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Synaptic Vesicles: Half Full or Half Empty?

The regulation of quantal size through pre- rather than postsynaptic mechanisms has recently received considerable attention as a potential mechanism for plasticity. Vesicular transporters catalyze the filling of synaptic vesicles with transmitter and are thus potential substrates for such presynaptic regulation. In this issue of *Neuron*, Prado et al. pursue this line of investigation and show that changes in transporter expression that alter quantal size can affect behavior.

The fundamental unit of synaptic transmission is the quantum—the postsynaptic response to the release of a single synaptic vesicle. Alterations in synaptic strength are thus typically thought to reflect either a presynaptic change in the likelihood of vesicle fusion (i.e., release probability) or a change in quantal size due to a postsynaptic modification in sensitivity (e.g., change in receptor density). However, it is now clear that at certain synapses, postsynaptic receptors are not saturated during release, and that a presynaptic change in vesicle filling can also modulate quantal size (Van der Kloot, 1990; Mainen et al., 1999).

What mechanisms might regulate the amount of neurotransmitter per vesicle? Transport into neurosecretory vesicles is catalyzed by proteins that couple the uptake of transmitter to the movement of H^+ in the opposite direction. A vesicular H^+-ATPase similar to the F0/F1-ATPase in mitochondria provides the H^+ electrochemical gradient that drives transmitter uptake. Thus, changes in the activity of the H^+-ATPase, the flux of other ions that influence the H^+ electrochemical gradient (e.g., chloride), and the cytoplasmic concentration of substrate will influence how much transmitter is stored in a vesicle. In addition, the expression and activity of transporter has been shown to influence the amount of transmitter per vesicle. Overexpression of the vesicular acetylcholine transporter (VAChT) or neuronal vesicular monoamine transporter (VMAT2) increases quantal size in culture (Song et al., 1997; Pothos et al., 2000). Homozygous knockouts of VMAT2 die within days after birth, but reduced expression in heterozygotes leads to deficits in dopamine release and modifies the behavioral response to amphetamine (Fon et al., 1997; Takahashi et al., 1997; Wang et al., 1997; Patel et al., 2003). Thus, modest changes in transporter expression have the potential to influence behavior. The question remains, when does the level of vesicular transport become limiting for a range of behaviors?

In this issue of *Neuron*, Prado and colleagues developed an in vivo mouse model of reduced VAChT expression (Prado et al., 2006). Since VAChT knockouts are likely to die at birth from respiratory failure, the authors generated a knockdown animal that produces less VAChT protein (50% less than wild-type for heterozygote, 70% less for homozygote). Studied at the neuromuscular junction, the mutants exhibit a moderate reduction in the amplitude of miniature end-plate potentials (quantal size), suggesting a reduction in vesicular content at steady state. It is also possible that the electrophysiologic analysis underestimates the magnitude of changes in quantal size due to homeostatic compensation in the postsynaptic cell. In addition, homozygous knockdown mice show increased synaptic depression to high-frequency stimulation, suggesting a more severe defect in filling recycled vesicles than spontaneously released vesicles, which presumably have more time to fill. However, the duration of the stimulus was extremely short (0.5 s), and it would be interesting to determine whether depression is more dramatic with prolonged stimuli that provide sufficient time for vesicle recycling. On the other hand, synaptic vesicle exocytosis appears to be normal even though the vesicles are not full, consistent with previous reports using the VAChT inhibitor vesamicol, studies using the VMAT2 knockout, and observations at glutamatergic synapses in the hippocampus (Parsons et al., 1999; Zhou et al., 2000; Croft et al., 2005). Using in vivo microdialysis, Prado et al. also find that central cholinergic neurotransmission is reduced (Prado et al., 2006). Thus, changes in VAChT expression lower the amount of ACh per vesicle, which leads to reduced quantal size and evoked release.

Acetylcholine signaling has long been associated with hippocampus-dependent learning, and reduced ACh is one of the hallmarks of memory-related illnesses such as Alzheimer's disease (Coyle et al., 1983). Because the genetic manipulation was made in mice, the authors were able to assay the consequences of reduced vesicle filling for learning and other behaviors. Homozygous VAChT knockdown mice display a severe neuromuscular phenotype that would confound the analysis of more complex behaviors, but heterozygotes exhibit limited, if any, neuromuscular deficit. On the other hand, heterozygotes differ substantially from wild-type on habituation tasks involving complex cues. The heterozygous mutant animals habituate more slowly to novel objects, including intruder mice, suggesting reduced ability to form or retrieve memories. Remarkably, this learning deficit was rescued by performing the experiments in the presence of acetylcholinesterase inhibitors, indicating that raising cholinergic tone compensates for the reduced quantal size. Interestingly, VAChT knockdown mice do not show a deficit in habituation to a simple olfactory cue. They also show no difference from wild-type in active avoidance learning to an unconditioned foot shock. These data suggest that some forms of learning are more dependent on ACh than others. Among those synapses dependent on ACh, some may also be more sensitive to changes in VAChT expression, perhaps due to the high rates of vesicle recycling required for behavior. However, future experiments using classical learning tasks such as the Morris water maze, fear conditioning, or operant responding will help determine the full extent of the deficit. The ability to rescue with acetylcholinesterase inhibitors should also prove a useful tool for characterizing the defect in learning through treatment

of the mice only during the acquisition or retrieval phases of the various tasks. Additionally, experiments in older animals may reveal age-dependent deficits resulting from reduced VAChT expression (Paban et al., 2005). The work also suggests a role for cholinergic transmission in behaviors involving social preference, independent of learning, and it will be interesting to explore this further.

Limitations of the knockdown mutation are that ACh release is not completely eliminated, and that the mutation affects release at all cholinergic synapses. Although acetylcholine has a role in behavior mediated by the hippocampus, it also acts in cortex and striatum, and its role in these locations is less well understood. It is thus important to note that the construct used by Prado et al. contains loxP sites flanking exon 1 (Prado et al., 2006), which should enable conditional deletion of the gene in specific central cholinergic populations.

To understand how the presynaptic regulaton of quantal size contributes to synaptic transmission, we must first understand the mechanisms responsible. If synaptic vesicles fill to an equilibrium dictated by the ionic coupling of the transporter, then having one transport protein per vesicle should produce the same quantal size as several transporters (Daniels et al., 2006). On the other hand, high rates of recycling may limit the time available for filling, making quantal size dependent on the number of transporters. Increased expression may also serve to offset a nonspecific leak through the vesicle membrane (the "leaky bathtub" model) (Williams, 1997). In addition, it is important to note that all postsynaptic measurements of quantal size involve spontaneously released vesicles, and that these may differ from vesicles capable of evoked release, particularly at central synapses (Sara et al., 2005). Further, there are no measurements of quantal size during high-frequency stimulation of neurons, so we still understand little about how vesicle filling contributes to synaptic depression. Nonetheless, Prado et al. demonstrate the potential for presynaptic regulation of quantal size to alter synaptic physiology in a behaviorally relevant context (Prado et al., 2006).

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Subplate Neurons Foster Inhibition

Previous work demonstrates an essential role of subplate neurons during ocular dominance (OD) column formation in the developing visual cortex. While inhibitory circuitry has also been shown to play an essential role in OD plasticity, the relationship between subplate neurons and the development of inhibitory circuits has been unclear. In this issue of *Neuron*, Kanold and Shatz provide evidence that maturation of inhibitory circuitry requires subplate neurons in the developing cortex.

Visual cortex is the first stage of the mammalian visual pathway where information from the two eyes, relayed through the thalamus, is combined. During brain development, thalamic axons from the two eyes are initially overlapped in layer 4 of the developing visual cortex, but during subsequent development, they are segregated into eye-specific patches (OD columns) (Figure 1A; Hubel et al., 1977; Levay et al., 1980). Prolonged monocular deprivation (occlusion of one eye) can cause a shift in ocular dominance, in which cortical neurons become responsive exclusively to the open eye (Wiesel and Hubel, 1963). In the visual cortex of the monocular deprived animal, the thalamic axons driven by the open (active) eye occupy a larger territory, while the territory occupied by the axons driven by the occluded (less active) eye shrink (Hubel et al., 1977; Levay et al., 1980). The OD shift can be induced only during a short period after the time of natural eye opening, called the critical period (Hubel and Wiesel, 1970). Thus, the OD plasticity is specific to a restricted period in early cortical development.