NEUROSCIENCE

Vesicular Glutamate Transporter—Shooting Blanks

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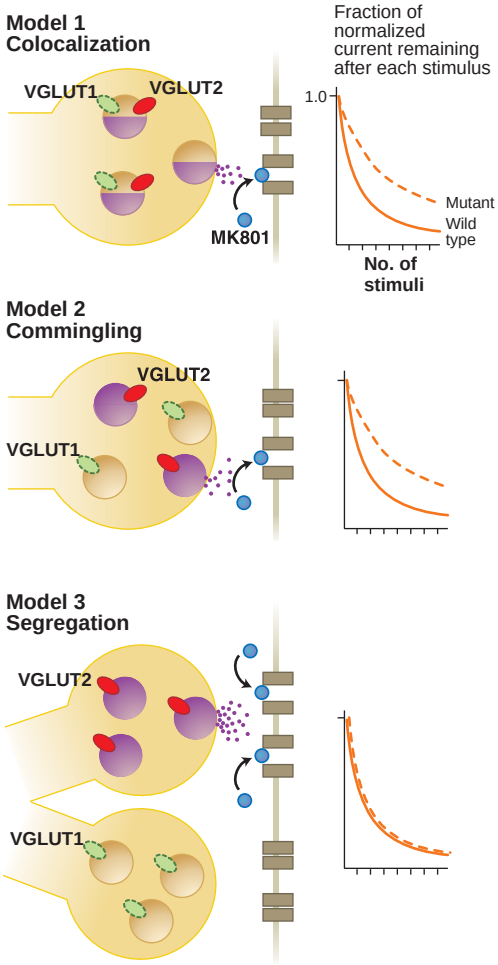
One would think that a mouse lacking the major excitatory neurotransmitter in the brain would be a dead mouse, and indeed it is—but it's not nearly as dead as one might have thought. Two groups have knocked out the gene for the main vesicular glutamate transporter, VGLUT1, and surprisingly, these mice live for several months. The studies by Fremeau *et al*. (*1*) on page 1815 in this issue and by Wojcik *et al*. in a recent issue of *Proceedings of the National Academy of Sciences U.S.A.* (*2*) both look at transporter expression, trafficking, and mechanisms of vesicle loading in VGLUT1 mutant mice. However, the two studies present different models for subcellular localization of the vesicular transporter and for vesicle loading.

Neurotransmitters are stored in synaptic vesicles at neuron terminals that contact other neurons. When the synaptic vesicle membrane fuses with the plasma membrane, the neurotransmitter is released into the synaptic cleft. The main excitatory neurotransmitter in the brain is the amino acid glutamate. It was recently found that the vesicular glutamate transporter, which had eluded researchers for many years, was actually disguised as a plasma membrane inorganic phosphate transporter (*3*–*6*). This transporter was named VGLUT1 and two additional glutamate transporters—VGLUT2 and VGLUT3—have since been identified (*7*–*11*). Deleting the gene for the vesicular glutamate transporter would prevent release of glutamate into the synaptic cleft, which could reveal the function of this neurotransmitter. The Fremeau *et al*. (*1*) and Wojcik *et al*. (*2*) studies both show that excitatory neurotransmission is greatly reduced in VGLUT1 knockout mice—as expected if neurons were releasing empty vesicles. However, analysis of the small amounts of current remaining lead to different interpretations.

Why are there three vesicular glutamate transporters? One possibility is that these transporters have different kinetics for neurotransmitter loading. However, the transporters exhibit very similar transport kinetics (*3*, *4*, *8*–*14*). A second possibility is that the three transporters are expressed in different parts of the brain. In fact, in adult mice, the distribution of the transporters is mostly nonoverlapping. Roughly, VGLUT1 is expressed in the cortex, VGLUT2 is expressed in the brainstem, and VGLUT3, suprisingly, is expressed in a small number of cells that are not thought to use glutamate as their neurotransmitter (*7*–*9*, *12*–*14*). However, Fremeau *et al*. (*1*) and Wojcik *et al*. (*2*) demonstrate that in newborn mice, both VGLUT1 and VGLUT2 are expressed in the cortex and hippocampus, with VGLUT2 expression in these regions disappearing over the next several weeks. Thus, promoter diversity seems to be developmental rather than just spatial. Coexpression of VGLUT1 and VGLUT2 in the same cells in newborn mice suggests that these two proteins might serve different functions within a cell. The cellular distribution of VGLUT1 and VGLUT2 might give clues to their function. There are three possible scenarios: The two transporters could colocalize on the same synaptic vesicles; separate VGLUT1 and VGLUT2 vesicles could commingle at the same synapse; or the two transporters could be segregated to different synapses (see the first figure). Based on three lines of evidence, Fremeau and colleagues (*1*) propose that VGLUT1 and VGLUT2 are segregated to separate synapses.

First, VGLUT1 and VGLUT2 are segregated to different parts of a cell. When both transporters are transfected into PC12 cells, VGLUT1 localizes to the periphery, whereas VGLUT2 is uniformly expressed in the cytoplasm (*8*). Similarly, immunolocalization shows no overlapping distribution of the two transporters in hippocampal cells (*1*).



Models for coexpression of VGLUT1 and VGLUT2. (Left) In Model 1, the two transporters are on the same synaptic vesicles. In the VGLUT1 mutant, the vesicles are partially filled. MK801 is an open-channel blocker that can only block a channel that has been activated. In the mutant, MK801 does not rapidly block all glutamate receptors at the synapse because only a few are activated after a stimulus. **(Right)** The fraction of the initial current remaining after each stimulus declines because MK801 blocks previously activated channels. The current is normalized to the initial current in the two genotypes. The absolute current is much less in the VGLUT1 mutant. The current remaining after each stimulation declines more slowly in the mutant (dashed line) than in the wild type (solid line). In Model 2, the two transporters are present on distinct vesicles, but these vesicles commingle at any particular synapse. Thus, a filled vesicle is only released once every two stimuli. In Model 3, the transporters are segregated to different synapses. All current comes from VGLUT2 synapses, and the time required for inhibition by MK801 is the same as that in the wild type. Results from Fremeau *et al*. (*1*) are most consistent with Model 3.

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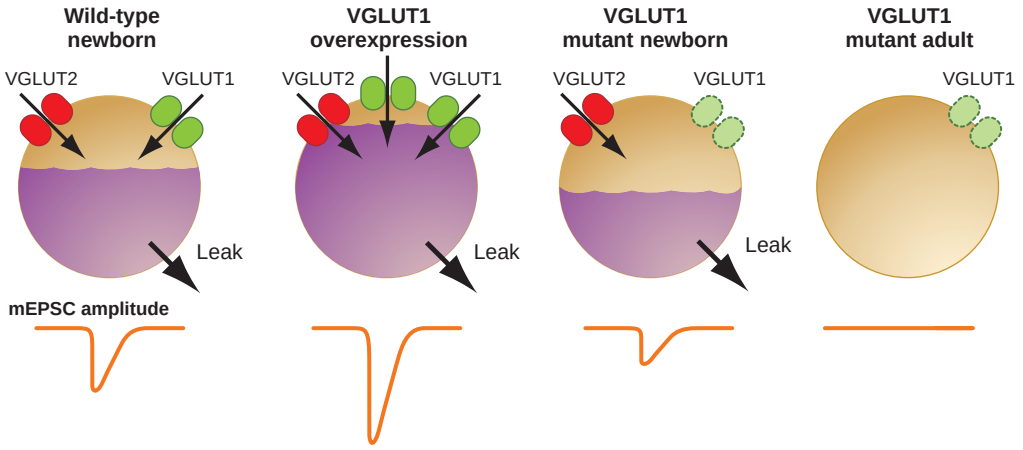
Second, VGLUT1 and VGLUT2 seem to segregate to different populations of vesicles. If VGLUT1 and VGLUT2 were present on the same population of vesicles, then deleting VGLUT1 transporters would lead to a reduction in neurotransmitter loaded into all vesicles. The amount of neurotransmitter in a vesicle fusing to the plasma membrane can be indirectly measured by determining the current in a postsynaptic cell, called a miniature excitatory postsynaptic current (mEPSC). Despite the severe reduction in the frequency of mEPSCs in the VGLUT1 mutant, Fremeau *et al*. found that the current amplitudes of the remaining events are the same as those in the wild type. Thus, rather than a reduction in loading over all vesicles, there appears to be normal loading of the remaining VGLUT2 vesicles.



Filling vesicles. The amount of neurotransmitter in a vesicle depends on the number of transporters on the vesicle (**above**). Greater levels of neurotransmitter in a vesicle will activate more postsynaptic receptors and cause a larger current in the postsynaptic cell (**below**). Empty vesicles can still fuse to the plasma membrane but cause no postsynaptic current (far right). Results from Wojcik *et al.* (*2*) are most consistent with the two transporters both contributing to filling of a single vesicle. mEPSC, miniature excitatory postsynaptic current.

Third, VGLUT1 and VGLUT2 seem to be segregated to different synapses. Fremeau *et al.* performed electrophysiological recordings on brain slices from the hippocampus of mutant mice lacking VGLUT1. Presynaptic cells were stimulated and currents from the postsynaptic cells recorded in the presence of a glutamate receptor channel blocker, MK801, that only blocks activated channels (see the first figure). The authors did not observe slower blocking kinetics, as would be predicted if the transporters were localized to the same synaptic vesicles, or if they were on commingling vesicles. Rather, the rate of blocking is the same as that in the wild type, as would be predicted if all the residual current in the VGLUT1 mutant strains came from specialized VGLUT2 synapses. To prove that wild-type cells express both VGLUT1 and VGLUT2 and that the currents seen in the slices do not arise from distinct populations of VGLUT2 cells, the authors cultured single cells from VGLUT1 mutant hippocampus and demonstrated that the cultured cells have reduced but residual neurotransmitter release. Thus, these cells must normally express both VGLUT1 and VGLUT2 in the wild type.

These results are directly contradicted by the results of Wojcik *et al*. These authors cultured VGLUT1 mutant neurons and found that their results are most consistent with synaptic vesicles containing a mix of VGLUT1 and VGLUT2 transporters. Specifically, they observed that partially filled vesicles are released from most neurons. These data are consistent with the idea that the transporters are co-expressed, but suggest that they are not segregated to separate synaptic vesicles. How can these contradictory results be resolved? Of note is that Fremeau *et al*. conducted their experiments on brain slices, whereas Wojcik *et al*. examined primary cultured cells, which do not have appropriate synaptic targets for vesicle segregation. The cultured cell cannot have VGLUT1 and VGLUT2 specialized synapses because all the synapses are the same—the cell forms synapses onto itself.

Regardless of the state of the cultured cells, Wojcik *et al*. answer a longstanding question about the mechanism of vesicle filling: Why does each synaptic vesicle contain the same amount of neurotransmitter? There are two models: Either the mechanism for filling is extremely reliable, or a checkpoint prevents unfilled vesicles from being released. To distinguish between these two models, Wojcik *et al*. used cultured neurons to look at single synaptic vesicle fusions. They found that the average current amplitude of mEPSCs is reduced in the VGLUT1 mutant cells; the vesicles are only partially filled by VGLUT2. Thus, fewer transporters on a vesicle result in less neurotransmitter loading (see the second figure). They conclude that partially filled vesicles can fuse. This is consistent with data showing that pharmacological perturbation of vesicle filling resulted in the fusion of partially filled vesicles (*15*). By contrast, Fremeau and colleagues demonstrate that VGLUT1 heterozygous mice that express half the amount of transporter display no electrophysiological defect. This suggests that synapses are releasing normal amounts of neurotransmitter despite the presence of fewer transporters. One could argue that in the heterozygote there are more than enough transporters to fill a vesicle. However, Wojcik and colleagues argue that vesicles are not even completely filled in the wild type, and in their studies overexpression of VGLUT1 increased the mEPSC amplitude, suggesting that *more* neurotransmitter can be loaded into synaptic vesicles. Thus, the results from cultured neurons indicate that the number of VGLUT molecules on a synaptic vesicle determines how much neurotransmitter is loaded into the vesicle. If there is no checkpoint for a filled vesicle, then perhaps even empty vesicles can fuse to the plasma membrane. To directly test this hypothesis, Wojcik *et al.* loaded vesicles with the lipophilic dye FM1-43, stimulated the cells, and assayed exocytosis. Dye unloading occurred at almost all mutant nerve terminals, even though the amount of neurotransmitter released was an order of magnitude smaller than that from wild-type terminals. The cells were shooting blanks! Thus, a quantal mEPSC arises because vesicle filling is a reliable process rather than because of a checkpoint for filled vesicles. Moreover, the amount of neurotransmitter depends on the number of transporters on a vesicle, and an equilibrium between loading and leaking maintains a consistent level of neurotransmitter in the vesicle.

Based on the expression pattern of VGLUT1 and the physiological experiments, we conclude that the mutant mice should display almost no cortical function. These mice should lack visual perception, sound discrimination, complex voluntary movement, and memory. Nevertheless, both studies note that the mutant mice can survive for several weeks. In the Fremeau study, mutant mice survived even to adult-

hood. This will come as a surprise to most. However, to those who are close observers of human behavior, life without cognition has always seemed possible.

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PHYSICS

Cooking a Two-Dimensional Electron Gas with Microwaves

Adam C. Durst and Steven M. Girvin

"Who ordered this?" That was the famous reaction of Nobel laureate I. I. Rabi upon learning of the discovery of a new particle (the muon) in 1947. At that time, the quantum physics of atoms and electrons was rather well understood, and the universe didn't seem to need another particle. Yet there it was—and its unexpected discovery helped shape the development of modern particle physics.

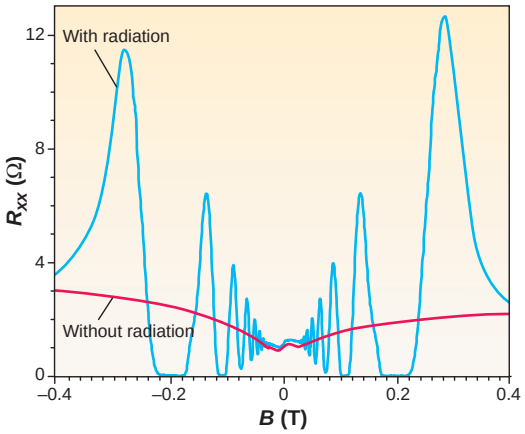
Those of us who work in condensed matter physics had a similar reaction when we first learned about a surprising new discovery in our field. Experimentalists in some very well respected research groups had recently discovered a new phenomenon in a very well studied system, the two-dimensional electron gas (2DEG) in a magnetic field. A 2DEG is a type of metal in which electrons are confined to move only within a two-dimensional plane formed at the interface between two semiconductors. At high magnetic fields, this system exhibits the quantum Hall effects, phenomena in which the transverse (Hall) component of the electrical resistance is quantized in integer or fractional units of a fundamental quantum of resistance. These effects have been studied exhaustively over the past two decades and were the subject of two Nobel Prizes.

In the new experiments which were conducted at much lower magnetic fields, experimenters subjected the system to microwave radiation and found that in the presence of microwaves of the right frequencies, the electrical resistance of the 2DEG would decrease (*1*). This is very surprising because one would expect that the absorption of microwaves would cause the system to heat up (just as food in a microwave oven does), exciting vibrational modes that scatter electrons and thereby increase the electrical resistance. More surprising still, they found that for a high enough radiation intensity, the resistance could be reduced nearly all the way to zero (*2*, *3*) (see the first figure). We got very curious very quickly, as did many others (*4*).

States of matter characterized by zero resistance hold a special place in the heart of a condensed matter physicist because zero resistance is often an indication that some interesting physics is afoot. Most materials exhibit some electrical resistance because the flow of electrons is inhibited by scattering from impurities, defects, and excited modes of the system. For a material in equilibrium to exhibit zero resistance, it must organize itself into some sort of collective state in which all of the electrons work together to make the state robust against these scattering mechanisms that degrade the electrical current. So one possibility is that this new microwave-induced zero-resistance state corresponds to a new collective state induced by the presence of the microwaves. This idea is appealing because in the absence of microwaves, and at much higher magnetic fields, this very system exhibits the quantum Hall effects, which are indeed characterized by collective states of matter. Note, however, that in quantum Hall systems, zeros in the longitudinal (dissipative) resistance are accompanied by plateaus in the transverse (Hall) resistance, which are crucial to the physics of the quantum Hall effects. Yet in the present case, no such plateaus are observed. Nonetheless, some other collective effect could be at work here, and ideas along these lines have been discussed (*2*).

However, there is another possibility. Because the system is pumped with microwaves, it is continuously supplied with energy from an outside source, and therefore is not in equilibrium. A pumped system can exhibit zero resistance, or even negative resistance, without forming a collective state, as long as the pump is able to overwhelm the effect of the probe used to measure the resistance (*5*). The probe in this case is the applied dc current. Without microwaves, a dc voltage builds up in response to the applied current as prescribed by the dark (no-microwaves) resistance. If the effect of the microwaves is to induce an additional dc voltage in the opposite direction, the measured resistance will decrease. If this voltage matches the dark voltage, the resistance will be zero. If it exceeds it, the resistance will be negative.

Soon after the experimental work was published, it was shown via a straightforward linear-response calculation that the microwaves can have precisely this effect (*6*–*8*). [Actually, unbeknown to the recent authors, similar ideas were reported in the Soviet literature (*9*, *10*) more than three decades ago—well before the ma-



Microwave surprise. Schematic depiction of the surprising data measured in recent experiments. Plotted is the magnetic field dependence of the low-temperature (~1 K) electrical resistance both in the absence (red) and presence (blue) of microwave radiation. The microwaves induce a new resistance oscillation controlled by the ratio of the radiation frequency to the magnetic field. For radiation of sufficient intensity, the minima are driven nearly all the way down to zero resistance. [Adapted from (*2*)]

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