Vesicle fiesta at the synapse

Gary Matthews

Communication between nerve cells is what makes our brains work. Several new studies offer us a close-up view of the chemical signals controlling these cellular conversations.

Nerve cells need to transfer information to each other, and researchers have been unravelling the secrets of how they do this for decades. Technical advances and human ingenuity have now combined to take us several steps further in this intellectual challenge. With the publication of two reports in this issue[1,2] (pages 849 and 889) and a third in *Science*[3], we can actually watch the intricate molecular dance that takes place when neurons talk to each other, and we learn more about the control mechanisms involved.

Neurons communicate at special junctions, known as synapses, where the transmitting cell releases a chemical signal into the small gap separating it from the receiving cell. When the transmitting neuron is stimulated, channels in its plasma membrane at the synapse open, allowing calcium ions to flood into the cell. This prompts sacks containing chemical neurotransmitters to fuse with the plasma membrane, releasing their contents — the 'signal' — into the synaptic gap. These neurotransmitters then diffuse across the gap to the neighbouring neuron, where they bind to receptors on the plasma membrane and trigger an electrical response. Zenisek *et al.*[1] have filmed the cellular sacks, known as synaptic vesicles, as they move to the membrane and fuse there in response to an influx of Ca^{2+}. Meanwhile, Schneggenburger and Neher[2] and Bollmann *et al.*[3] have found that the sensor that triggers this fusion is more sensitive to Ca^{2+} levels than was previously supposed.

Physiological studies indicate that synaptic vesicles prepare for rapid, Ca^{2+}-triggered fusion with the plasma membrane (exocytosis) through a sequence of docking and 'priming' stages (Fig. 1). These preparatory stages and exocytosis itself have been directly observed in other types of cell that secrete hormones[4]. But the small sizes of synaptic terminals (with diameters of about 1 μm) and synaptic vesicles (diameter about 30 nm) have made it difficult to observe the events preceding neurotransmitter release.

Zenisek *et al.*[1] overcame these problems by using total-internal-reflection microscopy to study the giant (roughly 10 μm) synaptic terminals of goldfish retinal bipolar neurons. Their technique exploits the way a neuron's synaptic terminal will stick tightly to a glass coverslip. Light reflected at the interface between the glass and the terminal

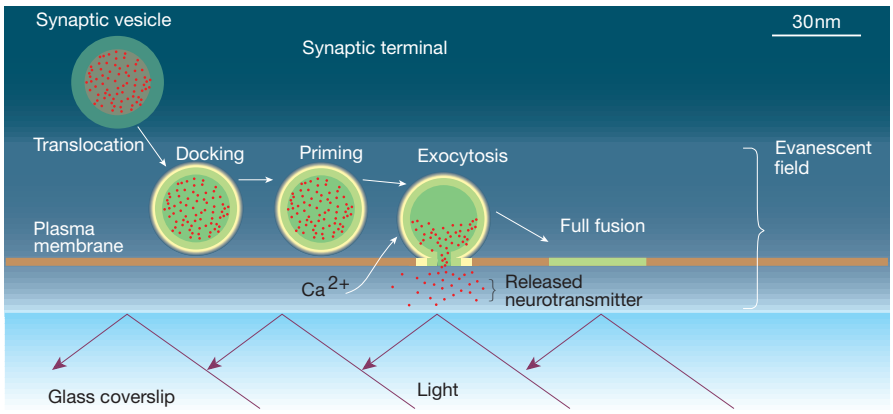

Figure 1 **Watching neurotransmitter release. Communication between nerve cells takes place across the synaptic gap between them. Neurotransmitter chemicals are sent from the terminal of the signalling neuron across the gap to its neighbour. When a neuron is stimulated, calcium channels in the plasma membrane of the nerve terminal open, allowing an influx of calcium ions. This causes vesicles loaded with neurotransmitter (red) to move to the plasma membrane, dock, and undergo 'priming' for fusion (exocytosis). Zenisek *et al.*[1] used total-internal-reflection microscopy to watch the dynamics of Ca^{2+}-driven fusion of single synaptic vesicles. Light reflects from the interface between a glass coverslip and the neuron, establishing a region of brightness (an evanescent field) that extends a short distance into the nerve terminal. The vesicles are labelled with a fluorescent marker, and begin to glow when they enter the evanescent field. During exocytosis, the fluorescence spreads into the plasma membrane of the synaptic terminal, allowing exocytosis to be seen.**

forms an evanescent field that excites any fluorescent molecules that come within about 100 nm of the interface[5] (Fig. 1). When a fluorescently labelled vesicle enters this field, its brightness increases as it moves nearer to the plasma membrane. And when fusion takes place, the fluorescence is incorporated into the membrane. By labelling only a small fraction of the vesicles in the terminal, Zenisek *et al.* were able to see single vesicles approaching, docking and fusing with the membrane during stimulation of the neuron.

Their work also reveals several new features of vesicle dynamics. Fusion occurred preferentially at specific 'hot spots' along the membrane. These probably correspond to active zones, where Ca^{2+} channels cluster and most neurotransmitter release happens. During an electrical stimulus, vesicles that were already near the plasma membrane fused rapidly with it. But vesicles arriving at the membrane a little later paused, on average, for a quarter of a second before approaching the membrane and fusing. This pause might represent the time needed for priming, during which interactions between proteins on the plasma membrane and the vesicle's own membrane (Fig. 2, overleaf) prepare the vesicle for exocytosis. During the pause, newly arrived vesicles often stopped about 20 nm away from the cell membrane. Active zones in retinal bipolar neurons are marked by synaptic 'ribbons', which tether numerous synaptic vesicles, and it may be these ribbons that hold the vesicles away from the membrane during the pause.

But there are some limitations to the microscopy technique. The closeness of the plasma membrane and the glass substrate might influence exocytosis. For example, as Ca^{2+} is taken up by the nerve terminal, the levels of Ca^{2+} available in the small space between the plasma membrane and the glass coverslip might drop, which could affect the kinetics of exocytosis for late-arriving vesicles. Also, Zenisek *et al.* detected fusion (and hence neurotransmitter release) by watching for fluorescence from the vesicle appearing in the plasma membrane, so only full fusion was detected. But neurotransmitters can sometimes be released from a vesicle that is not fully merged with the plasma membrane. Zenisek *et al.* saw some vesicles move to the membrane, pause, and then retreat without fusing. This behaviour might reflect the reversibility of docking, but it may also show neurotransmitter release without full fusion.

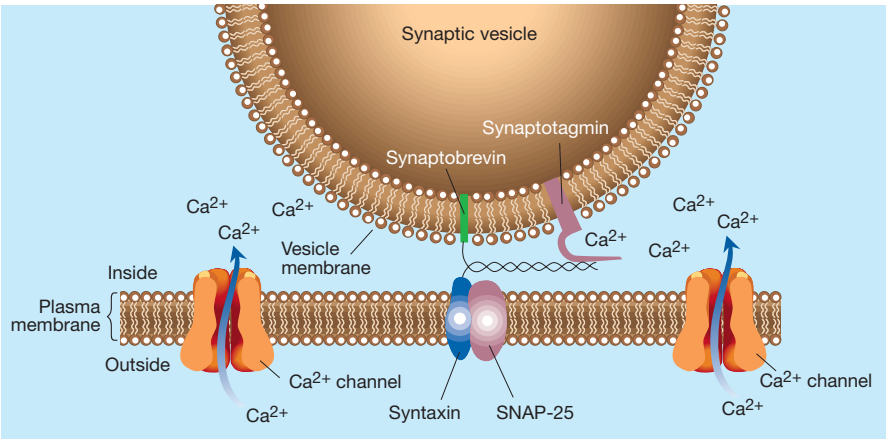

Figure 2 **Kinetics of calcium-dependent neurotransmitter release. This close-up view of a nerve terminal shows a synaptic vesicle docked at the plasma membrane near two Ca^{2+} channels. During priming, the plasma-membrane proteins syntaxin and SNAP-25 and the vesicle protein synaptobrevin form a complex required for vesicle fusion with the plasma membrane[9]. The Ca^{2+} sensor that triggers fusion is thought to be the vesicle protein synaptotagmin[9]. Schneggenburger and Neher[2] and Bollmann *et al.*[3] show that the Ca^{2+} concentration needed to trigger fusion is lower than previously thought. So the Ca^{2+} sensor does not need to be close to a single Ca^{2+} channel, where the Ca^{2+} concentration is very high. Instead it can be some distance away from the channels, where the Ca^{2+} concentration is lower as a result of diffusion. This means that more than one Ca^{2+} channel can influence neurotransmitter release. (Modified from ref. 10.)**

Meanwhile, Schneggenburger and Neher[2] and Bollmann *et al.*[3] have been looking at the way in which vesicle fusion depends on calcium. Averaged across the whole terminal, the concentration of Ca^{2+} inside the neuron rises from about 0.1 μM to just under 0.5 μM during neuronal stimulation. But vesicle fusion is thought to be triggered by the much higher Ca^{2+} concentrations (more than 100 μM) near the open mouth of individual Ca^{2+} channels[6] (Fig. 2). It now appears[2,3] that such high concentrations are not needed. Schneggenburger and Neher[2] and Bollmann *et al.*[3] released 'caged' Ca^{2+} to produce known levels of Ca^{2+} in the calyx of Held — a large synaptic terminal that allows nerve impulses in both the signalling and the receiving neurons to be recorded simultaneously. The authors found that a rise in Ca^{2+} concentration in the signalling neuron to 10 μM or less was enough to drive fast neurotransmitter release. They estimate that an action potential (the electrical change in a neuron that follows stimulation) activates the exocytosis of synaptic vesicles by raising Ca^{2+} levels to 10–25 μM for about 0.5 ms.

Apparently, it is not important for the exocytosis machinery and Ca^{2+} channels to be near each other to allow rapid neurotransmitter release (Fig. 2) — Ca^{2+} can diffuse some distance away from a channel into the cell, its concentration declining as it diffuses, and still trigger neurotransmitter release. This means that single vesicles might be influenced by Ca^{2+} ions entering through several channels. If so, and if the Ca^{2+} sensor is therefore not saturated by concentrated Ca^{2+} influx through a single open channel, greater control of neurotransmitter release becomes possible.

These results[2,3] differ significantly from those of similar experiments on retinal bipolar neurons[7], where increases in Ca^{2+} concentration to 10–100 μM were needed to drive exocytosis. But unlike neurons in the calyx of Held, bipolar neurons release neurotransmitters over relatively long periods and do not fire action potentials, which tend to be brief. The global Ca^{2+} concentration can exceed 1 μM in bipolar-cell synaptic terminals, even with moderate stimulation. So releasable vesicles would be exhausted rapidly if the exocytosis machinery here were as sensitive as that at the calyx of Held. Perhaps low Ca^{2+} affinity is a hallmark of sustained neurotransmitter release, whereas the brevity of Ca^{2+} signals driven by action potentials allows for higher Ca^{2+} affinity and greater integration of local Ca^{2+} signals.

It is almost 50 years since Katz and colleagues embarked on the work that still forms the cornerstone of our understanding of how neurons talk to each other[8]. But this field is as exciting as ever, and it is thrilling to be able to watch single vesicles as they fuse, to observe directly the stages before exocytosis, and to begin to glimpse the calcium signals that trigger fusion. ■

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Chemistry

Another noble gas conquered

Gernot Frenking

On page 874 of this issue Khriachtchev *et al.*[1] report the synthesis of the first compound containing the noble gas argon. This leaves only two stable elements in the periodic table — helium and neon — for which no neutral compound exists. But the experimental strategy that was successful for argon might also work for these elements.

One of the most fundamental questions in chemistry, which remained unanswered for a long time, was what drives the chemical elements to form stable molecules. Why are some elements very reactive, some less reactive, and a peculiar small group of elements, known as noble gases, not reactive at all? Quantum theory finally explained these observations by showing that the electrons within atoms are arranged in shells around the atomic nucleus. Each shell can only take a fixed number of electrons, and a completely filled shell is energetically very favourable. So the driving force behind chemical reactions

Figure 1 **Argon is no longer alone. The first neutral argon molecule, synthesized by Khriachtchev *et al.*,[1] is HArF, a stable hydride compound. The numbers give the calculated distances between the atoms in picometres.**

is the filling of electron shells by release or uptake of electrons, or by sharing electrons with neighbouring atoms, which leads to the formation of chemical bonds.

The noble gases have a completely filled outer electron shell, so there is no driving force for these elements to engage in chemical bonding, as the energetically most stable arrangement of electrons already exists in the neutral atoms. Many chemists believed that it was impossible to synthesize a stable molecule containing a noble gas element,