



### SEIZURES BEGET SEIZURES IN TEMPORAL LOBE EPILEPSIES: THE BOOMERANG EFFECTS OF NEWLY FORMED ABERRANT KAINATERGIC SYNAPSES

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*Do temporal lobe epilepsy (TLE) seizures in adults promote further seizures? Clinical and experimental data suggest that new synapses are formed after an initial episode of status epilepticus, however their contribution to the transformation of a naive network to an epileptogenic one has been debated. Recent experimental data show that newly formed aberrant excitatory synapses on the granule cells of the fascia dentate operate by means of kainate receptor-operated signals that are not present on naive granule cells. Therefore, genuine epileptic networks rely on signaling cascades that differentiate them from naive networks. Recurrent limbic seizures generated by the activation of kainate receptors and synapses in naive animals lead to the formation of novel synapses that facilitate the emergence of further seizures. This negative, vicious cycle illustrates the central role of reactive plasticity in neurological disorders.*

Whether or not seizures beget seizures in adult temporal lobe epilepsies (TLE) (1) has been debated for decades (2–4). Recent extensive experimental data have provided sufficiently convincing evidence to suggest that seizures indeed do beget seizures by means of a cascade of events that include various types of neuronal damage (5–10), sprouting of fibers, and new synapse formations that establish aberrant glutamatergic synapses (11–16). Central to this scenario is the observation

that in humans and experimental animals, mossy fibers sprout and establish novel synapses, including some that are only observed in epileptic networks (15–19). Whereas naive granule cell synapses operate only by means of AMPA receptors, newly formed aberrant mossy fiber synapses also rely on kainate receptors (12). In other words, the epileptic state is one that uses signaling cascades that are not found under ordinary conditions. Blocking kainate receptors in epileptic networks reduces seizures, providing direct evidence that kainate receptor signals play an active part in TLE (12). Therefore, reactive plasticity produces novel functional excitatory glutamatergic synapses that not only contribute to epileptogenesis but also are aberrant in that they rely on different signals than the naive ones. Taken altogether, these studies suggest that kainate receptor-operated synapses play a direct role in the induction and expression of TLE.

It took time for implications of the serendipitous observations and unexpected consequences of the acaricide agent, kainic acid (isolated from sea algae), to reveal themselves. The story began with a curious observation: administration of kainate generates seizures—in fact, typical limbic types of seizures—followed by a pattern of lesions that mimics that observed in TLE (2,3,20). Then kainate receptors were found in vulnerable regions of the brain (14,21,22); they were cloned and identified as being part of the family of glutamate receptors (23). Kainate receptor-operated synapses were identified next; they revealed that the release of glutamate activates kainate receptors as well as the conventional AMPA receptors (12,24–31). Parallel studies showed that mossy fiber synapses, instrumental in TLE and enriched in kainate receptors, sprout in humans and in animal models; the mossy fiber synapses also establish novel functional synapses, including aberrant ones (12,14). The loop was closed when newly formed synapses were found to operate partly via aberrant kainate receptors and that seizures are sensitive to kainate receptor antagonists (12). Thus, the negative loop is comprised of epileptogenic receptors located on strategic neurons that generate seizures when activated by exogenous kainate, which leads to the generation of status epilepticus that is associated with the formation of aberrant kainate receptor-mediated synapses that further contribute to the generation of additional seizures.

#### Kainate Receptors, Seizures, and Brain Damage

The identification of kainate, an amino acid that potently excites cortical neurons (3,4,32), was rapidly followed by the discovery of its high-affinity receptors, located in the brain and having an interesting distribution—notably in regions that are

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known to play a central role in seizures, particularly in the hippocampus (14,21,33). This finding was expectedly followed by studies aimed at determining the effects of kainate in animals. The era of kainate and TLE was initiated during the late 1970s with studies showing that systemic (or better yet, intra-amygdaloid administration) of kainate generates a syndrome of seizures and brain damage that mimics human TLE (3,20). Surface EEG and depth recordings demonstrated that the seizures are initiated in regions that also are highly excitable and central to TLE—notably, the CA3 region and the amygdala (3). The inaugurating status epilepticus exhibited clinical manifestations that clearly reflected the involvement of limbic structures, including rearing, paw, and facial movements (2,15), and were similar to those produced in kindling using electrical stimulation of the amygdala and/or the hippocampus (34,35). This outcome confirmed the correlation between activation of kainate receptors that lead to the generation of seizures and a limbic syndrome. The status epilepticus was associated with neuronal cell loss in the same vulnerable CA3 and CA1 regions, again suggesting that the recurrent activation of these synapses produces neuronal lesions by means of an excitotoxic process (3,36).

Parallel investigations then showed that in some regions, there is a direct link between the severity of seizures and neuronal loss: i) the extent of the CA3 lesion is directly correlated with the severity of the seizures (i.e., duration of the postictal depressions; duration and severity of the ictal events [2]); ii) lesions of the mossy fibers that innervate CA3 neurons reduce both the initial seizures triggered by kainate and the extent and severity of the subsequent lesions (37); and iii) neuronal damage is not seen at an early developmental stage when mossy fibers are not fully operative (38). Direct measures of blood flow as well as oxygen and carbon dioxide consumption in seizing animals confirmed that, at least in certain brain regions, damage is not due to an imbalance between oxygen supply and consumption but to more specific activity-dependent mechanisms (39). These and other observations suggest the presence of seizure-specific neuronal damage (i.e., directly caused by the activity). Therefore, kainate receptors and signals clearly confer a high degree of excitability and epileptogenesis to the neurons on which they are present.

### The Pharmacology and Molecular Biology Era

Glutamate, like other central neurotransmitters, activates several ionotropic receptors—in particular, AMPA receptors, which are associated with most fast-acting postsynaptic currents, and NMDA receptors, which are activated by strong stimuli and involved in neuronal plasticity (40). Are there kainate receptor-operated synapses, and if so, what do they do? The development of relatively selective pharmacological

tools permits an answer to these questions (41,42). Studies first identified that kainate receptor-operated synapses do exist, particularly on neuronal targets of mossy fibers, which again stresses the strong links between kainate and these synapses (12,24–26,30,31). Perhaps more interestingly, other investigations demonstrated that many glutamatergic synapses are mediated solely by kainate receptors with kainate-mediated EPSC, which are activated by quantal release of glutamate (12,30). Kainate receptor mediated EPSCs have small amplitudes, but because of the slow kinetics of kainate receptors, the total charge carried is, in fact, larger than that of the fast-acting AMPA receptor-mediated synapses. Thus, there are pure kainate receptor-mediated EPSCs, pure AMPA receptor-mediated EPSCs, and mixed kainate and AMPA receptor-mediated components of EPSCs, suggesting that the release of glutamate can generate a variety of signals with different properties and different consequences on network operations. In sum, kainate receptor signaling has unique properties in central synapses and appears to fill a role that is different from that of conventional AMPA receptor-operated synapses.

Parallel studies, using novel genetic tools, enabled investigators to identify families of glutamate receptors (notably, the subtypes of kainate receptors) and to determine their distribution (23). Two subtypes of kainate receptors are instrumental in that context: the GluR6 and GluR5 subtypes. The former is clearly related to limbic epilepsies because of its distribution, especially on the vulnerable CA3 pyramidal neurons (23), and because of the strong reduction in seizures that is produced by knocking out this subtype of subunit (43,44). GluR6 knockouts have a greatly reduced vulnerability to seizures and to kainate (43,44). In contrast, GluR5 subunits are enriched on interneurons and when activated, lead to a dramatic increase of the tonic and phasic inhibitions that impinge on the principal cells. Thus, it is presumed that kainate exerts a dual action, with proconvulsive consequences for principal neurons mediated by GluR6 and anticonvulsive effects mediated by the GluR5 subunits (44,45). Therefore, again in keeping with other transmitter-gated systems (via acetylcholine or catecholamines), the transmitter acts on different intracellular cascades to produce quite distinct consequences (31).

### Postseizure and Postlesion Reactive Plasticity Are Key Players in TLE

Studies performed during the last two decades have shown that once neurons degenerate, the environment does not remain idle: fibers sprout and establish novel synapses. This effect is best exemplified in TLE for which overwhelming numbers of observations suggest that the inaugurating status triggers a cascade of events that lead to reactive plasticity. Studies using kainate receptor binding, immunocytochemistry, electron microscopy,

and tracing techniques have shown that mossy fibers sprout and innervate novel targets (11,12,14–16,18,19,46). Some of these targets also are present in naive brains (e.g., additional CA3 pyramidal neurons); other targets are aberrant in that they are not present in naive brains (i.e., they stem from the innervations by mossy fibers of other granule cells of the fascia dentata from which they originated). This process requires time (about 2–3 weeks) and is triggered by the activation of thousands of genes (47,48), including immediate early genes, growth factors, cell adhesion molecules, guiding factors, and cytoskeleton proteins—ultimately leading to the formation of novel synapses.

In addition to the identification of anatomical and molecular signals involved in the formation of novel synapses, electrophysiological studies recording neurons in chronic foci confirmed the functionality of these synapses. Whereas granule cells rely exclusively on AMPA receptors for their glutamate signals, granule cells in the epileptic foci use both AMPA and kainate receptors; that is, they have both pure kainate receptor- and pure AMPA receptor-mediated components to the EPSCs (12). The reactive plasticity that leads to the formation of novel synapses is apparently largely confined to excitatory synapses. In contrast, GABAergic inhibitory synapses do not appear to sprout. Thus, recordings made weeks or months after the inaugurating status show a permanent loss of dendritic inhibition, suggesting that the loss of interneurons and inhibition in TLE is irreversible. The somatostatin dendrites projecting interneurons that are highly vulnerable to seizures degenerate and the inhibitory input they provide to pyramidal neurons is not replaced by other sources (6,7).

What are the links between seizures, aberrant sprouting and synapse formation, and the transformation of a naive network to one that will generate further seizures? Recordings from granule cells possessing aberrant kainate receptor-operated synapses revealed that, unlike naive neurons, they readily generate epileptiform bursts and that seizures in granule cells induced by electrical stimuli are largely blocked by kainate receptor antagonists (12). Therefore, seizures beget seizures by means of the lesion and postreactive plasticity associated with the formation of novel kainatergic synapses not present in controls. Taken altogether, the confirmation of all the elements of this sequence confirms that the formation of novel aberrant mossy fiber synapses is a hallmark of TLE and that reactive plasticity is not always for the best. The only experimental evidence that speaks against this scenario is that the administration of protein synthesis blockers prevents the sprouting of mossy fibers, however they do not prevent the occurrence of chronic seizures (49). Moreover, the complex and multiple effects of blocking protein synthesis should not be underestimated, nor should the alterations of the inaugurating status epilepticus that, in turn, could modify the activation of the triggering cascades. One cannot exclude the possibility that reactive plasticity is not the sole

mechanism that operates in TLE; according to the severity of the initial insult, other mechanisms also could contribute.

What are the links between this sequence and clinical data? The principal argument against the suggestion that seizures beget seizures in humans is that human TLE can recur for long-lasting periods, with little change in its clinical and electrographic manifestations (50). Removing the focus can alleviate the epilepsy, which speaks against the formation of a mirror focus in the other hemisphere by the inaugurating insult—in spite of the demonstration that sprouting of mossy fibers does occur in humans, as in rodents (17,18). However, several challenges can be raised to these human-specific findings. First, the presence of sprouting in humans as well as in experimental animals suggests that the cascade of events occurs in both, and the mossy fiber sprouting could contribute to the ongoing generation of seizures in human TLE patients. Second, rodents and humans have very different types of interhippocampal connections, and it is likely that reactive plasticity occurs in humans—as in experimental animals—in other important synaptic connections, for instance, in intrahippocampal or amygdalo–hippocampal connections. This occurrence, in turn, could explain why large resections are needed for a successful, antiepileptogenic surgical intervention. In other words, the process of reactive plasticity, but not necessarily the players could be applicable to humans. In addition, relapse as well as the need for antiepileptic therapy following surgery might reflect the persistence of a high degree of excitability of the epileptic network after the removal of the presumed focus. Another confounding possibility is that axons, other than mossy fibers, also sprout, resulting in a more complex picture with location and animal species-specific cascades. Yet, the evidence collected over the past decades on animal TLE is strong enough to assume that it is very likely that reactive plasticity also does occur in humans.

In conclusion, reactive plasticity appears to play a central role in engraving the long-term deleterious sequels of seizures. The data on TLE and kainate provide a reliable basis for a general scenario of the events triggered after an insult. The plasticity of the nervous system, which constitutes one of the most fundamental features of neurons and networks, provides evidence of the important events in the generation of seizures by an epileptic network. Since sprouting also is observed in other disorders, studies on the mechanisms of TLE and the development of novel therapies can only be performed in genuinely chronically epileptic animal models. Models using tissues acutely rendered seizure prone by short-acting convulsive drugs do not take into account the unique properties of epileptic networks and thus, are not necessarily informative in respect to the chronic property of epilepsies. Kainate receptor-mediated signals (particularly the GluR6 subunit-containing receptors) that appear to intervene significantly in developing and epileptic networks may be targets for antiepileptic drug investigations. The debate regarding

whether or not seizures beget seizures exemplifies how epilepsy studies enable major advances in understanding brain operation and integrative functions.

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