

## ARE ALTERED EXCITATORY SYNAPSES FOUND IN NEURONAL MIGRATION DISORDERS?

### Physiological and Morphological Characterization of Dentate Granule Cells in the p35 Knock-out Mouse Hippocampus: Evidence for an Epileptic Circuit

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There is a high correlation between pediatric epilepsies and neuronal migration disorders. What remains unclear is whether intrinsic features of the individual dysplastic cells give rise to heightened seizure susceptibility, or whether these dysplastic cells contribute to seizure activity by establishing abnormal circuits that alter the balance of inhibition and excitation. Mice lacking a functional *p35* gene provide an ideal model in which to address these questions, because these knockout animals not only exhibit aberrant neuronal migration but also demonstrate spontaneous seizures.

Extracellular field recordings from hippocampal slices, characterizing the input–output relation in the dentate, revealed little difference between wild-type and knockout mice under both normal and elevated extracellular potassium conditions. However, in the presence of the GABA<sub>A</sub> antagonist bicuculline, p35 knockout slices, but not wild-

type slices, exhibited prolonged depolarizations in response to stimulation of the perforant path. No significant differences were found in the intrinsic properties of dentate granule cells (i.e., input resistance, time constant, action-potential generation) from wild-type versus knockout mice. However, antidromic activation (mossy fiber stimulation) evoked an excitatory synaptic response in more than 65% of granule cells from p35 knockout slices that was never observed in wild-type slices. Ultrastructural analyses identified morphological substrates for this aberrant excitation: recurrent axon collaterals, abnormal basal dendrites, and mossy fiber terminals forming synapses onto the spines of neighboring granule cells. These studies suggest that granule cells in p35 knockout mice contribute to seizure activity by forming an abnormal excitatory feedback circuit.

### Prolonged NMDA-mediated Synaptic Response, Altered Ifenprodil Sensitivity, and Generation of Epileptiform-like Events in a Malformed Hippocampus of Rats Exposed to Methylazoxymethanol in Utero

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Cortical malformations are often associated with refractory epilepsy and cognitive deficit. Clinical and experimental studies have demonstrated an important role for glutamate-mediated synaptic transmission in these conditions. With whole-cell voltage-clamp techniques, we examined evoked glutamate-mediated excitatory postsynaptic currents (eEPSCs) and responses to exogenously applied glutamate on hippocampal heterotopic cells in an animal model of malformation (i.e., rats exposed to methylazoxymethanol [MAM] in utero). Analysis of eEPSCs revealed that the late *N*-methyl-D-aspartate

(NMDA) receptor-mediated eEPSC component was significantly increased on heterotopic cells compared with age-matched normotopic pyramidal cells. At a holding potential of +40 mV, heterotopic cells also exhibited eEPSCs with a slower decay-time constant. No differences in the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) component of eEPSCs were detected. In 23% of heterotopic pyramidal cells, electrical stimulation evoked prolonged burstlike responses. Focal application of glutamate (10 mM) targeted to different sites near the heterotopia also evoked epileptiform-like bursts on

heterotopic cells. Ifenprodil (10  $\mu$ M), an NR2B subunit antagonist, only slightly reduced the NMDA receptor-mediated component and amplitude of eEPSCs on heterotopic cells (MAM) but significantly decreased the late component and peak amplitude of eEPSCs in normotopic cells (Control). Our data demonstrate a functional alteration in the NMDA-mediated component of excita-

tory synaptic transmission in heterotopic cells and suggest that this alteration may be attributable, at least in part, to changes in composition and function of the NMDAR subunit. Changes in NMDA-receptor function may directly contribute to the hyperexcitability and cognitive deficits reported in animal models and patients with brain malformations.

## COMMENTARY

The discovery of an association between neuronal migration disorders and some forms of pediatric epilepsy has led to a dramatic increase in experimental epilepsy research on animal models with dysplastic cortex. An important goal of this research has been to determine how disorders of neuronal migration may lead to epileptogenesis. These two publications approach the issue from different perspectives and with different hypotheses, but the results may point to interrelated conclusions. The work of Patel and colleagues was based on a p35 knockout mouse and focused on the hypothetical formation of abnormal recurrent excitatory circuits in the dentate gyrus. The studies by Calcagnotto and Baraban demonstrated the capacity of prenatal administration of methylazoxymethanol to cause cortical malformations, and the investigators hypothesized that the long-duration excitatory postsynaptic responses of heterotopic CA1 pyramidal cells are due to alterations in *N*-methyl-D-aspartate (NMDA) receptors. Thus, both articles refer to developmental alterations of excitatory glutamatergic synapses, but the former work emphasizes the potential for a new epileptic circuit, whereas the latter focuses on the possibility of a molecular transformation of NMDA receptors. Yet the two series of experiments may include manifestations of similar mechanisms.

Not all humans with cortical malformations have seizures, and many of the animal models of cortical malformation also do not exhibit seizures. Therefore, an important advantage of the p35 knockout is that this animal model does have spontaneous recurrent seizures. Although overt tonic-clonic seizures are not seen after methylazoxymethanol treatment or in some of the other models of cortical malformation (e.g., prenatal irradiation), methylazoxymethanol-treated animals appear to have increased seizure susceptibility. It is possible that further analyses of these animal models may reveal electrophysiologic events reminiscent of seizure activity. However, the models support the human observations that cortical malformations do not necessarily lead to chronic seizures, although many cases of pediatric epilepsy are associated with them.

Both studies involve electrophysiologic experiments with hippocampal slices and use experimental designs that incorporate GABA<sub>A</sub>-receptor antagonists, which are necessary to exclude (or at least minimize) the effects of possible alterations of GABAergic neurons or synapses on the mechanisms under investigation. Numerous studies have shown that the presence of GABAergic synaptic systems in cortical structures can mask both the functional expression of recurrent excitatory circuits and the NMDA receptor-mediated components of excitatory postsynaptic responses to extracellular electrical stimulation. Thus, even small changes in the GABA-mediated synaptic systems can lead to apparent changes in recurrent excitation and/or NMDA receptors, and conversely, the presence of inhibitory systems can obscure changes in recurrent excitation and NMDA receptor-mediated synaptic responses. Similarly, changes in one of these two excitatory synaptic mechanisms can affect the appearance of the other.

The work of Patel and coworkers, involving the dentate gyrus from the p35 knockout model, used electrophysiologic, histopathologic, and ultrastructural techniques to provide evidence that Timm-stained mossy fiber terminals in the inner molecular layer form excitatory synapses on the dendritic spines of granule cells (and also on basal dendritic spines). Activation of these fibers caused abnormal excitatory responses, which were detected when inhibition was blocked with bicuculline. The similarity between these data and results from studies on hippocampal slices from animal models of temporal lobe epilepsy (e.g., kindled rats and rodents treated with kainic acid or pilocarpine) adds support to the concept that mossy fiber sprouting (also studied as Timm stain in the inner molecular layer) leads to formation of new recurrent excitatory circuits that are at least partially masked by synaptic inhibition. In the case of the p35 knockout, the abnormal recurrent excitatory circuits appear to arise from a developmental disorganization rather than from a seizure- or injury-induced reorganization of mature pathways. In both cases, however, the abnormal excitatory circuits may contribute to epileptic seizures, but probably not without a simultaneous depression of inhibition near seizure onset. Patel

and coworkers focused on recurrent excitation and, thus, did not study the issue of altered or enhanced NMDA-receptor function, which could also contribute to the increased synaptic excitation seen in this model.

The key results from the work of Calcagnotto and Baraban are the presence of prolonged excitatory responses in voltage-clamped CA1 pyramidal cells after single extracellular stimulations and the sensitivity of these responses to NMDA-receptor antagonists. As the authors point out, a characteristic of many developing neuronal circuits is the presence of increased NMDA receptor-mediated synaptic responses. Although the data in their articles support this hypothesis, it is possible that the pyramidal cells formed abnormal recurrent excitatory circuits in the CA1 area and that these late responses reflect, at least in part, the excitation from other multisynaptic pathways. No fewer than three independent laboratories have provided several lines of evidence from kainate- and pilocarpine-treated rats, demonstrating that CA1 pyramidal cells also form new axon collaterals after induction of status epilepticus and that this sprouting of axon collaterals leads to the generation of new recurrent excitatory circuits (1–5). If the NMDA-receptor component is important in the generation of action potentials by the intercalated CA1 pyramidal cells, the delayed synaptic responses would be sensitive to NMDA-receptor antagonists, in a manner similar to that described in this article. Thus, as the authors briefly explain, abnormal recurrent excitatory circuits could contribute to the results.

These studies highlight several important points. First, cortical malformations associated with neuronal migration disorders, similar to epilepsy-associated increases in recurrent excitation and NMDA receptors, do not necessarily lead to epilepsy. Many authors over the past several decades have emphasized that even small alterations in GABAergic inhibition (e.g., loss of only a small fraction of the inhibitory interneurons) could

contribute to epileptogenesis; small reductions in inhibitory circuits might be particularly epileptogenic if they were associated with reorganization of glutamatergic circuits. Similarly, it has been well documented that GABAergic synapses are depressed during repetitive activation, which could also unmask abnormalities of excitatory glutamatergic circuits. Thus, alterations in several interacting mechanisms could be a critical aspect of epileptogenesis associated with neuronal migration disorders. Finally, this constellation of alterations—cortical dysplasia, increased recurrent excitation and NMDA-receptor synaptic responses, and decreased GABAergic inhibition—could occur in any area of the cerebral cortex. Thus, it may be beneficial to consider that although the various regions of the cerebral cortex may show differences in regard to neuronal membrane and synaptic properties, similar molecular and cellular mechanisms of epileptogenesis possibly may be present in a combinatorial manner across the cortex.

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