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Editorial and abstracts

Editorial: Stiftung Michael and the Michael Prize

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What is the «Stiftung Michael», or, to put it in English, the «Michael Trust»? A short answer would be that it is a foundation with the specific aim to foster research into experimental and clinical epileptology, assist in epileptology training for physicians and support staff in less affluent countries, improve equipment of hospitals and outpatient departments, and provide logistic and financial support for patient groups. Its overall aim is to ameliorate the diagnosis, treatment and rehabilitation of epilepsy patients, which it has pursued since its beginning or founding in 1962.

A lengthier answer would dig deeper and include the biography of the trust's founder, Dr. Fritz Harzendorf. Harzendorf was a journalist and publisher from Konstanz, the city that also gave its name to the lake bordering on Austria, Germany and Switzerland. Born in 1889, Harzendorf was drafted into the army in World War I, during which he fought first as a soldier and later as an officer. After the war, he studied philosophy and political science, attained his PhD and worked as a journalist – a commendable feat, because all of this happened during the Great Depression and with practically no support from his family. During the early 30's he wrote in opposition of the National Socialist party, which started to gain momentum in Germany at that time. As a consequence of his critical voice, he later lost his position as an editor and was therefore forced to keep himself afloat by selling soap and insurance policies. After the war, Harzendorf became editor-in-chief of a local newspaper in his hometown, and in 1946 obtained permission by the American military government in the southern sector of Germany to publish a regional newspaper. Thus, he became cofounder and editor-in-chief of the *Neue Württembergische Zeitung*. What does this have to do with the Stiftung Michael? To protect the political independence of the newspaper, Harzdorf came up with the concept of a foundation. Simultaneously, it turned out that his recently born son Michael suffered from epilepsy. This experience, and the sad realization that the therapeutic scope of epileptology was very limited at that time, with many issues still unexplored, prompted him to divert his initial plans and to sell his stake in the newspaper to found the «Michael Trust». He conferred with the epileptologist Professor Dieter Janz about his plan for this donation to a foundation, and with legal assistance from Professor Konrad Duden, the trust came into being on September 5, 1962. Only two years later, Dr. Harzendorf succumbed to a severe disease and which he had borne with dignity and optimism.

The trust continues to be linked to the Harzendorf family: Dr. Harzendorf's daughter, Agathe Bühler, the sister of Michael, is actively involved in the work of the «Stiftung Michael» in her role as a member of the board of trustees headed by Dieter Janz. This board, together with the executive office, are steering the trust and managing its manifold tasks. The executive office, with its chairman Dr. Christe and former chairman Dr. Reith, is responsible for the operational work and enables the trust to continue to work for the aim of improving quality of life for epilepsy patients.

The «Michael Prize» was initiated to stimulate and support research in epileptology, both nationally, and since 1975, also internationally. Since 1980, this prize was awarded biannually to scientists below the age of 40, and it is now – supported by Novartis – one of the most highly endowed international prizes for epilepsy research. The prize has gone to groups in Austria, Brazil, Britain, Canada, Germany, Israel, Italy, Poland, Switzerland, and the USA, and among the winners are Massimo Avoli, Colin Binnie, Otto Creutzfeldt, Jerome Engel, Hans-Dieter Lux, Brian Meldrum, Solomon Moshé, Jeffery Noebels, Uwe Heinemann, Greg Holmes, and Yoel Yaari, just to name a few.

The first head of the Board of Trustees, Dr. iur. Scheidermann, forged the idea of regular scientific meetings of the Michael Prize winners, and thus the «Michael Forum» was conceived at a dinner in Jerusalem during the International Epilepsy Meeting in 1987. The «Michael Forum», first held in 1988, serves as an informal workshop to stimulate discussions among the laureates and to foster

productive exchanges of current ideas and concepts. During this meeting, the prize winners, together with the trustees and representatives of the sponsor (Novartis), discuss their recent scientific work illuminating causes, consequences, and the treatment of epilepsy. The «Michael Forum», however, is more than just a workshop. It always includes non-scientific lectures on art or architecture of the area. Because these meetings are held in Germany at changing sites, the participants get to know very different corners of that country. Informal get-togethers, excursions or barbecues are also fixtures of these gatherings. The scientific discussions, in turn, are always open, and interruptions are lively and welcome. And, as one of the participants pointed out, «the food is always exceptionally good». The most recent of these workshops in this series, organized by the executive office now under the auspices of Dr. Christe and Sabine Reith, the daughter of the former chairman of the office, took place in Potsdam near Berlin, on May 27-30, 2004, and the following papers summarize the talks given by the prize winners gathered on that occasion.

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Epilepsy in the developing brain: how do we get there from here?

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Status epilepticus (SE), which is arbitrarily defined as 30 minutes or more of continuous epileptic seizure activity, is a common neurological emergency in children associated with high morbidity and mortality. Although SE with or without recurrent seizures is associated with a wide variety of neuropsychological problems, memory deficits including impairment of episodic memory are especially prominent. It is important to identify the processes by which SE leads to permanently abnormal brain function, to prevent the operation of such processes or to reduce their effects afterwards.

Normal declarative memory, the ability to learn and recall specific information about people, places and events, depends on a properly functioning hippocampus [1, 2]. In light of the memory deficits seen after SE in humans, it is not surprising that SE is preferentially associated with histologically detectable damage of the hippocampus and related areas («mesial temporal sclerosis»). This form of damage is characterized by cell loss in CA1, CA3, the hilus and dentate gyrus and synaptic reorganization as evidence by sprouting of mossy fibers [3]. While the precise role of cell loss and synaptic reorganization in the sequelae of SE is unclear, there is evidence that other processes including recurrent seizures may play important roles in memory deficiencies [4, 5].

Extensive work in rodents has demonstrated strong behavioral correlates of hippocampal neuronal activity, the most robust of which is the selective activation of CA1 and CA3 pyramidal cells at particular locations (firing fields) in the space available to the animal [6, 7]. Thus, the action potential frequency of these «place cells» is high only when the head of a freely moving rat is in a cell-specific region of the space; when the head is outside the firing field the firing rate is virtually zero [8, 9]. In simple circumstances, the discharge of place cells is remarkably independent of aspects of the animal's behavior other than location [10]. Firing fields are stable over long periods of time if the environment remains constant, implying that the representation is recalled and not created *de novo* each time the rat enters a familiar environment [8]. Successful performance in spatial tasks appears to require the coordinated, location-specific firing of place cells, although the relationship between the place cell phenomenon and spatial memory needs further testing and clarification [11]. Changes of firing patterns in a novel environment are attributable to a rapid, learning-like, «remapping» process that persists over a long time [10, 12-15].

Place cells do not operate in isolation from the remainder of the hippocampal network. For an animal to determine his location, sensory information from the environment, primarily visual, is transmitted to the entorhinal cortex. This representation is then transformed by feedforward pathways (the direct perforant path connections to CA3 and CA1 and pathways through the dentate gyrus and then into CA3 pyramidal cells) [16]. Information is transmitted through recurrent connections between CA3 cells and to inhibitory interneurons which innervate both CA1 and CA3 principal neurons [17].

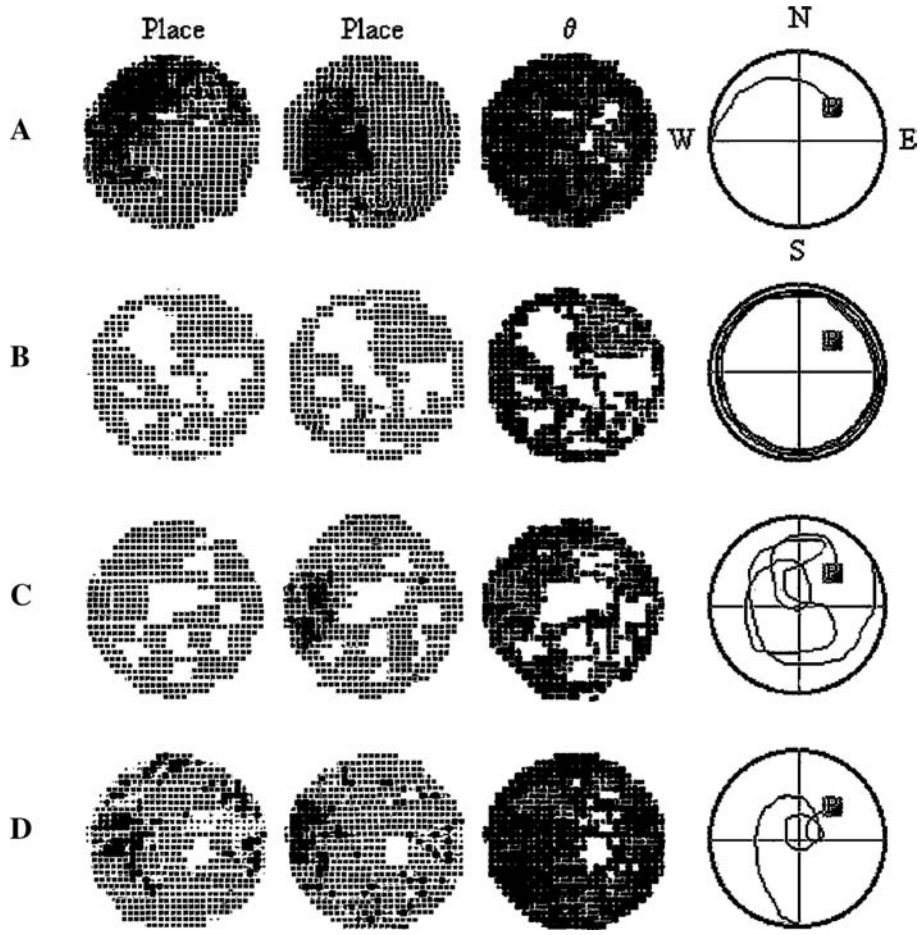
To directly address the cellular concomitants of spatial memory impairments, we recently recorded the activity of single hippocampal neurons in freely moving rats subjected to SE during early development and compared this activity to that in control rats [18]. Adult rats experiencing SE during early development showed deficient performance in a test of visual-spatial memory, the Morris water maze.

We found that the rats with SE had a similar number of place cells as control animals. CA1 pyramidal cells judged as place cells in SE rats appeared normal in several ways. Specifically, we saw no differences between control place cells and place cells from the SE rats in field area, field center firing rate, field firing rate or information content. Given these similarities, it was remarkable and exciting that place cells in the two groups differed strikingly ac-

cording to coherence which measures the local smoothness of spatial firing patterns. The mean coherence for control place cells of 0.81 ± 0.22 (n = 69) was reliably higher than the mean of 0.59 ± 0.22 for the place cells (n = 47) from the SE rats ($p < 0.0001$). Thus, firing field organization was weaker in adult rats subjected to SE in prepubescence. On the assumption that place cell activity is essential to normal navigational behavior, the decreased coherence helped account for the poorer water maze performance seen in the SE rats.

We then asked if the positional firing patterns of the place cells from the SE rats are stable over time. To this end, we ran four recording sessions for each control cell and cell from the SE rats within a 7-hour period. Place cells in the control rats appeared stable; their fields remained in approximately the same position and retained their shapes across the four recording sessions. In marked contrast, the positional firing patterns of SE rats often changed considerably over time. The poor stability of the place cell was akin to «forgetting» where the platform was located.

To determine if there was a relationship between place cell function and water maze performance we compared coherence, cell stability (angular displacement and maximal similarity scores) with results of the probe test in the water



Rapid, parallel changes of place cell firing patterns and water maze performance after flurothyl-induced generalized tonic-clonic seizures in a normal rat.

The rat is trained to run around a cylinder to chase food pellets. Action potentials from single place cells are recorded continuously. To track position the rat has a light-emitting diode (LED) on its head that is recorded by an overhead TV camera. The total time the LED is detected in each pixel and the number of action potentials in each pixel is recorded. A time-averaged firing rate distribution is calculated by dividing the number of spikes in each pixel by the dwell time in that pixel. Color-coded firing rate maps are used to visualize positional firing distributions. In this black and white figure, the darker pixels represent the firing field of the place cell. Unvisited pixels in the cylinder and pixels outside the cylinder are coded white. In this example, three cells were recorded simultaneously in all 3 sessions. ***A****) The firing patterns of 2 place cells (left and center columns marked «Place») and 1 theta cell (interneuron) (right column) prior to the flurothyl seizure. Shortly after this recording session water maze performance was accurate (trajectory of rat's swim to find the platform (P) in far right column).* ***B****) Thirty minutes after a seizure, both place cells were silent. The theta cell fired but at a lower rate than before the seizure. The rat swam in circles at the tank edge and could not find the platform.* ***C****) Two hours after the seizure, one place cell began to fire and the rat improved its performance in the water maze.* ***D****) Six hours after the seizure, both place cells discharged but at a lower frequency than in the pre-seizure state. By this time, water maze performance has substantially improved.*

maze in the large tank. There were significant correlations between coherence (p = 0.002), angular displacement at the short interval (p = 0.005), angular displacement at the long interval (p = 0.017), maximal similarity score at the short interval (p = 0.006), and maximal similarity score at the long interval (p = 0.004). These findings demonstrate a close relationship between place cell precision and stability, on a single cell level, with water maze performance.

In summary, these rats had abnormal place cells. The place cells from the status epilepticus rats were defective in two ways: 1) Their firing fields were less orderly than those of normal rats; and 2) Their firing fields were less stable than those of normal rats. There was a strong correlation between aberrant place cell firing patterns and water maze performance. Animals that had unstable place cells performed worse in the water maze than rats that had stable place cells.

In addition to examining place cells chronically in animals with a prior history of seizures, we measured place cells and water maze performance in normal adult rats who underwent acute seizures induced by flurothyl inhalation. Rats were previously trained in the water maze and swam directly to the platform when placed in the water maze *(figure A)*. After a brief seizure the place cell firing is suppressed and the rats cannot find the water maze platform *(figure B)*. As the place cells recover, the rat also takes less time to find the platform *(figures C and D)*. These acute studies provide additional proof that place cells have an important role in spatial memory in rats.

Seizures therefore have consequences that are reflected at the single cell level in the hippocampus, the brain region implicated in declarative memory in humans and spatial memory in rodents. These single cell recordings may serve as a surrogate marker for cognitive impairment, and as such provide a powerful tool for eliciting mechanisms responsible for seizure-induced cognitive impairment.

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Computational approaches to epilepsy diagnosis: exchanging cases and classifying diagnoses

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Epilepsy is one of the most difficult areas in medicine for diagnosis. Frankel [1] has estimated that there are about 1000 genes for which mutation can produce epilepsy. Since there are numerous situations in which alternate mutations of the same gene can produce different disorders [2], and environmental factors can interact with genes to produce disorders such focal epilepsy [3], it is likely that there are thousands of different types of epilepsy.

The International League against Epilepsy (ILAE) has approached two parts of this problem with well-accepted

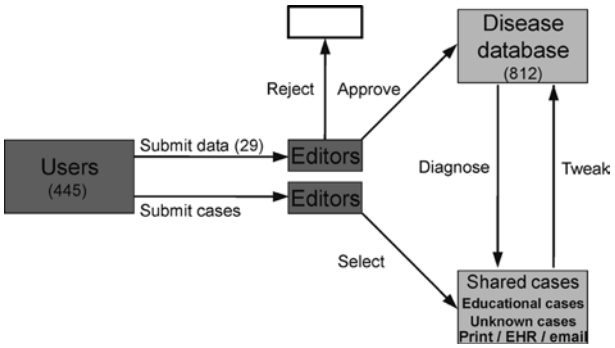
classifications: the *Classification of Seizure Types* [4], and the *Classification of Epilepsies & Syndromes* [5].

In 2001 the ILAE revisited the diagnosis problem with a new report [6], elaborated in detail at a Web site (http://epilepsy.org/ctf/). A different classification of seizure types was put forward, and the classification of epilepsies and syndromes was deferred, with focus on efforts to delineate individual epilepsies and syndromes. However, no new classifications were adopted in recognition of the following issues:

1. There was not a big enough paradigm shift to justify a new classification;

2. Knowledge is advancing so quickly that classification is a moving target;

3. Focusing on evidence-based diagnosis is a more urgent need than classification.



Schematic diagram of database structure.

We have been dealing with similar issues in the diagnosis of the Neurological Syndromes, using a computational approach to aggregate diagnostic evidence from 29 collaborators in the United States and Europe. Our current working model includes 812 Neurological Syndromes and over 13,000 data points of individual findings in individual diseases (http://SimulConsult.com/neurologicalsyndromes/). Our experience suggests a similar philosophy to the ILAE and provides a framework that may be helpful in approaching epilepsy diagnosis:

1. A paradigm shift to a computational approach could consider seizure types and EEG results as findings, epilepsies & syndromes as diseases, and disease categories that are recognized to be non-unique;

2. A collaborative internet-based approach allows the database to be changed continuously;

3. A model with an 'inference engine' that is based on hard data allows an evidence-based approach.

A second computational approach we have been taking to diagnosis is the «Case Sharing Project», an automated procedure for documenting and exchanging patient cases. A group of residency programs in the United States is beginning to use this approach to convert patient cases being run through the software to educational cases shared among the various programs. This provides a framework both for teaching diagnosis and for harnessing the efforts of a large group of doctors to add evidence to the diagnostic model, resulting in a database that improves in performance and keeps up with changes in diagnostic knowledge.

These approaches provide a working model of an approach that may be useful as we face the increasingly difficult task of dealing with the large amount of information on epilepsy diagnosis.

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Neuron loss and aberrant connectivity in the epileptic hippocampus: different expression of experimentally-induced ictaform activity *in vitro*

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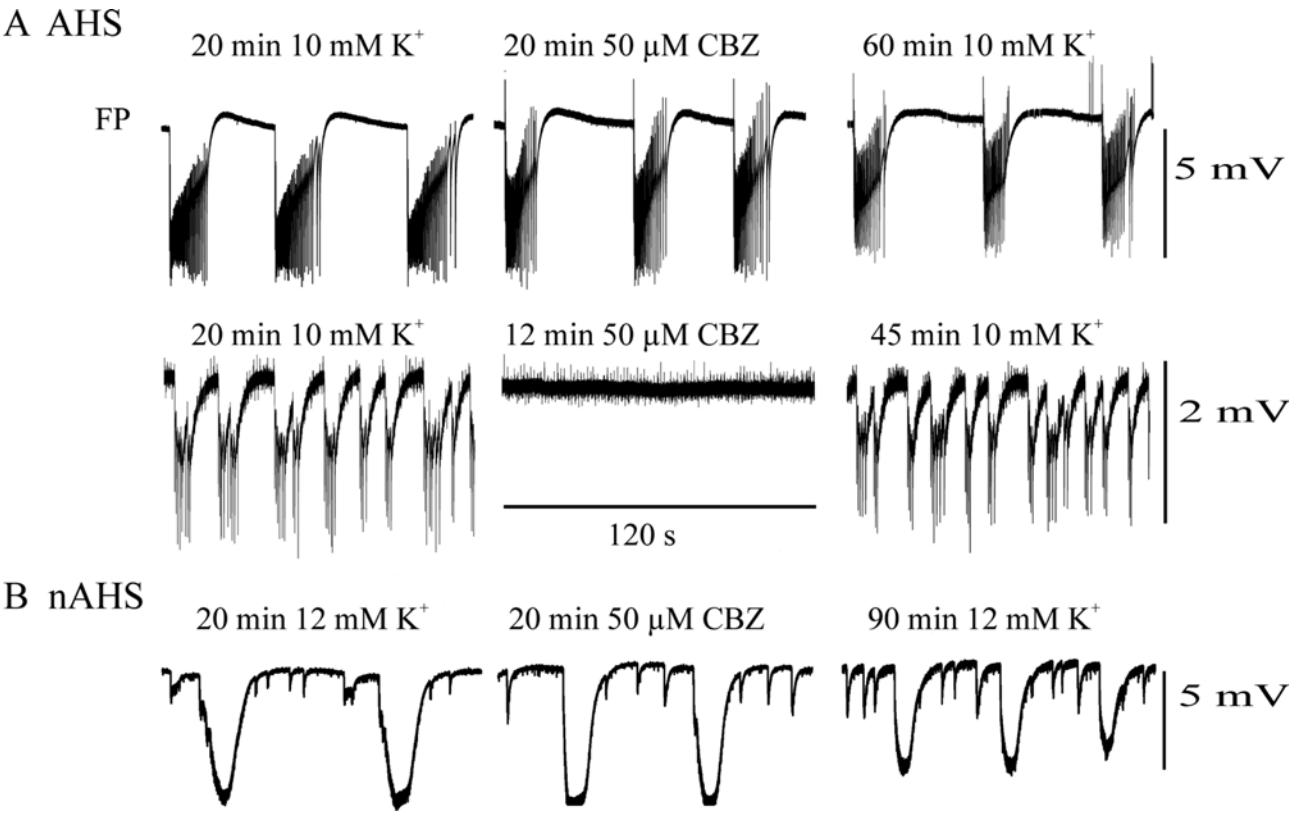
Reorganisation of the brain has been considered a hallmark of temporal lobe epilepsy (TLE). It includes neuron loss, gliosis, axonal sprouting, synaptogenesis, and neurogenesis [1-3], changes in the expression or function of receptors or transporters of neurotransmitters [4, 5], neuronal and glial ion channels [6, 7], matrix glycoprotein's, axon guidance molecules, and genes [8] encoding proteins, lipids and enzymes involved in signalling pathways. These alterations appear individually or in concert and vary in degree and localisation. However, it remains unknown whether and to what extent such alterations contribute to pathogenesis and pharmacoresistance of TLE. We focussed: (a) on the variance of hippocampal neuron loss and aberrant connectivity at the chronic state of temporal lobe epilepsy in the rat pilocarpine model of hippocampal sclerosis and in patients who underwent epilepsy surgery, (b) on the induction of ictaform activity in slices from resected hippocampal specimens, (c) on differences of ictaform activity between tissue characterised by neuron loss and aberrant connectivity and tissue apparently devoid of such alterations, and (d) on the sensitivity of experimental ictaform activity to treatment with the anticonvulsant drug carbamazepine. After listing the methods used we will give a short summary, while detailed descriptions of individual experiments and results were already published or will appear elsewhere.

All experiments were approved by local ethic committees and follow the rules of the European Communities Council. Adult and young adult male Wistar-rats were subjected to post-pilocarpine status epilepticus (SE) of 90-120 min duration [9, 10] and investigated 3-6 month after SE. Surgically resected human hippocampal specimens have been received in the operation theatre and transported to the laboratory. Hippocampal slices from both rat brain and human specimens were prepared and maintained as previously described [9, 11]. Cells were counted on representative sections stained with cresyl violet. Aberrant connec-



Effects of carbamazepine (CBZ) on experimentally induced ictaform activity in slices from human resected hippocampi.
A*) 43 year old patient, 21 years epilepsy, Ammon's horn sclerosis (AHS) Wyler grade 3, CBZ-resistant patient. At top: one of three slices (anterior corpus hippocampi) insensitive to treatment; below: one slice (8 mm further occipital) sensitive to treatment.* ***B****) 46 year old patient, 26 years epilepsy, focal cortical dysphasia, Wyler grade 1, CBZ resistant.*

tivity was determined using fluorescent dextran amine tracing [9] and Neo-Timm stain. In human hippocampal slices ictaform activity was induced by low frequency hilar stimulation in presence of high potassium (10-12 mM)-containing ACSF. Field potentials and $[K^+]o$ were recorded using K^+-sensitive/reference microelectrodes [11].

In both adult and young adult SE animals and in surgically treated TLE-patients with Ammon's horn sclerosis (AHS) hippocampal cell loss frequently coincided with appearance of aberrant supragranular mossy fibre terminals. These terminals are known to synapse on dendritic structures [12] forming a network of recurrently connected granule cells [13]. Likewise, increased interconnectivity between pyramidal cells of area CA1 occurred in rat and human tissue with AHS [9, 14, 15], as long as cell loss in area CA1 was not larger than about 70 %. This supports the view that structural network reorganisations in the dentate gyrus and in area CA1 are characteristics of AHS.

In order to test whether such network reorganisation might favour seizure generation in AHS tissue, we induced ictaform activity in the dentate gyrus of hippocampal slices prepared from human resected hippocampi with and without AHS. Long-time recordings of self-sustained ictaform activity were obtained in slices from both AHS- and nAHS hippocampi *(figure)*. However, in AHS-slices the potassium concentration mandatory for induction of such activity was lower than in nAHS-slices. Additionally, seizure like events *(figure A)* and periodic ictal spiking were predominantly observed in AHS-tissue while atypical negative field potential shifts occurred in nAHS-tissue *(figure B)*. The findings suggest that dentate structural network reorganisation, possibly in concert with other AHS-specific reorganization processes, increases susceptibility and determines pattern of experimental ictaform activity in the dentate gyrus of sclerotic tissue. The mechanisms have to be specified by further experimentation.

The time course of experimental ictaform activity permits to study mechanisms of pharmacoresistance or to test whether newly developed drugs are superior to presently available anticonvulsants. We could show that the antiepileptic drug carbamazepine had predominantly no effect on ictaform activity in resistant patients (*figure*: upper traces of A and B) while it blocked such activity in sensitive patients (not shown), thereby supporting the hypothesis that loss of sodium channel drug-sensitivity constitutes a novel mechanism underlying the development of drug-resistant epilepsy [16].

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Mechanisms of antiepileptic drug resistance in models of intractable epilepsy

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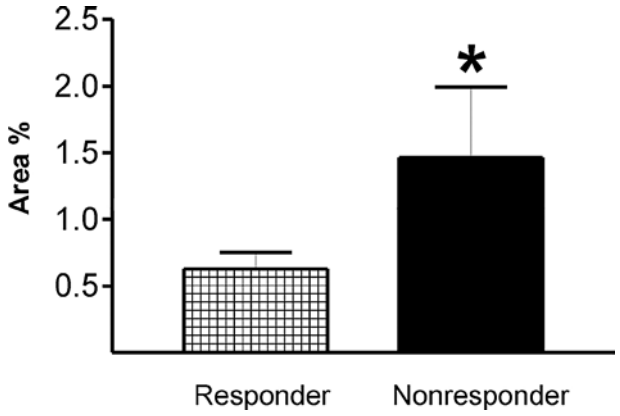
A significant proportion of individuals with epilepsy suffer from pharmacoresistant epilepsy despite early treatment and an optimum daily dosage of an adequate antiepileptic drug (AED) [1]. Thus, there is a clear need for new drugs or new strategies of therapeutic management. Although surgical treatment of epilepsy may be an option if AEDs fail, surgery for epilepsy might not be needed if we knew more about ways to prevent medical intractability or if we had more effective and less toxic AEDs. Pharmacoresistant epilepsy represents a challenge for both experimental and clinical research to gain new insights and perspectives of its mechanisms in order to improve future rational management approaches. One of the major unsettled questions is whether patients are predetermined to become refractory at the time of their first seizure, or whether they progress to a refractory state over time. Most likely, both scenarios exist. In patients in which refractoriness is not predetermined, it might be possible to intervene by aggressive therapy at an early stage and prevent disease progression [1]. However, for such intervention AEDs with a disease-modifying effect are needed. Unfortunately, such an effect has not been clearly demonstrated for any of the clinically established AEDs. Furthermore, there is as yet no sensitive or specific marker that enables the prediction whether a newly diagnosed patient will become refractory to AED treatment. To improve this situation, investigators have begun to examine the genetics associated with drug resistance. It is becoming increasingly clear that genetic polymorphisms play an integral role in the variability in AED efficacy, so that advances in pharmacogenetics and pharmacogenomics may allow developing strategies by which refractory epilepsy can be prevented. Several genes have been identified that influence responsiveness to many drugs, including AEDs. One of these, the multidrug resistance gene (*MDR1*), encodes the multidrug transporter P-glycoprotein (P-gp) that exports a variety of lipophilic drugs, including AEDs, back from the endothelial cells of the blood-brain barrier (BBB) into the blood, thereby restricting drug entrance into the brain [2, 3]. An overexpression of *MDR1* and P-gp has been found in epileptogenic brain tissue of patients with refractory epilepsy, suggesting that these patients may be AED resistant because they cannot concentrate the drug in brain parenchyma despite blood levels within the «therapeutic range» [2, 4]. Thus, pharmacological inhibition of P-gp in the BBB could form a strategy to prevent and overcome drug resistant epilepsy. Another mechanism involved in pharmacoresistant epilepsy is alteration of AED targets in epileptogenic brain tissue. For instance, recent observations indicate that reduced pharmacosensitivity of voltage-gated sodium channels in epileptogenic brain tissue may contribute to the development of drug resistance in epilepsy [5]. In order to develop strategies for overcoming pharmacoresistance, future studies need to dissect the relative importance of altered drug targets in the brain *versus* altered regulation of brain drug concentrations for each AED in order to understand what relation these two candidate mechanisms may have to the development of drug resistance.

Animal models of epilepsy allowing selection of drug-refractory (pharmacoresistant) and pharmacosensitive subgroups of animals are a valuable tool to study mechanisms of drug refractoriness and to develop more effective treatment strategies. Only few models are available which mimic patterns of drug resistance in humans with epilepsy [6]. One model seems to be particularly interesting in this regard, i.e., amygdala-kindled Wistar rats. In this model of temporal lobe epilepsy (TLE), animals which do not respond to repeated or chronic administration of AEDs (non-responders) can be separated from animals in whom AEDs are effective (responders) [6]. Hence, pharmacoresistant subgroups of kindled rats provide a unique tool to study why seizures become intractable, particularly because pathophysiologically processes in these resistant rats can be directly compared with those of kindled rats which respond to treatment. By using this model, we have recently shown that both the individual genetic background and kindling-induced brain alterations determine whether a rat becomes a responder or non-responder to anticonvulsant treatment after kindling [6]. We currently study the cellular and molecular mechanisms underlying the development of drug-refractory kindled seizures. With respect to the «target hypothesis» of pharmacoresistant epilepsy, evaluation of phenytoin's effects on voltage-dependent sodium channels of hippocampal neurons did not show any difference between phenytoin-responders and non-responders [7].

As mentioned above, one novel and reasonable hypothesis to explain drug-refractoriness in epilepsy is overexpression of multidrug transporters such as P-gp in the BBB, thereby reducing drug entry into the brain. Such overexpression would explain why phenytoin-refractory kindled rats are refractory to most major AEDs. We recently found [8] that phenytoin-non-responders have a marked overexpression of Pgp in their kindled focus (i.e., the basolateral amygdala) compared to responders *(figure)*, substantiating the multidrug transporter hypothesis of pharmacoresistant epilepsy. Thus, with respect to development of new AEDs, drugs not transported by multidrug transporters expressed in the BBB could have advantages toward available AEDs. Furthermore, Pgp can be blocked by specific inhibitors [3, 9], which raises the option to use such inhibitors as adjunctive treatment for medically refractory epilepsy. This possibility is currently evaluated in phenytoin-resistant kindled rats. However, although overexpression



Expression of P-glycoprotein (P-gp) in the basolateral amygdala (BLA) of BLA-kindled rats.
Fully kindled rats were repeatedly treated with phenytoin and selected into responders and nonresponders as described previously [6]. Nonresponders did not show any anticonvulsant effect of phenytoin, whereas phenytoin reproducibly exerted such an effect in responders. After this selection, rats were killed and P-gp expression was determined in the ipsilateral BLA by immunohistochemistry. The figure shows the area (x ± SEM) labeled by Pgp in the ipsilateral BLA of responders (n = 7) and nonresponders (n = 6). Pgp labeling exclusively occurred in capillary endothelial cells. In nonresponders, significantly more endothelial cells expressed P-gp compared to responders (p < 0.05). Data are from Potschka et al. *[8].*

of multidrug transporters is a novel and reasonable hypothesis to explain multidrug resistance in epilepsy, further studies are needed to establish this concept. With respect to animal models of intractable epilepsy, the similarities between drug refractoriness in the kindling model and patients with TLE indicate that such a model can be used to predict the clinical refractoriness to novel compounds in humans and may serve to develop new strategies for treatment of drug-refractory patients.

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ELB139 – a new anxiolytic and anticonvulsant compound

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Benzodiazepines are still regarded as the most effective drugs for the treatment of anxiety disorders and are potent anticonvulsants. However, the clinical use, both for the treatment of epilepsy and anxiety, is limited by side-effects, such as sedation, tolerance and the potential for drug abuse Costa and Guidotti [1]. Both, partial and subtype selective agonists are discussed to have advantages in this regard [2]. ELB139 (1-(p-chlorphenyl)-4-piperidin-1-yl-1,5-dihydro-imidazo-2-on) is a novel low affinity agonist at the benzodiazepine-binding side with characteristics of a partial or subtype selective agonist [3, 4]. In the present study, its anticonvulsant and anxiolytic potential was determined in rodent models.

To characterize the anticonvulsant potential, the compound was tested in standard seizure models, i.e. the MES (maximal electroshock) and PTZ (pentylenetetrazole) seizure test using the rotarod prozedure to determine the side effect potential. In addition we used two genetic models for generalised seizures and the amygdala kindling as a model of partial seizures. To determine the anxiolytic potential, the effect of ELB139 after acute and subchronic administration was tested in the elevated plus maze, the light and dark box, the Vogel conflict test and the open field examining anxiety- and motor activity-related parameters. To get more insight in the mechanism of action, the reversibility of anxiolysis by the benzodiazepine antagonist flumazenil was examined and catecholamines were determined in the rat striatum using microdialysis. Diazepam was used as reference compound.

ELB139 showed potent effects against electrically and chemically induced seizures in rats. The ED50s determined in the MES – and in the PTZ test, were 10.8 mg/kg and 19.3 mg/kg, resp., after oral administration. Further characterisation in WAG rat and the DBA/2 mouse as a genetic models of epilepsy as well as in the kindling model revealed a broad anticonvulsant activity against both, generalised and partial seizures. In amygdala kindled rats we observed an elevation of seizure threshold at 1 mg/kg i.p. and a reduction in seizure severity at 3 mg/kg i.p.. Antiepileptogenic effects which persisted even after termination of the treatment after 22 days, were present at 30 mg/kg i.p. In the rotarod test ELB139 was well tolerated (TD50 265.2 mg/kg p.o. rat).

In all models of anxiety, ELB139 at 30 mg/kg p.o. showed a potent activity in all tests. The extend of activity was similar compared with diazepam at maximal doses, however in contrast to diazepam without significantly affecting motor activity. While the anxiolytic activity of diazepam at 4 mg/kg p.o. bid was lost after subchronic administration, no development of tolerance was observed for ELB139 at 30 mg/kg p.o. bid even after treatment for 4 weeks. The anxiolytic effect of ELB139 in the elevated plus maze was almost completely antagonized by flumazenil indicating that the low affinity benzodiazepine interaction indeed contributes to the pharmacological effect. However, using microdialysis we could show that in addition ELB139 at 30 mg/kg i.p. but not diazepam induced a significant increase of serotonin levels in the rat striatum hinting at an additional mechanism.

The results indicate that ELB139 being significantly effective in different rodent models of epilepsy and anxiety at a non-sedative dose may be a promising approach for a new treatment of both, epilepsy and anxiety disorders without the major side-effects of the classical benzodiazepines including development of tolerance. The compound is currently undergoing phase I clinical testing.

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The substantia nigra and seizures: sex- and age-specific aspects, and the role of sex hormones

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Epileptic seizures are influenced by age and sex. In fact, the immature brain is more susceptible to seizures than the mature brain [1], and the incidence of epilepsy or unprovoked seizures is higher in boys than in girls. For example, multifocal infantile epileptic syndromes are 1.5-2 times commoner in males, especially those syndromes without a proven underlying pathology [2]. This increased susceptibility may be related to sex-specific maturational patterns in the development of neuronal networks that can suppress seizures.

The substantia nigra pars reticulata (SNR) is a brain nucleus important in seizure control, as observed in many different experimental models [3]. It is mainly composed of GABAergic neurons, and $GABA_A$ receptors contribute to its modulatory effects on seizures.

This laboratory has shown that SNR controls seizures in age- and sex-specific manner. In postnatal day (PN) 15 rats, bilateral infusions of muscimol (a $GABA_A$ receptor agonist) in the SNR have a proconvulsant effect in male (a «male phenotype») but no effect in female rats (a «female phenotype»), on clonic seizures induced by a volatile convulsant flurothyl [4].

Based on these findings, we hypothesized that the presence of testosterone in males may be responsible for these differences in GABA-mediated seizure control by the SNR. During development, circulating sex hormones have organizing effects producing permanent differences between males and females in distinct brain structures [5]. Specifically, the presence or absence of testosterone determines the future male or female phenotypic changes in different brain regions, and these changes persist into adulthood [5]. Testosterone either acts on androgen receptors (AR) or it is aromatized to estrogen and interacts with estrogen receptors (ER) to alter the development of a brain structure [6]. Both AR and ER are present in the SNR at PN0 [7].

We recently showed that the presence of postnatal testosterone determines the SNR sex-specific effects on seizures as the neonatal castration changes the «male» phenotype of SNR seizure control to a «female» phenotype [4]. Thus, in neonatally castrated male rats or females with daily systemic testosterone supplementation from PN0 to PN14 and tested at PN15 for seizure susceptibility, muscimol infusions in the SNR were proconvulsant in both groups, confirming the role of postnatal testosterone in modulation of the SNR seizure-controlling effects. However, a single dose of testosterone, administered 24 hours prior to seizure testing, did not induce the proconvulsant effect in PN15 female rats. These results suggest that sustained levels of testosterone, from PN0 on, completely reverse the effects of neonatal castration and produce the «male» type of SNR responses in female rats at PN15, and that this phenomenon is due to persistent modifications rather than to acute «pharmacological» effects of testosterone. Therefore, the presence of testosterone during the early postnatal period is the crucial factor concerning the development of functional differences in the SNR.

	Male rat				Female rat			
	PN15		PN30		PN15		PN30	
	$SNR_{anterior}$	$SNR_{posterior}$	$SNR_{anterior}$	$SNR_{posterior}$	$SNR_{anterior}$	$SNR_{posterior}$	$SNR_{anterior}$	$SNR_{posterior}$
Muscimol infusion	proconv	proconv	anticonv	proconv	no effect	no effect	anticonv	no effect
Muscimol infusion after castration at PN0	no effect	no effect	anticonv	anticonv	- - [a]		- - [a]	
Muscimol infusion after TP administartion for 14 days	- - [b]		- - [b]		proconv	proconv	anticonv	proconv
GABAA receptor a1 subunit mRNA	low	low	↑	low	moderate	moderate	↑	moderate
Neuronal content of GABA	low	low	↑	low	moderate	moderate	↑	moderate
KCC2 mRNA expression	low	low	↑	low	moderate	moderate	↑	moderate
SNR neuronal effect of muscimol application	depolarization		- - [c]		hyperpolarization		- - [c]	

Abbreviations: PN = post-natal day; $SNR_{anterior}$ = rostral part of substantia nigra pars reticulata; $SNR_{posterior}$ = caudal part of substantia nigra pars reticulata; TP = testosterone propionate; proconv = proconvulsant in the flurothyl seizure model; anticonv = anticonvulsant in the flurothyl seizure model; ↑ = representative increase as compared to the same area in the same gender at PN15.
[a] = only testicular castration was performed, therefore females could not be used
[b] = group not prepared because male rats already have natural testosterone
[c] = group not assessable because intracellular recordings from SNR neurons could be performed only in animals up to PN17 for technical reasons

A schematic representation of sex- and age-related differences in SNR as assessed in vivo and in vitro.

These earlier studies, however, did not define whether the very first days of postnatal life are the critical time window during which testosterone alters the maturation of the SNR. Determination of the presence of such an early critical period would allow for the delineation of the time during which a «therapeutic agent» could be administered to prevent the development of the proconvulsant SNR region. Moreover, the previously mentioned study did not address the issue whether the dimorphic maturation of SNR effects on seizures are due to testosterone itself, or to its estrogen or androgen metabolites. Our recent data show that testosterone presence is necessary in the very first postnatal days to induce the «male» phenotype of the SNR muscimol response, and that this effect is mediated by both AR and ER.

It has been recently shown that the sex- and age-specific effects of muscimol infusions in the SNR of developing rats upon the threshold to flurothyl-induced seizures, correlate with distinct molecular and electrophysiologic phenotypes of the GABAergic SNR neurons.

We analyzed the temporal pattern of the expression of several GABAergic markers within the SNR in both sexes. Our data using muscimol infusions show that the maturation of the SNR finishes around PN 30. This age marks a peripubertal stage, and the cyclical changes in sex hormone levels are still not present; thus in our further studies we refer to the mature SNR state in rats PN 30 [4]. In PN 30 male rats, there is a subregional compartmentalization of the effect of muscimol infusions in the SNR, by which it is anticonvulsant when infused in the rostral portion of SNR (SNRanterior), but proconvulsant when infused in the caudal part of the SNR (SNRposterior)[4]. Interestingly in PN30 females, muscimol infusions into the SNRanterior are anticonvulsant, but have no effect in the SNRposterior. Recently, we evaluated the GABA content, $GABA_A$ receptor a1 subunit mRNA [8] and KCC2 [9] in the SNR. KCC2 is a neuronal-specific potassium chloride co-transporter, whose expression determines the Cl^- content of neurons.

Our data showed that in PN15 males, low GABA content, low $GABA_A$ receptor a1 subunit mRNA and low mRNA expression for KCC2 in GABAergic SNR neurons predict a proconvulsant effect of intranigral muscimol infusions [8, 9]. In PN 30 male rats, the expression of all three markers increased in the SNRanterior (where muscimol infusions are anticonvulsant) more than in the SNRposterior (where muscimol infusions remain proconvulsant). In PN 15 females, we found higher levels of GABA content, $GABA_A$ receptor a1 subunit mRNA and mRNA expression for

KCC2 compared to PN 15 males and accordingly, muscimol infusions revealed no effect on seizures. The increase of these markers by age PN 30 in the SNRanterior corresponded with an anticonvulsant effect of muscimol infusions in this region, while in the SNRposterior, the moderate levels of all three GABA markers indicated no effects of muscimol infusions.

Finally in PN15 rat SNR, the low expression of KCC2 is associated *in vitro* with depolarizing responses of SNR neurons to bath application of muscimol [9]. Apart from the immediate effects on seizure threshold/spreading, this cellular effect might have long-term consequences. In fact, the persistence of muscimol-induced depolarizing responses is associated with calcium entry into the SNR neurons leading to upregulation of calcium-regulated genes and ongoing maturation of the SNR.

The data obtained *in vivo* on SNR role in seizures after perinatal hormonal treatments need to be confronted to the effects of the same treatments on the expression of the above mentioned markers in the SNR. This will allow to determine whether early hormones may play a role in the SNR-mediated seizure controlling effects.

In conclusion, the data summarized above might have clinical implications. The fact that the presence of natural testosterone in the early postnatal life in males is sufficient to induce the development of the muscimol-sensitive proconvulsant region in the SNR at PN15 may permit the institution of therapeutic approaches to alleviate a sex-specific endogenous proconvulsant system. Moreover, the recognition that the SNR has sex- and age-related features can be translated into the development of specific and effective treatments of other disorders affecting the SN, such as Parkinson disease and Tourette syndrome. Indeed, there is ample clinical evidence that gender and age may influence the expression of both disorders [10-12].

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Excitability changes in the parahippocamapal region during temporal lobe epileptogenesis

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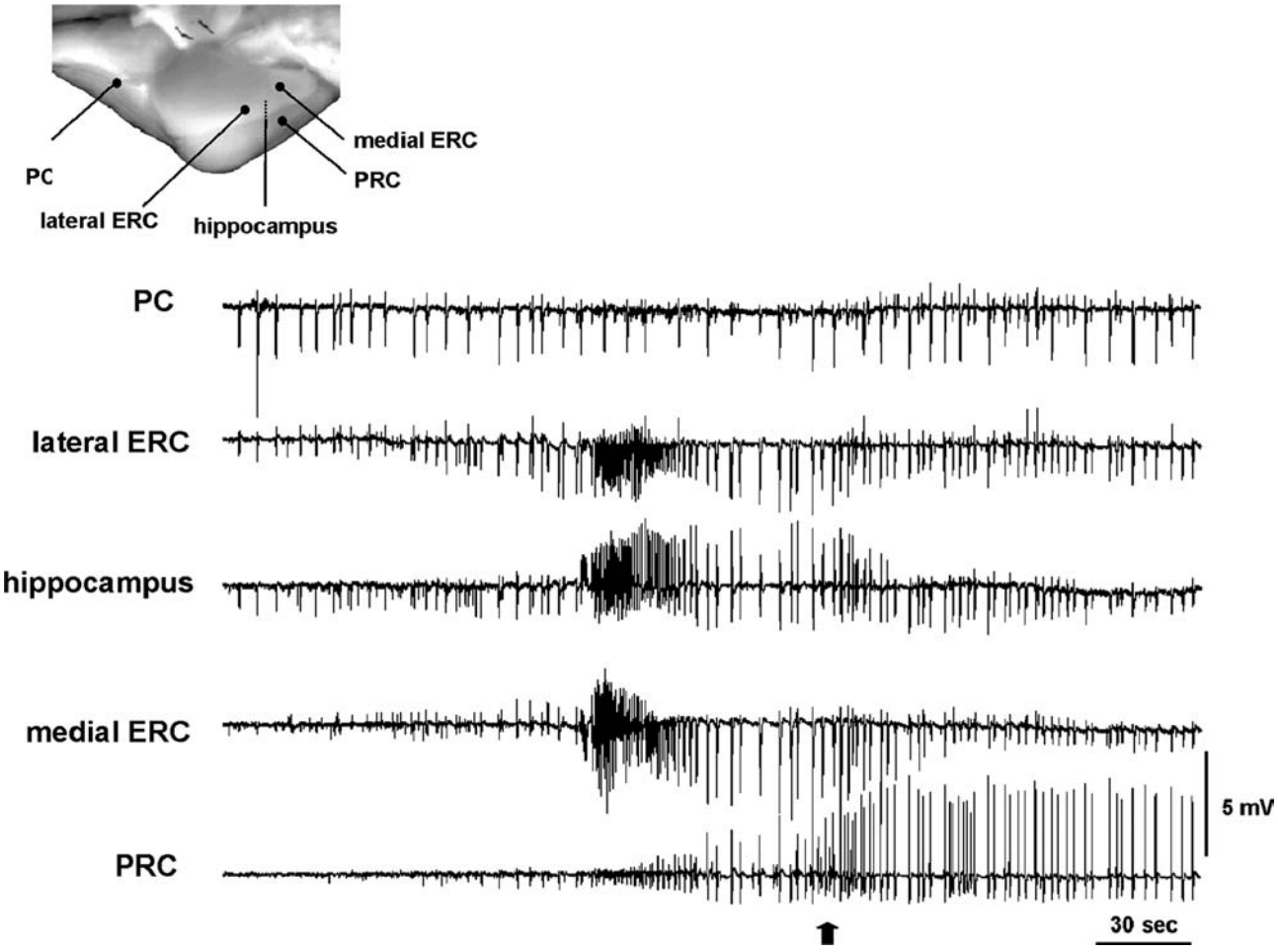
Temporal lobe epilepsy (TLE) is often complicated by a severe clinical condition characterized by frequent, invalidating and pharmaco-resistant seizures. Clinical and experimental findings suggest that the severity of the disease depends on the presence of a structural damage of the mesial portion of the temporal lobe that includes the hippocampus and the parahippocampal region (PHR). In a number of experimental models and in human TLEs, indeed, the presence of neuronal depletion in the CA3/CA1 region associated to gliosis and to an abnormal arrangement of synaptic interactions within the dentate gyrus of the hippocampus has been demonstrated [1]. Such neuropathological alterations, designated as mesial temporal sclerosis (MTS) [2] have been proposed to favor the generation of hypersynchronous epileptiform discharges in the hippocampal formation. The demonstration that the close interaction between hippocampus and PHR plays a crucial role in several physiological functions once attributed to the hippocampus exclusively, such as memory formation/retrieval and contextual learning, suggested

that the PHR could also be considered in the pathogenesis of TLE.

The parahippocampal cortex is the gate region to the hippocampal formation, critically involved in complex functions such as memory, object recognition, sensory representation and spatial orientation and learning. In mammals, the PHR is divided in three neighbouring subareas, namely the perirhinal (PRC) and the entorhinal cortex (EC), positioned lateral and medial to the rhinal fissure respectively, and the postrhinal cortex (POR), also referred to as parahippocampal cortex or area TH and TF in primates, located lateral and caudal to the EC. The connections between the temporal neocortex, the PHR and the hippocampus have been extensively studied with neuroanatomical techniques [3, 4]. Such interactions have been analyzed with electrophysiological techniques in the isolated guinea-pig brain preparation [5-7] and in PHR rat slices [6, 8, 9]. A new picture is emerging according to which longitudinal bands of PRC and EC are efficiently interconnected, whereas the propagation of activity from the NC to the EC is under an efficient inhibitory control that regulates the flow of information to the hippocampus.

Recent intraoperative stereo-electroencephalographic observations in TLE patients demonstrated that EC is involved in mesial temporal lobe seizures [10, 11]. These findings are not unexpected since the EC is strongly reciprocally connected with the hippocampus. *In vivo* and *in vitro* experimental reports demonstrated that tetanic stimulation of the EC and local EC injection of convulsants evoke ictal discharges that secondarily entrain the dentate gyrus and promote the re-entrant activation of the EC-hippocampal-EC loop, therefore facilitating the initiation of self-sustained epileptiform discharges in the hippocampal-parahippocampal region [12-14]. Recent experiments performed in complex *in vitro* slices that



Simultaneous recordings performed in the piriform cortex (PC), in the medial and lateral entorhinal cortex (EC), in area CA1 of the hippocampus and in the perirhinal cortex (PRC) of the isolated guinea pig preparation maintained in vitro by arterial perfusion, after arterial perfusion with 50 µm bicuculline. The position of the recording electrodes is illustrated in the upper panel. As previously demonstrated [27], interictal spikes are generated in the PC and propagate to the m-EC, but not to the hippocampus and PRC. Ictal discharges originate in the hippocampus/EC and propagate after several seconds to the PRC (arrow).

include the EC and the hippocampus demonstrated that seizure-like activity induced by acute exposure to epileptogenic solutions (such as high potassium solution, application of 4-amino-pyridine or exposure to a low-magnesium solution) is generated primarily in the EC and from there propagates to the hippocampal subfields CA1 and CA3 [15-17]. These findings suggest that the dentate gyrus, which is recognized as an inhibitory gate that prevents hippocampal hyperexcitation [18], is bypassed by the propagation of the epileptiform discharge generated in the EC directly to the CA1/CA3 region, along the temporo-ammonic pathway mediated by the EC neurons of layer III.

Following these experimental indications, the focus of the clinical studies on human TLE has been recently shifted from the hippocampus to the PHR. The analysis of extra-hippocampal temporal cortices with magnetic resonance in patients suffering of TLE (with or without demonstrable MTS) demonstrated that the EC is markedly reduced in volume [19-21]. The above findings suggest that changes in EC excitability and network interactions may act as trigger elements in the development of TLE and may precede the direct involvement of the hippocampus proper. The role of the PRC and its dynamic interaction with the EC and the hippocampus is still obscure. The physiology of this region has been only recently investigated. Experimental findings suggest that the NC-to-PRC-to-EC pathway is under the control of a powerful inhibition. Similarly, the propagation of activity from the EC to the PRC is also hindered in conditions of normal excitability [6, 7]. McIntyre and coworkers demonstrated that the rostral part of the PRC strictly connected to the olfactory cortex at the border of the insular cortex is critically involved in the development of kindling [22, 23], suggesting that this region may be involved in the early stages of TLE. It can be hypothesized, indeed, that a breakdown of feedforward inhibition that characterizes the interactions between the PRC and both the EC and the hippocampus may initiate hyperexcitability phenomena that promote limbic epileptogenesis.

To study the role of the PHR in temporal lobe epileptogenesis, extracellular recordings were performed on an acute model of epileptogenesis in the whole guinea-pig brain isolated and maintained *in vitro* by arterial perfusion [24-26]. Epileptiform discharges in the temporal lobe were obtained either by local application of bicuculline in the piriform cortex or in different parts of the PHR (but not in the hippocampus proper) or by arterial perfusion of the same compound. Epileptiform activity was analyzed for several hours, in order to identify the patterns of generation and propagation of interictal and ictal discharge and to characterize the dynamic changes that occur with time in the network under study. As illustrated above, interictal spikes generated in the olfactory cortex do not propagate to the PRC and to the medial/caudal EC unless a single ictal discharge is generated in the hippocampus/lateral EC. This priming ictal event is followed by recruitment of PRC that may assume a leading role in the generation of further epileptiform afterdischarges during the late phase of the ictal event (arrow). These preliminary findings demonstrate that the connectivity between EC and PRC within the PHR shows a «high resistance» to activity propagation, that is abated during ictal seizure activity generated in the hippocampus. The data obtained in an acute model of temporal lobe epileptogenesis suggest that the PHR is a low excitability element in the limbic area.

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The role of neurogenesis in forming hilar basal dendrites on rat dentate granule cells after seizures

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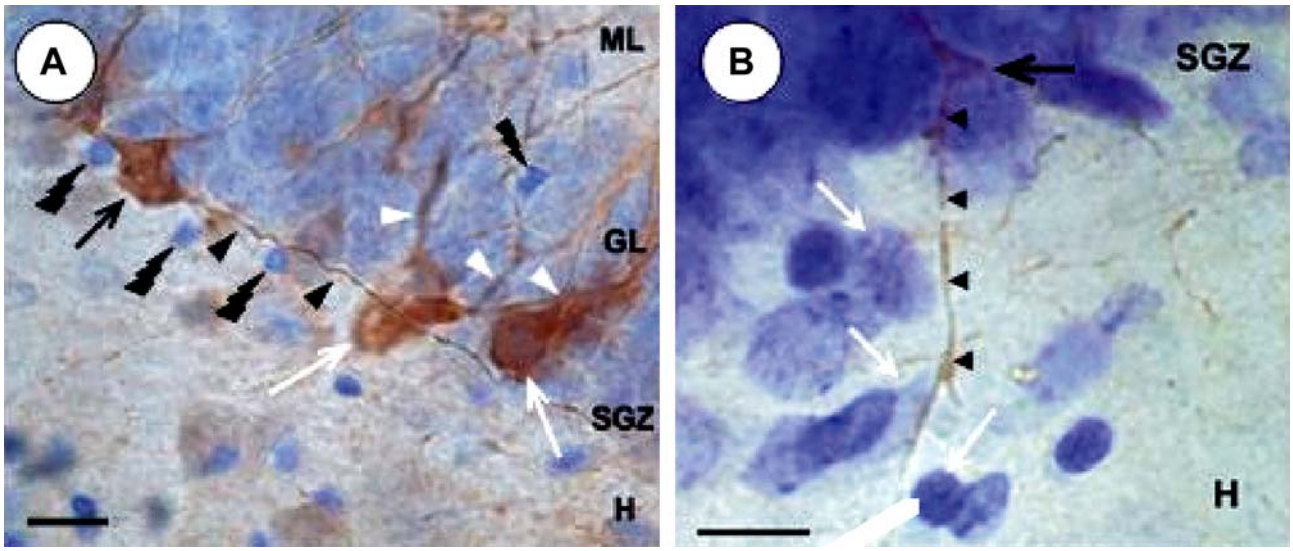
Several neuroplastic changes occur in the dentate gyrus after epileptic seizures. These include mossy fiber sprouting [1, 2], granule cell layer dispersion [3], increased granule cell neurogenesis [4], the formation of hilar basal dendrites [5, 6] and the appearance of hilar ectopic granule cells [4, 7]. The focus of this presentation is on the formation of hilar basal dendrites on rat granule cells following seizures.

Spigelman *et al.* [6] observed that after perforant path-induced seizures, some (6-15 %) granule cells exhibit hilar basal dendrites. These granule cells are located in the granule cell layer at the hilar border. They have also been demonstrated in two other models of temporal lobe epilepsy, kainic acid and pilocarpine [5, 8]. Ribak *et al.* [8] later showed using electron microscopy that these hilar basal dendrites are postsynaptic to mossy fibers. This finding indicated that hilar basal dendrites contribute to additional recurrent excitatory circuitry in the epileptic dentate gyrus. More recently, Austin and Buckmaster [9] observed increased bursts from cells in this recurrent excitatory circuit.

Developmentally, basal dendrites have been shown to be a transient feature of newly born granule cells [10, 11]. More recent data showed that many newly born granule cells in adult rats also exhibit basal dendrites, and these basal dendrites frequently curve into the granule cell layer as recurrent basal dendrites [12]. Dashtipour *et al.* [13] used biocytin labeling to demonstrate that after seizures, granule cells exhibit a temporal profile of hilar basal dendrite formation. These cells were found 7 days after status epilepticus at the hilar border suggesting that hilar basal dendrites arose from newly born neurons (NNs). Therefore, we used an antibody to the early neuronal marker doublecortin (DCX) to study NNs in the dentate gyrus of control and epileptic rats.

In the control animals we observed DCX-labeled NNs with basal dendrites at the hilar border *(figure A)*. In the control animals this basal dendrite from the NN appears to either retract or curve into the granule cell layer [12]. Thus, as in development, the basal dendrite exhibited on NNs in the adult is presumably a transient structure. However, our preliminary data from epileptic rats indicate that the basal dendrites from NNs are longer than those in the control animals, and that these elongated basal dendrites extend deep into the hilus. Other preliminary data indicate that these elongated hilar basal dendrites from NNs are adjacent to glial cells in the hilus *(figure B)*.

Based on these results we hypothesize that an ectopic glial scaffold forms after seizures and contributes to the formation of hilar basal dendrites. In this hypothesis, we propose that epileptic seizures cause hilar cell loss [14] which results in gliosis [14] and neoneurogenesis [14]. As glial cells move to clean up the infarct debris in the hilus, they may maintain an angiogenic association [15] thus contributing to an ectopic glial scaffold. A possible result of this ectopic glial scaffold in the hilus is aberrant dendritic growth of basal dendrites from NNs. Such hilar basal dendrites were shown to receive mossy fiber synapses and



Light photomicrographs of DCX-labeled NNs in the dentate gyrus from adult control and pilocarpine-induced epileptic rats.
*In **A**, DCX-labeled NNs from a control rat are shown at the border between the subgranular zone (SGZ) and the granule cell layer (GL). The NN to the left of the figure (black arrow) has a long dendritic process (black arrowheads) extending horizontally along the base of the granule cell layer. The other NNs (white arrows) have apical dendrites (white arrowheads) extending toward the molecular layer (ML). Note the glial cells (lightning bolts) adjacent to various processes. In **B**, a DCX-labeled NN (black arrow) from an epileptic rat is shown. This NN has a basal dendrite (black arrowheads) that extends into the hilus. This is an abnormal feature of NNs and we propose that the hilar basal dendrites found in epileptic rats grow along an ectopic glial scaffold. Note that the hilar basal dendrite is adjacent to glial cells (white arrows) in the hilus. Scale bar = 10 µm in A and 10 µm in B.*

therefore contribute to recurrent excitatory circuitry [8]. Additional support for this hypothesis comes from preliminary data that show NNs in the normal adult dentate gyrus have apical and recurrent basal dendrites that are aligned along radial glial cells in the granule cell layer. Because we observe elongated hilar basal dendrites aligned adjacent to glial cells in the hilus, we hypothesize that the hilar basal dendrites grow along an ectopic glial scaffold.

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Functional differentiation along the *longitudinal axis of hippocampus* and its possible relevance to *epileptogenesis*

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The Hippocampus consists of lamellae of similar histology repeated across its long axis. Epileptic foci are more often found in the anterior part of hippocampus as opposed to the rest. We wondered whether some functional differences at the synaptic and circuit level could account for this difference in epileptogenicity.

We studied *in vitro* transverse hippocampal slices taken from the ventral and dorsal parts of rat hippocampus which respectively correspond to the anterior and posterior parts in human hippocampus.

Circuits in the CA1 field of ventral slices were found to have relatively higher excitatory transmitter release probability and weaker recurrent inhibition. Ventral but not dorsal slices were observed to spontaneously generate and maintain almost unchanged for hours a synchronous activity consisting of sharp field potentials recurring 1-6 times per second. Finally ventral slices displayed stronger and more frequent epileptiform activity when perfused in Mg^{2+} free medium. Finally only ventral slices maintained a robust epileptiform activity for hours after being reperfused with normal medium.

These findings indicate that a differentiation along the longitudinal axis of hippocampus with regards to excitability and propensity of intrinsic circuits for synchronization may contribute to the relatively higher epileptogenicity of the anterior part of hippocampus.

Temporal lobe simple and complex partial seizures are the most common types of epilepsy. Such seizures most often have EEG onset in anterior hippocampus and surrounding mesial structures of temporal cortex (MTLE). Excision of anterior hippocampus along with entorhinal cortex often suffices to ameliorate drug resistant seizures. Hippocampal sclerosis, which often underlies MTLE, usually affects CA1 principal cells, but also dentate inhibitory interneurons and other parts of mesial temporal lobe. Hypotheses on the pathogenesis of MTLE include neuronal and glial cell death by glutamate excitotoxicity and/or mitochondrial dysfunction, immune reactions, genetic predisposition, developmental disorders, febrile seizures and other factors. A picture of a multifactorial condition emerges with clear need for several predisposing factors in the presence of a precipitant one. The pathophysiological mechanisms underlying MTLE are still elusive and the interpretation of current research findings is still debated, i.e. it is difficult to decide whether the observed loss of GABAergic interneurons in anterior hippocampus – presumably leading to reduced inhibition and unmasking of (NMDA dependent) hyperecitability - is a consequence or a cause of seizures. Similarly hippocampal sclerosis is reported by most (but not all) to be more severe in anterior hippocampus, but atrophy is not considered to be the direct cause of epileptogenesis and certainly it is not an independent predictor of the site of epileptogenesis [1].

In lower mammals like rats and cats the long axis of hippocampus is dorso-ventral. In analogy to human anterior hippocampus, ventral hippocampus (VH) *in vivo* is more seizure prone than the dorsal one (DH) [2, 3]. VH but not DH presents epileptiform bursting upon TTX infusion in DH [4] and shows greater increase of seizure induced mitotic activity [5]. Furthermore VH slices display more epileptiform bursting upon high $[K+]_o$, disinhibitory or opioid provocation [6-9]. What makes anterior and VH (in humans and rats respectively) the part of the brain most vulnerable to epileptogenesis? Answering this question appears as a logical experimental approach, which could help us understand the pathophysiology of MTLE.

Although hippocampus consists of histologically similar lamellae apparently repeating themselves from end to end, the differentiation across the long axis of hippocampus with regards to its external connections is well established, although poorly characterized electrophysiologically [10]. Differentiation with regards to behavior has been recently forthcoming [11, 12]. In animals DH is more critical for spatial memory, whereas VH is more critical for imformation related to internal state. In humans PET and fMRI studies indicate that posterior hippocampus is activated by familiar presentations with behavioral relevance, whereas anterior hippocampus may respond to novel presentations and register mismatches between expectation and experience. Neurochemically VH in comparison to DH is poorer in NMDA receptors (in st. radiatum), in adenosine A1 receptors and muscarinic receptors, but richer in aminergic (NA, DA, 5-HT) and peptidergic innervation. VH is also relatively less susceptible to ischemia [13]. In recent *in vitro* experiments we found that VH slices' CA1 area displays weaker short-, long-term and frequency potentiation [14, 15]

In spite of these findings the factors underlying the vulnerability of anterior/VH to epileptiform activity are not yet clear. We decided to examine local functional differences independently of mutual influences by comparing homologous circuits from the two ends of hippocampus in slices isolated and maintained *in vitro*.

We obtained 550 µm thick transverse slices from adult male Wistar rats (21 days – 4 months) and we maintained them in an interface recording chamber at 32 ± 0.5 °C, perfused with normal TENY in mM: 124 NaCl; 4 KCl; 2 MgSO4; 2 CaCl2; 1.25 NaH2PO4; 26 NaHCO3; 10 glucose; at pH 7.4, and 95 % O^2 and 5 % CO^2. We recorded extracellularly with single carbon fiber electrodes and 16-channel multielectrode arrays as well as intracellularly with glass micropipettes (50-120 MΩ, 2M K+- acetate). We took all precautions to treat slices taken from the two ends of hippocampus *(figure A)* in an identical manner

1. Passive membrane properties and basal synaptic excitatory responses in the CA1 area were not different in slices taken from DH and VH. Field EPSPs from VH slices however were not enhanced by elevations of [Ca + +]o from 2 to 5 mM *(figure C)*. This along with the lack of short term plasticity [14] suggests that CA3 to CA1 synapses in VH may have a relatively higher release probability.

2. Paired pulse inhibition *(figure B)* and evoked early as well as late IPSPs in ventral slices were significantly weaker compared to those in dorsal slices, i.e. both the amplitude and duration of intracellularly recorded fast-IPSPs were found significantly smaller in VH neurons (5.2 ± 0.6 mV, 54.8 ± 5.8 ms) than in DH ones (11.2 ± 1.1 mV, 105 ± 10 ms), *figure D* [16].

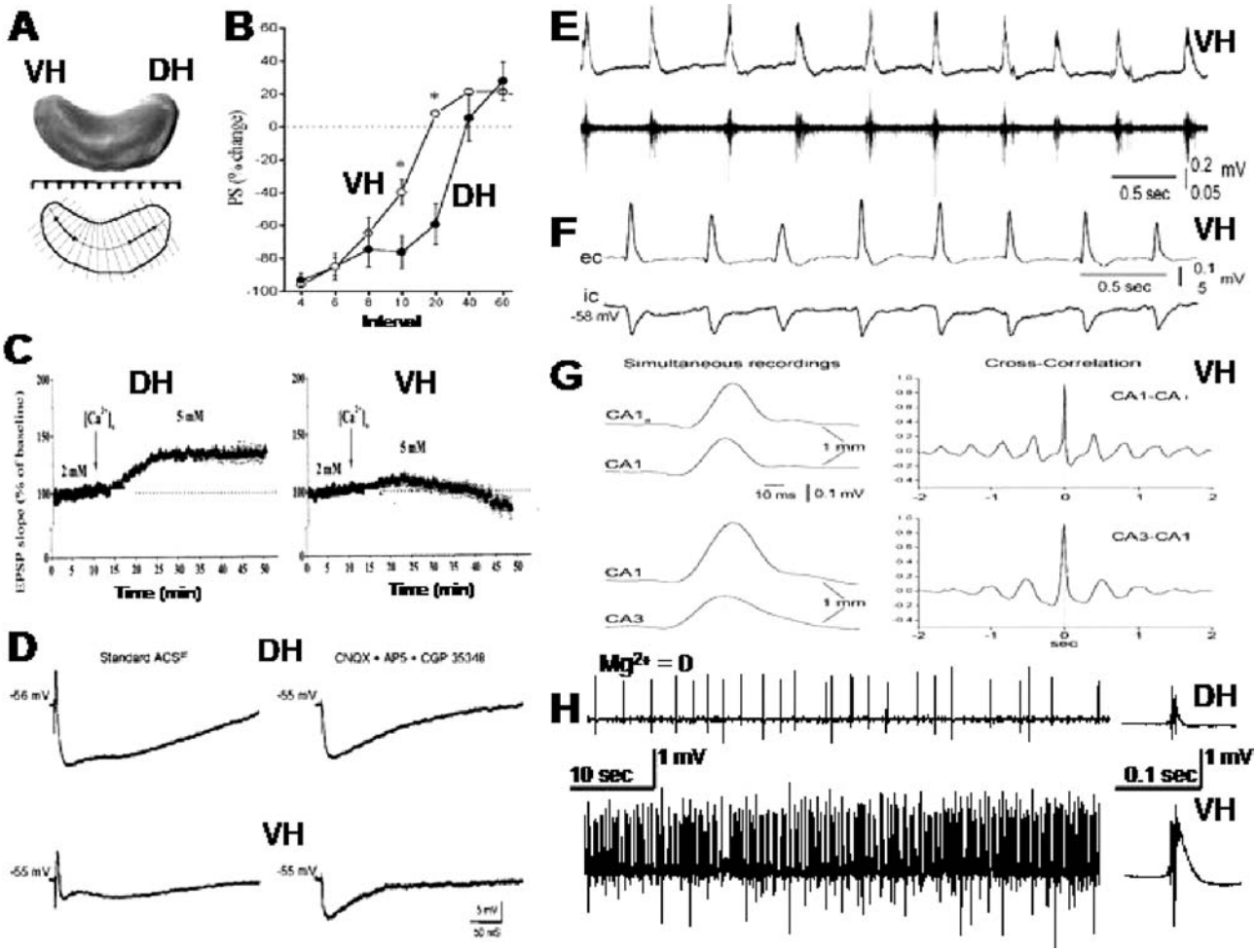
3. We have demonstrated that transverse slices taken from VH (but not DH) of adult rats can spontaneously organize synchronous activity under standard conditions of *in vitro* perfusion/maintenance [17]. The activity consists of field potentials resembling «sharp waves» regularly recurring about 1-6 times/sec in different slices; in each slice the activity was maintained for hours with the amplitude and inter-wave interval kept within narrow limits. CSD showed that these field potentials were generated by one main current source, which appeared highly synchronous across the entire CA stratum pyramidale. This source was identified as synchronous GABAA-mediated IPSPs in pyramidal neurons (*figure F*, chloride mediated reversing at 70 mV). In simultaneous recordings from the CA3 and CA1 regions the activity in CA3 preceded in time (within a few msec, *figure G*) the activity in CA1. However, mini-slices containing only the CA1 region could still support the generation of these field waves. The pacing mechanism depends on the integrity and normal activation of GABAA and AMPA/kainate receptors as well as several other factors. Spontaneous non synchronous excitatory events such as depolarizations, orthodromic spikes and ectopic-antidromic spikes, which were frequently observed in pyramidal neurons in association to the field waves, presumably participated in the still undisclosed mechanisms triggering the field waves [18]. Electrical coupling through gap junctions participated actively in the latter mechanisms as revealed by the presence of spikelets and the pharmacological abolishment of the activity by gap junction blockers. This remarkable ability to generate synchronous rhythmical activity was reversibly sensitive to several neurotransmitters and drugs.

4. Ventral slices were found more prone than dorsal slices to develop epileptiform discharges when perfused with Mg^{2+} free ACSF and upon addition of NMDA in the perfusing medium (ictal as well as interictal-like bursting appeared larger and more frequently, see *figure H*). Most interestingly, upon reperfusion with normal ASCF spontaneous field epileptiform discharges persisted for several hours in ventral but not dorsal slices. This activity coexisted independently of the spontaneous field waves.

The observations broaden the range of findings supporting the concept of differentiation between DH and VH at the level of local synaptic circuits [11, 12]. The preservation of the same lamellar structure along the longitudinal axis does not preclude fundamental diversification at the functional level. In all tests DH characteristics resembled those already established by work in many labs as characteristics of hippocampus. We therefore considered the differences as due to some aberration in VH deviating from what is considered usual. Many properties of synapses have been shown to differ between pathways and brain regions, with age and with pathology. Here we show distinct functional differences in adult histologically similar parts of the same region under normal *in vitro* conditions. The findings provide a physiological basis able to explain, to some extend, the propensity of VH for epileptic activity and may help us understand hippocampal function and malfunction. The demonstration of several qualitative differences in two histologically similar isolated and fully controlled circuits also offers a new convenient experimental tool to study mechanisms underlying excitability, inhibition, plasticity/learning, rhythmogenesis and epileptogenesis.

The observation that VH CA3 to CA1 field EPSPs are not enhanced by raising $[Ca^{++}]o$ appears to be the most basic observed aberration of VH. Along with the lower ability for short term plasticity it suggests synapses of relatively higher release probability in VH. Taken together with the observed relatively weaker evoked inhibition, it could contribute to lower seizure initiation threshold. Consistent with this notion is the observation that VH is more vulnerable to NMDA dependent provocation of epileptiform discharges. It is important that VH can also maintain robust field epileptiform activity long after the provocation is lifted (for the «life of the slice»). This kind of *in vitro* kindling additionally implies some form of plasticity in need of explanation since ventral slices show minimal ability to sustain LTP [15].

Besides hyperexcitability low seizure threshold requires also an ability to recruit large parts of neuronal assemblies into synchronous activity. In VH (but not DH) slices we observed a remarkable ability to organize robustly recurrent synchronous activity under normal perfusion condi-



Electrophysiological differences between slices taken from dorsal (DH) and ventral hippocampus (VH), which may underlie the higher vulnerability of VH to epileptogenesis.

A*) Excised whole rat hippocampus (with a mm scale), direction of cutting transverse slices and areas of the two poles of hippocampus from which slices were taken (double headed arrows).* ***B****) Paired-pulse inhibition is significantly weaker in VH at stimulation intervals of 10 and 20 msec.* ***C****) Field EPSPs are not enhanced in VH slices upon increase of $[Ca^{2+}]o$ from 2 mM to 5 mM.* ***D****) In standard ACSF both early GABAA-dependent and late GABAB-dependent evoked IPSPs are expressed, while in ACSF containing CNQX, AP5 and CGP 35348 (blocking glutamate ionotropic and GABAB receptors) only early IPSPs are present. Both early and late evoked IPSPs are weaker in VH.* ***E****) Only in ventral slices we observed a regularly persistent activity consisting of synchronous waves appearing positive in the somatic layer of CA fields. Lower trace is high pass filtered to show concomitant extracellular bursting activity.* ***F****) The field potentials are always accompanied by hyperpolarizations of principal neurons.* ***G****) By averaging individual waves (left) or cross-correlating (right) activity in simultaneous double recordings demonstrates a remarkable coherence across CA fields with CA3 field usually preceding CA1 by a few msec.* ***H****) VH slices show much stronger epileptiform activity upon perfusion with medium deprived of Mg^{2+}.*

tions. Similar activities have been recorded under nominally normal conditions in epileptic human and monkey tissue [19, 20] and recently in intact hippocampus [21] and in mice hippocampal slices [22, 23]. The latter authors have likened this *in vitro* activity to the sharp waves recorded in the DH of rats during slow wave sleep and some phases of awake immobility [24]. However several important characteristics differ in the above activities recorded in diverse preparations and a final association between them appears premature. The mechanism of the synchronous activity we observed in VH appeared to demand the cooperation of several network properties rather than a single membrane pacemaker. The smallest possible assembly of neurons isolated *in vitro* (CA1 mini-slice) may generate rhythmical activity. The remarkable ability of relatively restricted local circuits contained in transverse VH slices to spontaneously generate synchronized semi-periodic activity reflects on the notion of lamellar internal organization of the hippocampal system and its sufficiency not only to serially advance incoming messages but also to organize population rhythmic activity under normal conditions. The propensity for synchronization may be contributive to the factors making hippocampus more epileptogenic. Although PRSA is expressed as

IPSPs in pyramidal neurons it appears to result at least in part from a relatively richer excitatory background in both principal neurons and interneurons, possibly related to the observed relative weakness of evoked inhibition on pyramidal neurons.

The findings that VH appears to be characterized by high transmitter release probability, weak inhibition and an ability for periodic synchronization are obviously relevant to low seizure threshold. The question however remains as to how they relate to human MTLE. Since the observations were made in slices all above reflect properties of intrinsic hippocampal circuits. To what extend the phenomena observed depend on the *in vitro* conditions and their relevance to phenomena observed in whole hippocampus *in vivo* needs further experimentation. Several putative mechanisms could underlie the observed characteristics of VH, which should be pursued in depth in multidisciplinary experiments. The need for confirmation of these observations and comparisons in whole hippocampus *in vivo* and possibly in humans cannot be overemphasized. Optimism is however justified that possible mechanisms of epileptogenesis may be revealed by examining the functional differentiation along the longitudinal axis of hippocampus.

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Single pulse electrical stimulation (SPES) in presurgical assessment of epilepsy

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Despite technical advances in surgical procedures and presurgical assessment over recent decades, the overall success rate of resective surgery of epilepsy remains at about 75 %. It may be a tautology to state that the objective of such surgery is to identify and if possible remove the «epileptogenic zone», that region of tissue the removal of which is necessary and sufficient to relieve the epilepsy. An important cause of poor outcome is probably failure to identify the epileptogenic zone. No single set of criteria defines this zone; its location is inferred from clinical, imaging and electrophysiological findings. Both ictal and interictal electrographic abnormalities may be found; sometimes these are of localising value, sometimes not, probably because the characteristic epileptiform activity is rapidly propagated from one region to another [1].

An alternative approach to electrophysiological investigation is to attempt to map the excitability of the cortex, in order to identify an hyperexcitable area that corresponds to the epileptogenic zone. We have attempted this by the simple technique of direct cortical electrical stimulation.

Brain stimulation has long been used in presurgical assessment, but for different reasons: to map after-discharge thresholds, to elicit habitual seizures and to identify eloquent regions that may not be resected. For these purposes, repetitive stimulation has been used; in the present investigation electrically evoked responses to single pulses were studied. We have employed single pulses at 8-sec intervals to study the electrically evoked potentials as a measure of cortical excitability.

Single pulse electrical stimulation (SPES) has been performed in patients undergoing subacute investigation with intracranial electrodes, either depth or subdural in type. Stimuli were presented between adjacent electrodes at 5-8 sec intervals. Seizures were captured by video-EEG telemetry. The results of stimulation were compared with seizure onsets located by EEG telemetry, surgical outcome, MRI findings and pathology. These findings were also used, together with ictal semiology, to determine the epileptogenic zone. For details of patient selection and evaluation, and stimulation methods see [2].

Three type of response have been found. When normal cortex was stimulated, an «*early response*» (ER) was elicited consisting of one or more sharp and slow transients, lasting less than 300ms. Stimulation in the epileptogenic zone typically elicited a «*delayed response*» (DR), lasting or commencing more than 300 ms after the stimulus. Often this closely resembled in morphology and topography the patient's spontaneous interictal discharges. At frontal sites only, stimulation within the epileptogenic lobe (often the epileptogenic zone could not be more precisely determined) sometimes elicited a «*repetitive response*» (RR), resembling a train of repeated early responses, lasting 1s or longer.

Location of seizure onset: In an initial study of mainly temporal lobe epilepsy [2], a close relationship was found between the electrographic site of seizure onset and the location of delayed responses *(table 1)*. Focal seizure onsets were invariably associated with DRs of similar topography. Patients with regional onsets showed corresponding regional DRs, focal DRs within the same region, or no DR. No patient with a diffuse seizure onset exhibited a DR.

Table 1. Topographic relationship of localisation of delayed responses and seizure onset

Delayed Responses	*Patients by onset type*				
	Focal	Focal/ Regional	Regional	Diffuse	Total
Focal	12	1	2	0	15
Focal/Regional	0	1	1	0	2
Regional	0	1	6	0	7
Bilateral	0	1	2	0	3
None	0	0	12	6	18
Total	12	4	23	6	45

A further study of 30 patients with frontal electrodes (with either frontal or extra-frontal seizure onsets) showed an RR or a frontal DR exclusively in 15 patients with frontal seizure onset. Nine of 13 patients with extra-frontal onsets showed extra-frontal DRs.

Prediction of outcome: Twenty-eight patients have so far undergone temporal lobe resections and completed 12 months follow up. In 21, a DR had been recorded within the area of resection; 20 had a favourable result (grade I or II on the outcome classification of [3]). Of 7 without a DR in the resected zone, only 3 had a favourable outcome ($p < 0.01$) *(table 2)*.

In frontal lobe surgery procedures are less anatomically standardised than for temporal lobe epilepsy. Consequently, determination of the required extent of resection presents a problem. When the area generating DRs or RRs was entirely excised a favourable outcome was achieved. This was never obtained when tissue exhibiting DRs or RRs was incompletely removed.

Table 2. Association of resection of tissue giving abnormal SPES response with favourable outcome (good = Engel grade I/II; poor Engel grade III/IV)

$p < 0.01$ for temporal, $p < 0.05$ for frontal resection.

	Good outcome	Poor outcome	Total
Temporal resections n = 23			
DR in resected area	20	1	21
No DR in resected area	3	4	7
Frontal resections n = 8			
DR/RR area completely resected	4	0	4
DR/RR area not completely resected	0	4	4

In all but two cases where an area displaying DRs was excised, neuropathological examination showed structural abnormality, although in 5 subjects the MRI findings had been negative. No adverse effects resulted from the SPES procedure.

Single pulse electrical stimulation is a safe and, on present evidence reliable, technique for identifying the epileptogenic zone, as evidenced by the close relationship between the topography of abnormal responses and location of electrographic seizures onset, surgical outcome, and pathology. At the least, by providing additional evidence it may reduce the need to capture seizures, allowing invasive EEG telemetry to be performed for shorter periods. It may in some instances even replace ictal recording as a method of locating the epileptogenic zone, allowing this to be identified intraoperatively by SPES immediately before resective surgery. The technique may also prove of value during electrode implantation to determine whether the sites chosen are likely to lie within the epileptogenic zone.

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GABA-mediated pathological synchronisation

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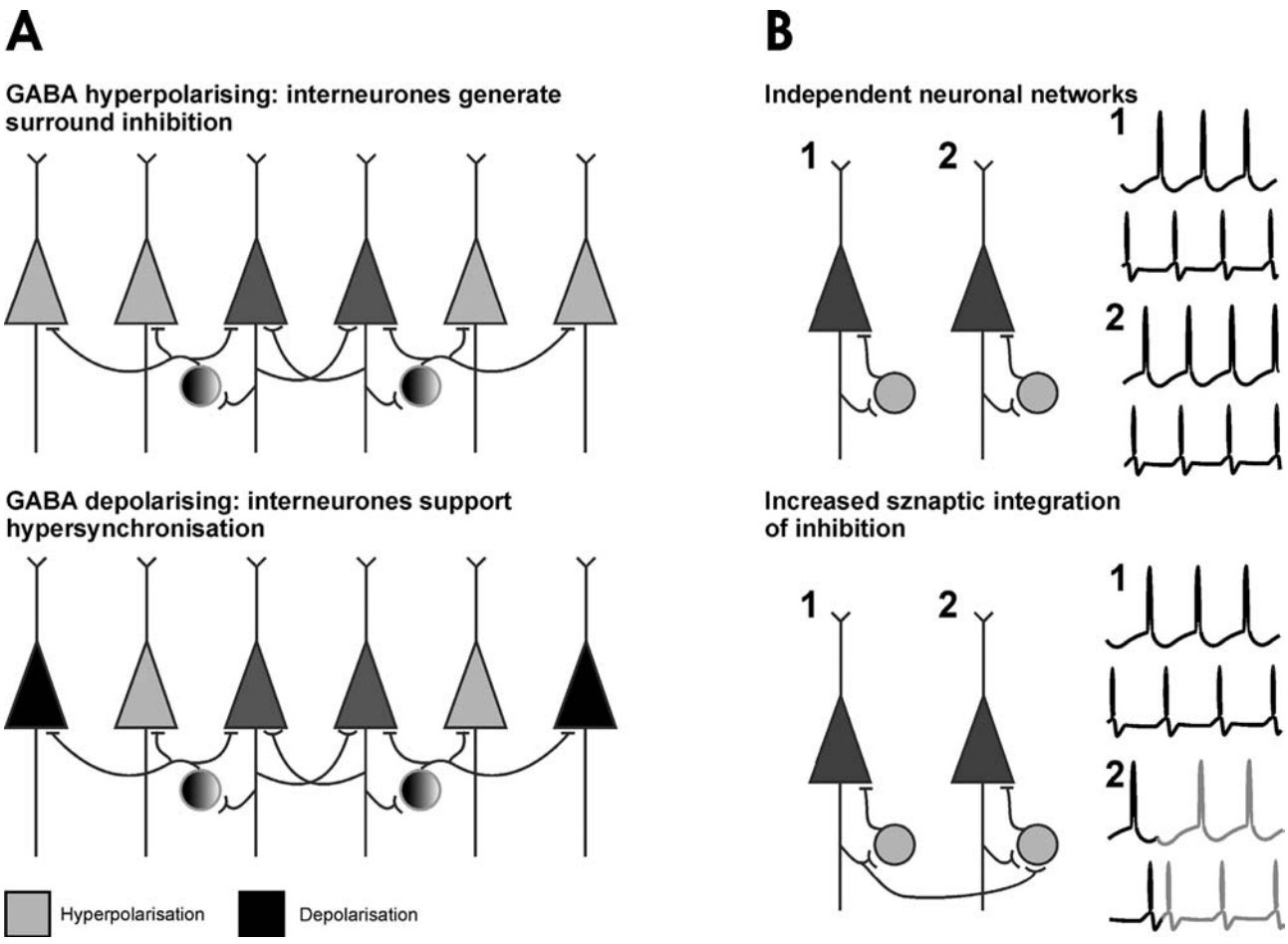
The idea that an «imbalance» of excitation and inhibition in the brain causes seizures is appealing for its simplicity and immediate plausibility. Thus, it has been propagated as standard concept of the pathomechanism of epilepsy. However, increasing evidence is mounting suggesting that this «imbalance» theory of seizure generation does not hold in many circumstances, both regarding animal models of epilepsy and findings in human epileptic tissue. In particular, the role of GABAergic transmission, generally deemed to be inhibitory, is becoming ambiguous. This paper summarises experimental data generated over the last ten years that show GABA to be instrumental in pacing, or indeed initiating seizures or rather seizure-like activity.

The experiments disclosing a pro-epileptic role of GABAergic transmission were performed on *in vitro* brain tissue slices, both from experimental animals and from human tissue resected during epilepsy surgery, making use of artificially induced epileptiform discharges and different model epilepsies, but also analysing spontaneous activity arising in chronically epileptic human tissue. Using pharmacological tools to block GABAergic transmission and field potential recordings, and employing intracellular recordings to gauge single postsynaptic events and optical methods to apprehend spatio-temporal changes of network excitability, different groups could show, in different models, that GABA may either pace, or even trigger epileptic discharges.

Among the first investigations disclosing the pro-epileptic role of GABA were are a series of studies on the mechanisms of 4-aminopyridine (4-AP)-induced epileptiform activity in combined hippocampal-entorhinal cortex slices of rats. In this model, and subsequently also with acute application of pilocarpine, it could be demonstrated that of the three types of discharges emerging under these conditions, two, namely a so-called slow interictal and a prolonged, ictal type appear to have the same pathomechanism. Indeed, ictal-type discharges were regularly initiated by the slow interictal events. These ionic currents underlying these events, in turn, were shown to be most likely chloride-mediated [1, 2]. Even though a substantial component driving neurones into discharges was demonstrated to consist of an elevation of extracellular potassium (supporting neuronal depolarisations), the precipitating factor – as also indicated by the chloride dependence – were GABAergic synaptic events. Consequently, a block of GABAergic transmission by the GABAA – antagonist bicuculline (normally acting as epileptogenic agent), suppressed ictal epileptiform discharges [2, 3].

This finding is not limited to the above-mentioned models. Using isolated hippocampal slices and the so-called 0-Mg^{2+} model, stimulus-induced epileptiform ictal events in the CA1 region of adult rat preparations were shown to be regularly initiated by field and membrane potential oscillations in the gamma frequency range (> 30 Hz) [4]. These oscillations were identical to tetanus-induced gamma oscillations in many respects, including concomitant massive (≈ 80 %) reduction of neuronal input resistance and sensitivity to alterations of extracellular volume fraction *via* osmolality changes. Most importantly, however, they could again be blocked by suppressing GABAergic synaptic transmission with bicuculline which led to a subsequent ablation also of ictal-type epileptiform discharges [4]. One of the mechanisms responsible for the epileptogenic action of GABAA-receptor activation under these conditions appeared to be the conversion of inhibitory postsynaptic potentials (presumably mediated by GABA) from hyperpolarising to depolarising with continued wash-out of Mg^{2+} [4]. This notion is indeed supported by recent findings showing a down-regulation of the neuron-specific K^+/Cl^- co-transporter KCC2 [5], which results in an intracellular accumulation of chloride and hence in a positive shift of the chloride reversal potential and thus depolarising actions of GABA *(figure A)*. Other depolarising components of GABA-receptor activation support this excitatory drive: Under similar epileptogenic conditions, Perez-Velazquez (2003) [6] showed that depolarisations could also be mediated by bicarbonate flux *via* GABA-receptor associated channels. Lastly, the activation of GABA receptors and the resulting Cl^- and HCO^{3-} flux give rise to a counter-transport of K^+, which, under epileptogenic conditions, substantially contributes to neuronal depolarisation [7].

Does such a pro-epileptic role of GABA also apply for human epilepsy? Investigations on human chronically epileptic brain tissue, both from neocortical and hippocampal areas obtained during epilepsy surgery, suggest this. In studies on human neocortical resectates, spontaneous



*Schematic diagram illustrating how depolarising actions of GABA can render presumably inhibitory interneurones (circles) depolarising for some principal neurones (black) and hence excitatory (**A**) and how GABAergic inhibition, even when remaining hyperpolarising and inhibitory, can synchronise neuronal networks by resetting the rhythm in case of e.g. an increase in synaptic integration of interneurones (B). (Part **A** modified from Köhling: GABA becomes exciting.* Science *2002; 298: 1350-1).*

interictal-like field potential discharges persist *in vitro* [8]. These discharges are accompanied by hyperpolarising, and not depolarising, potentials in most neurones, suggesting that GABAergic transmission plays a role. Indeed, the spontaneous potentials, as their artificially-induced counterparts in animal experiments, could be abolished by GABAA-receptor blockade [8]. The fact that suppression of glutamatergic transmission elicits the same result points to a slightly different function of GABAergic transmission in this case: GABA, at least in most neurones, did not appear to be outright depolarising and excitatory under these conditions. Rather, rhythmic GABAergic input seems to provide a reset and thus pacing mechanism for network discharges *(figure B)*. Such a pacing function of GABA for epileptiform activity has recently been suggested also in animal models [9]. Is this an exclusive mechanism, or might GABA additionally be depolarising also in human epilepsy? Findings in hippocampal slices suggest this to be the case: In the investigations by Cohen *et al.* [10], a subset of neurones, incidentally those firing prior to the network discharge and thus speculated to act as pacemakers, were demonstrated to have a more depolarised reversal potential of GABAergic potentials. As a consequence, these were de- rather than hyperpolarising [10], rendering interneuronal activity pro-epileptic rather than inhibitory in case of interneurones projecting onto this subset of neurones *(figure A)*. This finding in the hippocampus does not preclude a similar action of GABA also in the neocortex. Although there was no indication for depolarising, GABA-mediated potentials in neurones being activated with the entire network, the fact that the initiating foci of the spontaneous discharges described above were very heterogeneously distributed and restricted to minimal foci (< 200 µm) [11] may be reflecting also only a subset of neurones to function as pacemakers [12]. Such neurones, in turn, might yet be shown to display depolarising GABA-reactions.

In summary, the experimental findings obtained in animal models and in human epileptic tissue suggest that GABA, instead of being inhibitory, may subserve at least two epileptogenic functions under certain conditions eliciting 1) a direct depolarising and excitatory reaction or 2) acting as a reset and pacing mechanism making synchronous network activity particularly efficient.

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