Neurosciences

The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge

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Abstract

Although there are many types of epilepsy of both genetic and acquired forms, temporal lobe epilepsy (TLE) with hippocampal sclerosis is probably the single most common human epilepsy, and the one most intensely studied. Despite a wealth of descriptive data obtained from patient histories, imaging techniques, electroencephalographic recording, and histological studies, the epileptogenic process remains poorly understood. Progress toward understanding the etiology of an acquired neurological disorder is largely dependent on the degree to which experimental animal models reflect the human condition. Recent observations suggest that significant disparities exist between the features of human TLE with hippocampal sclerosis and those of animal models that involve prolonged *status epilepticus* to initiate the epileptogenic process. TLE most commonly involves patients with focal seizures who exhibit limited and often asymmetrical brain damage, did not experience *status epilepticus* prior to the onset of epilepsy, and who appear relatively normal on neurological examination. Conversely, animals subjected to prolonged *status epilepticus* exhibit severe brain damage, behavioral abnormalities, and frequent generalized seizures. In addition, although many TLE patients exhibit an atrophic hippocampus that may, or may not, be a source of spontaneous seizures, hippocampal damage in animals subjected to *status epilepticus* is an inconsistent and often minor part of a much greater constellation of damage to other brain structures. Furthermore, many patients exhibit developmental structural abnormalities that presumably play a role in the clinical etiology, whereas most animal models involve severe insults in initially normal laboratory rats. Although much has been learned using the current animal models, the available data suggest the need for a critical reappraisal of the assumptions underlying their use, and the need to develop experimental preparations that may more closely model the human epileptic state. ***To cite this article: R.S. Sloviter, C. R. Biologies 328 (2005).***
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Résumé

Bien qu'il y ait de nombreuses épilepsies, à la fois de formes génétique et acquise, l'épilepsie du lobe temporal (ELT) avec sclérose hippocampique est probablement l'épilepsie humaine la plus commune, et celle qui est étudiée le plus intensément. Malgré une abondance de données descriptives obtenues d'après l'historique des patients, par des techniques d'imagerie, d'en-

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registrement électroencéphalographiques et par des études histologiques, le processus épileptogène demeure mal connu. Les progrès dans la compréhension de l'étiologie d'un désordre neurologique acquis dépendent largement du degré auquel les modèles expérimentaux animaux reproduisent les conditions du patient. Des observations récentes suggèrent que des différences significatives existent entre les caractéristiques de l'ELT humaine avec sclérose hippocampique et celles observées sur les modèles animaux qui exigent un *status epilepticus* pour créer le processus épileptogène. L'ELT concerne le plus communément des patients développant des crises focales traduisant une lésion cérébrale limitée et souvent asymétrique, qui n'ont pas traversé de *status epilepticus* avant que ne se déclare leur épilepsie, et qui apparaissent relativement normaux à l'examen neurologique. Inversement, des animaux soumis à un *status* prolongé montrent de sévères lésions cérébrales, des anomalies comportementales ainsi que de fréquentes crises généralisées. De plus, bien que de nombreux malades atteints d'ELT montrent un hippocampe atrophique qui peut, ou non, être une source d'attaques spontanées, des lésions hippocampiques chez des animaux présentant un *status* ne sont en revanche qu'une partie souvent mineure d'une constellation beaucoup plus grande de lésions impliquant d'autres structures cérébrales. De plus, nombre de patients montrent des anomalies structurales du développement, qui jouent probablement un rôle dans l'étiologie clinique, alors qu'un grand nombre modèles animaux impliquent des lésions sévères réalisées chez des rats de laboratoire initialement normaux. Quoiqu'on ait appris beaucoup en utilisant les modèles animaux courants, les données disponibles suggèrent qu'un réexamen critique des hypothèses sous-jacentes à leur usage doit être effectué, et qu'on doit développer des protocoles expérimentaux qui puissent modéliser de manière plus fidèle l'état épileptique humain. ***Pour citer cet article : R.S. Sloviter, C. R. Biologies 328 (2005).***

1. Introduction

Temporal lobe epilepsy (TLE) is a common and often medically intractable neurological disorder that is possibly unique in terms of the wealth of descriptive data that have been obtained from historical anatomical studies, electroencephalographic recording methods, modern imaging techniques, depth recording before and during surgery, and from histological study of surgical and autopsy tissues [1]. The sheer mass of data now available on the subject of temporal lobe epilepsy is emblematic of the modern problem of having so much information on any given subject that it is a significant conceptual challenge to separate facts from notions, associations from causes, and to discriminate between the possibly important and the probably unimportant. The enormous amount of information now available to us, taken together with the natural desire we have to get clear and unambiguous answers to all of the questions we ask, makes simple answers appealing, and may explain, in part, the general hesitancy we have to admit that we may know far less than we actually do.

In the search for real understanding of the epileptic process, it may be useful conceptually to contrast TLE, a neurological disorder in which the nature of the network defect is largely unknown, with Parkinson's disease, a disorder in which the loss of identified dopaminergic neurons disinhibits a known network, resulting in the clinical behavioral signs of the disorder [2]. Like patients with Parkinson's disease, those with TLE exhibit neuronal loss and a network imbalance that presumably causes the clinical condition. Unlike the identified network defect of Parkinson's disease, however, the neuronal loss that presumably produces a network imbalance in the temporal lobe remains unidentified. We do not really know precisely which cell populations, when lost, cause the network imbalance in TLE, or which cells generate the seizure discharges. Nor do we have an effective drug treatment, like levodopa, that both points to the identity of the defective component and corrects the network imbalance to an extent that produces symptomatic improvement. Thus, in TLE, both the cause and the cure remain unknown, and we primarily utilize drugs that suppress the clinical manifestations, but probably do not directly target the underlying network defect.

A logical assumption that we can make about the etiology of temporal lobe epilepsy is that there is a derangement of excitatory and inhibitory mechanisms that, in some way, causes abnormal network discharges that define the clinical epileptic state. Un-

fortunately, the extraordinary progress made in our understanding of the neurophysiology of excitatory and inhibitory mechanisms has not yet been translated into an understanding of the pathophysiology of these systems at the network level, where they presumably relate to epilepsy. Although we can imagine and propose a myriad of genetic, developmental, and acquired factors that might influence the excitatory/inhibitory balance, how do we determine which network defects actually occur in human patients, and how do we establish a causal relationship between any given mechanism or pathological feature, and the clinical state, particularly for the vast majority of patients who do not have a strictly familial neurological disorder [3]? For this answer, and to find a cure, we are largely dependent upon deductive inferences drawn from studies of human patients, and on the experimental results obtained from studies of animal models of genetic and acquired epilepsy. Thus, a critical re-examination of the data from which many of the currently prevailing assumptions have arisen seems warranted.

2. The current state of knowledge

A clear and thorough description by Engel [1] of the features of human TLE summarizes the rationale for believing that when the hippocampus is found to be asymmetrically atrophic on initial imaging, the shrunken hippocampus is a likely source of epileptic seizures. If this is true, it is logical to presume that hippocampal changes constitute the primary epileptogenic process in a significant proportion of patients. Conversely, if the shrunken hippocampus is a predictive indicator of a good surgical outcome [4], but not a common primary source of spontaneous seizures, much needs to be reconsidered. The case for the hippocampus as a primary epileptogenic structure can be summarized as follows. Firstly, temporal lobe epilepsy with hippocampal sclerosis is the single most common form of human epilepsy [1], and the presence of a shrunken hippocampus is a predictive indicator of a medically refractory state [4]. Secondly, depth electrode recordings demonstrate hypersynchronous electrical activity in the hippocampus that is often associated with auras that can spread to cause clinical seizures [1]. Thirdly, surgical removal of the hippocampus and adjacent medial temporal structures

effectively reduces seizure frequency [1]. If the assumption that the hippocampus is a frequent source of seizures is correct, the observation that typical hippocampal sclerosis involves an extensive loss of dentate hilar neurons and CA1 and CA3 pyramidal cells [5], logically focuses attention on surviving dentate granule cells [6,7] and subicular neurons [8] as likely candidates for neurons that become the seizure generators.

The conspicuous survival of dentate granule cells in most surgical hippocampal samples [5] has been a primary driving force behind the conceptually compelling, but experimentally unconfirmed idea that the injury-induced formation of recurrent excitatory connections among normally unconnected dentate granule cells transforms granule cells into spontaneously discharging seizure generators [9,10] or an intrinsically quiet syncytium of abnormally interconnected cells that responds excessively to afferent excitation [6,7]. This idea is appealing, in part, because a time-dependent formation of abnormal, recurrent excitatory connections could explain the "latent" period between an initial injury and the emergence of clinical seizures [11]. However, despite the undisputed and frequent presence of a shrunken and synaptically reorganized hippocampus in human TLE, the functional consequences of cell loss and abnormally redirected granule cell axons remain unclear.

One perspective ("epileptogenic" mossy fiber sprouting) posits that a trauma- or seizure-induced loss of vulnerable dentate hilar neurons causes granule cells to redirect their axonal output to each other, resulting in a recurrent excitatory network [6,7]. A second perspective ('inhibitory' mossy fiber sprouting) focuses on the postsynaptic targets of vulnerable hilar neurons, which include both granule cells and inhibitory neurons. According to this "inhibitory" hypothesis, the degeneration of vulnerable hilar mossy cells denervates the dendrites of both granule cells and inhibitory neurons, resulting in the aberrant reinnervation of both target cells. When inhibitory neurons are reinnervated by redirected granule cell axons [12], the resulting inhibition may predominate functionally over the reinnervation of granule cell spines in a limited segment of the granule cell dendritic tree. According to this hypothesis, mossy fiber sprouting may cause the dentate gyrus to become hyperinhibited rather than hyperexcitable [12]. Thus, post-traumatic synaptic reorgani-

zation may be predominantly compensatory, rather than epileptogenic. Although evidence of hippocampal hyperinhibition exists in experimental animals [12] and epileptic patients [13], and this point can be cited selectively to support the second hypothesis, this question remains an area of unresolved controversy because it can also be hypothesized that interictal hippocampal hyperinhibition collapses just before a seizure [3,14,15], allowing an underlying hyperexcitability to initiate granule cell epileptiform discharges [6,7,9,10]. The question is: does that happen? I do not think we know. On a more fundamental level, it is often stated that hippocampal cell loss and synaptic reorganization constitute the epileptogenic process, and that the hippocampus becomes "epileptic". But is that true? I do not think we know that either.

3. How do we differentiate ideas, assumptions, and suppositions from knowledge?

The reason why this question is so important to ask is that if the currently prevailing perspectives are fundamentally incorrect, but generally accepted as being correct, new questions are not asked, and progress is delayed until the prevailing hypotheses are disproved. This is one of the greatest challenges in experimental science because it is often difficult or technically impossible to disprove a hypothesis. Therefore, if hypotheses attain the status of dogma undeservedly and prematurely, the creative questioning process suffocates. Perhaps a greater degree of skepticism is needed to prevent notions from becoming dogma in the first place. Regardless, the questioning of hypotheses is always healthy because, if a prevailing perspective is essentially correct, then intensive questioning will strengthen the hypothesis as attempts to disprove it fail. Thus, critical examination is not an assault on a hypothesis or its proponents, but rather, a required process for either confirming a hypothesis, or learning that we need to find a different path.

In deciding whether we know a lot or a little about TLE, we need to ask a question that applies to all hypotheses based on empirical observations and experimental results: does the theory in question closely account for all of the available data, or has the hypothesis both arisen, and been maintained, by a selective inclusion of observations and experimental results that

support the hypothesis, and an active exclusion of results that contradict it? The process of sifting through the mass of available clinical information unavoidably involves the subjective selection of certain pieces of information that seem of particular importance to us, and the exclusion of data that we subjectively perceive as being relatively unimportant by comparison. Thus, if subjectivity is unavoidable in deciding which information we will consider and assess, all of the available data at least need to be considered initially without a priori regard for whether they support a particular view. When this is done for any question, the picture becomes much less clear. For example, although many TLE patients exhibit asymmetric hippocampal sclerosis that is generally assumed to be epileptogenic [1], other patients exhibit apparently normal hippocampal structure despite having a clinically similar presentation [16]. In addition, many surgical patients who exhibit typical hippocampal sclerosis have no history of febrile seizures or any other brain insult, which raises questions about why and how cell loss has occurred in these cases, or whether histories are reliable. Furthermore, recent depth electrode studies indicate that although synchronous hippocampal activity can be recorded in epileptic patients, these events often occur without clinical consequences, whereas discharges apparently arising from the neighboring amygdala or parahippocampal gyrus more frequently spread to cause clinical seizures [17]. Clearly, much depends on which brain regions are chosen for study, whether discharges in patients can be accurately localized to one of several closely neighboring structures, and whether high-amplitude electrical activity truly reflects propagating neuronal population discharges (i.e., seizures), rather than field depolarizations or some other form of non-propagating, but nonetheless visually striking, electrical activity. Also, the importance of the hippocampus in the epileptogenic process may have been repeatedly overemphasized because of our tendency to confer significance on histologically dramatic hippocampal changes, and to de-emphasize the possible importance of more subtle, but consistently accompanying pathologies in other structures [18,19]. Thus, if an atrophic hippocampus is consistently accompanied by more subtle damage in a related structure, any clinical event caused by damage in the related structure will nonetheless be correlated with the presence of hippocampal sclerosis, seemingly sup-

porting the 'hippocampocentric' perspective. Clearly, associations are not causes.

The natural human tendency to imbue associations with causal qualities has hampered research on the mechanisms of epileptogenesis because the structures undergoing the epileptogenic process must be accurately identified before the nature of the epileptogenic process can be studied. That is, if surviving dentate granule cells in a sclerotic human hippocampus are hyperinhibited, and not a source of spontaneous seizure discharges, the changes that they undergo after injury are unlikely to constitute the epileptogenic process. Of course, the conditions of each clinical and pathological study, the seizure types included and excluded, the anatomical approaches taken, and the methods used in each study, are always open to question. The process of collecting and analyzing clinical and pathological data is therefore something like being dealt a large hand of cards and then trying to arrange them in different patterns to see what kind of a hand you have. Different observers will arrange the same cards in different ways with different results, all based on differences in how each person subjectively sees patterns emerging from the same flood of information. This problem, which is inherent in the analysis of non-experimental clinical and pathological observations, highlights the importance of using experimental animal models to support or disprove hypotheses drawn from observational data. Based on the available data from a variety of experimental animal models, and their overall lack of similarity to the human condition, I would suggest that much of what is assumed to be 'settled' is, in fact, open to question and essentially still unknown. This crossroad of confusion is common to every scientific subject at a particular stage, and it is worthwhile to remind ourselves of the words of Rene Descartes (from *Meditation I* of *Meditations on the First Philosophy*, 1641):

> *"It is now some years since I detected that I had accepted many false beliefs as truths, and how doubtful was everything I had since constructed on this basis; and from that time I became convinced that I must rid myself of all opinions formerly accepted, and commence to build anew from the foundation, if I wanted to establish any firm and permanent structure in the sciences."*

Assuming that we are at that stage in epilepsy research, and it should be acknowledged that many will not agree with this assertion, it is worth asking: what do we know and how do we know it?

4. How well do animal preparations that utilize *status epilepticus* as the initiating injury 'model' human TLE?

The rate of progress in research on any acquired neurological disorder is largely determined by the degree to which the animal models we use mirror the human condition. In this regard, it is my view that the most frequently used animal models, which involve prolonged and often lethal *status epilepticus* to initiate the epileptogenic process, do not closely mirror the human condition, and that the popular belief to the contrary has delayed the development of better animal models. Several significant disparities between the features of the human disorder and those of the currently used animal models may have been insufficiently considered, and include the following observations. Firstly, human temporal lobe epilepsy often involves patients who have brief focal seizures, who exhibit usually limited, asymmetric brain damage, and, most importantly, who appear relatively normal on routine neurological examination. In contrast, normal animals subjected to prolonged and generalized *status epilepticus* exhibit severe, widespread, bilateral brain damage, frequent generalized seizures, and severe behavioral and cognitive abnormalities *if* they survive the severe initial insult. Secondly, although an apparent majority of TLE patients exhibit an atrophic hippocampus upon initial imaging [1,4], hippocampal damage in normal animals subjected to prolonged *status epilepticus* is an inconsistent and often minor part (Fig. 1) of a much greater constellation of damage to other brain structures. Thirdly, many patients exhibit evidence of pre-existing, presumably developmental abnormalities that are likely to play a role in the clinical etiology [20], whereas most animal models involve severe insults in initially normal laboratory rats. Thus, the most widely used animal models do not involve pre-existing defects, rarely exhibit the typical pattern of human hippocampal sclerosis (despite general impressions to the contrary), and suffer a much more extensively brain-damaging insult than most hu-
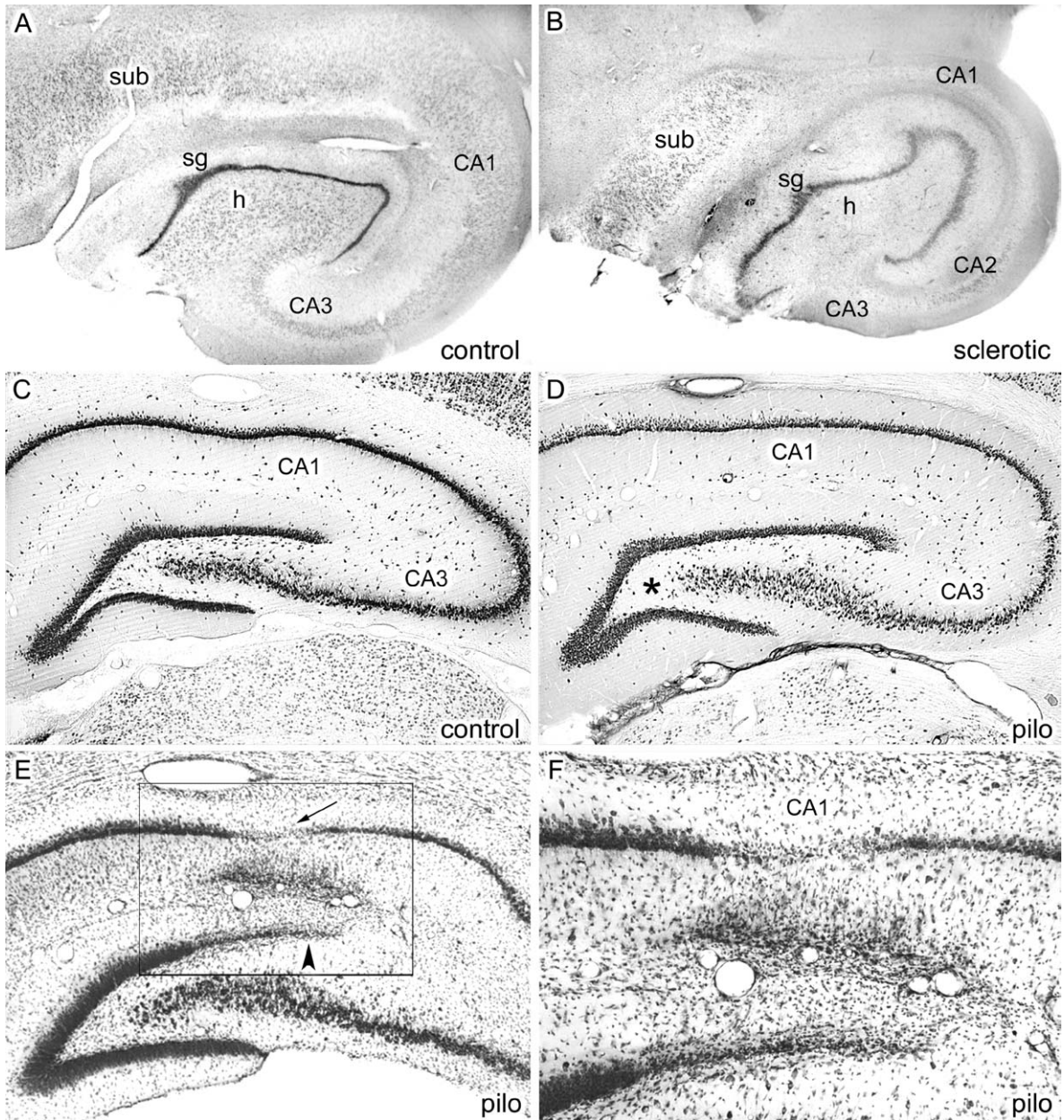
Fig. 1. Human hippocampal sclerosis compared to post-pilocarpine *status epilepticus*-induced damage in rats. (**A**) Autopsy control specimen showing normal human hippocampus. (**B**) Hippocampal sclerosis in a surgical specimen. Note extensive neuron loss in the dentate hilus (h) and in areas CA3 and CA1, and survival of CA2 and subicular (sub) neurons. (**C**) Normal rat hippocampal structure in a coronal section two months after subcutaneous saline injection. (**D**) Neuronal loss in the rat hippocampus two months after prolonged (> 3 h) *status epilepticus* induced by subcutaneous pilocarpine (350 mg kg^{-1}) given 30 min after atropine methylbromide (1 mg kg^{-1} sc). Note that obvious cell loss is restricted to the dentate hilus (asterisk), and unlike in the human sclerotic hippocampus, pyramidal cells are minimally affected by prolonged generalized *status epilepticus*. (**E**) In some identically pilocarpine-treated rats, damage is present in the pyramidal cell layer (arrow) and adjacent dentate granule cell layer (arrowhead). (**F**) Higher magnification view of the outlined area in (**E**). Note that neuronal loss is accompanied by pathology in the region between the cell layers, adjacent to capillaries of the hippocampal fissure. Stain: 1% cresyl violet in (**A**), (**B**), (**E**), and (**F**); NeuN immunoreactivity in (**C**) and (**D**). Magnifications: 8× (**A** and **B**); 24× (**C–E**); 48× (**F**).

man patients with TLE ever experience. These animals do, however, exhibit frequent seizures, which is their principal appeal.

Although much has been learned using the current animal models, the available information suggests the need for a reassessment of the assumptions underlying the use of the current animal models, and the need for the development and study of experimental preparations that may more closely model the human epileptic state. In this conference presentation, and as an illustration of how the underlying assumptions of the current animal models need to be carefully re-examined, I focus on two points that have received minimal consideration: (1) the fact that experimental *status epilepticus* models rarely involve a pattern of hippocampal damage similar to that seen in human epilepsy patients; and (2) the assumption that when hippocampal pyramidal layer cell loss does occur in experimental animals following *status epilepticus*, it results from the same excitotoxic mechanism [21] that presumably causes human hippocampal neuron loss.

4.1. Human vs. rat hippocampal sclerosis

Extensive analysis of human surgical hippocampal specimens has confirmed that most specimens exhibit hippocampal atrophy that is caused by the extensive loss of dentate hilar neurons and cells of the CA1 and CA3 pyramidal cell layers (Fig. 1B). In most cases that we and others have examined [22–24], few pyramidal cells or hilar neurons remain, although the extent of cell loss certainly varies [5]. Conversely, the vast majority of animals given pilocarpine or kainate systemically to induce prolonged *status epilepticus* exhibits extensive extrahippocampal damage (Fig. 2), but inconsistent hippocampal damage, a point that is rarely illustrated or emphasized in published experimental studies. In pilocarpine-treated rats, for example, the only consistent hippocampal neuron loss is in the hilus of the dentate gyrus (asterisk in Fig. 1D). Unlike most human hippocampal specimens removed surgically, most animals subjected to prolonged *status epilepticus* exhibit minimal loss of hippocampal pyramidal cells (Fig. 1D). Even highly vulnerable dentate hilar neurons, which are often nearly completely absent in sclerotic human hippocampi [23], and after prolonged excitation delivered under controlled experimental conditions [25,26], are often only partially affected in animals subjected to prolonged *status epilepticus* [27,28].

The fact that only a fraction of CA3 and CA1 pyramidal layer neurons die after hours of behavioral *status epilepticus* [27,28] seems paradoxical, but this issue has been clarified in recent studies of chronically-implanted, awake rats. During kainate-induced *status epilepticus*, hippocampal granule cells often do not discharge continuously, despite the severe generalized seizures, and, as a result, their target cells do not die [25]. Thus, in normal rats, severe *status epilepticus* produces widespread, bilateral injury to numerous extrahippocampal cortical and thalamic structures, but relatively limited damage to the hippocampus (Fig. 2A). We do not believe that this dissimilarity between rats subjected to *status epilepticus* and humans with TLE is due to rats having less vulnerable hippocampal neurons than humans. To the contrary, prolonged unilateral excitation of CA3 pyramidal cells *in vivo* produces extensive, bilateral loss of CA3 and CA1 pyramidal cells [29]. Thus, prolonged *status epilepticus* in normal rats is apparently so severe that it fails to excite most hippocampal pyramidal neurons effectively. Whether this is due to *status epilepticus*-induced inhibition of the hippocampus, depolarization block, or some other mechanism, is unknown. Given the significant differences between normal rats subjected to *status epilepticus* and the pattern of pathology in human TLE, it is questionable whether it can be assumed that the hippocampus in these animals has undergone the epileptogenic process, or is a source of spontaneous seizures. Remarkably, despite 20 years of research since the first suggestion that post-injury hippocampal synaptic reorganization may be an epileptogenic mechanism [6], it has never been demonstrated *in vivo* that any identified hippocampal neurons generate spontaneous epileptiform discharges that initiate the spontaneous behavioral seizures that develop in these animals.

4.2. Status epilepticus – *induced hippocampal CA1 pyramidal cell loss in rats: excitotoxicity, or something else?*

Although few pilocarpine- or kainate-treated animals exhibit the human pattern of extensive CA1 pyramidal cell loss, we have observed in some animals analyzed long after *status epilepticus* that neuronal
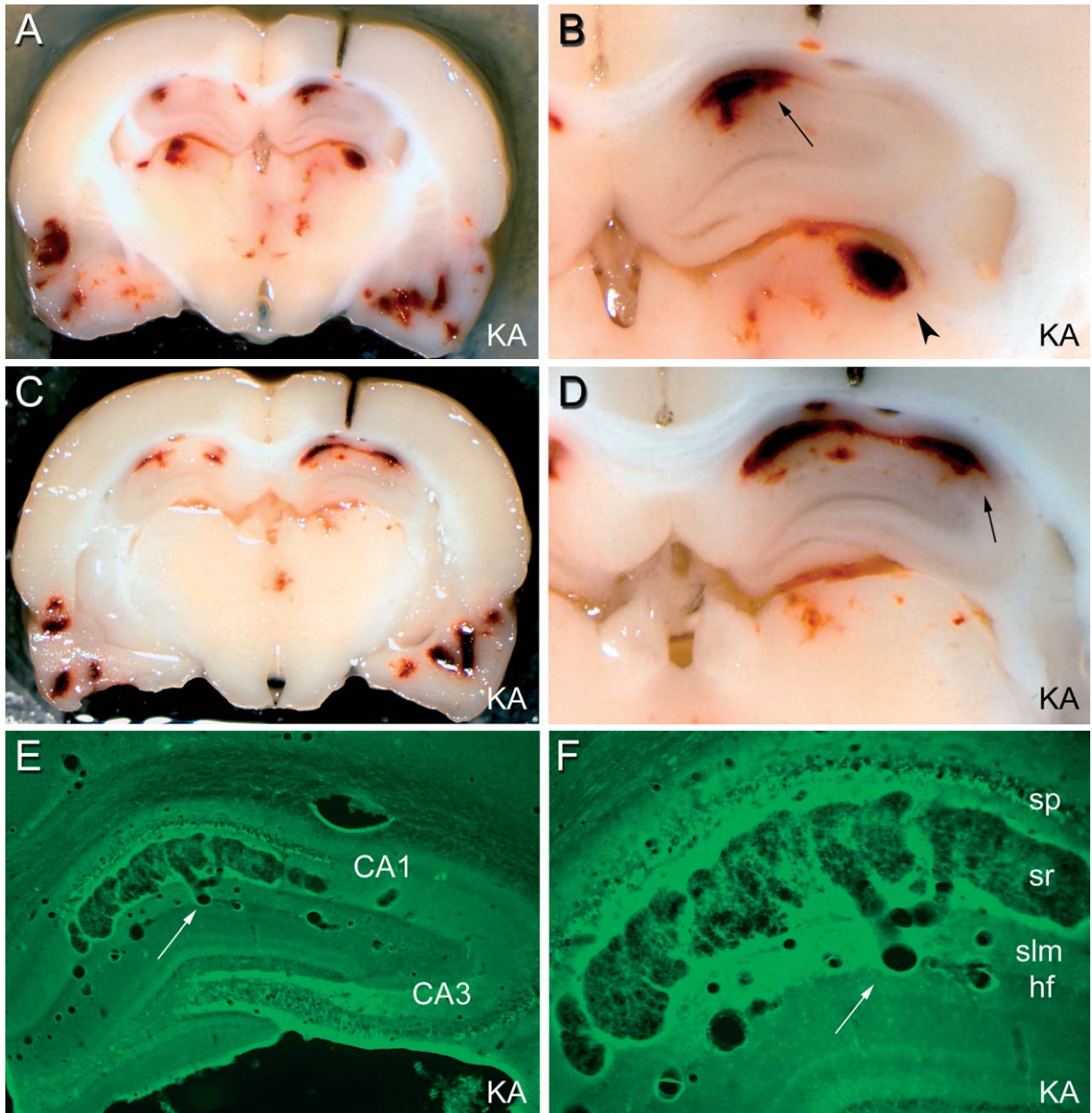
Fig. 2. Brain structure 3 days after *status epilepticus* induced by systemic kainic acid. In some animals given kainate or pilocarpine systemically, prolonged *status epilepticus* caused apparent hemorrhages in a multitude of brain structures that were not cleared by vascular perfusion-fixation. (**A**, **C**) Two coronal views of the same brain during the sectioning process. Note apparent hemorrhagic foci in the hippocampi, thalamus, and temporal cortices. (**B**, **D**) Foci preferentially involve the CA1 pyramidal cell layer (arrow) and the dorsolateral thalamus (arrowhead). (**E**, **F**) In a Fluoro Jade B-stained section from the brain shown in (**A**)–(**D**), degenerating neurons are fluorescent. Note that the CA1 pyramidal cell layer pathology consists of a vascular expansion that is continuous with a capillary in the hippocampal fissure (hf; arrow). (**G**) In a different kainate-treated rat, smaller focal vascular expansions occurred within the stratum radiatum (arrows). (**H**) At higher magnification, degenerating CA1 pyramidal cell somata are evident only adjacent to the vascular pathology. These results suggest that, in some cases, CA1 pyramidal cell layer injury in rats subjected to prolonged *status epilepticus* may be ischemic in nature, rather than excitotoxic. Abbreviations: sp: stratum pyramidale; sr: stratum radiatum; slm: stratum lacunosum-moleculare; hf: hippocampal fissure. Magnifications: 5× (**A** and **C**); 13.5× (**B** and **D**); 22× (**E** and **G**); 55× (**F** and **H**).
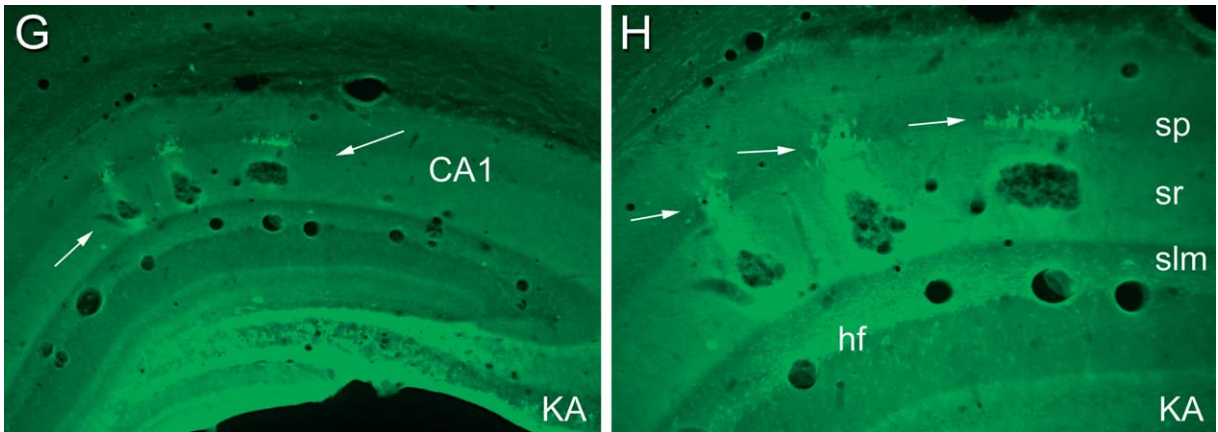
Fig. 2. (*Continued*)

loss was evident in the pyramidal layer, as shown in Fig. 1E and F. However, analysis of acute pathology only days after *status epilepticus* revealed a pattern of neuronal degeneration in area CA1, and in the adjacent granule cell layer, that is apparently related to an undescribed vascular phenomenon. We recently studied this phenomenon because we detected in a number of animals that there was also an uncharacteristic focal loss of granule cells at the lateral tip of the inner granule cell layer, and that both the granule cell and CA1 pyramidal cell injury was accompanied by a previously unreported and apparently vascular pathology in the dendritic regions between the two cell layers (Fig. 1F).

When rats were perfusion-fixed 1–3 days after prolonged *status epilepticus*, areas of apparent hemorrhage were frequently evident during the sectioning process in multiple thalamic and cortical areas, including the hippocampus (Fig. 2A–D). Although we initially suspected an extravascular hemorrhage, under the assumption that extravascular blood would not have been cleared by the perfusion process, closer examination revealed an apparent expansion of blood vessels originating from capillaries of the hippocampal fissure (arrow, Fig. 2F). This apparent pooling of blood within abnormal vessels, which was not removed during the perfusion process, suggests that, in life, affected regions were not being perfused and oxygenated, and that this resulted in an anoxic/ischemic insult to area CA1 that was unrelated to excitotoxic injury.

Fluoro Jade B staining of degenerating neurons revealed a close relationship between the focal areas of vascular pathology and the CA1 pyramidal cell layer injury (Fig. 2G and H). Fig. 2H shows small vascular expansions in the stratum radiatum (sr), and precisely corresponding clusters of degenerating CA1 pyramidal cell somata (arrows, Fig. 2G and H). Thus, although most rats exhibit minimal CA1 pyramidal cell loss after prolonged *status epilepticus* (Fig. 1D), when it does occur, it apparently involves a previously undescribed vascular phenomenon unrelated to excitotoxic CA1 cell loss [29]. These observations indicate that rats subjected to prolonged *status epilepticus* rarely exhibit CA3 and CA1 pyramidal cell loss that is in any way similar to that seen in human hippocampal sclerosis, or produced by electrical stimulation in urethane-anesthetized rats [29]. To the contrary, prolonged *status epilepticus* in normal rats apparently triggers an anoxic/ischemic insult, in addition to the widespread excitotoxicity originally described by Olney and colleagues [21].

Clearly, electrophysiological data obtained in area CA1 of pilocarpine-treated rats [30] are unlikely to reflect the functional state of the epileptic human hippocampus because the usually undamaged CA1 pyramidal cell layer of epileptic rats is not known to be "epileptic", and the damaged CA1 pyramidal region in rats probably has little in common with "epileptic" tissue. Thus, *status epilepticus*-based models may be poorly suited for testing hypotheses generated in other experimental models [29]. Nor can it be assumed that simply because an animal is epileptic, hip-

pocampal abnormalities must be causally related to the epileptogenic process [9,14,27,30]. These considerations clearly indicate the need for careful histological analysis of each experimental animal in experimental studies, and a renewed appreciation that different animal preparations cannot be used interchangeably to address hypotheses generated from data obtained in other animal models.

5. Synthesis

These observations, and the issues that they raise, have implications for experimental studies that use prolonged *status epilepticus* in normal rats to address hypotheses of human epileptogenesis (for a thorough and well-balanced review, see Morimoto et al. [31]). An important question facing us is whether currently used animal models resemble the human condition in any significant way. If they do, then continued research utilizing initially normal animals subjected to prolonged *status epilepticus* is clearly indicated. However, if rats subjected to prolonged *status epilepticus* do not closely resemble the human condition, and the hippocampus is not the source of the spontaneous seizures that define these animals as "epileptic", new animals models need to be developed and carefully characterized. It is my contention, and the main point of this presentation, that we know much less about both the human condition and the current animal models than is generally assumed. Thus, a number of outstanding questions need to be considered:

1. Do human 'hippocampal-onset' seizures, which have been recorded in depth electrode studies [17], truly originate in hippocampal cell populations, and are the high amplitude electrical events that have been recorded really propagating population discharges? If not, hippocampal events may be non-propagating depolarizations produced in the hippocampus by seizures that arise elsewhere.
2. Does the severe and widespread brain damage caused by prolonged *status epilepticus* in initially normal animals result in spontaneous seizures that are similar in location to those observed in human TLE patients?
3. Do the frequent spontaneous seizures that occur in animals after prolonged *status epilepticus* come from, or involve, dentate granule cells or hippocampal pyramidal cells? If not, can it be assumed that the hippocampus has undergone the epileptogenic process?
4. Why do most human surgical specimens exhibit extensive loss of CA3 and CA1 pyramidal cells, whereas normal animals subjected to prolonged *status epilepticus* do not? Is there a particular "window" of seizure activity that damages pyramidal cells, which is exceeded during pilocarpine- or kainate-induced *status epilepticus*?
5. Can it be assumed that TLE patients possessed a structurally and functionally 'normal' hippocampus prior to an initial epileptogenic insult? If not, hippocampal surgical specimens cannot be assumed to have initially had the structure or neuron number of autopsy specimens, which are often used for control comparison.
6. Exactly how vulnerable is the normal brain, and do pre-existing defects make normally innocuous insults more injurious [32]? If they do, normally innocuous insults might cause hippocampal sclerosis without inducing clinically detectable seizures or an episode of *status epilepticus*, consistent with many patient histories indicating no obvious risk factors.

Finally, the prospect that much of what we assume to be 'known' may only be supposition, might be viewed as disheartening. Conversely, critical re-evaluation of the available data, and having greater doubt about what is really 'known', might be a fruitful development because new questions could then be asked. Recognition of the need for new questions and new answers might be the first step toward a renaissance in epilepsy research.

Acknowledgements

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