a result, the RGC's are substantially less light sensitive than the intact retina. These are solvable problems. This publication is clearly a significant first step into this new field of re-engineering retinal interneurons as genetically modified "prosthetic" cells.

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Kissing and Pinching: Synaptotagmin and Calcium Do More between Bilayers

Building on recent findings that synaptotagmin (Syt) participates in synaptic vesicle endocytosis, Poskanzer et al., in this issue of *Neuron*, show distinct mechanisms by which Syt functions in this process. Most significantly, they show (1) that calcium binding to Syt determines the rate but not fidelity of vesicle recycling and (2) that mutations in a different Syt domain affect the shape but not rate of formation of recycled synaptic vesicles.

Calcium triggering of synaptic vesicle exocytosis is mediated by calcium binding to the synaptic vesicle membrane protein, synaptotagmin (Koh and Bellen, 2003). Following exocytosis, synaptic vesicles are retrieved by an endocytic mechanism. Two interesting features of the latter process are that (1) in order to maintain vesicle pools and plasma membrane morphology, the rate of synaptic vesicle endocytosis must be regulated to match the rate of exocytosis; and that (2) endocytic proteins must associate with cytoplasmic domains of synaptic vesicle proteins in the context of plasma membrane, but not in the context of synaptic vesicles. Thus, there are at least two conceptually different regulatory steps: one determines the *rate* of endocytosis and the other the *need* for endocytosis of vesicle proteins. Experiments by Poskanzer et al. (2006) indicate that synaptotagmin participates in both of these regulatory steps, by distinct molecular mechanisms.

Syt's involvement in endocytosis has long been suspected, based not only on its biochemical interactions with several endocytic proteins, but also on phenotypes of *C. elegans* mutants lacking synaptotagmin (Jorgensen et al., 1995). However, strong, direct evidence for Syt function in synaptic vesicle reformation comes from recent, parallel studies of Syt 1 mutant synapses, both at the *Drosophila* neuromuscular junction and in cultured mouse cortical neurons (Nicholson-Tomishima and Ryan, 2004; Poskanzer et al., 2003). These analyses measured kinetics of synaptic vesicle reformation using a pH-sensitive green fluorescent protein (pHluorin) targeted to the luminal domain of synaptic vesicles (Miesenbock et al., 1998). Thus, the differential fluorescence of pHluorin exposed to pH 7 after exocytosis compared to pH ~6 within synaptic vesicles reports on the fraction of vesicles in mature synaptic vesicles. Both papers used clever methods to separate and measure rates of exocytosis and endocytosis that occur simultaneously at nerve terminals. For example, Nicholson-Tomishima and Ryan (2004) used bafilomycin to block reacidification of synaptic vesicles after exocytosis and thus measure the rate of exocytosis under given experimental conditions. Poskanzer et al. (2003) used conditional temperature shifts of *shi*^{ts} mutants or temporally controlled photoinactivation of FlAsH-tagged synaptotagmin to isolate and analyze the role for Syt I in synaptic vesicle endocytosis. Strikingly, both *Drosophila* and mouse studies concluded that Syt I was necessary for normal endocytosis of synaptic vesicles.

Here, exploiting previous structure-function analyses of Syt (Chapman, 2002), Poskanzer et al. analyze how Syt variants, with defects in specific molecular interactions, function in synaptic vesicle recycling. First, they make the very interesting observation that mutations in the Ca^{2+}-coordinating aspartate residues of the C_2B domain (Syt-D3,4N), but not C_2A, inhibit synaptic vesicle recycling. This observation implicates for the first time the calcium-binding function of Syt in vesicle recycling. Direct evidence that the effect of these C_2B mutations is through altered calcium binding is provided by an additional experiment. Increased extracellular calcium substantially enhances recycling rates of synapses

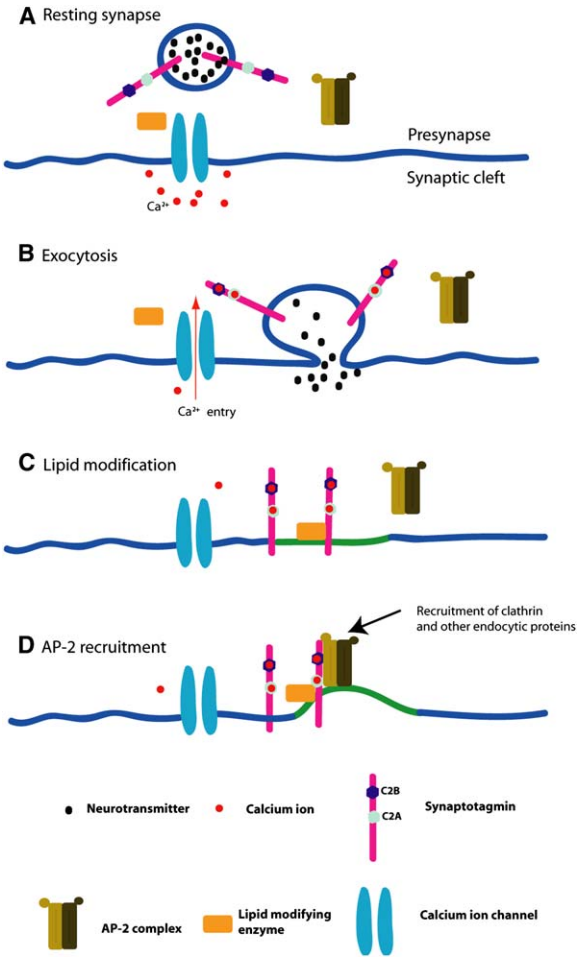

Figure 1. A Model to Explain Two Activities of Synaptotagmin

The figure above proposes that calcium binding to Syt promotes (but may be dispensable for) activation of a lipid-modifying enzyme (Wenk and De Camilli, 2004). Lipid modification enables coat protein (e.g., AP-2) interactions with synaptic vesicle membrane, a process facilitated through a second activity of synaptotagmin. A postulate that the stage prior to the entry of coat proteins is rate determining (at least in the specific mutants examined) explains the effect of Syt-D3,4N on the rate of endocytosis. Subsequent correct assembly of a clathrin coat around synaptic vesicle proteins determines the fidelity of vesicle internalization. This assembly is compromised in Syt-KQ. For simplicity, additional and potentially contributing processes, e.g., V-SNARE/T-SNARE association and different routes of calcium entry (Kuromi et al., 2004) are not included in this model.

expressing Syt-D3,4N; in contrast, it has no effect on the rate of recycling of synapses expressing wild-type Syt. Thus, a low calcium binding affinity of this Syt-D3,4N accounts for the observed slow rate of recycling.

That calcium binding influences rate, but not fidelity, of the process is suggested by EM analyses of Syt-D3,4N mutant and control synapses, performed 1 min after cessation of tetanic 50 Hz stimulation. Recovered synaptic vesicles are found to have normal (control) vesicle size and distribution. This effect on rate but not form contrasts nicely with the effect of Syt-KQ-expressing synapses, in which a lysine motif that forms the site of interaction between mammalian Syt I and the clathrin adaptor protein AP-2 has been mutated. Remarkably, this mutation causes a specific defect in the size of vesicles reformed after 50 Hz stimulation, but does not affect the rate of endocytosis. Thus, for the first time, a mechanism that controls endocytic rate has been separated from one that regulates morphology and/or composition of synaptic vesicles.

Figure 1 attempts to place these physiological observations in a mechanistic framework. Elaborated more completely in the figure legend, the model, in gist, proposes an early rate-determining step promoted by calcium binding to Syt and a later, fast step that governs the high-fidelity process of vesicle internalization. To appreciate the limitations of the model, it is worthwhile to consider what significant issues remain to be completely understood in the wake of the progress represented by the work of Poskanzer and colleagues.

First, while the rate of synaptic vesicle recycling is shown to depend on calcium binding by Syt, it remains unclear whether calcium-dependent acceleration of vesicle recycling is mirrored by the calcium dependence of Syt binding. Given that endocytosis occurs over a time scale substantially longer than rapid high-amplitude calcium transients that drive vesicle exocytosis, it is more than a formal possibility that slower or higher affinity calcium sensors may be involved in determining rates of compensatory endocytosis. Second, despite the allure of an all-encompassing model, it is important to appreciate that calcium may have multiple and diverse effects on the process of endocytosis. For instance, molecular explanations for phenomena such as calcium repression of vesicle endocytosis in goldfish bipolar neurons (von Gersdorff and Matthews, 1994) or the rapid calcium triggering of endocytosis in mast cells remain poorly understood (Henkel and Almers, 1996). Finally, but no less important, the molecular consequences of calcium binding to Syt on the molecular processes driving endocytosis remain to be discovered.

Thus, this study from Graeme Davis's group serves not only to inform us of mechanisms of synaptic vesicle recycling, but also drives us to deeper and wider consideration of mechanisms that regulate endocytosis in eukaryotic cells.

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Shining Light on Spike Timing-Dependent Plasticity

Although spike timing-dependent plasticity has been well-characterized in vitro, it is less clear to what degree spike timing-dependent plasticity contributes to shaping visual system properties in vivo. In this issue of *Neuron*, two papers by Vislay-Meltzer et al. and Mu and Poo provide evidence that STDP contributes to the effects of sensory stimuli in refinement of the retinotectal system in *Xenopus*.

The N-methyl-D-aspartate glutamate receptor (NMDAR) is the Dr. Jekyll and Mr. Hyde of molecular neurobiology, being both an agent of adaptive plasticity and a killer in stroke and some neurodegenerative diseases. However, one of the most recently recognized (Markram et al., 1997), perhaps most ubiquitous, and still most mysterious aspects of this receptor concerns its role in spike timing-dependent plasticity (STDP), through which the strength of synaptic contacts are up- or down-regulated depending on the timing of presynaptically driven NMDAR current and a postsynaptic membrane depolarization, frequently but not always a spike (Lisman and Spruston, 2005). While it has long been known that fast, strong activation of synaptic NMDARs produces synaptic potentiation and that slow and/or weak activation results in depression, in STDP the timing of a postsynaptic response relative to presynaptically driven NMDAR current is critical. Synaptic activation preceding a postsynaptic spike by ~<40 ms is potentiated, while synapses that are active following a postsynaptic spike by <100–40 ms are depressed. STDP has been hailed as the synaptically controlled associative signal for Hebbian plasticity (Magee and Johnston, 1997), and it has added considerable computational power to models of how small timing differences can determine the strengthening or weakening of synapses (Abbot and Nelson, 2000). In general terms, STDP has allowed the popular idea of reinforcement of correlated pre- and postsynaptic firing to be implicated in hypotheses arising from empirical observations that only certain temporal patterns of input activity are effective in "training" or entraining postsynaptic cell firing (Froemke and Dan, 2002; Wang et al., 2005). Although still controversial in terms of mechanistic detail (Lisman and Spruston, 2005), there is broad agreement that NMDAR activity and timing events frequently less than a spike train in length are generated by natural stimuli and generate long-term changes in synaptic strength. Beyond this near consensus many issues arise, including how, or even if, true STDP is involved when neurons in the intact brain are activated by natural relatively nondiscrete stimuli.

There have been several studies of intact visual cortex (Fregnac and Shulz, 1999; Schuett et al., 2001; Yao and Dan, 2001) using natural stimuli and demonstrating changes in visual neuron response properties fully consistent with STDP. Nevertheless, there has been no preparation in which quantifiable single-cell responses to spike timing-dependent stimulation could be studied at a single-neuron level while natural stimuli (as opposed to electrically induced spikes) produced the postsynaptic response. Two papers in this issue of *Neuron* (Vislay-Meltzer et al., 2006; Mu and Poo, 2006) now make this essential jump between in vitro demonstration of STDP at the single-cell level and in vivo proof that the same outcome is observed when natural stimuli drive the postsynaptic response. Both papers use perforated patch-clamp recording from single tectal neurons of anesthetized *Xenopus* larvae in a preparation in which a light-emitting grid is placed on the retina to deliver computer-controlled stimulation via the retinal ganglion cell projection to the region of the tectum in which a target cell held under perforated patch-clamp recording conditions resides. As has been done before, in both papers visual stimuli are applied to the retina at various times before or after a spike is generated through the patch electrode on the postsynaptic neuron. STDP is demonstrated either as a change in the size or position of the neuron's receptive field (Vislay-Meltzer et al., 2006) or in the neuron's direction selectivity for a moving bar stimuli (Mu and Poo, 2006).

One problem with using natural stimulation and the polysynaptic inhibitory and excitatory responses recorded in the target is defining a precise relationship between the visually evoked spike train input and the complex postsynaptic current generated in the target. In the Vislay-Meltzer et al. (2006) report, effects of polysynaptic responses were minimized by restricting analyses to the initial excitatory portion of the response. The question asked was whether the timing of a suprathreshold retinal visual stimulus presented in the center of the receptive field of the tectal neuron could either enhance or depress the neuron's response to a training stimulus presented within a subregion of the receptive field and, therefore, change the boundaries of that receptive field. Initially, the authors mapped the receptive field of the target neuron using a standard procedure: small spots of randomly presented light were generated on the visual array while voltage-clamp recordings measured the current response as the integrated area under the curve. Subsequently, to obtain a precise and rapid input/output relationship between the visual stimulus and the position-dependent response of the target neuron, flickering, band-pass filtered white noise stimuli were presented to the retina, and for each stimulated pixel a reverse correlation analysis was applied to characterize the input and the cell's response to it (De Boer and Kuyper, 1968). The procedure yields a response-weighted average of the output to stimulation of every