Vesicles: Equal in Neurotransmitter Concentration but Not in Volume

The quantal nature of neurotransmitter release is often considered to provide the elemental unit for information processing by the nervous system. Changes in synaptic strength are generally thought to involve the probability of exocytotic release and the postsynaptic response, rather than the amount of neurotransmitter per vesicle. And if postsynaptic receptors were invariably saturated by the neurotransmitter released from a single synaptic vesicle (SV), it would not matter how much each vesicle contains. However, considerable evidence indicates that postsynaptic receptors are not saturated at many synapses. Neurotransmitters also spill over to activate adjacent synapses. Thus, changes in the amount of transmitter released per vesicle (*quantal size*) clearly have the potential to alter the postsynaptic response. In addition, the amount of transmitter released per SV appears to vary considerably, even at single synapses (Liu et al., 1999; Van der Kloot, 1990). Thus, quantal release of neurotransmitter is not the invariant, elemental unit of synaptic transmission it is often considered. So what determines the amount of neurotransmitter per vesicle?

Very similar to the inwardly directed Na^+ gradient across the plasma membrane that drives neurotransmitter reuptake from the synaptic space, a H^+ electrochemical gradient drives the transport of transmitter into secretory vesicles. Transport into vesicles involves H^+ exchange, and the stoichiometry of ionic coupling influences the concentration gradients produced across the vesicle membrane. The magnitude of the outwardly directed proton electrochemical gradient and the cytoplasmic concentration of transmitter also determine the luminal concentration finally achieved. In the case of monoamines, the stoichiometry and driving force together predict concentration gradients of 10^4–10^5, and luminal concentrations in the low molar range have been demonstrated in large dense core vesicles (LDCVs). However, the dense core of LDCVs contains insoluble monoamine, which can greatly increase the amount of transmitter released without altering the concentration gradient of soluble transmitter across the vesicle membrane. Unlike LDCVs, which also contain neural peptides, SVs that release only classical small molecule transmitters do not exhibit an obvious dense core. They may contain other luminal components that reduce the solubility of the transmitter and hence increase its potential concentration, but these are certainly not as conspicuous as in LDCVs.

In this issue of *Neuron*, Bruns and colleagues use amperometry to measure the release of serotonin from the SVs and LDCVs of leech Retzius neurons (Bruns et al., 2000). Amperometry relies on the electrochemical detection of monoamines, due to their oxidation at characteristic potentials, to measure the amount of transmitter released directly and in real time, independent of the saturation or kinetics of postsynaptic receptors (Michael and Wightman, 1999). Bruns and Jahn previously used amperometry to characterize the release of serotonin from Retzius neurons and identified two modes of re-

lease that correspond to release from two distinct populations of secretory vesicles, LDCVs and SVs (Bruns and Jahn, 1995). As observed in other neurons and secretory vesicles, the two types of release differ in site and speed as well as amount (Sulzer and Pothos, 2000). In the Retzius cell, LDCVs release 10^5–10^6 molecules of monoamine over several milliseconds whereas SVs release $\sim 10^4$ molecules in less than a millisecond.

The correlation of careful morphometric analysis by electron microscopy with amperometry allows the authors of the new study to draw some very surprising conclusions (Bruns et al., 2000). First, LDCVs and SVs fill with roughly the same concentration of transmitter (270 mM). Differences in the amount of transmitter released thus primarily reflect the differences in vesicle volume, not the presence of the dense core. The correlation between monoamine release and vesicle size also holds between smaller LDCVs observed at the nerve terminal and larger LDCVs at the cell body. Second, the correlation between the size of the amperometric events and vesicle volume holds even within the population of SVs. The size distribution of small amperometric events corresponds very well to the distribution of the cubed vesicle radii, indicating that all the SVs fill to roughly the same concentration with neurotransmitter. However, the variation in large amperometric events exceeds that predicted by the variation in volume of LDCVs. In this case, fluctuation in the size of the dense core may contribute more or less insoluble monoamine that perturbs the otherwise tight correlation between soluble transmitter and vesicle size. Alternatively, vesicles may not always release their total transmitter content. Nonetheless, the results strongly suggest that all SVs and LDCVs achieve very similar luminal concentrations of releasable transmitter. These similar concentrations presumably reflect a stable equilibrium determined by the cytoplasmic concentration of transmitter, the H^+ electrochemical driving force, and the ionic coupling of the vesicular monoamine transport protein.

If vesicle filling reaches a stable equilibrium based on the transport mechanism, the number of transport proteins should make little difference to the luminal concentration of transmitter. Surprisingly, a number of observations have indicated that the level of transporter expressed even under physiological conditions limits the storage and release of both monoamines and acetylcholine (Reimer et al., 1998). How can we reconcile the apparently stable filling equilibrium achieved by secretory vesicles of leech Retzius neurons with the effect of transporter expression on quantal size? First, Retzius neurons in culture are quiescent, whereas the ongoing release of monoamines by mice in vivo may require higher rates of filling and hence higher levels of transporter expression. If the rate of SV recycling exceeds that of vesicle filling, the level of transporter expressed could dramatically affect the concentration of monoamine achieved (see figure, panel A). In this case, transporter expression affects only the rate of vesicle filling, not the equilibrium that would ultimately be achieved in quiescent neurons. The very slow turnover reported for vesicular monoamine and acetylcholine transporters (1–5/s) and the effect of activity on dopamine release by vesicular monoamine transporter 2 (VMAT2)–deficient
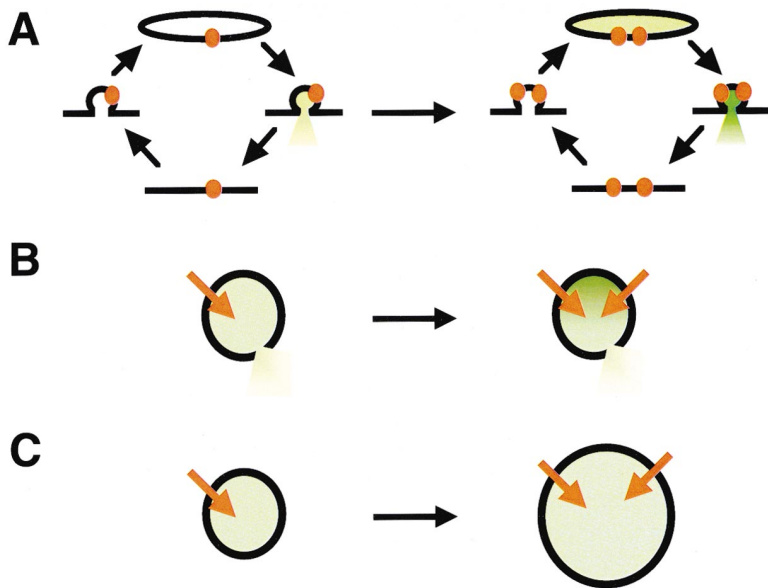
Mechanisms by which the Level of Transporter Expression May Influence Vesicle Filling

(A) If synaptic vesicles cycle faster than they fill with transmitter (green), increases in transporter expression (red balls), which increase the rate of filling, would result in higher luminal amounts at the time of exocytosis. If vesicles cycle more slowly than they fill, increases in transporter expression should not increase luminal amounts because filling would have already reached equilibrium before exocytosis.

(B) If synaptic vesicles exhibit a constant, nonspecific leak of neurotransmitter (the *leaky bathtub model)*, increases in the rate of filling due to increased transporter expression (red arrows) will result in higher luminal concentrations.

(C) Increases in transporter expression increase the amount but not the concentration of transmitter per vesicle by increasing vesicle volume.

mice support a role for this mechanism (Reimer et al., 1998).

Second, filling may oppose a nonspecific efflux of monoamine from the vesicle (the *leaky bathtub model*) (see figure, panel B). By increasing the amount of transmitter accumulated in the presence of a relatively fixed leak, increased transporter expression alters the equilibrium ultimately reached, not simply the rate at which it is achieved. Third, vesicles may sense filling with transmitter and shut down transport at a particular luminal concentration. Interestingly, the heterotrimeric G protein G_{o2} has been observed to associate with secretory vesicles and to inhibit VMAT2 function (Höltje et al., 2000). However, loading with L-DOPA, as well as increases in VMAT2 expression, also increases quantal monoamine release (Pothos et al., 2000), suggesting that a sensor with a specific threshold does not exist. Rather, the close correlation between variation in amperometric events and SV volume observed by Bruns and colleagues suggests a surprising alternative mechanism for the apparent uniformity of transmitter concentration.

LDCVs and SVs may fill to a limit set by osmolarity as well as to an equilibrium governed by the cytoplasmic concentration of transmitter, the driving force, and the ionic coupling of transport. Consistent with this possibility, the luminal concentration of monoamine estimated by Bruns et al. (270 mM) is very close to isotonic. But how can the amount of transmitter per vesicle be increased? If the concentration of transmitter remains constant, this predicts that the vesicles swell. Remarkably, two recent papers provide direct support for this hypothesis (see figure, panel C). Using voltammetry to measure the *concentration* of catecholamine very close to release sites on rat PC12 cells (amperometry measures total *amount* rather than concentration), Ewing and colleagues show that although L-DOPA increases the number of transmitter molecules released per quantum, it does not increase the resulting transmitter concentration (Kozminski et al., 1998). More recently, the group has provided direct evidence that LDCVs swell

as the quantal size is increased by L-DOPA (Colliver et al., 2000). The halo of soluble material surrounding the dense core increases in volume. Conversely, the VMAT inhibitor reserpine reduces vesicle volume. Further, these observations suggest an explanation for the close correlation between variation in quantal size and SV volume observed by Bruns et al.—increased filling presumably distends the vesicle rather than increasing the luminal concentration of transmitter. The energy provided by the driving force that would otherwise have increased the concentration gradient across the vesicle membrane may instead distend the wall of the vesicle.

It is remarkable that secretory vesicles can increase dramatically in volume (up to 4-fold) without rupture. Changes in biogenesis have been observed to alter both synaptic vesicle volume and quantal size (Zhang et al., 1998), but the changes observed after loading monoamine cells with L-DOPA presumably involve vesicles that have already formed. Perhaps a characteristic composition of lipids helps the vesicles to accommodate such large increases in volume.

In summary, recent work indicates the potential for changes in quantal size to contribute to synaptic plasticity. However, the mechanism may involve changes in vesicle volume rather than transmitter concentration. In addition, we do not yet understand how the rate of vesicle cycling compares to filling with transmitter, and this may provide an additional constraint on quantal size. Finally, neurosecretory vesicles contain more than just neurotransmitter: what happens to other contents during the process of filling? For example, rapidly cycling synaptic vesicles contain extracellular concentrations of Na^+ and Cl^-, and we do not know the fate of these ions. These questions are particularly important because, unlike massive ionic shifts at the plasma membrane that do not perturb bulk phase concentrations in the cytoplasm or extracellular space, translocation across the vesicle membrane dramatically affects the luminal concentration of ions as well as neurotransmitter.

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Sound Amplification in the Inner Ear: It Takes TM to Tango

Sound impinging upon a mammal's ear must travel along a wonderfully elaborate series of membranes, levers, and tubes to reach its cellular receiver within the cochlea, the mechanosensory hair cell. After coursing down the ear canal and deflecting the tympanic membrane, each compression cycle of a sound wave causes the piston-like stapes to push on a window into the cochlea, thereby increasing the pressure in a fluid-filled tube called the scala vestibuli (see figure, panel A). Increased pressure in the scala vestibuli and its neighboring tube, the scala media, pushes down on the organ of Corti, a ribbon of sensory hair cells and supporting cells that runs the length of the cochlea. The organ of Corti is suspended on the basilar membrane, a trampoline-like array of elastic fibrils that deflects under pressure, so that it bounces slightly with each cycle of sound (see figure, panel B). Adjacent to the organ of Corti, the greater epithelial ridge synthesizes and secretes a diaphanous extracellular matrix—the tectorial membrane—which is coupled at its far end to the tips of the hair cells' mechanosensory stereocilia (Kimura, 1966; Lim, 1987). Because the tectorial membrane and basilar membrane are essentially hinged at different points, there is shear between them at the level of the stereocilia. When the basilar membrane moves down, the stereocilia are pulled one way (to the left in the figure); as it moves up in the next half-cycle, the stereocilia are pushed to the right. Stereocilia deflection opens transduction channels to cause a receptor potential in all hair cells, but only the inner hair cells use their receptor potentials to modulate neurotransmitter release onto the postsynaptic spiral ganglion neurons. Although they sense the vibration, outer hair cells do not seem to make functional synapses on postsynaptic neurons.

But then something magic happens. Somehow the outer hair cells respond to the vibration of the basilar membrane by pushing back on it, exerting force with just the right amplitude and phase to amplify the vibration, especially for faint sounds, by 100-fold or more. The movement of the basilar membrane is amplified from hundredths of nanometers to around a nanometer for the quietest perceptible sounds, from a nanometer to several nanometers at conversational level, but not much at all for loud sounds. In a healthy ear, movement is therefore not linearly proportional to sound level, but shows a compressive nonlinearity. Immediately postmortem, or even with acute occlusion of cochlear blood flow, the active amplification disappears and the basilar membrane movement is proportional to sound level. Moreover, outer hair cells in each region of the long organ of Corti only amplify sound of a particular frequency, so that each region is exquisitely tuned to a characteristic frequency (CF) and not to other frequencies. Inner hair cells then sense the amplified vibration, and send a frequency-specific signal to spiral ganglion neurons of the eighth nerve (Hudspeth, 1997).

A new study from Guy Richardson, Ian Russell, and their colleagues (Legan et al., 2000 [this issue of *Neuron*]) explores the role of the tectorial membrane in active tuning of the basilar membrane. Over the last decade, Richardson's group has made the major contribution to our understanding of the structure of the tectorial membrane (TM). The TM is composed of collagens—types II, V, and IX—and of collagenase-insensitive glycoproteins. The collagen fibrils run radially across the TM and are embedded in a striated-sheet matrix composed of two types of 7–9 nm filaments. Two of the major glycoproteins—α- and β-tectorin—were purified from chick TM and cloned from chick and mouse by Richardson's group (Legan et al., 1997). α-tectorin is a large protein with an N-terminal entactin G1 domain, a central region with five full or partial von Willibrand factor type D repeats, and a C-terminal zona pellucida domain (Legan et al., 1997). Mutations in human α-tectorin underlie two dominantly inherited human nonsyndromic deafnesses, DFNA8 and DFNA12 (Verhoeven et al., 1998). Richardson's group has now generated a targeted deletion in the mouse α-tectorin gene (*Tecta*), and finds, remarkably, that the mouse has no functional TM. This genetic decoupling of basilar membrane vibration and outer hair cell feedback provides a physiological system in which to study the amplification.