The α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type glutamate receptors (AMPAR) are normally assembled from GluA subunits 1–4 into tetrameric heteromers containing GluA2 within the endoplasmic reticulum. Prior to their assembly, GluA2 mRNA is edited by ADAR2 at a critical region within the pore. Editing is thought to be complete from birth onward. Edited GluA2 subunits, when associated with other GluA subunits, confer calcium impermeability while GluA2-lacking receptors flux calcium. Calcium-permeable AMPAR—GluA2-lacking or GluA2-unedited—can be detected by their resulting inward rectification (relative lack of current at positive versus negative holding potentials) conferred by endogenous or exogenous polyamines such as spermine, unique sensitivity to externally applied polyamine toxins such as PhTx433, faster kinetics and larger single-channel conductance (1, 2).

Following assembly and packaging into endosomes, AMPAR are trafficked to synapses where they exist in three simplified pools: synaptic, extrasynaptic, and endosomal (sub-synaptic) pools. Synaptic GluA2-lacking AMPAR are generally a feature of early development, largely disappearing after the first post-natal week in CA1 hippocampus (3); later > 80% of synaptic and > 95% extrasynaptic receptors contain GluA2 (4). However, enrichment of synaptic GluA2-lacking receptors (presumably GluA1 homomers) has been transiently detected in both “normal” and pathological conditions. Post-synaptic changes in GluA subunit numbers or properties are thought to underlie synaptic modification in long-term potentiation, and depression (LTP, and LTD) (5). This has resulted in postulated AMPAR “subunit rules”: 1) synaptic removal of GluA2 subunits underlies LTD, 2) GluA1 not associated with GluA2 act independently, and 3) insertion and/or modification of GluA1 underlies LTP (6).

A rapid, selective trafficking of GluA2-lacking receptors into synapses has been shown to participate in the early phase of hippocampal CA1 LTP (7). Calcium influx through these receptors triggers a later phase swap of GluA2-lacking for GluA2-containing receptors. This swap appears to be critically dependent on the nature—size, extent, and temporal
features—of the calcium accumulation. The swap is mediated in part by the calcium sensor PICK1. This feature is crucial, as alternative calcium accumulations may trigger PICK1 to remove GluA2s in LTD (8).

Calcium permeable AMPAR are also a pathological feature. Down-regulation of GluA2, up-regulation of GluA1, loss of GluA2 editing, and selective GluA1 trafficking could each potentially lead to more calcium-permeable AMPAR. The former contributed to the “GluA2 hypothesis” (9) whereby preferential decrease in expression and subsequent loss of synaptic GluA2 (with no changes in GluA1) could lead to AMPAR that flux calcium. GluA1 up-regulation has been found in an adult model of electroconvulsive therapy (10) and after hypoxic seizures in immature rats (11). GluA2 knock-down studies have shown that down-regulation of GluA2 can lead to seizures and hippocampal injury (12). Clinical evidence from pathological studies might support up-regulation of GluA1 in epileptic tissue (13). Loss of GluA2 mRNA editing by down-regulation of the editing enzyme ADAR2, leading to greater calcium permeability, has been demonstrated after hypoxia (14). The present study now sheds light on alterations in GluAs associated with status epilepticus.

Refractory status epilepticus (RSE) (longer than 60–90 minutes) is a worst-case clinical scenario. Patients experience prolonged seizures, and nothing short of pentobarbital-induced coma may (or may not) stop the seizures and prevent the associated morbidity and mortality. Despite the clinical impact of 60,000–150,000 patients per year with approximately 55,000 deaths (15), the mechanisms underlying the transition from self-limited seizure to prolonged, medically refractory seizure are not fully understood. Utilizing animal models, prior work has detailed alterations in GABA receptors that begin to explain the resistance of RSE to benzodiazepines (16). Here, the picrotoxine model of status epilepticus has been used as a rodent model of RSE. The picrotoxine model is especially attractive in this regard as it represents the severe end of the spectrum of rodent models of status epilepticus; it is associated with significant mortality and morbidity, including later epilepsy.

The authors prepared hippocampal slices from rats during RSE. Using whole-cell patch-clamp recordings in CA1 and dentate gyrus, they found electrophysiological evidence for synthetically activated GluA2-lacking/calcium-permeable AMPAR. Using a biochemical technique, they found a nearly twofold loss of surface (synaptic plus extrasynaptic) GluA2 and a twofold gain of surface (synaptic plus extrasynaptic) GluA1. Given that the time course and nature of these alterations could not be readily studied in an intact preparation, the authors used hippocampal cultures. Brief treatment of hippocampal cultures with low magnesium resulted in sustained burst-firing, thought to represent an in vitro correlate of RSE. Here, the authors measured the rate of decline of surface GluA2 which paralleled the internalization (endosomal) and accumulation of GluA2 with a time-constant of ~6 minutes. Further, the authors measured calcium accumulations in burst-firing cultures and determined that these could be attenuated with selective antagonism of GluA2-lacking receptors. The authors did not investigate whether synaptic GluA2-lacking receptors were present, nor did they investigate accumula-

tion of surface GluA1 receptors in burst-firing cultures. These findings might be necessary to further tightly link their in vivo findings with their in vitro model of RSE. Nevertheless, these findings highlight the role of AMPAR in RSE. To strengthen this point, the authors found that the selective AMPAR antagonist GYKI-52466 stopped RSE in a dose-dependent manner in vivo.

Thus, the authors provide strong evidence for the therapeutic benefit of AMPAR antagonism to combat RSE. The authors’ findings do raise several important questions to be addressed by future work to better understand the impact of AMPAR in mediating RSE: While synaptic pools of AMPAR are affected by RSE, it is not clear whether extrasynaptic pools are altered as well. In other words, not all shifts in surface AMPAR impact the synapse. This distinction is important in RSE, as the normally tight control of glutamate within the synaptic cleft is potentially lost by alterations in glutamate uptake (17). Activation of extrasynaptic AMPAR may lead to activation of additional signaling pathways that mediate RSE, as is the case for extrasynaptic NMDA-receptor activation in models of hypoxia (18) and Huntington disease (19). To make parallel comparisons to LTP, understanding why synaptic GluA1 homomers stay increased without a switch back to GluA1/2 heteromers is important. While transient calcium-mediated PICK1 signaling mediates the normal late phase subunit switch, prolonged signaling may underlie the further removal of GluA2 in a parallel comparison to LTD. This may further exacerbate calcium accumulations in a positive feedback loop. This is also important, as it represents perhaps another therapeutic target for RSE. Further, does RSE actually lead to a potentiation of synaptic responses? Does GluA1-mediated excessive synaptic drive further underlie RSE? In other words, how do all of the changes in AMPAR mediate RSE? Has ADAR2 modulation been ruled out?

Finally, GluA2-lacking receptor function is further modulated by associated proteins called TARPs; alterations in TARP-AMPAR interactions could contribute to some of the changes in rectification seen with RSE (20).

by Tim Benke, MD, PhD

References

7. Plant K, Pelkey KA, Bortolotto ZA, Monita D, Terashima A, McBain CJ, Collingridge GL, Isaac JTR. Transient incorporation of native GluR2-
lacking AMPA receptors during hippocampal long-term potentiation. 


<table>
<thead>
<tr>
<th>Type</th>
<th>1. Money to Your Institution</th>
<th>2. Consulting Fee or Honorarium</th>
<th>3. Support for Travel to Meetings for the Study or Other Purposes</th>
<th>4. Fees for Participating in Review Activities Such as Data Monitoring Board, Statistical Analysis, End Point Committee, and the Like</th>
<th>5. Payment for Writing or Reviewing the Manuscript</th>
<th>6. Provision of Writing Assistance, Medical Equipment, or Administrative Support</th>
<th>7. Other</th>
<th>Comments **</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Money</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Complete each row by checking "No" or providing the requested information. If you have more than one relationship, just add rows to this table.

* This means money that your institution received for your efforts on this study.
** Use this section to provide any needed explanation.
Use this section to report other relationships or activities that readers could perceive to have influenced, or that could appear to have influenced, what you wrote in the submitted work.

3. Relevant financial activities outside the submitted work

- Please list all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all sources of support, whether from public or private entities, including grants or contracts, or honoraria paid for consultation or other services. Please note that this section asks about your financial relationships with entities in the bio-medical arena that could be relevant to the submitted work.

4. Other relationships

When a perspective or interpretation of your work could be influenced by your relationship with another party, please disclose that relationship. Examples include professional or commercial relationships, financial interests, or other relationships in your personal or professional life that could create, or appear to create, a conflict of interest. Please list any positions or relationships that could be perceived as influencing your work.

For example, if you were provided by a pharmaceutical company to write an article on a particular drug, you should disclose this information. If you have received travel expenses to attend a conference or given a presentation, you should disclose this as well. If you have a financial stake in a company that would benefit from your work, it is important to disclose this information.

Disclosures of potential conflicts of interest should be included in the submitted work. This helps readers understand potential biases or influences that might affect the research or conclusions presented.

If you have any questions about what should be disclosed, please contact the journal's editor or the society's ethics committee.

American Epilepsy Society
<table>
<thead>
<tr>
<th>Type of Relationship (in alphabetical order)</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (oo on the side of full disclosure)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** This means the amount of compensation received for your efforts.
** For example, if you report a consultancy, there is no need to report travel related to that consultancy on this line.

Section #2 Relevant financial activities outside the submitted work.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship as you need by clicking the "Add" box. You should report relationships that were present during the 36 months prior to submission.

Thank you for your assistance.

Page 3