

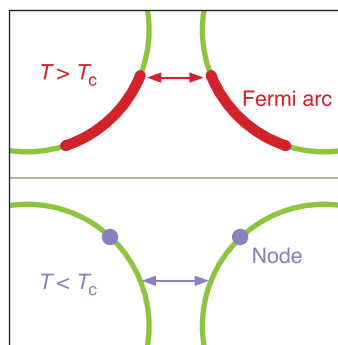
PERSPECTIVES

would be expected of coherent electrons scattering off defects in the crystal (7, 8). One limitation of these studies is that they have been confined to low temperatures. But Vershinin *et al.* have now produced Fourier maps above T_c , the temperature at which the material is superconducting.

In their study (2), they looked at a so-called underdoped sample that exhibits an unusual state of matter above T_c known as the pseudogap phase, characterized by a partially gapped Fermi surface. What they found in this pseudogap phase is the presence of a nondispersive Fourier peak with a wave vector similar to that associated with magnetic vortices seen in an earlier STM study below T_c (9). This wave vector corresponds to a weak charge density wave modulation of the electrons having a checkerboard pattern in real space, although the Kapitulnik group (who found a similar wave vector in their own STM studies) has claimed a one-dimensional aspect to the pattern, which would support the existence of stripes (6).

However, the Fourier peak found above T_c by Vershinin *et al.* was only seen for energies within the pseudogap. Outside this energy range, no detectable peak was seen. Although they did not study this sample below T_c , they did study another sample, a so-called optimal doped sample with maximal T_c , and found that at low temperatures they no longer observed this nondispersive peak, but only a dispersive peak seen in the earlier work of the Davis group (7, 8). On the basis of these findings, the authors suggest that the nondispersive peak above T_c is replaced by a dispersive peak below T_c .

What could be going on? One possibility can be seen in the figure. Below the temperature at which the pseudogap forms, the Fermi surface (green) becomes partially gapped in energy, leading to the formation of Fermi arcs (red). The wave vector found by Vershinin *et al.* (red arrow) is remarkably close to the distance separating the tips of the Fermi arcs, as measured independently by angle-resolved photoemission measurements (10). Now consider what happens below T_c . A superconducting energy gap with d -wave symmetry opens up along the Fermi arcs. That is, the Fermi arcs are gapped out, leaving only single gapless points known as nodes (blue dots). As a consequence, this wave vector (now shown as a blue arrow) only occurs at an energy equal to the superconducting gap at that particular location on the Fermi surface. That is, because the energy gap below T_c changes along the Fermi arcs, the wave vector that was nondispersive above T_c is replaced by a dispersive wave vector below T_c . This model is supported by a new STM Fourier study below T_c by the Davis group (11). On the other hand, Vershinin *et al.* have tested this



Momentum space for cuprates. The green curve is the Fermi surface of the normal state, and the red segments are Fermi arcs due to partial gapping of the Fermi surface in the pseudogap phase (10). Below the superconducting transition temperature T_c , these Fermi arcs are gapped, with only single gapless points (nodes) surviving, as indicated by the blue dots. The wave vector connecting the tips of the Fermi arcs (red arrow) is remarkably close to that identified by Vershinin *et al.* (2) in a scanning tunneling microscopy experiment in the pseudogap phase of a cuprate superconductor. Below T_c , this wave vector disappears and is replaced by a wave vector dispersing in energy (blue arrow), which is thought to be associated with the variation of the superconducting energy gap along the Fermi arcs (7, 8).

model above T_c in unpublished calculations and find that it has problems—the old story of the “devil being in the details.” They conclude by listing a variety of other scenarios, all of which remain to be worked out.

An alternate to this “replacement” picture has been proposed by the stripes advocates (1, 5, 6). In their picture, the nondispersive wave vector is associated with stripe formation and is always present. Below T_c , a new dispersive feature due to coherent electrons appears, which coexists with the original feature. But neither the results of the Davis group (7–9, 11) nor that of Vershinin *et al.* (2) are in support of one-dimensional stripes. Rather, what is found is a weak charge density modulation with a two-dimensional checkerboard pattern. So, although the cuprates have certainly earned their checks, it remains to be seen whether they have earned their stripes.

In any case, these new STM results, coupled with earlier studies, indicate that the true theory for cuprate materials may reside somewhere in the murky regime between where a localized (real space) picture applies and where an itinerant (momentum space) picture applies. Constructing a proper theory in this region will be a major challenge for those seeking to solve the high- T_c puzzle.

References and Notes

1. S. A. Kivelson *et al.*, *Rev. Mod. Phys.* **75**, 1201 (2003).
2. M. Vershinin *et al.*, *Science* **303**, 1995 (2004); published online 12 February 2004 (10.1126/science.1093384).
3. S. H. Pan *et al.*, *Nature* **413**, 282 (2001).
4. K. M. Lang *et al.*, *Nature* **415**, 412 (2002).
5. A. Cho, *Science* **297**, 499 (2002).
6. C. Howald *et al.*, *Phys. Rev. B* **67**, 014533 (2003).
7. J. E. Hoffman *et al.*, *Science* **297**, 1148 (2002).
8. K. McElroy *et al.*, *Nature* **422**, 592 (2003).
9. J. E. Hoffman *et al.*, *Science* **295**, 466 (2002).
10. M. R. Norman *et al.*, *Nature* **392**, 157 (1998).
11. K. McElroy *et al.*, in preparation.
12. I thank J. C. Davis for helpful discussions.

NEUROSCIENCE

Synaptic Vesicles in the Fast Lane

Matthew Holt and Reinhard Jahn

Neurons transmit signals to each other at specialized junctions called synapses. Here, electrical impulses are converted into chemical signals through the release of specific molecules called neurotransmitters. On page 2037 of this issue, Rizzoli and Betz (1) investigate how a subset of synaptic vesicles carrying neurotransmitter are recaptured after they have released their contents into the synapse.

Synapses vary in size and shape, but they are all thought to operate in a similar way. In the presynaptic nerve terminal, neurotransmitter is stored in synaptic vesicles and released into the synapse when the vesicles fuse with the nerve-terminal membrane during calcium-dependent exocytosis (see the

figure). The cytoplasm of the presynaptic nerve terminal is filled with synaptic vesicles. A subset of these vesicles is docked at specific sites along the presynaptic membrane (the active zones), which are characterized by an accumulation of electron-dense material. Neurotransmitter is released from synaptic vesicles only at these sites.

Stimulation by electrical impulses leads to an influx of calcium ions (Ca^{2+}) into the presynaptic nerve terminal through voltage-gated Ca^{2+} channels. This results in release of neurotransmitter at a high rate that then declines. Apparently, a subset of synaptic vesicles is ready to discharge its contents. Exocytosis of this pool of vesicles accounts for the initial high rate of neurotransmitter release. At rat hippocampal synapses, the amount of neurotransmitter released during this rapid phase approximately matches the amount that is stored in docked vesicles, suggesting that

The authors are in the Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany. E-mail: rjahn@gwdg.de

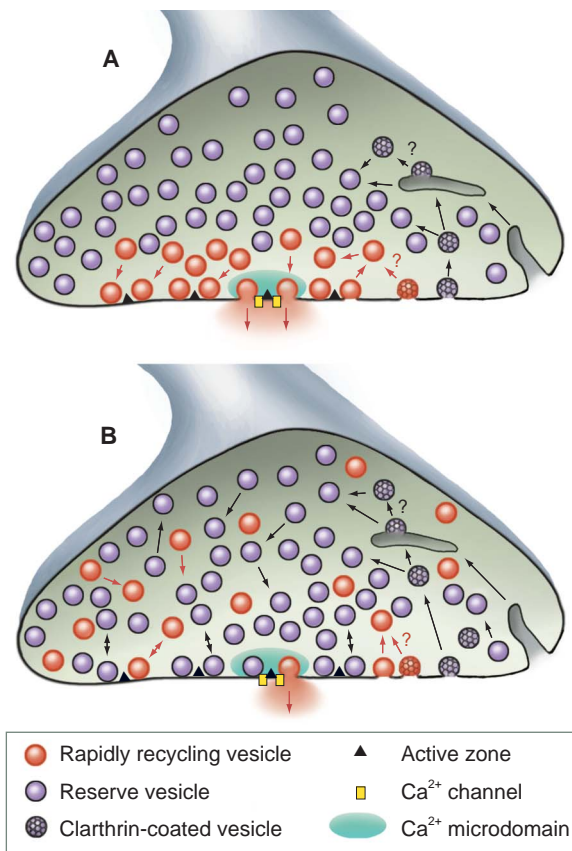
these docked vesicles account for the rapidly released vesicle pool (2).

Surprisingly, vesicles that have just undergone exocytosis are preferred for the next round of neurotransmitter release. Initially supported by the finding that vesicles recently refilled with neurotransmitter are among the first to undergo exocytosis (3), the introduction of FM fluorescent dyes has provided direct evidence for a distinct pool of rapidly recycled vesicles. FM dyes are amphipathic molecules that reversibly partition into, but do not pass through, lipid membranes. During endocytosis, these dyes are taken up into vesicles that are being recycled and are retained there until the next round of exocytosis. By applying such dyes during defined time windows, in combination with precise stimulation protocols, specific pools of endocytosed vesicles can be labeled and their release kinetics can be measured upon subsequent stimulation.

How are rapidly recycled vesicles selected from among seemingly identical organelles? In the 1970s, two alternative endocytic pathways were put forward for presynaptic nerve terminals (see the figure). The "classical" Heuser-Reese model proposed that endocytosis occurs at sites far away from the active zones through a clathrin-dependent mechanism. Vesicles are returned to the synaptic vesicle pool after loss of clathrin and passage through an endosomal compartment (4). In contrast, Ceccarelli and co-workers suggested that at least some vesicles fuse only transiently with the presynaptic membrane during exocytosis and are immediately recaptured (5), a mechanism now referred to as "kiss-and-run." Compared to the Heuser model, the "kiss-and-run" mechanism provides a simpler explanation for rapid recycling of active vesicles. Irrespective of the pathway, however, vesicles that are rapidly recycled are assumed to remain close to sites of neurotransmitter release, but so far no one has directly tested this assumption.

Enter Rizzoli and Betz (1), who address the fate of rapidly recycled vesicles and come up with surprising results. Using the frog neuromuscular junction, they com-

bined FM dye labeling with photoconversion, allowing labeled vesicles to be viewed by electron microscopy. Immediately after loading with dye, vesicles were localized at the plasma membrane of the presynaptic nerve terminal, but not at active zones. More surprisingly still, if the preparation was left to recover for just 2 minutes before photoconversion, labeled vesicles were distributed randomly throughout the nerve terminal, including the active zones (see the figure) but excluding the center of vesicle clusters at the active zones. Similar randomization has also been observed in a large central synapse called the Calyx of Held (6). Apparently, there is no special highway for guiding rapidly recycling vesicles back to release sites. Yet, despite random intermixing, labeled vesicles preferentially underwent another round of exocytosis when nerve terminals were loaded with FM1-43 and then partially destained by stimulation.



Fast-track synaptic vesicles. (A) In this model, rapidly recycled synaptic vesicles (red) dock preferentially at active zones of the presynaptic nerve terminal. After Ca^{2+} -triggered exocytosis, the vesicle membrane is recovered through endocytosis, by recapture either at sites of neurotransmitter release ("kiss-and-run") or away from active zones in a clathrin-mediated pathway. (B) In the Rizzoli and Betz model (1), rapidly recycled vesicles mix randomly with other (reserve-pool) vesicles both at active zones and elsewhere in the nerve terminal. However, rapidly recycling vesicles, through an unknown mechanism, have a higher probability of exocytosis in response to Ca^{2+} influx.

These exciting findings have major implications for our concepts of synaptic vesicle recycling. First, they refute the notion that rapidly recycled vesicles must remain close to the active zones. Furthermore, they exclude "kiss-and-run" as a major recycling pathway, in agreement with a recent study in retinal bipolar cells (7). Even more surprisingly, rapidly recycling vesicles do not dock preferentially at active zones. Apparently, these vesicles receive no special treatment at any other step during recycling, including endocytosis, uncoating, and intracellular transport. Thus, the only difference between rapidly recycling and reserve-pool vesicles is the increased release probability of the former after docking with the presynaptic membrane. A third, inevitable conclusion is that synaptic vesicles are much more mobile in the nerve terminal than previously envisioned. This again agrees with a recent study on vesicle dynamics in retinal bipolar cells, suggesting that the marked mobility of synaptic vesicles is not confined to ribbon synapses (8). Furthermore, synaptic vesicles can dock and undock within seconds.

If it is not their location in the synapse, then it must be the vesicles themselves that "remember" their release history, perhaps by carrying a "tag" that marks them for release after docking. The identity of this tag remains a mystery. Complementary work from Betz's group has shown that rapidly recycling vesicles persist as a distinct vesicle pool over periods of hours, clearly excluding fast, transient molecular changes such as protein phosphorylation.

It remains to be seen whether these conclusions can be extended to small central synapses. In hippocampal neurons, the "kiss-and-run" mechanism of endocytosis is thought to dominate under conditions of moderate activity (9, 10). Considering the findings of Rizzoli and Betz, a fresh look at these experiments should be taken to determine whether there are fundamental differences in the recycling pathways of large and small synapses.

References

1. S. O. Rizzoli, W. J. Betz, *Science* **303**, 2037 (2004).
2. P. De Camilli, V. Haucke, K. Takei, E. Mugnaini, in *Synapses*, W. M. Cowan, T. C. Südhof, C. F. Stevens, Eds. (John Hopkins Univ. Press, Baltimore, 2001), pp. 89–133.
3. I. von Schwarzenfeld, *Neuroscience* **4**, 477 (1979).
4. J. E. Heuser, T. S. Reese, *J. Cell Biol.* **57**, 315 (1973).
5. B. Ceccarelli, W. P. Hurlbut, A. Mauro, *J. Cell Biol.* **57**, 499 (1973).
6. R. P. de Lange, A. D. de Roos, J. G. Borst, *J. Neurosci.* **23**, 10164 (2003).
7. D. Zenisek, J. A. Steyer, M. E. Feldman, W. Almers, *Neuron* **35**, 1085 (2002).
8. M. Holt, A. Cooke, A. Neef, L. Lagnado, *Curr. Biol.* **14**, 173 (2004).
9. A. M. Aravanis, J. L. Pyle, R. W. Tsien, *Nature* **423**, 643 (2003).
10. S. P. Gandhi, C. F. Stevens, *Nature* **423**, 607 (2003).