GABA synapses enter the molecular big time

Activity-dependent changes in the number and composition of $GABA_A$ receptors provide the basis for inhibitory synaptic plasticity and may be a key feature of the chronic epileptic state (pages 1166–1172).

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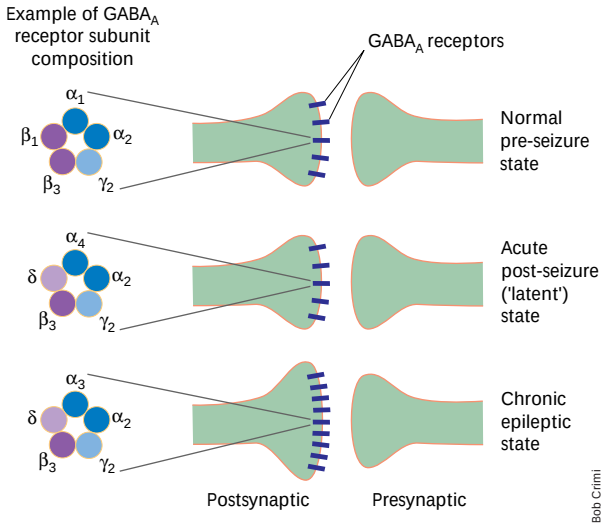
INVESTIGATIONS OF THE central nervous system (CNS) at the cellular and molecular levels have generated some of the most exciting breakthroughs in neuroscience. They have revealed the brain to be a dynamic and constantly changing organ that is characterized by neural plasticity: the capacity of neural elements to alter their electrical properties, grow new processes, and/or modify connections and circuits in order to adapt to an ever-changing world. Many of these studies have focused on the synapse—that micro-molecular point of contact through which one neuron communicates with the next. Interest in synaptic plasticity has grown, particularly with the demonstration that the strength of synaptic connections can be altered by the degree of activity at the synapse. This property is thought to provide the basis for normal brain functions such as learning and memory, as well as for neurological disorders such as epilepsy. Two papers, by Brooks-Kayal *et al.*[1] on page 1166 of this issue and Nusser *et al.*[2] in *Nature*, suggest that hyperactivity at inhibitory synapses—junctions that use the neurotransmitter GABA (γ-aminobutyric acid)—leads to both an increase in the number of $GABA_A$ receptors in the postsynaptic membrane and an alteration in their subunit composition. These results shed light on the involvement of GABAergic transmission and associated inhibitory synaptic activity in epileptic disorders.

In a widely studied example of plasticity, called long-term potentiation (LTP), co-activation of pre- and postsynaptic cells gives rise to a long-lasting increase in the ability of the presynaptic cell to excite the postsynaptic neuron. Many laboratories are now attempting to elucidate the mechanisms underlying the LTP phenomenon[3], and have concentrated particularly on excitatory synapses where glutamate is the neurotransmitter. Meanwhile, there has also been a quiet revolution in our thinking about inhibitory systems and how they contribute to many forms of synaptic plasticity. GABA is the major inhibitory neurotransmitter in the CNS, and much of the research into inhibitory activity has involved the identification and description of neurons that use GABA as their means of communication. These cells—often called interneurons because they tend to communicate primarily with other cells in their local environment—are thought to modulate brain excitability and shape the output of the excitatory cells with which they interact. Investigation of the hippocampal formation, a structure known for its involvement in learning and memory as well as its key role in temporal lobe epilepsy, reveals a rich complexity of GABAergic cell types[4], each with its own unique structure, connectivity and (presumably) function.

Perhaps not surprisingly, much of the interest in GABAergic function has arisen from research into the basic mechanisms underlying epileptic disorders[5], for the epileptic state presents apparently dramatic changes in the balance between excitatory and inhibitory activity. The Brooks-Kayal and Nusser studies are a continuation of efforts to decipher the basis of epilepsy. They both use a sophisticated combination of electrophysiological and molecular analyses that provides new insights into the plasticity of GABA synapses. Much work has shown that seizure activity results in a change in $GABA_A$ receptor subunit expression[6] and that the pharmacologic and kinetic properties of these receptors depend on subunit composition[7]. However, these two groups provide for the first time a convincing molecular/mechanistic link between activity-dependent receptor changes and the level of functional inhibition in an intact CNS structure.

Their results bring interesting new 'grist to the mill' with respect to our understanding of epileptogenesis (the development of a chronic seizure condition) and the nature of the epileptic state. Although much work has centered on the relative loss of inhibition in the epileptic brain, we have known for some time that inhibition is not always decreased during seizures. In some brain regions, and in some epileptic states, inhibition seems to be increased. This has been observed not only in animal models but also in human (temporal lobe) epilepsies. Both of the present studies investigate the dentate region of the hippocampal formation in which inhibition increases after treatments—electrical stimulation (kindling) or drugs (pilocarpine)—that lead to a chronic seizure state. That there



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In the normal (pre-seizure) state (top), the $GABA_A$ receptor population on hippocampal dentate granule cells is dominated by α_1, β_1 and γ_2 subunits. Shortly after an acute episode of intense seizure activity, but before the animal exhibits spontaneous chronic seizure behavior (that is, in the latent period, middle), the composition of the receptors shifts: α_1 and β_1 are reduced relative to other α and β subunits, and α_4 and β_3 are increased along with δ and ε subunits (no significant change in γ_2). In this period, GABA efficacy is not changed, suggesting that the number of available receptors at the synapse has not been affected. In the chronically epileptic animal (bottom), the altered subunit composition is maintained, and there is also an increased number of $GABA_A$ receptors per synapse (both an increased density of receptors and an increased area of the synaptic junction)—a change that presumably gives rise to increased GABA efficacy in these neurons[1,2].

is an increase in inhibition in the dentate region at the same time that the brain (as a whole) shows enhanced excitability may seem paradoxical. But this observation reminds us of the extreme complexity of seizure states: clearly, seizures involve many cellular/molecular variables and numerous interactions between many areas of the brain.

What part do altered $GABA_A$ receptors play in epileptogenesis? Brooks-Kayal and co-workers attempt to address this question by examining changes in the molecular make-up of $GABA_A$ receptors at different times during the epileptogenic process. They do this using a combination of patch-clamp and RNA amplification techniques, which allows them to study both electrical and molecular changes in a given neuron. The authors describe a change in the subunit composition of the $GABA_A$ receptors—a decrease in α_1 and β_1 subunits relative to other α and β subunits, respectively, and a concomitant increase in δ and ε subunits (see figure)—weeks before the animal demonstrates spontaneous seizure activity. They speculate that these changes are involved in the evolution of the chronic seizure state. However, the strength of response of single neurons to GABA (that is, GABA efficacy) increases much later, after spontaneous seizures have occurred. From the timing of these changes, one might view the later increase in GABA inhibitory power as a compensatory reaction to the high level of excitation associated with the seizures, but not as a mechanism of seizure development itself.

Although no comparable data are available from the Nusser study (which examined GABA receptor responsiveness only after establishment of the epileptic state), it is tempting to correlate the late increase in GABA efficacy with the increased number of GABA receptors. Whether changes in $GABA_A$ receptor subunit composition and/or number are early events in the epileptogenic process, or whether they are, indeed, compensatory and triggered by the repeated occurrence of spontaneous seizures, remains a crucial question. Some recent efforts to develop more effective and specific antiepileptic drugs are directed towards targeting the molecular subunit composition of $GABA_A$ receptors. Thus, we need to know whether to enhance or to counteract the influence of new receptors found in the epileptic brain.

The Brooks-Kayal and Nusser reports, in their focus on the postsynaptic GABA receptor changes associated with seizures, also offer interesting commentary on the question of whether pre- or postsynaptic changes are responsible for LTP-like synaptic plasticity—long a controversial issue in the excitatory (glutamatergic) synapse literature. At least under the experimental conditions described in the new experiments, it seems clear that postsynaptic changes in molecular structure contribute—and perhaps explain entirely—seizure-related plasticity (although presynaptic contributions have not been absolutely ruled out).

Whether such a conclusion can also be drawn for increased physiologic plasticity at GABAergic synapses in the absence of the seizure state—indeed, whether LTP can be induced at GABAergic synapses—awaits further experimentation. It is tempting to speculate, however, that just as the nature of the postsynaptic glutamate receptors (the relative contribution of NMDA and non-NMDA receptor subtypes) seems essential for a synapse's potential for plasticity, so too might GABAergic synaptic plasticity depend on the subunit composition of the receptors at the synapse. The insertion of different GABAergic receptor subunits into the postsynaptic membrane, and the increase in the number of receptors at the postsynaptic site, are reminiscent of recent results, which suggest that glutamatergic receptor number and subtype might be influenced by activity yielding changes in synapse strength[8].

It is important to emphasize that the demonstration of GABA receptor plasticity has implications for more than our understanding and treatment of epilepsy. Indeed, these two new reports suggest that the properties of inhibitory (GABAergic) synapses can be altered by activity—as has been shown for excitatory (glutamatergic) synapses in studies of LTP. Although the model systems described in both papers involve seizure (a pathological extreme), the demonstrations of plasticity at GABAergic synapses suggest that inhibitory points of contact must be included in any analysis of a change in brain excitability.

How common this GABAergic plasticity might be, and under what physiological conditions it is induced, remain key issues for investigation. The dramatic GABA receptor plasticity induced in association with seizures strongly suggests that we should revisit previous attempts to demonstrate plasticity at the level of inhibitory (GABAergic) interneurons[9]. Although these earlier studies suggested that excitatory synaptic contacts impinging on certain interneurons undergo LTP-like potentiation—and thus indirectly promote inhibition mediated by the GABAergic cells—the possibility that neuronal activity produces potentiation directly at GABAergic synapses has rarely been investigated (but see ref. 10). GABA synapses, and in particular the $GABA_A$ receptors themselves, may provide more than gentle modulation of excitatory neurotransmission—they, too, may be important points of synaptic plasticity.

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