A New Animal Model of Temporal Lobe Epilepsy*

Jean-Marc Fritschy, Institut für Pharmakologie und Toxikologie, Universität Zürich

Summary

Temporal lobe epilepsy (TLE) with hippocampal sclerosis is a predominant form of acquired epilepsy, characterized by profound neuronal loss and gliosis in the hippocampal formation and by recurrent complex partial seizures. Retrospective studies suggest that the disease is related to an initial precipitating event, such as febrile seizures in early childhood. In rodents, TLE can be modeled by inducing a status epilepticus, either by prolonged electrical stimulation or by administration of drugs, such as pilocarpine or kainic acid, which produce a strong activation of the hippocampal formation. These treatments produce extensive bilateral lesions and lead to recurrent, generalized seizures following a latent period of several weeks. In this review, a new animal model is described, in which kainic acid is injected unilaterally into the hippocampus of adult mice to cause a small initial lesion. Thereafter, the lesion leads to a gradual, unilateral degeneration of the hippocampus CA1 area and to a marked dispersion of dentate gyrus granule cells, two features of the human pathology. Following a latent period of about 2 weeks, the mice develop focal, recurrent seizures interspaced with interictal spikes-and-waves lasting for the rest of the life of the animal. The clear temporal separation between the acute lesion, the latent phase, and the chronic seizures allows investigating morphological and functional changes underlying epileptogenesis and generation of recurrent focal seizures. We show that while kainic acid injection initially affects specific populations of GABAergic interneurons, there is a pronounced up-regulation of GABAergic transmission in the dentate gyrus during the chronic phase. The plasticity of the surviving GABAergic neurons might represent a compensatory adaptation to recurrent seizures.

Ein neues Tiermodell der Temporallappenepilepsie

Temporallappenepilepsie (TLE) mit Hippokampussklerose ist eine häufige Form der erworbenen Epilepsien. Sie ist durch einen ausgeprägten Neuronenzellverlust und eine Gliose im Hippokampus gekennzeichnet, darüber hinaus auch durch das Auftreten von chronischen partiell-komplexen Anfällen. Retrospektive Studien lassen vermuten, dass die TLE auf ein Ereignis im Kleinkindalter, wie einen komplizierten Fieberanfall, zurückzuführen sein könnte. Bei Nagetieren kann die TLE durch das Auslösen eines Status epilepticus modelliert werden, entweder mittels eines starken elektrischen Reizes oder einer systemischen Behandlung mit Substanzen wie Pilocarpin oder Kainat. Diese Behandlungen führen zu grossen Läsionen im Hippokampusbereich und lösen, nach einer Latenzzeit, spontan-generalisierte Anfälle aus. Im Tiermodell der TLE, das hier beschrieben wird, wird eine winzige Menge von Kainat unilateral in den Hippokampus von erwachsenen Mäusen eingespritzt, um eine kleine lokale Läsion zu erzeugen. Im Laufe der Zeit führt diese Läsion zu einer ausgeprägten unilateralen Degeneration des CA1-Bereiches des Hippokampus und zu einer Dispersion der Körnerzellen im Gyrus dentatus, zwei Merkmalen der Hippokampussklerose. Nach einer Latenzzeit von zwei Wochen entwickeln die behandelten Mäuse spontane fokale Anfälle und interiktale Aktivitäten, die während ihres ganzen Lebens zu beobachten sind. Die deutliche zeitliche Trennung der initialen Läsion, der Latenzzeit und der chronischen Phase erlaubt die Untersuchung der Mechanismen der Epileptogenese und der Aufrechterhaltung von wiederkehrenden Anfällen. Wir zeigen, dass die chronische Phase durch eine ausgeprägte Erhöhung der GABAergen Neurotransmission im Gyrus dentatus gekennzeichnet ist, obwohl Kainat spezifische Typen von GABAergen Neuronen zerstört. Die Plastizität der überlebenden GABAergen Zellen könnte als kompensatorischer Mechanismus gegen die chronischen Anfälle angesehen werden.

Un nouveau modèle animal de l'épilepsie du lobe temporal

L'épilepsie du lobe temporal (ELT) avec sclérose hippocampale est une forme fréquente d'épilepsie acquise. Elle se caractérise par une perte importante de neurones et une forte gliose dans l'hippocampe, ainsi que par des crises partielles complexes. Des études rétrospectives suggèrent que l'ELT soit liée à un événement déclenchant lors de la petite enfance, tel que la survenue d'une crise fébrile. Chez les rongeurs, il est possible de modéliser l'ELT en induisant un status epilepticus par des stimulations électriques répétées ou par l'injection systémique de substances excitotoxiques telles que la pilocarpine ou l'acide kaïnique. Ces traitements provoquent de fortes lésions dans l'hippocampe et déclenchent, après un temps de latence, des crises épileptiques généralisées spontanées. Dans le modèle d'ELT décrit ici, tout commence par une petite lésion initiale produite par une injection unilatérale d'une quantité minime d'acide kaïnique directement dans l'hippocampe de la souris adulte. Cette lésion augmente graduellement en raison d'une dégénérescence progressive de la

région CA1 de l'hippocampe et d'une dispersion des cellules en grain du gyrus dentelé, deux phénomènes typiques de l'ELT. Après une latence de deux semaines environ, des crises focales se produisent spontanément, en alternance avec des activités intercritiques, qui peuvent être observées tout au long de la vie des souris traitées. La claire séparation temporale entre la lésion initiale et la phase chronique permet d'étudier à la fois les mécanismes de l'épileptogenèse et de la répétition des crises spontanées. En particulier, il est démontré que la phase chronique est marquée par une forte augmentation de la neurotransmission GABAergique dans le gyrus dentelé, bien que l'injection d'acide kaïnique détruise sélectivement certains types de neurones GABAergiques. La plasticité des cellules épargnées par la lésion initiale pourrait donc représenter un mécanisme compensateur activé par la survenue des crises focales récurrentes.

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Acquired epilepsies are caused by multiple factors, including traumatic brain injury, brain tumors, cerebrovascular disease, developmental malformations, central nervous system infections, perinatal insults, febrile seizures, and status epilepticus. Several of these conditions can be mimicked experimentally, offering the possibility to study causal mechanisms of epileptogenesis and both short and long-term adverse consequences of seizures [1]. Temporal lobe epilepsy (TLE) is of particular interest because it can be modeled relatively easily in laboratory animals, and because the final stages of the disease can be studied after surgical resection of the epileptic focus, thereby offering the invaluable possibility to validate the experimental models [2].

Which aspects of temporal lobe epilepsy can be studied in animal models?

Although the heading "temporal lobe epilepsy" covers all seizure types originating from the temporal lobe, one of the most frequent forms of TLE and which constitutes a large number of medically refractory epilepsies, is TLE with "hippocampal sclerosis". This term refers to a typical lesion of the hippocampal formation and was related to epilepsy more than 150 years ago [3]. It is characterized by extensive loss of neurons and gliosis, most severe in the CA1 and CA3 regions of the hippocampus and in the hilus of the dentate gyrus [4]. The neurodegeneration also affects, to a variable degree, granule cells of the dentate gyrus and CA2 pyramidal cells. The profound neuronal loss results in a marked volume reduction of the hippocampal formation, detectable by magnetic resonance imaging. While the cause of hippocampal sclerosis is not known, retrospective studies of TLE patients who underwent a surgical resection for intractable seizures have shown a correlation between febrile seizures in early childhood and the development of TLE with hippocampal sclerosis [3]. Although this correlation is disputed, it is very likely that an initial precipitating event predisposes certain patients to the development of TLE with hippocampal sclerosis.

In rodents, insults to the hippocampus induced by a status epilepticus also lead to a condition with spontaneous, unprovoked limbic seizures that mimic clinical findings. Histologically, these insults resemble hippocampal sclerosis, pointing to a selective vulnerability of certain types of hippocampal neurons, notably in the hilus of the dentate gyrus. Although the etiology underlying TLE with hippocampal sclerosis and experimentally-induced hippocampal lesions is very different, it is generally believed that common mechanisms might underlie the generation of spontaneous seizures in man and rodents [5]. Therefore, animal models constitute a valuable tool to study the pathophysiology of TLE, in spite of the inherent differences between the human and the rodent brain. In particular, experimental studies allow determining the type of lesion that might contribute to epileptogenesis, observing and manipulating the disease process before the onset of spontaneous seizures, assessing the effects of seizures on neuronal survival, and examining the chronically epileptic brain with a battery of anatomical, biochemical, pharmacological, and electrophysiological techniques [1].

TLE is a chronic disorder, which manifests itself by the sporadic apparition of seizures and other symptoms, such as the auras. It implies a permanent change in brain structure and/or function, which remains unnoticed between these episodes but is necessary for triggering seizures. Several animal models, in particular the "kindling" model of epilepsy, have been designed to investigate the nature and location of these changes. More recently, a model of febrile seizures was developed in young rats to study the long-term effects of a single episode of seizures occurring in juvenile brain [6,7].

There is no single animal model that reproduces faithfully all features of TLE with hippocampal sclerosis, and therefore each model has to be selected for addressing a specific set of questions. The mouse model described in this review is characterized by a pattern of neuronal loss comparable to hippocampal sclerosis and by the occurrence of spontaneous focal seizures that resemble the complex partial seizures seen in many patients with TLE. It presents with a unilateral hippocampal lesion, offering the advantage to investigate whether compensatory changes in the contralateral hippocampus represent endogenous anticonvulsant mechanisms preventing the spread of seizures to the intact side of the brain.

Finally, animal models have a good predictive value for the efficacy of antiepileptic drugs and for studying the mechanisms of drug resistance affecting a large

proportion of patients with partial complex seizures [8]. Again, the mouse model described here appears to be very valuable in this respect, since it was shown to be resistant to classical anti-epileptic drugs [9].

Classical animal models

It has been observed long ago that electrical stimulation of the brain can cause a seizure. Several regions of the temporal lobe, including the entorhinal cortex, amygdala, and hippocampal formation, are particularly sensitive to electrical stimulations, and low intensity currents are sufficient to induce epileptic seizures in these regions. The kindling model of TLE is derived from this observation (see [2] for review). Kindling is induced by repeated applications of low intensity stimuli, which initially cause only a local excitation, without any behavioral effect. Gradually, however, each subsequent stimulation recruits a large neuronal population, resulting in electrographic seizures and ultimately in a generalized convulsion. When rats or mice are stimulated once daily, it takes about three weeks to achieve a generalized tonic-clonic seizure episode (grade 5 seizure on a scale from 0 to 5). Once established, the kindled state is permanent. Renewed application of the low intensity electrical stimulus, even after a delay of several weeks or months, will suffice to provoke a grade 5 seizure. The kindling procedure therefore demonstrates that repeated electrical stimulations of sensitive areas in the temporal lobe induce irreversible changes in the brain that greatly lower the threshold for seizures. The nature of these changes is unknown but could be related to the plasticity phenomena discussed in the accompanying articles by Stoop and McKinney. Kindling causes only moderate neuronal damage and does not lead to spontaneous seizures. It is therefore not a "state of epilepsy". Recently, two lines of rats have been bred, exhibiting "slow" and "fast" development of the kindled state [10]. This study demonstrates a clear genetic basis for differences in susceptibility to kindling, supporting the notion of a genetic predisposition for development of TLE in man following an early precipitating event.

To produce a "state of epilepsy", characterized by the occurrence of spontaneous seizures, it is necessary to induce structural damage in the temporal lobe, in particular in the hippocampal formation. This can be achieved either by prolonged electrical stimulation, resulting in a status epilepticus, or following injection of specific drugs that produce a strong stimulation of the vulnerable regions [2]. For example, a systemic injection of the muscarinic acetylcholine receptor agonist pilocarpine to adult rats induces a severe status epilepticus, lasting for several hours unless terminated with diazepam. A latent, seizure-free period of several weeks occurs after the status epilepticus, eventually leading to the onset of recurrent seizures, which are then observed for the entire life of the animal. Unlike in most patients with TLE, these seizures are generalized, reflecting the widespread bilateral lesions caused by pilocarpine treatment in numerous brain areas, notably the entorhinal cortex, dentate gyrus, hippocampus, and several thalamic nuclei.

Instead of pilocarpine, similar effects can be produced by a potent excitatory neurotoxin, kainic acid (KA) [11]. This substance, which activates glutamate receptors, causes widespread neuronal loss in the hippocampus due to overexcitation and excessive influx of calcium, a phenomenon called "excitotoxicity". Like pilocarpine, systemic or intracerebroventricular administration of KA causes a severe status epilepticus followed after several weeks by spontaneous recurrent seizures. KA treatment causes severe neuronal loss in the hippocampal formation, especially in the hilus of the dentate gyrus and in the CA3 area. There is less extra-hippocampal neuronal damage than after pilocarpine treatment, supporting the view that lesions of the hippocampus are important for epileptogenesis.

These models have been extensively studied because they mimic, to some extent, TLE with hippocampal sclerosis. The main differences, however, are that the drugs cause bilateral lesions and induce generalized seizures. A major finding was that chronic epilepsy is accompanied by the formation of aberrant neuronal circuits, notably due to sprouting of granule cells axons so-called mossy fibers because of their unique morphology into the molecular layer of the dentate gyrus, a feature never seen in normal brain [12]. Mossy fiber sprouting is a prominent feature of TLE with hippocampal sclerosis and has been proposed to provide a substrate for enhanced excitability in the dentate gyrus. Recent studies have shown, however, that this form of structural plasticity is not required for the onset of spontaneous recurrent seizures in animal models of TLE [13]. Its significance in human TLE remains debated.

The mouse kainate-model of temporal lobe epilepsy

This model was developed following the chance observation that injection of a very small dose of KA directly into the hippocampus of adult mice causes striking morphological changes over the course of several weeks **(figure 1)** without producing generalized seizures [14]. The authors of the initial report observed a profound degeneration of the CA1 region of the hippocampus and a striking enlargement of the dentate gyrus, due to the hypertrophy and dispersion of granule cells. It was shown in a subsequent study that these mice develop spontaneous focal seizures in the lesioned hippocampal formation [15]. Finally, a detailed histological and functional characterization of KA-treated mice demonstrated that this model replicates several major features of TLE with hippocampal sclerosis [16], and it is now being extensively used to study experimentally pathophysiological mechanisms of TLE *in vivo*.

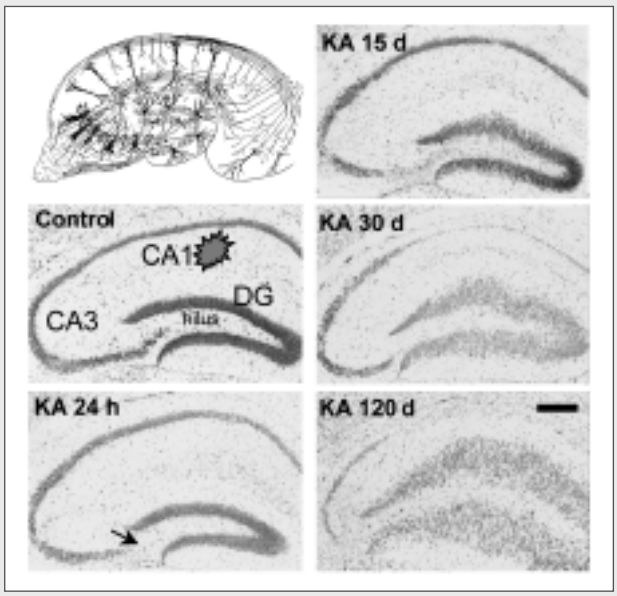

Figure 1: Gradual degeneration of the hippocampus and hypertrophy of the dentate gyrus following a unilateral injection of kainic acid (KA) in adult mouse. A diagram (adapted from [17]) depicting the main types of neurons and the circuitry of the hippocampus is given for orientation. Photomicrographs of Nissl-stained sections are shown to illustrate the cytoarchitecture of the mouse hippocampal formation in control (untreated mouse) and at the given time-points following KA injection (indicated by the orange symbol). Note the loss of neurons in the hilus and CA3 region (arrow) as soon as 1 day after the lesion, and the gradual disappearance of the CA1 area over the next few months. Starting at 2 weeks post-KA, the dispersion of granule in the dentate gyrus (DG) becomes evident. After 4 months, the enlarged dentate gyrus occupies most of the volume of the original hippocampal formation. Scale bar, 200 µm.

The main features of the model

One of the most interesting features of this model is the slow, but gradual progression of neurodegeneration in the KA-injected hippocampal formation, leading to a profound atrophy of the hippocampus [16, 18] **(figure 1)**. The time course of neurodegeneration, along with the changes in EEG patterns, allows distinguishing three phases in the disease process [9] **(figure 2)**: The initial phase, characterized electrophysiologically by a non-convulsive status epilepticus **(figure 2B)**, corresponds to the acute excitatory and neurotoxic effects of KA. When examined histologically after 24 h, the treatment causes a local lesion in CA1 around the injection site and an extensive loss of neurons in the hilus and in the distal (hilar) part of CA3 **(figure 1)**. The second phase lasts about 2 weeks and corresponds to the latent phase of the pilocarpine model. EEG recordings of the injected hippocampus revealed a disappearance of the normal activity pattern, in particular, the occurrence of theta rhythms during exploration and active behavior **(figure 2A)**. Instead, it is replaced by a low amplitude activity interrupted at irregular intervals by isolated spikes and waves, but no seizures **(figure 2C)** [9]. During this phase, there is a slow progression of the lesion in CA1, evidenced by increasing neuronal loss in the pyramidal cell layer **(figure 1)**. Finally, the chronic phase, which starts by the occurrence of the first recurrent seizure and which leads after about four months to a complete degeneration of the CA1 region and to the hypertrophy of the dentate gyrus noted in the initial report **(figure 1)**. During the chronic phase, mice experience frequent recurrent seizures interspaced by isolated spikes and waves of large amplitude, typical for interictal activity [9] **(figures 2D, E)**. Most strikingly, seizures are unilateral, restricted to the lesioned hippocampal formation, whereas intericatal spikes and waves can be recorded also contralaterally. Only occasionally do seizures spread to the cerebral cortex and to the contralateral hippocampus, resulting in secondary generalization [9]. In mice recorded up to one year after KA injection, the length and frequency of recurrent seizures did not change over time, indicating that the chronic state is stable [15].

The three distinct stages of the model allow several conclusions. First, while the initial lesion in CA1 and the loss of neurons in the hilus and CA3 result from the neurotoxic action of KA, the delayed degeneration of the entire CA1 region most likely has another cause. It is not known why these cells die, or whether recurrent seizures contribute to neurodegeneration. For instance, inflammatory mechanisms triggered by the KA-induced lesion might also contribute to the delayed loss of neurons in CA1. The mouse KA model therefore offers an excellent opportunity to study the mechanisms of neuronal and to develop therapeutic strategies based on neuroprotection. Secondly, the acute damage induced by KA is not sufficient for triggering the onset of recurrent seizures. Additional mechanisms, which develop during the latent phase, are necessary. Thirdly, recurrent seizures start before the degeneration of the CA1 area is much advanced. Hippocampal sclerosis might therefore be a consequence rather than a cause of recurrent seizures in TLE.

The hypertrophy and dispersion of granule cells in the dentate gyrus is another striking feature of this model **(figure 1)**. This process starts towards the end of the latent phase and evolves gradually over a period of about two months [14]. At the end, granule cells are dispersed and markedly enlarged in volume. Their dendritic tree is also expanded and becomes covered by numerous spines [19]. As a result, the volume of the dentate gyrus is significantly increased. The onset of recurrent seizures matches the beginning of the dispersion, but it is not clear how the two phenomena are related. Granule cell dispersion is also a feature of human TLE with hippocampal sclerosis [20], but it is not observed in all patients and it is much less pronounced than in the KA-mouse model. The hypertrophy of granule cells is

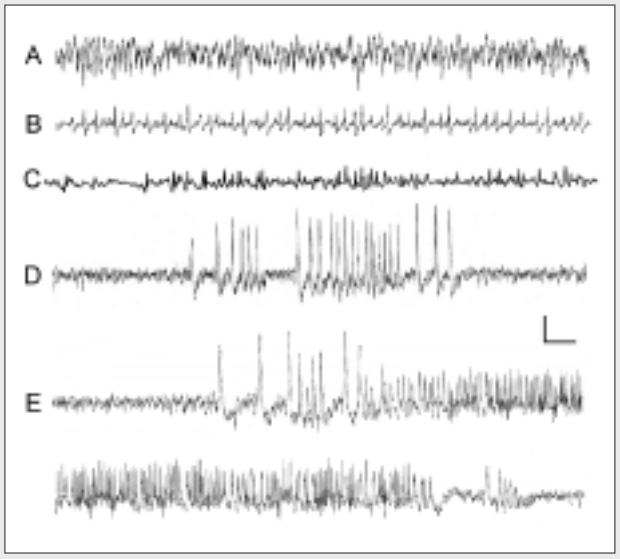

Figure 2: Traces from intrahippocampal EEG recordings taken at various stages after KA treatment with a bipolar electrode implanted in the hippocampus (see [9] for details). A) Typical theta-like activity in a control mouse during exploratory behavior; B) Status epilepticus recorded upon recovery from anesthesia shortly after the KA injection; C) Irregular spiking activity during the latent phase. Note the overall decrease in amplitude and the absence of rhythmic activity compared to control; D) Large amplitude, low frequency spikes and waves characteristic of interictal activity during the chronic phase. These spikes and waves were seen either isolated or grouped in short trains as depicted; E) Example of a recurrent seizure during the chronic phase. Typically, seizures started with a few large amplitude spikes and waves, followed by regular spikes with higher frequency and gradually decreasing amplitude. Such seizures were seen at variable frequencies (up to several per hour), mainly during the resting phase of the mice. Horizontal scale: 1 second (A-C) ; 1.5 second (D-E); vertical scale : 1 mV (A-C) ; 1.5 mV (D-E).

accompanied by numerous neurochemical changes in these neurons (see next section). They are therefore likely to become functionally altered during the chronic phase of the model.

Finally, during the entire disease process, the contralateral hippocampal formation, which is not lesioned by the KA injection, remains structurally largely intact [16]. In spite of the numerous synaptic connections between the two sides of the brain, the repeated occurrence of seizures in the KA-treated side does not seem to induce major alterations on the other side. It will therefore be of major interest to study the endogenous adaptations preventing the spread of seizures to the contralateral hippocampus.

Mechanisms of epileptogenesis

Similar to the classical rat models of TLE discussed above, vulnerable populations of neurons, notably in the hilus of the dentate gyrus, are irreversibly destroyed during the KA-induced status epilepticus. As noted above, this lesion does not result in spontaneous seizures, suggesting that additional adaptation mechanisms are required. Since seizures generally originate from an imbalance between excitatory and inhibitory inhibition, a detailed morphological analysis of KA-treated mice was undertaken to determine whether a change in inhibitory neurons, the so-called GABAergic interneurons, underlies the onset of recurrent seizures. In the hippocampal formation, interneurons are highly specialized cells, which use the inhibitory neurotransmitter GABA to control the activity of dentate gyrus granule cells and CA1-CA3 pyramidal cells. These cells can be visualized in brain sections with several markers, which label each a distinct subpopulation of interneurons. To determine whether some interneurons are destroyed by KA treatment, brain sections from control and KA-treated mice were examined at different time-points after the injection with markers of interneurons. These investigations revealed an irreversible disappearance of several of these markers within 1 day after KA treatment, even in hippocampal regions where the pyramidal cells did not appear to be lesioned by the neurotoxin **(figures 3A-B)** [16]. This finding suggested that certain interneurons are highly vulnerable to the acute toxic action of KA, resulting in a reduction of GABAergic inhibition at the onset of the latent phase. Pronounced changes were observed also in dentate gyrus granule cells, notably a strong overexpression of a neuropeptide, named NPY **(figure 3C**), which has been suggested to have an anticonvulsant action in the brain [21]. It is not known whether NPY is involved in seizure suppression during the latent phase, but this possibility is currently being investigated.

Compensatory adaptations during the chronic phase

TLE with hippocampal sclerosis offers the advantage, as noted in the introduction, that final stages of the disease can be studied in tissue resected from patients suffering from intractable seizures. Numerous studies have reported changes in the expression or distribution of neurotransmitters, their receptors, ions channels, and other components of interneurons signaling in TLE with hippocampal sclerosis. The significance of these changes for the pathophysiology of TLE has remained uncertain, because it is not known whether they are established during the latent phase of the disease, or whether they occur as a consequence of seizures. In the mouse KA model, the clear separation between the two phases allows to distinguish causes and consequences.

We have focused our attention primarily onto the GABAergic system to investigate the long-term consequences of the loss of GABAergic neurons occurring during the acute phase and to determine whether

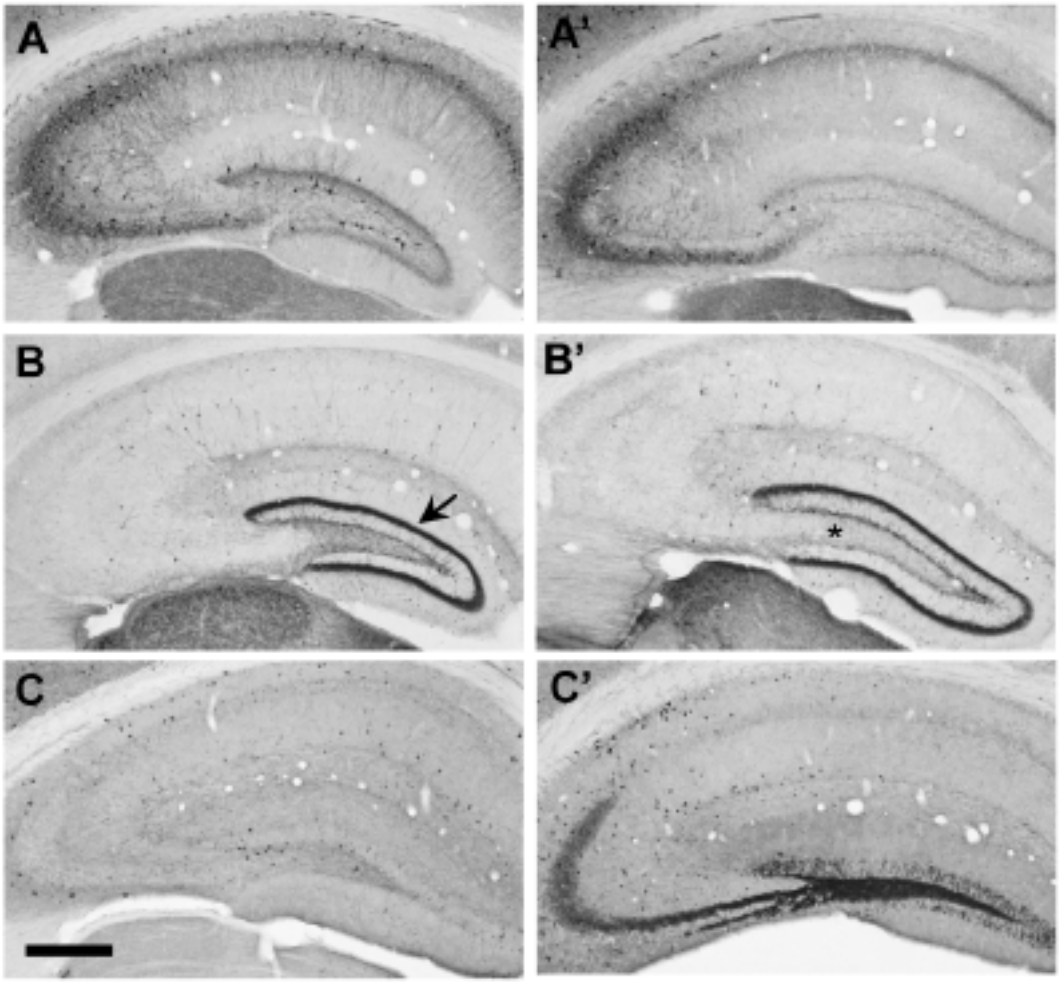

Figure 3: Neurochemical changes during the latent phase after unilateral intrahippocampal KA injection. A-A': Immunohistochemical staining for the calcium-binding protein parvalbumin, which marks a subpopulation of interneurons in the hippocampus and dentate gyrus (A). This marker labels their cell body, dendrites, and axons that form a dense plexus in the granule cell layer of the dentate gyrus and in the CA3-CA1 pyramidal cell layer. Note that most parvalbumin-positive cells have disappeared in the dentate gyrus and in CA1 at 24 h after KA injection (A'). B-B': Unchanged staining for calretinin, another calcium-binding protein that marks a distinct population of interneurons, as well as a very dense layer of axons in the molecular layer of the dentate gyrus (arrow). Few changes are visible in KA-treated hippocampus (B'), except for the reduced staining of the hilus (*), reflecting the acute loss of cells in this area. C-C': Up-regulation of NPY, a neuropeptide, in granule cells and in their axon innervating the CA3 pyramidal cells (mossy fibers), as seen at 24 hours after KA injection. Note that NPY also is present in isolated interneurons, which appear more strongly stained that in control. Scale bar, 100 µm.

changes in $GABA_A$ receptors, which have been observed in human [22], also occur in the KA-model of TLE. $GABA_A$ receptors are formed by the assembly of five proteins, so-called subunits, which form an ion channel permeable for chloride ions in the membrane of neurons. They play an essential role in the control of neuronal excitability and a reduction in $GABA_A$ receptor function can induce seizures, as discussed in the accompanying articles. However, GABAergic transmission also contributes to synchronize the activity of neurons. Enhancing $GABA_A$ receptor function above a certain level causes hypersynchronicity and seizure-like discharges. Therefore, either too little or too much GABAergic transmission can lead to seizures.

The analysis of $GABA_A$ receptors in the hippocampal formation of KA-treated mice using immunohistochemistry revealed a marked increase in the dentate gyrus **(figure 4A)**, selectively during the chronic phase [16,18]. Therefore, the hypertrophy of granule cells is accompanied by a higher amount of $GABA_A$ receptors in these neurons. The increase was particularly marked in the cell body layer **(figure 4A)**, where numerous GABAergic synapses are normally formed. This result was unexpected, in view of the partial loss of interneurons that characterizes this model, but it suggested that the remaining GABAergic cells form novel axons and synapses

during the chronic phase, possibly to compensate against the increased activity during the seizures. This possibility was examined using a marker of GABAergic axons, the enzyme "glutamic acid decarboxylase", which is responsible for GABA synthesis and is localized selectively in GABAergic neurons. This study indicated that the density of GABAergic axons, which is dramatically reduced after KA-injection, gradually increases during the chronic phase **(figure 4B)**, especially in the

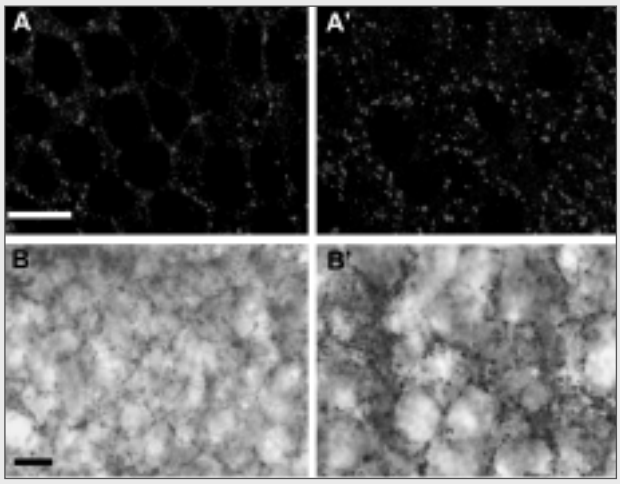

Figure 4: Up-regulation of $GABA_A$ receptors (A-A') and GABAergic axons (B-B') in the granule cell layer during the chronic phase after KA treatment. $GABA_A$ receptors were labeled by immunofluorescence using an antibody against the $\alpha 2$ subunit and visualized by confocal laser scanning microscopy (see [18] for details). They appear as brightly labeled dots, representing individual synapses around the cell body of granule cells. Note that the density of these dots is markedly increased four weeks after KA treatment. GABAergic axons were stained by immunoperoxidase staining with antibodies against glutamic acid decarboxylase and visualized by light-microscopy. They appear as dark brown dots on the surface of unlabeled granule cells. The size and density of these dots, which represent synaptic contacts matching the receptors depicted in A-A', are increased in KA-treated dentate gyrus granule cell layer. Scale bars 10 µm.

granule cell layer of the dentate gyrus. This effect was seen after the onset of recurrent seizures and is therefore a consequence rather than a cause of epilepsy. It is not known, however, whether this abnormally high number of GABAergic axons and synapses represents a protective mechanism against seizures, or whether it facilitates additional seizures due to hypersynchronization.

The observation that the GABAergic system is increased during the chronic phase of TLE, both in human tissue and in sections from KA-treated mice strongly reinforces the validity of this model for studying TLE with hippocampal sclerosis [23]. It was unexpected, because it makes it difficult to explain why hippocampal neuron becomes more excitable when a transmitter system, which normally serves as a "brake" in the brain, is increased. The response to this paradox will require further analysis. Given the numerous parallels between the model and TLE with hippocampal sclerosis, we can expect the answer to this question to help us understand the pathophysiology of the disease.

The possibility of modeling TLE with hippocampal sclerosis in a mouse offers novel avenues for genetic analyses, because of the availability of numerous lines of mice carrying specific mutations. The KA-model of TLE will therefore represent an important tool for future research aiming at understanding the role of specific genes in epilepsy. Finally, as outlined in the following article, this model is well suited for testing novel therapeutic strategies.

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Korrespondenzadresse:
Prof. Dr. Jean-Marc Fritschy
Universität Zürich
Institut für Pharmakologie und Toxikologie
Winterthurerstrasse 190
CH 8057 Zürich
Tel. 0041 1 635 59 26
Fax 0041 1 635 68 74
fritschy@pharma.unizh.ch