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## **Hippocampus and epilepsy: findings from human tissues.**

### **Hippocampe et épilepsie : données issues des tissus humains**

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## **Abstract**

Surgical removal of the epileptogenic zone provides an effective therapy for several focal epileptic syndromes. This surgery offers the opportunity to study pathological activity in living human tissue for pharmacoresistant partial epilepsy syndromes including temporal lobe epilepsies with hippocampal sclerosis, cortical dysplasias, epilepsies associated with tumors and developmental malformations. Slices of tissue from patient with these syndromes retain functional neuronal networks and may generate epileptic activities. The properties of cells in this tissue may not be greatly changed, but excitatory synaptic transmission is often enhanced and GABAergic inhibition is preserved. Typically epileptic activity is not generated spontaneously by the neocortex, whether dysplastic or not, but can be induced by convulsants. The initiation of ictal discharges in neocortex depends on both GABAergic signaling and increased extracellular potassium. In contrast, a spontaneous interictal-like activity is generated by tissues from patients with temporal lobe epilepsies associated with hippocampal sclerosis. This activity is initiated, not in the hippocampus but in the subiculum an output region which projects to the entorhinal cortex. Interictal events seem to be triggered by GABAergic cells which paradoxically excite about 20% of subicular pyramidal cells while simultaneously inhibiting the majority. Interictal discharges thus depend on both GABAergic and glutamatergic signaling. The depolarizing effects of GABA depend on a pathological elevation in levels of chloride in some subicular cells, similar to those of developmentally immature cells. Such defect is caused by a perturbed expression of the cotransporters regulating intracellular chloride concentration, the importer NKCC1 and the extruder KCC2. Blockade of NKCC1 actions by the diuretic bumetanide, restores intracellular chloride and thus hyperpolarizing GABAergic actions so suppressing interictal activity.

## **Résumé**

La résection chirurgicale de la zone épileptogène est la procédure thérapeutique de choix de multiples épilepsies focales. Elle permet d'étudier les activités pathologiques dans du tissu humain maintenu en vie in vitro pour divers syndromes épileptiques pharmacorésistants dont les épilepsies temporales avec sclérose hippocampique, dysplasies corticales, autres malformations développementales ou tumeurs. In vitro, dans des tranches de tissu issus de pièces opératoires, le réseau épileptique est conservé et des activités épileptiques sont produites. A l'échelle neuronale, les propriétés intrinsèques semblent peu modifiées, certaines composantes synaptiques excitatrices glutamatergiques apparaissent renforcées et l'inhibition GABAergique est maintenue. Dans le néocortex, qu'il soit dysplasique ou non, une activité synchrone épileptiforme n'est généralement pas enregistrée spontanément mais doit être induite pharmacologiquement. L'initiation des décharges ictales implique alors la signalisation GABAergique et une augmentation de la concentration extracellulaire en potassium. Au sein de tissus provenant de patients souffrant d'épilepsies mésio-temporales associées à une sclérose hippocampique, une activité épileptiforme est recueillie spontanément. Il s'agit de bouffées interictales générées non pas dans l'hippocampe mais dans le subiculum, sa région de sortie interfacée avec le cortex entorhinal. Cette activité est initiée par la décharge d'interneurones qui excitent paradoxalement par le GABA libéré environ 1/5 des cellules pyramidales, hyperpolarisant les autres. Les décharges sont donc sous-tendues tant par la signalisation GABAergique que glutamatergique. L'origine des réponses dépolarisantes au GABA est une perturbation de l'homéostasie du Chlore, secondaire à une modification de l'expression des co-transporteurs régulant sa

concentration intracellulaire, NKCC1 et KCC2, évoquant un retour à un phénotype neuronal immature. La restauration d'une concentration normale en Chlore en bloquant NKCC1 par le diurétique bumétanide permet ainsi de supprimer les activités interictales.

## Introduction

Epilepsy is an emblematic brain disease. Epileptic activities depend on a mode of neuronal discharge, the burst of action potentials as well as a mode of collective activity, neuronal synchrony [1], both of which are physiological. Epileptic discharges therefore replay an excessive, dysregulated brain function. Explaining epileptic activities requires understanding neuronal functions at levels from single ion channels and neurotransmitter receptors, through the cellular and synaptic properties of single neurons to the collective activities of groups of interconnected neurons modulated by glial cells. Epileptic syndromes are thus diseases of the entire nervous system as it interacts with the environment and electrophysiology is a key discipline to understand epileptic activities and their relations and boundaries with physiological function.

The epilepsies are a diverse and distributed group of diseases. Numerous natural or engineered models have been used to reproduce epileptic features in animals. Clinically human epilepsies are typically first explored by electrophysiological recordings. Electroencephalography (EEG) records field potentials produced by the synchronization of synaptic potentials in large cortical areas (several  $\text{cm}^3$ ) and transmission can vary between different brain regions [2-4]. Intracranial recordings improve the quality and spatial precision of the data, and permit sampling of higher frequency oscillations but usually cannot resolve the mechanisms of epileptic activities. Recent use of intracranial records to detect discharges of single neurons in situ may help understanding the genesis of epileptic synchrony [5].

Animal models have been used to study epileptogenic alterations of brain tissues that lead to seizures, but less attention has been paid to ictogenesis, or mechanisms controlling the emergence of seizures from the remodeled brain. In vivo models, even if they do not replicate all aspects of a disease, have provided much of our data on epilepsy. Finally, in vitro work, especially from rodent hippocampal slices, has advanced understanding on potential mechanisms of neuronal synchrony. Neural networks are maintained intact and epileptiform activities are induced by blocking  $\text{GABA}_A$  receptors or by increasing neuronal excitability. These models can provide precise data on ionic conductances, synaptic events and neuronal firing even if they are a compromise between levels of integration in a slice versus a whole brain and a healthy animal tissue rather than a diseased human brain.

In vitro studies on human epileptic tissue provided a privileged window onto human epileptic activities [6]. They permit studies on the behaviors of single cells and neuronal ensembles and can be combined with imaging and genetic explorations. Finally, tissue slice microenvironment can be modified to mimic changes in brain extra-cellular medium to induce specific activities or for pharmacological investigation.

Here we review data from electrophysiological studies on human mesial temporal epileptogenic tissue in vitro. Mesial temporal lobe epilepsies associated with hippocampal sclerosis (mTLE-HS) are relatively frequent and homogeneous [7]. Slices with intact connections between the hippocampus, subiculum and entorhinal cortex, can be prepared from surgically removed tissue [8]. There are, however, two limitations [9]. Connections to and from other brain regions are cut and no satisfactory control tissue is available. With critical functional roles, non-pathologic mesial temporal tissue is never resected, so data is compared, at best, to healthy tissue from animals or internal controls can sometimes be devised. This review describes initial work on human temporal epileptic tissue and examines more recent suggestions of a causative role for a defect in inhibitory  $\text{GABA}_A$ ergic signaling in generating interictal activity.

### **Neuronal properties of the epileptogenic hippocampus.**

Studies on human epileptic tissue show that single neurons are physiologically quite similar to those of healthy rodents [10-13]. Some neurons discharge bursts of action potentials, a potentially 'epileptic' property' [14] but not in greater proportions than in healthy rodent tissue [11,15]. Afferent fiber stimulation evoked similar responses in cortical neurons of epileptic patients and in epileptic animals: a large, long lasting 'depolarization shift' was initiated at long, variable latencies in an all-or-nothing threshold behavior [16]. Some differences may exist. Atypical, persistent sodium conductances which might account for burst discharges, have been detected in epileptic subicular neurons [17]. Equally the hyperpolarization-activated cation current  $I(h)$  seems to be upregulated by 80% in dentate granule cells in hippocampi with sclerosis compared to cases without [18].

Synapses made in slices of human epileptic tissue have been studied by stimulating afferent fibers. At low intensities mono-synaptic EPSPs of varying amplitude are evoked in pyramidal cells. Beyond a threshold, possibly of population firing, 'all or nothing' bursts of post synaptic potentials with an NMDA receptor component and variable latency were recruited in tissue from epileptic temporal or frontal neocortex [11]. In the hippocampus, prolonged responses with after-discharges are initiated in the dentate gyrus by perforant path stimuli. In tissue from healthy animals such responses occur only when GABA<sub>A</sub> receptors are partly blocked, pointing to defects in epileptogenic human tissue [19]. Stimulation of granule cells via the perforant path generates prolonged excitatory postsynaptic potential, depending on polysynaptic circuits and NMDA receptors [20,21]. Such amplified responses may depend on an upregulation of certain ionotropic glutamate receptors (NMDA R2, GluR3 AMPA) [22], and kainate receptors [23], in the epileptic dentate gyrus of children [24]. The control of release due to mGluRs is reduced at synapses terminating on epileptic dentate granule cells [25].

High frequency stimulation of the perforant path generates GABAergic inhibition in human dentate cells that may be less effective in tissue from mTLE patients with a sclerosis than those without HS [26,27], supporting the idea that deficient inhibition contributes to hyperexcitability. Reduced GABAergic currents in dentate granule cells of patients with HS, are prolonged in duration perhaps due to a reduced GABA reuptake [28]. Finally, Menendez de la Prida has shown that interneurons of epileptic human neocortex, retain a 'regular spiking' discharge pattern and glutamatergic synaptic inputs [29].

Some properties of hippocampal cells may also be related to functional cognitive defects. The dentate gyrus can generate new neurons throughout life. The ability of human postoperative dentate gyrus to display neurogenic properties has been correlated to presurgical memory performances. Patients with low proliferation capacities had poor memories while those with many proliferative cells did not have memory defects [30]. Memory impairment be mediated via a reduced long-term plasticity at dentate synapses excited by the perforant path [31].

### **Induced population activities in the epileptic hippocampus.**

Post-operative tissues from cortex or hippocampus very rarely generate spontaneous synchronous activity. Almost all work, on temporal neocortex, rather than the hippocampal formation, has concerned pharmacologically induced epileptic activities

'Epileptiform' activities in human tissue have frequently been induced by blocking GABA<sub>A</sub> receptors as in rodent studies. Suppressing fast inhibition generates interictal-like

population bursts [11,32-35] associated with an intracellular 'Paroxysmal Depolarization Shift'. Bicuculline doses needed to induce this form of synchrony in the dentate gyrus were lower in tissue associated with a HS, possibly reflecting a hyperexcitability of this region resulting from aberrant recurrent connexions due to mossy fiber sprouting [32]. Presumably the need to block GABAergic transmission signaling indicates that synaptic inhibition normally controls recurrent excitation in epileptic tissue.

Application of 'zero'  $Mg^{2+}$  perfusion media induces prolonged ictal events as well as interictal bursts both in neocortical slices [36-39] and hippocampus [40]. This action may result from a relief of NMDA receptor blockade [41] an increase in neuronal excitability due to a membrane surface charge effect [6,42] as well as a suppression of inhibition. Postsynaptic GABA<sub>A</sub> potentials are recorded during the interictal phase, but are reduced and disappear at the transition to ictal discharges [43]. Ictal events are sustained by a NMDA signaling.

4-aminopyridine (4-AP), which blocks some  $K^+$  channels so enhancing release of excitatory and inhibitory neurotransmitters, also induces both ictal and interictal discharges in human epileptic temporal neocortex [44]. In dysplastic cortical tissue, ictal discharges depend not only on NMDA and AMPA excitatory transmission but also on signals mediated by GABA [36]. GABAergic preictal potentials, which precede seizure onset, are associated with increases in external  $K^+$  levels from 3.2 to 4.5 mM [45]. At the transition to ictal discharges, the increase reaches 6.4 mM, suggesting external  $K^+$  may contribute to seizure initiation. Such a role is emphasized by several studies. Using barium to block delayed rectifier  $K^+$ -channels, may impair  $K^+$  buffering by astrocytes which express these channels at high levels. Elevations of external  $K^+$  in response to fiber stimulation are much enhanced [46].  $K^+$  buffering seems to be depressed in reactive astrocytes from temporal lobe epilepsy tissues due to a down regulation of these inwardly rectifying  $K^+$  channels [47-49]. However, GABAergic signaling remains fundamental to the generation of epileptiform activities since the  $\mu$  opioid receptor agonist DAGO, which hyperpolarizes interneurons and so reduces GABA release, transforms ictal events into shorter interictal-like events [45]. GABA<sub>B</sub> signals play a protective role since baclofen blocks seizures, which are then re-induced by a GABA<sub>B</sub> antagonist. Epileptiform discharges induced by 4-AP are also suppressed by blocking gap junctions [50].

The human epileptic dentate gyrus also generates little spontaneous activity. Increasing extracellular  $K^+$  concentration to 10-12 mM evokes ictal-like discharges [51,52] which depend on AMPA receptors but remain unchanged when NMDA-mediated signals are suppressed.

Neocortical tissues obtained after mTLE surgery generate theta and gamma rhythms in vitro in response to kainate and carbachol application [53]. These activities are very similar to those recorded in vivo, suggesting that they are locally generated and do not depend on afferent connections with the other brain structures.

### **Spontaneous epileptiform population activities in human hippocampus.**

Post-operative tissue rarely generates epileptiform activity. However, a year after finding no activity [54], Schwartzkroin and colleagues described interesting spontaneously occurring synaptic events in tissue from mesial and lateral temporal cortex of epileptic patients [55]. It consisted of complex synaptic events, with depolarizing and hyperpolarizing components and sometimes firing, that was detected in pairs of pyramidal cells. Such activity suggested that groups of pyramidal cells and interneurons were firing synchronously

without external stimuli. These complex events recurred at about 2 Hz, and were recorded from cells in a range of 500-700  $\mu\text{m}$ . While the reversal potential of these composite synaptic events was not measured at first, later work [56] confirmed that the events included a GABAergic component since they were blocked by bicuculline. Intracellular records showed that interneurons fired both before and during synchronous synaptic events.

Field potentials associated with spontaneous activity generated by temporal neocortex from epileptic patients in vitro were first described by Kohling in the late 90s [38]. These events, which permit direct comparisons with in situ EEG traces, recurred at about 1 Hz, lasted 20-200 milliseconds and had an amplitude of 20-300  $\mu\text{V}$  (Figure 1). They were synchronized over areas of 100s of  $\mu\text{m}$  and several autonomous foci could coexist in the same slice. Intracellular record described depolarizing (37%), hyperpolarizing (44%) or mixed (19%) postsynaptic potentials associated with extracellular fields. Field events were suppressed by antagonists at both AMPA and GABA<sub>A</sub> receptors. The authors noted that blocking GABA<sub>A</sub> receptors could initiate synchrony in slices that were originally silent, while bicuculline blocked spontaneously occurring fields. The authors termed this activity 'sharp waves', and suggested they might 'facilitate the initiation or propagation of seizures'. Similar 'sharp waves' are recorded in neocortical tissue from mTLE-HS patients [53].

FIGURE 1

### **Interictal activities generated in the human epileptic hippocampus.**

Tissue slices containing the hippocampus, subiculum and entorhinal cortex, obtained after surgery on patients with defined mTLE-HS have provided data on human epileptiform activities [57,58]. Field potentials of duration 20-50 msec, recurring at 0.5-2 Hz are generated, without stimulation, by a region of 3-6 x 1-3 mm in the subiculum (Figure 2). These events are comparable to interictal discharges recorded in situ with intracerebral electrodes and were termed interictal-like activity. They were generated locally in isolated slices of the subiculum. Similar activity was not detected in the dentate gyrus, CA3, CA1 or entorhinal cortex. Subicular interictal fields were suppressed by antagonists at glutamatergic AMPA and also GABA<sub>A</sub> receptors. The role of GABAergic circuits was highlighted by the observation that interneurons fired just before and during interictal-like discharges. Most (~80%) subicular pyramidal cells were hyperpolarized during interictal events, however a minority (~20%) received depolarizing synaptic events and could sometimes discharge. Measurements of reversal potentials for isolated GABAergic synaptic events confirmed they were sometimes depolarizing from pyramidal cell at resting potential. Cells hyperpolarized during interictal discharges had a GABAergic reversal potential of  $72 \pm 7$  mV (rest potential:  $-62 \pm 8$  mV), while those associated with a depolarizing response reversed at  $-55 \pm 12$  mV (rest:  $67 \pm 10$  mV). These data were interpreted as resulting from a disturbed Cl<sup>-</sup> homeostasis in a minority of subicular pyramidal cells. It was suggested that the depolarizing GABAergic response might be linked to a deafferentation due to neuronal loss in the sclerotic CA1 region. Possibly, it was argued, the loss of innervation from CA1 might cause post-synaptic cells to regress to earlier stages of development when GABAergic reversal potentials are relatively depolarized [59].

This activity differs drastically from that of temporal cortex where no interictal-like activity is observed and where GABA<sub>A</sub> antagonists induce [11,33,34] epileptiform activity rather than block it as in human subiculum.



## FIGURE 2

These data from Parisian patients were confirmed in patients suffering from mTLE treated in Berlin. Wozny and colleagues showed the subiculum generated a spontaneous interictal activity [60,61] both in patients with and those without hippocampal sclerosis. This data challenges the notion that deafferentation contributes to events in the subiculum. However the category 'without sclerosis' was defined to include patients with 0-50% neuronal loss in CA1. In tissue studied in Berlin, interictal-like events were also blocked by GABA<sub>A</sub> receptor antagonists. Subicular cell excitability was found to be enhanced with reduced after-hyperpolarizations detected in cells that fired with interictal-like events. Most cells recorded in the epileptogenic focus discharged regularly, about 25% fired in bursts.

After in vitro data pointed to the subiculum, multi-electrode records were made from this zone in situ. Several forms of interictal spikes were detected, with distinct sites of origin and propagation patterns confirming a role in epileptogenesis for this site [62].

The cornu ammonis 2 (CA2) region of the human hippocampus is resistant to the cell loss associated with mTLE-HS. It also generates spontaneous interictal-like discharges in slices, which are completely dissociated from those generated in subiculum [63]. However cellular behavior differs. Most pyramidal cells fire during interictal-events in CA2, and the efficacy of GABAergic inhibition, mediated by parvalbumin containing cells, seems to be reduced.

Subsequent studies found no clear anatomical differences between pyramidal cells with perturbed and those with normal Cl<sup>-</sup> homeostasis [58]. The morphology of cells that were depolarized and those hyperpolarized during interictal bursts was comparable (Figure 3). Neuronal physiology was not dramatically different either in terms of resting potential, input resistance, firing pattern or the existence of an I<sub>h</sub> current.

## FIGURE 3

A detailed study examined the state of Cl<sup>-</sup> homeostasis in the epileptic subiculum. When GABA<sub>A</sub> receptors are activated, an anion-permeable channel opens with effects that vary according to the electrical and ionic environment. GABA channels are mainly permeable to Cl<sup>-</sup> ions. The direction and strength of Cl<sup>-</sup> movement depends in part on concentrations on both sides of a membrane. Since extracellular Cl<sup>-</sup>-concentration ( $[Cl^-]_e$ ) is higher than intracellular ( $[Cl^-]_i$ ), Cl<sup>-</sup> anions tend to enter the cell, provoking a hyperpolarization. A second force controlling Cl<sup>-</sup> ion movements is the transmembrane electrical gradient. Negatively charged Cl<sup>-</sup> anions are repelled by the negatively polarized cell. Charge and concentration combine to generate an electrochemical gradient for Cl<sup>-</sup> movement. For given concentrations of internal and external Cl<sup>-</sup>, there is a reversal potential ( $E_{INV}$ ) where charge and concentration gradients are equal and opposed. Then at any membrane potential,  $V_m > E_{INV}$  Cl<sup>-</sup> flows inward and the cell is hyperpolarized. Inversely, if  $V_m < E_{INV}$ , Cl<sup>-</sup> moves outward to depolarize the cell. Depolarizations beyond neuronal threshold could even cause cell firing.

In human, epileptic pyramidal cells sampled from the subiculum, the reversal potential of GABA<sub>A</sub> responses formed a distribution centered around -70 mV. But, it extended asymmetrically to values as depolarized as -45 mV: a skewed continuum of reversal potentials rather than two distinct cell populations. About 20% of pyramidal cells were depolarized by GABA; that is the reversal potential for GABA was more depolarized

than the resting membrane potential. A similar proportion to that of the cells depolarized during interictal discharges. In neocortical tissues, interictal-like discharges were associated with synaptic inputs reversing at a mean  $-62 \pm 2$  mV also suggesting depolarizing GABA signaling may be involved [53].

Regulation of  $[Cl^-]_i$  depends on multiple molecules including the Na-K-Cl (NKCC1) and K-Cl (KCC2) cotransporters [64]. NKCC1 loads the cell with  $Cl^-$ , using the electrochemical gradient of  $Na^+$  ions. KCC2 extrudes  $Cl^-$  from the cell, with energy derived from the chemical gradient of  $K^+$  ions. In normal and mature tissues, KCC2 is highly expressed while NKCC1 expression may be repressed [65].  $[Cl^-]_i$  is very low,  $\sim 4$  mmol/L, compared to  $[Cl^-]_e$ , close to 147 mmol/L, resulting in a reversal potential of  $-72$  mV. Adult neuron resting potentials are more depolarized than the reversal potential for GABA which thus hyperpolarizes and inhibits. In contrast during early development, KCC2 is not expressed and NKCC1 acts to charge immature neurons with  $Cl^-$  [66]. The reversal potential for GABA is more depolarized than resting potential, so GABA depolarizes [67].

The hypothesis of a loss of expression of KCC2 within deafferented cells of the subiculum [68,69] was tested using in situ hybridization to evaluate expression of mRNA for KCC2 (Figure 4). Nearly all cells in the CA2 region and the dentate gyrus expressed message for KCC2. These cells served as non-deafferented controls. In contrast only 60-80% of subicular pyramidal cells expressed KCC2 message. As for the KCC2 protein, immunohistochemistry showed that KCC2 was expressed by  $\sim 90\%$  of subicular pyramidal cells compared to 97% in dentate gyrus and almost 100% in the subiculum of control monkeys.

FIGURE 4

Single neuron expression of KCC2 was compared to the hyper- or depolarizing nature of potential changes during interictal discharges (Figure 5). All hyperpolarized pyramidal cells, expressed KCC2. However, not all cells that were depolarized, presumably with higher levels of internal  $Cl^-$ , lacked KCC2 expression. Possibly immuno-labelled KCC2 may not be functional due to posttranslational regulation [70-72]. Alternatively distinct  $Cl^-$  homeostatic pathways could be involved. NKCC1, the transporter which tends to import  $Cl^-$ , was functional in that the antagonist bumetanide, hyperpolarized GABA-mediated responses.

FIGURE 5

Selective blockade of NKCC1 by low doses of loop diuretic bumetanide hyperpolarized the GABAergic reversal potential by about 10 mV and blocked epileptiform activity over 10s of minutes, kinetics consistent with a reduced internal  $Cl^-$  (Figure 6). Work on animal models confirms the antiepileptic potential of this diuretic [65].

FIGURE 6

These studies have validated a role of an impaired  $Cl^-$  homeostasis in epileptiform activity of the human subiculum. They identified a continuum of depolarizing responses to GABA associated with a disturbed expression and / or function of the co-transporters KCC2 and NKCC1. Identification of these defects opens new therapeutic avenues by restoring  $Cl^-$  homeostasis. Possibly depolarizing responses to GABA are a common feature to

epileptogenic tissue. A recent study of human neocortex surrounding epileptic brain gliomas shows this tissue also generates interictal-like discharges. In the same way, interneurons initiate population events which depend on depolarizing responses to GABA. Cl<sup>-</sup> homeostasis in a majority of pyramidal cells is perturbed due to both a down regulation of KCC2 and an up regulation of NKCC1 [73].

What causes these disturbances in Cl<sup>-</sup> homeostasis in human epileptic tissue? In the subiculum of sclerotic hippocampus, it may result from deafferentation due to CA1 pyramidal cell loss. Deafferentation depolarizes GABA<sub>A</sub> responses by repressing KCC2 expression or function in several animal models [68,69,74]. After a delay, cortical transection can lead to ictal activities in deafferented cortex of the cat [75]. Perturbed Cl<sup>-</sup> homeostasis after deafferentation in the spinal cord may be mediated via BDNF release from activated microglia [76]. BDNF affects post-synaptic KCC2 via trkB receptors on neurons [77]. Epileptic activity itself may prolong disturbances in Cl<sup>-</sup> homeostasis [78]. It remains puzzling though that Cl<sup>-</sup> homeostasis is perturbed in only about 20% of subicular pyramidal cells in spite of severe neuronal loss and gliosis in the CA1 region. A limited cell loss in layer III of the entorhinal cortex [79] may reduce afferents from this region [80] to the subiculum. These changes in afferent connectivity may reorganize recurrent synaptic circuits between subicular cells [81-83]. Enhanced recurrent loops would tend to generate epileptic activity and provoke cognitive disturbances in patients.

Changes in Cl<sup>-</sup> homeostasis after deafferentation, recall early stages of neuronal development. During development, GABAergic precedes glutamatergic signaling, at a stage of neuronal migration, synaptogenesis and circuit formation [67]. Possibly interictal discharges in epileptic subiculum should be compared to the synchronous Giant Depolarizing Potentials [84], emblematic of this period which also depend on depolarizing GABAergic signals. Synaptogenesis in the human deafferented subiculum, could favor the genesis of synchronous activities. Neuronal density in the subiculum increases by ~50% during CA1 sclerosis [85]. However changes in recurrent excitatory connections have not been demonstrated and subicular axonal sprouting seems to be absent in animal models of epilepsy associated with CA1 sclerosis [86].

Further work is needed to define connectivity and post-synaptic actions for interneurons of human epileptic subiculum. It is not clear which type of interneuron discharges before interictal events. Possibly chandelier cells which make divergent contacts with proximal pyramidal cell axons, a site devoid of KCC2 [87], but connectivity of these cells seem to be unchanged in human tissue [88]. In contrast, parvalbumin-positive basket interneurons contacts with proximal pyramidal cells in the subiculum apparently involve large numbers of contacts [89]. Numbers of these cells are reduced in epileptic subiculum with or without HS [85] but no data is yet available on their discharge pattern during interictal-like activities.

In addition to a role in generating interictal events, GABAergic signaling affects their spread. Antagonists suppress variability in propagation pathways [90].

### **Genesis of ictal activities within the human epileptogenic subiculum.**

In vitro, human postoperative tissues generate interictal activity with no stimulation. Ictal like events must be induced. Effective stimuli have combined an increase in cellular excitability (increased external K<sup>+</sup> or reduced Ca<sup>2+</sup> and Mg<sup>2+</sup>) with an alkalinization, 4-AP or a reduced external Mg<sup>2+</sup> [91]. Ictal-like events generated by the subiculum typically comprise low-voltage fast activities followed by larger rhythmic oscillations (Figure 7). Ictogenesis

mechanisms can be studied with these pro-ictal stimuli. In vitro the transition from interictal to ictal-like events is accompanied by the emergence of larger, preictal recurrent population bursts.

#### FIGURE 7

Preictal discharges arise progressively during a long lasting transition period from the interictal state to the first seizure and coexist with interictal discharges (Figure 8).

#### FIGURE 8

Preictal discharges seem to depend on exclusively glutamatergic signals in contrast to the implication of both GABAergic and glutamatergic mechanisms in interictal events. Multiple observations support this point. In pyramidal cells, synaptic events associated with preictal bursts are exclusively depolarizing, with no trace of the hyperpolarization recorded from most pyramidal cells during interictal bursts (Figure 9A). Pre-ictal bursts are blocked by AMPA receptor antagonists, but unchanged by blockers at GABA<sub>A</sub> receptors (Figure 9B). The reversal potential of synaptic events associated with preictal discharges is -10 to -20 mV, while that for synaptic events associated with interictal discharges is more negative at -50 to -80 mV, compatible with mixed glutamate and GABA signals (Figure 9C).

#### FIGURE 9

Juxtacellular recordings confirm that preictal bursts were preceded, and presumably initiated, by firing in a subset of pyramidal cells, while interictal discharges were preceded by interneuron firing. The buildup of preictal discharges was associated with an increased cellular excitability mediated by NMDA-dependent processes of plasticity.

Finally, preictal discharges have been recorded from patients with mTLE-HS with in situ stereoelectroencephalography (Figure 10). As in vitro, they precede seizure onset, their amplitude clearly differs from that of interictal discharges. In vivo their sites of generation are restricted to areas of seizure initiation.

#### FIGURE 10

Ictal discharges have been induced in several ways. Zero Mg<sup>2+</sup> media, which increase local excitability and enhance NMDA mediated excitation, induce seizure like events in neocortical temporal tissue [37,38,92] and in hippocampal slices [40]. 4-aminopyridine (4-AP), a K<sup>+</sup> channel blocker which increases neuronal excitability and transmitter release, induces ictal events in neocortical slices [36,45]. Equally, increasing extracellular potassium (to 8-12 mM), which increases excitability and probably affect Cl<sup>-</sup> regulation, induces seizure-like events in hippocampal tissue [51]. Similar effects of these diverse stimuli highlight phenomena that may participate in seizures: activation of NMDA receptors [43], release of adenosine [92] which controls synaptic liberation of glutamate, reductions in external Ca<sup>2+</sup> [43], defects in GABA<sub>B</sub> control of inhibitory synapses [45]. However, two major ictogenic mechanisms predominate. During preictal discharges, extracellular K<sup>+</sup> increases transiently, then increases more dramatically to a plateau at seizure onset [45] (Figure 11). It is not clear if this increase is a cause or a consequence of neuronal firing at seizure onset. The second

key mechanism is GABAergic signaling [45,91]. Interneurons fire at high frequency at seizure onset, leading to a dynamic depolarizing switch of GABAergic signaling, dependent on Cl<sup>-</sup> dysregulation in pyramidal cells.

FIGURE 11

**Conclusion: practical benefits for the care of patients.**

The cellular basis of human epileptic activities may be better understood from work on slices of surgical specimens since they permit the use of recording techniques and pharmacological manipulations that are not possible in situ. This approach has revealed a role for depolarizing GABA responses in epileptiform activities in mTLE-HS and peritumoral tissue that could underlie, at least in part, a poor seizure control with GABAergic drugs. They assign a significant role to the subiculum which has been shown to generate both interictal and ictal epileptic activities. Slice data points to new therapeutic options to restore inhibitory effects of GABA and suppress epileptic activities by targeting Cl<sup>-</sup> regulators.

Human postoperative tissue may also help address the problem of pharmacoresistance. Multidrug transporter proteins are suggested to block antiepileptic drug access to the epileptic focus [93] while Na<sup>+</sup> channels targeted by specific drugs may be modified consequently reducing drug efficacy [52]. Human tissue is evidently useful to test anti-epileptic drugs and verify new therapeutic targets. Distinctions must be made between interictal and ictal discharges, since in rodents most current drugs block seizures but not interictal events [45]. Recently a microRNA (small non-coding RNA exerting a post-transcriptional regulation of coding mRNAs), miR-487a, was shown to be down regulated in TLE tissues with dentate gyrus pathology [94], which may affect the expression of the gene ANTXR1, a promoter of cell migration.

Work on slices also has limitations. Tissue is certainly damaged by surgery and slicing [95]. Axons must frequently be cut, leading to a possible dys-regulation of cellular Cl<sup>-</sup> and thus GABAergic signaling [96]. Long-term organotypic culture of human epileptic slices [97] could assist recovery from these traumas, and also permit long-term manipulations such as transfection. However the activity after several weeks differs from that of acute slices. In addition to subicular events, interictal-like bursts are also generated in the dentate gyrus and propagate to CA3. As in acute slices temporal neocortex in culture did not generate interictal events, although ictal-like firing was induced by GABA<sub>A</sub> receptors antagonists or 4-AP, indicating distinct features of mesial and lateral temporal areas.

Several novel techniques are being applied to human tissue slices. Microelectrode arrays [98,99] permit records from up to 100s of sites at high spatial and temporal resolution. Fluorescent dye techniques permit the activity of many cells to be combined with electrical records at cellular and population scale paving the way for exciting multimodal investigations [100].

## Figure legends

### Figure 1.

Spontaneous Interictal activity recorded in the temporal neocortex (based on [38]). Synchronous spontaneous interictal field potentials (FP) (A) are associated with a postsynaptic potential which reverses at a membrane potential (MB) near -70 mV (B) and blocked by the GABA receptor antagonist bicuculline (C).

### Figure 2.

Interictal activity generated in vitro by the subiculum obtained from mTLE-HS patients (from [57]).

A. Multiple extracellular recordings showing spontaneous synchronous population activity within the subiculum.

B. Pharmacological characterization of interictal activities: suppression by the GABA<sub>A</sub> receptor antagonists (bicuculline) and glutamate AMPA and NMDA receptors antagonists (NBQX + APV).

C. Behavior of different cell types during interictal activities. The top trace is an intracellular recording, the lower one an extracellular recording. Interneurons (top trace) discharge before and during the first phase of the burst. Pyramidal cells are hyperpolarized in the usually hot (middle trace) but can also be depolarized (bottom traces).

### Figure 3.

Interictal epileptiform activity recorded in vitro from the human subiculum obtained from surgical specimens of patients with mTLE-HS (from [58]).

A: A photograph of the block of postoperative tissue.

B: Tissue slices including the subiculum in the interface recording chamber

C: Records of intracellular (top) and extracellular (bottom) of a pyramidal cell hyperpolarized during interictal discharges, rebuilt after biocytin filling.

D: same as C for a depolarized cell.

### Figure 4.

Regulation of the intracellular Cl<sup>-</sup> concentration by the cotransporters KCC2 & NKCC1 and effects on GABAergic responses in mature (left) and immature and pathological tissues (right). Within pyramidal cells (triangles), Cl<sup>-</sup> concentration is represented by the height of the filling in yellow. V<sub>m</sub>: membrane potential, E<sub>Cl<sup>-</sup></sub>: reversal potential of Cl<sup>-</sup> currents.

### Figure 5.

Correlation between the behavior of a pyramidal cell during interictal discharges (hyperpolarization in A vs depolarization in B) and the expression of KCC2 (based on [58]). Electrophysiological trace is shown on the left (top: intracellular recording - bottom: extracellular). The recorded cell is then filled with biocytin and KCC2 is labeled by immunohistochemistry.

### Figure 6.

The diuretic Bumetanide blocks interictal activity (based on [58]).

A: Measurements of the GABAergic reversal potential in a pyramidal cell, before (-58 mV) and after applying Bumetanide (-70 mV).  
B: Bumetanide blocks interictal activity (extracellular recording)

#### Figure 7.

Ictal-like discharges induced in vitro in the epileptic human subiculum (from [91]).

A: Multiple extracellular recordings of an ictal event in a slice containing the hippocampus, subiculum and entorhinal cortex. Electrode locations: 1, dentate gyrus; 2, CA2; 3, CA1; 4–5–6, subiculum; 7, presubiculum; 8, entorhinal cortex.

B: Ictal event structure. Top, pre-ictal discharges (larger events, filled circles) recurred before a fast low-voltage activity (gray line) at seizure onset followed by rhythmic bursts (black line). Seizure onset is indicated by the dotted arrow. Middle, time frequency representation of the local field potential (LFP). Bottom, multi-unit activity (MUA) revealed recurring PIDs of short duration followed by the rapid unpatterned action potential discharges at seizure onset and then by oscillatory bursts.

#### Figure 8

Preictal discharges progressively emerge during the transition to the seizure (from [91]).

A: Extracellular recordings of the transition to seizure-like activity. E1 and E2 are recordings from two subicular electrodes. MUA frequency (upper trace) and the extracellular signal from E2 (lower trace) are shown.

B: Amplitude measurements for all field potentials recorded by electrode E2 during the transition show the emergence of larger PIDs, whereas the amplitude of inter-ictal events did not change.

C: Dual extracellular recordings showing IIDs (open circles, left) before convulsant application and coexpression of PIDs (filled circles, right) with IIDs during the transition.

D: Amplitude distribution for all field potentials during the 35-min transition period showed IIDs of amplitude 10–50  $\mu$ V and PIDs of amplitude 125–175  $\mu$ V.

#### Figure 9

Synaptic characteristics of interictal (IID) and preictal discharges (PID) (from [91]).

A: Intracellular recording from a subicular pyramidal cell (I) with a local extracellular recording (E). This cell received hyperpolarizing synaptic inputs during IIDs (open circle) both before and after convulsants were added. In contrast, it was depolarized during PIDs (filled circle) in the presence of convulsants.

B: IIDs and PIDs coexisted during the transition to ictal discharges (left trace). Bicuculline (bic) blocked IIDs, but not PIDs (middle left). The NMDA receptor blocker D,L-AP5 (100  $\mu$ M) did not change PIDs (middle right), but the AMPA receptor antagonist NBQX (10  $\mu$ M) suppressed them (right).

C: Distinct reversal potentials for synaptic events associated with IIDs and PIDs.

#### Figure 10.

In vivo StereoElectroEncephaloGraphic (SEEG) recording of preictal discharges (PID) (from [91]).

A: Stereo EEG recording (electrode HipAg1) showing activity of the subiculum and head of the hippocampus at seizure onset. The seizure was preceded by recurring IIDs (blue circles)

and PIDs (pink circles). It began (arrow) with fast low-voltage activity and continued with oscillatory rhythmic bursts.

B: Recordings from multiple electrode contacts on a referential montage. Traces from contacts with no epileptic activity are shown in green, those with isolated IIDs in blue, and those recording both IIDs and PIDs in pink. Left, IID sample (blue circle). Middle, PID sample (pink circle). Right, seizure onset (thick arrow). Seizure onset is highlighted in yellow. Electrodes are identified according to the recorded area.

Figure 11 :

A: Extracellular  $K^+$  concentration ( $[K^+]_o$ ) dynamics during epileptic activities (From [45]).

$[K^+]_o$  increases up to 4.5 mM accompany the isolated negative field events (asterisk), while an elevation up to 6.4 mM characterizes the field potential leading to the ictal discharge onset (arrow). The insert, which represents three graphically superimposed ictal onsets at faster time base, demonstrates that the initial increase in  $[K^+]_o$  occurs at the same time as the initial positive–negative field event, while the slower secondary negative field event follows the peak of the elevation in  $[K^+]_o$ .

B: Quantitative summary of the maximal elevations in  $[K^+]_o$  associated with the isolated negative-going events, those occurring during the negative-going events preceding ictal discharge and during the ongoing ictal activity (identified as ictal peak).



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