

gland cells by small hairpin RNAs, relieved the repression of NRSF-target genes by 2DG, implicating the NRSF:CtBP complex in 2DG-induced changes in gene expression. Together, these findings support a model in which inhibition of glycolysis by 2DG results in an NADH-labile association of the NRSF:CtBP complex with NRSF target genes such as BDNF and TrkB, and their reduced expression results in the 2DG-mediated antiepileptic effects (Fig. 1).

The new study by Garriga-Canut *et al.* is of interest for a number of reasons. First, it provides a plausible mechanism for how the ketogenic diet exerts its antiepileptic effects, namely by inhibition of glycolysis. It also provides one potential mechanism by which the inhibition of glycolysis exerts these beneficial effects, namely by reduced expression of BDNF and its receptor, TrkB. Finally, the work provides a plausible mechanism by which the inhibition of glycolysis reduces the

expression of BDNF and TrkB, namely by recruitment of the co-repressor CtBP to the promoters. That said, some cautionary notes are in order. Was glycolysis actually inhibited *in vivo* by 2DG under the conditions of this experiment? If so, was inhibition of glycolysis the mechanism by which the 2DG exerted these antiepileptic effects? Given the evidence that glycolysis occurs predominantly in astrocytes, followed by transfer of lactate to neurons to fuel their energy demands<sup>6</sup>, might the antiepileptic effects of 2DG be mediated by inhibition of glycolysis in astrocytes? Furthermore, alteration of energy metabolism will almost certainly have pleiotropic effects. For example, modifying dendritic mitochondrial activity affects the number of spines and the plasticity of synapses<sup>7</sup>, suggesting that synaptic energy metabolism may locally influence synaptic plasticity. These mechanisms could influence antiepileptic effects detected *in vivo*. It is therefore crucial that

future follow-up experiments determine whether the reduced expression of BDNF and TrkB is really sufficient to explain the antiepileptic effects of 2DG.

These caveats notwithstanding, the authors should be congratulated for providing a strong and original rationale for how inhibition of glycolysis could reduce neuronal excitability *in vivo*. Moreover, this work advances small-molecule inhibitors of glycolysis as a new and potentially powerful pharmacological approach for the treatment of drug-resistant epilepsy.

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## Long-distance signaling via presynaptic glutamate transporters

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Glutamate transporters have long been thought to help terminate the synaptic response through neurotransmitter binding and reuptake, but a new report in this issue identifies a role for their anionic current in information transmission in the retina.

Glutamate is the most common excitatory neurotransmitter in the brain, crucial for communication throughout the nervous system. However, rapid removal of glutamate following its release at the synapse is essential, because it is not subject to extracellular enzymatic degradation, and so without alternative mechanisms for its removal, it would be impossible to control the specificity of its effects. The canonical view of glutamate transporters is that they mediate the reuptake of this transmitter, thereby limiting the potentially damaging effects of lingering glutamate. However, careful study of these transport proteins has suggested that they also mediate

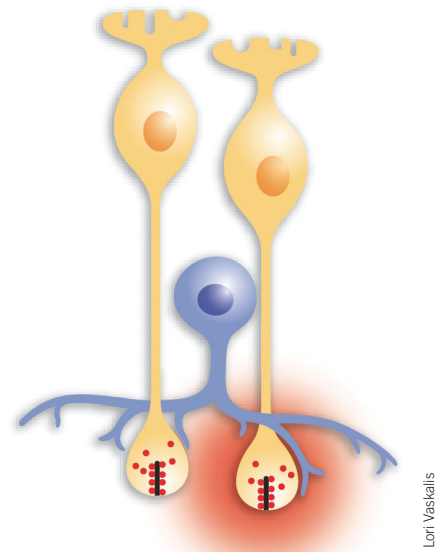
an anion conductance that is uncoupled from uptake<sup>1–3</sup>. Since its discovery, the anion current through glutamate transporters has been used as an assay for transport activity, but its physiological function in the cerebellum<sup>4,5</sup> and in the retina<sup>6–8</sup> has remained under debate. A new study by Veruki and colleagues in this issue identifies a novel role for this anion current in information transmission in the retina<sup>9</sup>.

To date, five excitatory amino acids transporters (EAATs) have been molecularly isolated. EAATs 1 and 2 are found in glial membranes, whereas EAATs 3–5 are found in neurons. The significance of neuronal uptake is not obvious, as the majority of glutamate uptake is the domain of glial transporters. Unlike that of other neurotransmitters, recycling of vesicular glutamate by neurons is thought to occur through a baroque glutamate-glutamine cycle that uses glial glutamate uptake and glutamine release followed by neuronal glutamine uptake<sup>10</sup>. It is thought that neuronal transporters use their density and location to terminate glutamate signaling.

Besides differences in their location, the fraction of the transporter-mediated current carried by anions also varies among EAAT proteins. EAATs 4 and 5 possess the largest anion conductance among this subfamily, but the functional relevance of this observation remains unclear. The retina-specific EAAT5 is expressed on photoreceptor and bipolar cell terminals, and this strategic location could be revealing because glutamate is released continuously in the dark. However, expression of EAAT5 in heterologous systems is associated with a substantial anion conductance but only modest glutamate uptake<sup>3</sup>. Supporting this notion, transporters from salamander photoreceptors and goldfish bipolar cell terminals boast an anion conductance equivalent to that of small ion channels<sup>6,7,11</sup>. Thus, it has been proposed that the function of EAAT5 transporters lies in their ion channel properties rather than their conventional glutamate transporter activity<sup>7,11</sup>.

The tour-de-force study by Veruki *et al.*<sup>9</sup> augments the functional repertoire of glutamate transporters. With a masterful use of paired

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**Figure 1** Bipolar cells communicate with each other via glutamate spillover. A rod bipolar cell terminal releases glutamate (red) from its ribbon-type active zone. This glutamate can bind to glutamate transporters on the same terminal and activate a chloride-mediated current. This is a form of presynaptic negative feedback, or self-inhibition. Released glutamate can also diffuse out of the synaptic cleft and thus inhibit nearby bipolar cell terminals by way of their glutamate transporter anion currents. This is a form of lateral inhibition between nerve terminals. Glutamate can also depolarize postsynaptic amacrine cells (blue) that release GABA back onto the same terminal (by way of reciprocal synapses) or onto nearby terminals (a GABA-mediated form of lateral inhibition).

recordings in different configurations, the authors convincingly show that the rod bipolar cell terminals in the inner plexiform layer (IPL) of the retina directly communicate with each other by way of the anion conductance of their glutamate transporters (Fig. 1). The authors characterized a conductance with pharmacology, permeability, affinity and kinetics that match well with glutamate transporters. Then paired recordings from presynaptic bipolar cells and postsynaptic AII amacrine cells showed that synaptic release of glutamate could activate the presynaptic transporters and excitatory postsynaptic conductances (EPSCs). Glutamate release could be detected by way of spontaneous transporter currents or through evoked transmission. Simultaneous recordings from two neighboring bipolar cells revealed self- and lateral-inhibitory responses as well as transporter currents that rose and decayed slowly. This implies that the release of glutamate from a single retinal bipolar cell terminal can control the release of glutamate from nearby bipolar cell terminals.

The new results are surprising given that bipolar cell terminals are not in direct contact or even very tightly packed within the IPL. In addition, the study by Veruki *et al.*<sup>9</sup> and another recent paper<sup>12</sup> show that glutamate transporters on mammalian bipolar cells can function as autoreceptors that inhibit further glutamate release. This negative-feedback mechanism is also present in photoreceptor terminals<sup>7</sup> and in goldfish bipolar cell terminals<sup>11</sup>, and it may prevent excessive glutamate release at these tonically releasing ribbon synapses. Presynaptic glutamate transporters may thus operate as sensors of local glutamate release, as well as sensors of more distal glutamate release by way of the diffusion and spillover of glutamate from nearby terminals.

These results are consistent with the spillover of glutamate between ribbon synapses, but could also be due to the ectopic release of glutamate<sup>13</sup>. Distinguishing between these phenomena remains a future challenge. Curiously, Müller glial cells, whose processes span the retina and possess a high density of transporters<sup>14</sup>, were apparently not able to prevent glutamate spillover. Assuming glial uptake remains intact in the reduced slice preparation, this suggests that Müller glial cells cannot prevent glutamate spillover or that glial transporters may be transiently overwhelmed.

An important question in the new study<sup>9</sup> was whether the presynaptic transporter, acting as an autoreceptor, could inhibit synaptic transmission. To address this, the authors first showed that local glutamate applications sufficiently altered the membrane potential of axon terminals. The authors then bypassed the

potentially confounding effects of postsynaptic desensitization using dynamic current clamp. The excitatory postsynaptic potential (EPSP) was substituted with an injected conductance, and the resultant voltage changes were measured directly from the axon terminal. The authors found that either pharmacological block of transport or reduction of transporter activity with a paired-pulse stimulation protocol enhanced the voltage response. This increase in the voltage response led to EPSCs in AII amacrine cells that were larger and rose more rapidly than when transport was intact. The authors concluded that inhibition of presynaptic transporter-mediated conductances led to larger postsynaptic responses through increased transmitter release rather than through changes in postsynaptic receptor efficacy. Thus, glutamate transporter anion conductance acts as a brake to inhibit further glutamate release through terminal hyperpolarization or shunting inhibition.

These findings raise the question of why a transporter is needed to fulfill such a role, which is normally performed by GABA receptors. However, the charge carried by the presynaptic transporters may be larger than that of the inhibitory GABAergic inputs<sup>12</sup>, although one should bear in mind that the slicing procedure may have damaged amacrine cell processes, which could have resulted in the underestimation of GABA feedback. Ultimately, the inhibition provided by presynaptic bipolar cell transporters can be effective only if intracellular chloride concentration is low in terminals, so determining the mechanisms that control chloride concentration in nerve terminals will be an important topic of future investigations.

In summary, glutamate transporters are thought to control the extracellular glutamate concentration through buffering and uptake. However, glutamate transporter activity is also associated with a thermodynamically uncoupled anion conductance. This conductance might be an evolutionary remnant from the transporters' phylogenetic origin, or it might be an epiphenomenon associated with the transporter's tertiary or quaternary structure. Arguing against these ideas is data from dopamine transporters that suggest that uncoupled currents can increase the excitability of midbrain neurons<sup>15</sup>. The new work<sup>9,12</sup> has now elegantly demonstrated that the EAAT5 transporter expressed at the rod bipolar cell terminal is a bona fide ligand-gated ionotropic receptor, and the amplitude and time course of its anion conductance is sufficient to mediate self- and lateral inhibition in retinal slices. It remains to be determined whether the anion conductances of other synaptic glutamate transporters have similar physiological roles.

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