Journal Club

Editor's Note: These short reviews of a recent paper in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to mimic the journal clubs that exist in your own departments or institutions. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Synaptotagmin: Is 2 Better than 1?

Katherine Leitzell
Neuroscience Graduate Program, University of Southern California, Los Angeles, California 90089
Review of Pang et al. (http://www.jneurosci.org/cgi/content/full/26/52/13493)

The synaptotagmin protein family, characterized by two C-terminal calcium-binding motifs, is expressed throughout the brain (Grise et al., 2007). Synaptotagmin-1, the best characterized member, acts as a calcium sensor in the Ca^{2+}-dependent release of neurotransmitter vesicles (Geppert et al., 1994) and is necessary for calcium-dependent exocytosis in invertebrates (Littleton et al., 1993). However, knock-out studies in mice demonstrated that synaptotagmin-1 was necessary for one type of Ca^{2+}-dependent exocytosis (fast, synchronous release), but not for another type (asynchronous release) (Geppert et al., 1994). Of the synaptotagmin family, synaptotagmin-2 exhibits the highest degree of homology with synaptotagmin-1. Indeed, synaptotagmin-2 can rescue synaptotagmin-1 deficiency, and its expression overlaps partially but not completely with synaptotagmin-1 (Geppert et al., 1994; Nagy et al., 2006).

In a recent *Journal of Neuroscience* article, Pang et al. (2006) generated a synaptotagmin-2 knock-out mouse to examine the expression and function of synaptotagmin-2. Pang et al. (2006) characterized the expression pattern of the protein, the consequences of deletion on survival, and the electrophysiological properties of synaptotagmin-2-deficient neurons in the forebrain and neuromuscular junction (Table 1). The authors inserted LacZ in the exon-2 region of the synaptotagmin-2 gene, allowing them to simultaneously examine the expression of synaptotagmin-2 and the consequences of its deletion. The expression of other synaptic vesicle proteins was not altered except for a slight upregulation of synaptotagmin-1 in the spinal cord [Pang et al. (2006), their Fig. 1 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F1), Table 1 (http://www.jneurosci.org/cgi/content/full/26/52/13493/T1)].

The knock-out mice exhibited motor dysfunction and reduced body weight and growth and survived no longer than 24 d after birth, a survival length similar to that of synaptotagmin-1-deficient mice [Pang et al. (2006), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F2)]. Pang et al. (2006) suggest that expression of synaptotagmin-1 at the neuromuscular junction is initially sufficient to maintain synaptic transmission in the absence of synaptotagmin-2. However it would be interesting to know the time course of expression of the two proteins as the mice mature. For example, early expression of synaptotagmin-1 could initially compensate for the absent synaptotagmin-2 but then be switched off later in development.

Synaptotagmin-2 was expressed heavily in the brainstem and spinal cord but only weakly in forebrain. Expression was confined to only a few areas in the forebrain, including the striatum, hypothalamus, and reticular nucleus of the thalamus, whereas expression in the cerebellum, brainstem, and spinal cord was more robust [Pang et al. (2006), their Figs. 3 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F3), 4 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F4)]. Immunostaining confirmed that synaptotagmin-1 and -2 are coexpressed at neuromuscular junctions and that synaptotagmin-1 expression was upregulated in synaptotagmin-2-deficient endplates [Pang et al. (2006), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F5)]. The authors note that expression seemed to be confined to inhibitory neurons in the forebrain and cerebellum.

The authors examined the function of synaptotagmin-2 in forebrain neurons, as well as at the neuromuscular junction. In inhibitory neurons in the striatum, deletion of synaptotagmin-2 did not alter the amplitude or charge transfer of evoked IPSCs, but it did delay the time course of release, effectively slowing down the slow component of Ca^{2+}-dependent release [Pang et al. (2006), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F6)]. This suggests that synaptotagmin-2 is not necessary for calcium-dependent release but can affect the slow component of release. Spontaneous release, as measured by miniature endplate potentials, was not affected.

At the neuromuscular junction, at which synaptotagmin-2 is highly expressed, deletion altered spontaneous release, evoked release, and high-frequency stimulation. The frequency of spontaneous release was increased, both in the presence of calcium and when calcium chelators were used [Pang et al. (2006),

Received Feb. 14, 2007; revised Mar. 5, 2007; accepted Mar. 6, 2007.
Correspondence should be addressed to Katherine Leitzell, Neuroscience Graduate Program, University of Southern California, 3641 Watts Way, Los Angeles, CA 90089. E-mail: kleitzel@usc.edu.
DOI:10.1523/JNEUROSCI.0668-07.2007
 0270-6474/07/274231-02$15.00/0

Table 1. Summary of electrophysiological findings

Measurement	Striatal neurons	Neuromuscular junction
Spontaneous release (mEPPs)	Unchanged	>10-fold increase in mEPP frequency, independent of calcium
Evoked synaptic responses	Slow component of IPSC slowed	Slow component of synaptic response slowed
	Amplitude and charge transfer not altered	Amplitude and quantal content decreased
Paired-pulse facilitation	Not measured	Decrease in paired-pulse ratio
High-frequency stimulus trains	Not measured	Desynchronization of vesicle release
		Facilitation of synaptic response (10 or 20 Hz)

mEPP, Miniature endplate potential.

their Fig. 7 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F7)]. Thus, synaptotagmin-2 may have a function independent of Ca^{2+} sensing. Evoked synaptic responses and release probability were also decreased [Pang et al. (2006), their Figs. 8 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F8), 9 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F9)]. High-frequency stimuli led to an increase in failure rate [Pang et al. (2006), their Fig. 10 (http://www.jneurosci.org/cgi/content/full/26/52/13493/F10)]. This finding correlates well with the reduced evoked response and release probability. High-frequency stimuli also produced asynchronous vesicle release in synaptotagmin-2-deficient neuromuscular junctions and delayed release for several seconds after a stimulus train [Pang et al. (2006), their Fig. 11*A*,*B* (http://www.jneurosci.org/cgi/content/full/26/52/13493/F11)]. Stimulus trains (10–20 Hz) facilitated release in mutant neurons [Pang et al. (2006), their Fig. 11*F*,*G* (http://www.jneurosci.org/cgi/content/full/26/52/13493/F11)], which the authors suggest could indicate that a buildup of Ca^{2+} during the stimulus trains partially rescues the deficiency (Pang et al., 2006).

These experiments demonstrate that synaptotagmin-2 is not necessary for Ca^{2+}-dependent release at forebrain and spinal cord neurons but that it can regulate synaptic transmission. It is interesting that the deletion of synaptotagmin-2 produces such disparate phenotypes in striatal and spinal neurons. The reasons for this disparity remain unclear but could involve other members of the synaptotagmin family. For example, synaptotagmin-6, -7, and -9 have similar Ca^{2+}-sensing functions, suggesting that this family of molecules may work together to regulate synaptic transmission (Grise et al., 2007; Lynch and Martin, 2007; Monterrat et al., 2007).

Although previous studies suggested that synaptotagmin-2 has a similar function to synaptotagmin-1, Pang et al. (2006) demonstrate that synaptotagmin-2 plays additional, nonredundant roles in regulating synaptic transmission. Synaptotagmin-1 is known to act primarily as a Ca^{2+} sensor for fast synchronous release. Pang et al. (2006) show that, like synaptotagmin-1, synaptotagmin-2 plays an important role in Ca^{2+}-triggered release. However, they also demonstrate that the deletion of synaptotagmin-2 does not entirely block release and also increases spontaneous release independently of calcium. Therefore, synaptotagmin-1 and -2 do not function in an identical manner, and the expression of one or the other at a specific synapse may be important in determining how those neurons fire.

References

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

Grise F, Taib N, Monterrat C, Lagree V, Lang J (2007) Distinct roles of the C 2A and the C 2B domain of the vesicular Ca2+ sensor synaptotagmin 9 in endocrine beta-cells. Biochem J, in press.

Littleton JT, Bellen HJ, Perin MS (1993) Expression of synaptotagmin in *Drosophila* reveals transport and localization of synaptic vesicles to the synapse. Development 118:1077–1088.

Lynch KL, Martin TF (2007) Synaptotagmins I and IX function redundantly in regulated exocytosis but not endocytosis in PC12 cells. J Cell Sci 120:617–627.

Monterrat C, Grise F, Benassy MN, Hemar A, Lang J (2007) The calcium-sensing protein synaptotagmin 7 is expressed on different endosomal compartments in endocrine, neuroendocrine cells or neurons but not on large dense core vesicles. Histochem Cell Biol, in press.

Nagy G, Kim JH, Pang ZP, Matti U, Rettig J, Sudhof TC, Sorensen JB (2006) Different effects on fast exocytosis induced by synaptotagmin 1 and 2 isoforms and abundance but not by phosphorylation. J Neurosci 26:632–643.

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