytosis. Proc. Natl. Acad. Sci. U. S. A. 102:5210–4.

Huntwork S, Littleton JT. 2007. A complexin fusion clamp regulates spontaneous neurotransmitter release and synaptic growth. Nat. Neurosci. 10:1235–1237.

Jackman SL, Turecek J, Belinsky JE, Regehr WG. 2016. The calcium sensor synaptotagmin 7 is required for synaptic facilitation. Nature 529:88–91.

Katz B, Miledi R. 1967a. A study of synaptic transmission in the absence of nerve impulses. J. Physiol. 192:407–436.

Katz B, Miledi R. 1967b. The timing of calcium action during neuromuscular transmission. J. Physiol. 189:535–44.

Katz B, Miledi R. 1970. Further study of the role of calcium in synaptic transmission. J. Physiol. 207:789–801.

Liu H, Bai H, Hui E, Yang L, Evans CS, Wang Z, Kwon SE, Chapman ER. 2014. Synaptotagmin 7 functions as a Ca2+-sensor for synaptic vesicle replenishment. Elife 3:1–18.

Matthew WD, Tsavaler L, Reichardt LF. 1981. Identification of a synaptic vesicle-specific membrane protein with a wide distribution in neuronal and neurosecretory tissue. J. Cell Biol. 91:257–269.

Miledi R. 1973. Transmitter release induced by injection of calcium ions into nerve terminals. Proc. R. Soc. London B Biol. Sci. 183:421–425.

Miledi R, Slater CR. 1966. The action of calcium on neuronal synapses in the squid. J. Physiol. 184:473–498.

Pang ZP, Melicoff E, Padgett D, Liu Y, Teich AF, Dickey BF, Lin W, Adachi R, Südhof TC. 2006. Synaptotagmin-2 is essential for survival and contributes to Ca2+ triggering of neurotransmitter release in central and neuromuscular synapses. J. Neurosci. 26:13493–13504.

Pang ZP, Südhof TC. 2010. Cell Biology of Ca2+-Triggered Exocytosis. Curr. Opin. Cell Biol. 22:496–505.

Perin MS, Brose N, Jahn R, Sudhof TC. 1991. Domain structure of synaptotagmin (p65). J. Biol. Chem. 266:623–629.

Perin MS, Fried VA, Mignery GA, Jahn R, Südhof TC. 1990. Phospholipid binding by a synaptic vesicle protein homologous to the regulatory region of protein kinase C. Nature 345:260–263.

Perin MS., Johnston PA., Özcelik T., Jahn R., Francke U., Südhof TC. 1991. Structural and functional conservation of synaptotagmin (p65) in Drosophila and humans. J. Biol. Chem. 266:615–622.

Poirier MA, Xiao W, Macosko JC, Chan C, Shin YK, Bennett MK. 1998. The synaptic SNARE complex is a parallel four-stranded helical bundle. Nat. Struct. Biol. 5:765–769.

Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE. 1993. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75:409–418.

Südhof TC. 2012. Calcium control of neurotransmitter release. Cold Spring Harb. Perspect. Biol. 4:a011353.

Sugita S, Han W, Butz S, Liu X, Fern??ndez-Chac??n R, Lao Y, S??dhof TC. 2001. Synaptotagmin VII as a plasma membrane Ca2+ sensor in exocytosis. Neuron 30:459–473.

Sutton RB, Davletov BA, Berghuis AM, Sudhof TC, Sprang SR. 1995. Structure of the first C2 domain of synaptotagmin I: A novel Ca2+/phospholipid-binding fold. Cell 80:929–938.

Sutton RB, Fasshauer D, Jahn R, Brunger AT. 1998. Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. Nature 395:347–353.

Thomson AM. 2000. Facilitation, augmentation and potentiation at central synapses. Trends Neurosci. 23:305–312.

Ullrich B, Li C, Zhang JZ, McMahon H, Anderson RGW, Geppert M, Südhof TC. 1994. Functional properties of multiple synaptotagmins in brain. Neuron 13:1281–1291.

Wang P, Chicka MC, Bhalla A, Richards D a, Chapman ER. 2005. Synaptotagmin VII is targeted to secretory organelles in PC12 cells, where it functions as a high-affinity calcium sensor. Mol. Cell. Biol. 25:8693–8702.

Weber JP, Toft-Bertelsen TL, Mohrmann R, Delgado-Martinez I, S??rensen JB. 2014. Synaptotagmin-7 is an asynchronous calcium sensor for synaptic transmission in neurons expressing SNAP-23. PLoS One 9:1–22.

Wen H, Linhoff MW, Mcginley MJ, Li G, Corson GM, Mandel G, Brehm P. 2010. Distinct roles for two synaptotagmin isoforms in synchronous and asynchronous transmitter release at zebra fish neuromuscular junction. Proc. Natl. Acad. Sci. 107:13906–13911.

Zhou Q, Lai Y, Bacaj T, Zhao M, Lyubimov AY, Uervirojnangkoorn M, Zeldin OB, Brewster AS, Sauter NK, Cohen AE, et al. 2015. Architecture of the synaptotagmin-SNARE machinery for neuronal exocytosis. Nature 525:62–67.