Limbic Networks and Epileptiform Synchronization: The View from the Experimental Side

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Abstract

In this review, we summarize findings obtained in acute and chronic epilepsy models and in particular experiments that have revealed how neuronal networks in the limbic system—which is closely involved in the pathophysiology of mesial temporal lobe epilepsy (MTLE)—produce hypersynchronous discharges. MTLE is often associated with a typical pattern of brain damage known as mesial temporal sclerosis, and it is one of the most refractory forms of partial epilepsy in adults. Specifically, we will address the cellular and pharmacological features of abnormal electrographic events that, as in MTLE patients, can occur in *in vivo* and *in vitro* animal models; these include interictal and ictal discharges along with high-frequency oscillations. In addition, we will consider how different limbic structures made hyperexcitable by acute pharmacological manipulations interact during epileptiform discharge generation. We will also review the electrographic characteristics of two types of seizure onsets that are most commonly seen in human and experimental MTLE as well as in *in vitro* models of epileptiform synchronization. Finally, we will address the role played by neurosteroids in reducing epileptiform synchronization and in modulating epileptogenesis.

1. BACKGROUND

The limbic system—as first described by Broca, followed by Papez and McLeans—includes the hippocampus proper and several parahippocampal areas such as the subiculum, the entorhinal and perirhinal cortices, and the amygdala complex. It is involved not only in physiological processes such as learning and memory (Gloor, 1997) but also in pathological conditions such as MTLE. With up to 80% of patients not achieving adequate seizure control, MTLE is one of the most refractory adult forms of epilepsy (Spencer, 2002; Wiebe, Blume, Girvin, Eliasziw, & Effectiveness and Efficiency of Surgery for Temporal Lobe Epilepsy Study Group, 2001). It consists of seizures that originate from the hippocampus, entorhinal cortex, or amygdala, often many years after an initial brain insult such as status

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epilepticus (SE), encephalitis, or febrile convulsions, but also subacutely as a result of autoimmune encephalitis. MTLE is often associated with a pattern of brain damage known as mesial temporal (also termed *Ammon's horn*) sclerosis, which is characterized by a selective neuronal loss in the CA3/CA1 region of the hippocampus and the hilus, along with granule cell dispersion and aberrant mossy fiber sprouting in the molecular layer of the dentate gyrus (Berkovic et al., 1991; Buckmaster, 2012; Du, Eid, Lothman, Kohler, & Schwarcz, 1995; Gloor, 1997). Anterior temporal resection will reduce seizure occurrence, but still approximately 30% of patients are not seizure-free after surgery, most certainly due to insufficient resection of the epileptic tissue.

MTLE can be reproduced in laboratory animals by topical or systemic injection of chemoconvulsants, such as kainic acid or pilocarpine, as well as by repetitive electrical stimulation (Ben-Ari, 1985; Curia, Longo, Biagini, Jones, & Avoli, 2008; Gorter, van Vliet, Aronica, & Lopes da Silva, 2001; Lévesque & Avoli, 2013). In adult animals, these experimental procedures induce an initial SE and lead to a chronic condition of recurrent seizures within 1–4 weeks; remarkably, a similar, seizure-free, latent period can be identified in MTLE patients who develop partial seizures in adolescence or early adulthood after having suffered an initial insult (e.g., complex febrile convulsions) in childhood (French et al., 1993; Salanova, Markand, & Worth, 1994). As illustrated in Fig. 4.1, similar electrographic patterns of epileptiform activity can also be induced *in vitro* in brain slices or in whole brain preparations following “acute” pharmacological manipulations that aim at increasing neuronal excitability (Avoli & de Curtis, 2011; Avoli, Panuccio, et al., 2013); it is important to emphasize that these “acute” experiments, which were originally made in *in vivo* models during 1960s, have provided relevant information regarding the fundamental mechanisms underlying epileptiform synchronization. However, their involvement in the pathophysiology of seizures occurring in MTLE must be taken with caution as they were obtained from normal, control brain tissue.

Understanding the pathophysiology of limbic seizures and thus of MTLE remains an open challenge in epilepsy research. In this review, we will mainly focus on the brain processes that lead to the initiation of seizure activity (a process that is also termed ictogenesis). Specifically, we will summarize the cellular and pharmacological features of synchronous, abnormal events that, as in MTLE patients, can occur in *in vivo* and *in vitro* animal models; these include interictal and ictal discharges along with high-frequency oscillations (HFOs). In addition, we will consider how different limbic structures made hyperexcitable by acute pharmacological manipulations do interact during epileptiform discharge generation. We will also review the electrographic characteristics of two types of seizure onsets that are most commonly seen in human and experimental MTLE as well as in *in vitro* models of epileptiform synchronization. Finally, we will address the role played by neurosteroids in reducing epileptiform synchronization and in modulating epileptogenesis.

2. MECHANISMS UNDERLYING EPILEPTIFORM SYNCHRONIZATION

For almost a century, interictal spikes (Fig. 4.1, asterisks) have been a key feature in the noninvasive diagnosis of partial epilepsy. They also contribute to the presurgical evaluation, helping to define the epileptogenic zone by delineating the so-called “irritative zone”

(Rosenow & Lüders, 2001). MTLE, however, differs from other partial epilepsies due to the deep topography of limbic structures. As a result, despite being often associated with interictal spikes, sharp waves, SW complexes, as well as slow activity, electrophysiological localization of the “irritative zone” in MTLE can be equivocal (Niedermeyer, 2004). Additionally, bilateral interictal findings are not rare but do not always preclude a lateralized underlying pathology.

Ictal activity on scalp EEG is usually characterized by rhythmic discharges, at a frequency of around 5 Hz, limited to temporal regions or involving more widespread networks, and starting before the occurrence of clinical symptoms (Fish, 1996; Fig. 4.1, dotted lines). In some cases, stereo-EEG is required to properly lateralize the seizure-onset zone (Diehl & Lüders, 2000) and when recorded using this method, mesial temporal seizures can be divided into two onset types: low-voltage fast onset (LVF) and hyper-synchronous onset (HYP) types (Spencer, Kim, & Spencer, 1992; Velasco, Wilson, Babb, & Engel, 2000; see also Section 4). More recently, HFOs have been recorded in MTLE patients using microelectrodes (Staba, Wilson, Bragin, Fried, & Engel, 2002) and macroelectrodes (Crépon et al., 2010; Jacobs et al., 2008), and they were proposed as better markers of the epileptogenic zone than interictal spikes (Jacobs et al., 2008).

2.1. Interictal spikes

Interictal spikes consist of a large-amplitude, rapid component usually followed by a slow wave. Intracellular recordings obtained from cortical neurons in acute *in vivo* and *in vitro* brain slice models have demonstrated that interictal spikes induced by GABA_A receptor antagonists correspond to paroxysmal depolarizing shifts of the membrane potential with sustained action potential firing that can be followed by hyperpolarization (Ayala, Dichter, Gumnit, Matsumoto, & Spencer, 1973; Dingledine & Gjerstad, 1980; Schwartzkroin & Prince, 1980; Fig. 4.2A). These studies have also shown that interictal synchronization results from enhancement of glutamatergic excitation (presumably caused by the pharmacological weakening of inhibition), high-threshold Ca²⁺ currents leading to action potential bursting, and nonsynaptic interactions such as intercellular gap junctions and ephaptic interactions (De Curtis, Jefferys, & Avoli, 2012; Jefferys, Jiruska, de Curtis, & Avoli, 2012).

Successive studies have, however, demonstrated that interictal activity induced by applying K⁺ channel blockers or medium containing low Mg²⁺, low Cl⁻, or high K⁺ are also contributed by GABA_A receptor mechanisms (Avoli et al., 2002; Avoli & de Curtis, 2011). In particular, application of the K⁺ channel blocker 4-aminopyridine (4AP) induces, in addition to frequently occurring interictal events that are driven by the CA3 network and are abolished by AMPA receptor antagonists (Fig. 4.2B, left sample), “slow” interictal discharges that (i) continue to occur and to propagate even in the presence of ionotropic glutamatergic receptor blockers and (ii) are abolished by drugs that interfere with GABA_A receptor signaling (Fig. 4.2B, right sample). These “slow” interictal spikes are associated with a long-lasting depolarization that can be preceded by a fast hyperpolarizing component (depending upon the resting membrane potential of the recorded cell; Fig. 4.2B, right sample) and often trigger ectopic action potentials that are presumably due to local, transient

elevations in extracellular $[K^+]$ (Fig. 4.2C; Avoli, Methot, & Kawasaki, 1998). Slow interictal spikes are indeed accompanied by sizeable increases in extracellular $[K^+]$ even during blockade of ionotropic glutamatergic transmission (Fig. 4.2D). Several studies have identified 4AP-induced glutamatergic-independent spikes in several structures (e.g., neocortex, amygdala, entorhinal, perirhinal, insular, and piriform cortices) maintained *in vitro* both in the slice and in the isolated guinea pig brain preparation (Avoli, de Curtis, & Köhling, 2013). In addition, similar network-driven events have been reported to occur following application of the muscarinic agonist carbachol (Dickson & Alonso, 1997).

The diverse contribution of distinct mechanisms to interictal spikes as demonstrated in *in vitro* experimental models of epileptiform synchronization is in line with the diversity in shape that is seen during *in vivo* EEG recordings of MTLE patients or of chronically epileptic animals (Fig. 4.2E; Bortel, Lévesque, Biagini, Gotman, & Avoli, 2010; Chauvière et al., 2012). In addition, by recording single unit activity from epileptic patients, Keller et al. (2010) have reported high levels of heterogeneity in the firing patterns of single units during interictal spikes. Single units in the seizure-onset zone could either show an increase or a decrease in firing rates at different time points before, during, and after the interictal spikes. It should be emphasized that interictal spikes may play divergent functional roles with respect to ictogenesis (see Section 3) as well as epileptogenesis as recently suggested by Chauvière et al. (2012).

2.2. High-frequency oscillations

HFOs (80–500 Hz) represent a new type of EEG activity that is captivating neuroscientists, as well as clinical and fundamental epileptologists. Oscillations at frequencies higher than what is normally considered standard in EEG recordings (i.e., <60 Hz) were initially reported in physiological studies that have demonstrated the presence of gamma (up to 80 Hz) and ripples (80–200 Hz) during visual perception in the neocortex, during consummatory behaviors, slow-wave sleep, and memory consolidation in the hippocampus (Buzsáki & da Silva, 2012; Buzsaki, Horvath, Urioste, Hetke, & Wise, 1992; Jefferys, Menendez de la Prida, et al., 2012). Approximately 15 years ago, HFOs (ripples: 80–200 Hz, fast ripples: 250–500 Hz) were recorded from patients with MTLE and in animal models mimicking this condition; these findings have suggested that these oscillatory events represent biomarkers of epileptogenic neuronal networks (Staba, 2012).

HFOs are not visible in standard intracranial EEG recordings and can only be extracted by amplifying the appropriately filtered signal. As it will be discussed in Section 4, ripples and fast ripples have been recorded with depth electrodes in chronic epileptic rodents and in epileptic patients both in coincidence with interictal events and in their absence (Staba, 2012). These pathological HFOs presumably reflect the activity of dysfunctional neural networks sustaining epileptogenesis, and they are now considered better markers than interictal spikes to identify seizure-onset zones (Staba, 2012). Experimental studies have highlighted several mechanisms that may contribute to physiological and pathological HFOs. These include kinetics of intrinsic neuronal currents such as after-hyperpolarizing currents (I_{AHP}) or fast potassium currents (I_A), as well as excitatory currents and recurrent inhibitory connectivity in combination with fast timescales of inhibitory postsynaptic

potentials (IPSPs), out-of-phase firing in neuronal clusters, and interneuronal coupling through gap junctions (Jefferys, Jiruska, et al., 2012; Jefferys, Menendez de la Prida, et al., 2012, Kohling & Staley, 2011). The exact role played by these mechanisms in pathologic HFOs remains undefined, but it has been proposed that ripples may represent population IPSPs generated by principal neurons entrained by synchronously active interneuron networks (Buzsáki & Chrobak, 1995; Ylinen et al., 1995); in contrast, fast ripples would mirror synchronous in-phase or out-of-phase firing of abnormally active principal cells, and would be independent of inhibitory neurotransmission (Bragin, Benassi, Kheiri, & Engel, 2011; Dzhala & Staley, 2004; Engel, Bragin, Staba, & Mody, 2009; Foffani, Uzcatagui, Gal, & Menendez de la Prida, 2007; Ibarz, Foffani, Cid, Inostroza, & Menendez de la Prida, 2010).

2.3. Ictal discharges

The process leading to seizure generation is termed ictogenesis; the underlying mechanisms make neuronal networks generate prolonged periods of hypersynchronous activity that disrupt normal brain function. Seizure termination and postictal state complete the sequence of ictogenesis. As shown in Figs. 4.1 and 4.3, the electrographic counterpart of a seizure (or ictal discharge) consists of a prolonged series of fast EEG transients that can vary in amplitude, and is easily distinguished from the background activity as well as from the interictal spikes. In the case of a prototypical tonic-clonic seizure, the clonic phase will follow the tonic one, mirrored on the EEG by an increasing amplitude and a decreasing frequency. Interspersed periods of “suppression” of increasing duration will occur and diffuse until finally reaching a typical postictal depression state (Engel, Dichter, & Schwartzkroin, 1997). Intracellular recordings have shown that ictal discharges are associated with sustained depolarizations capped by sustained action potential firing (Fig. 4.3A). It has also been established that neuronal network synchronization during ictal activity depends on glutamatergic ionotropic neurotransmission (particularly that mediated by the NMDA receptor; Köhr & Heinemann, 1989; Löscher and Hönack, 1991; Traynelis & Dingledine, 1988) and on persistent Na^+ currents that are fundamental to maintain the sustained action potential firing (Mantegazza, Curia, Biagini, Ragsdale, & Avoli, 2010). In line with this view, many of the most widely used antiepileptic drugs, including phenytoin, carbamazepine, lamotrigine, and, perhaps, topiramate, inhibit voltage-gated Na^+ channels (Mantegazza et al., 2010).

However, GABA_A receptor signaling is also required for ictogenesis and under specific *in vitro* pharmacological manipulations it can be the sole synaptic mechanism responsible for the occurrence of seizure-like events (Uusisaari, Smirnov, Voipio, & Kaila, 2002). The role of GABA_A receptor signaling in initiating and sustaining ictal discharges has been clearly defined in several studies performed in the *in vitro* 4AP model (Avoli & de Curtis, 2011). These experiments have shown that ictal activity disappears in several limbic and extralimbic structures during bath application of GABA_A receptor antagonists or of a μ -opioid receptor agonist. In addition, it has been found that inhibitory currents at the onset of and/or during *in vitro* ictal activity may overload the ability of the neuronal Cl^- transporter KCC2 to maintain a low intracellular Cl^- concentration thus disclosing depolarizing, and potentially excitatory, GABA_A receptor-mediated conductances. These events lead to

transient increases in extracellular $[K^+]$ that are caused by the activity of transporters responsible for reuptake of GABA and Cl^- extrusion (Viitanen, Ruusuvuori, Kaila, & Voipio, 2010) and that are larger in size during preictal spikes as compared to those seen during interictal discharges (Fig. 4.3B, arrow). However, the role played by synaptic mechanisms during seizure discharges should be considered with caution as epileptiform activity is accompanied by decreases in extracellular $[Ca^{2+}]$ that are not compatible with efficient transmitter release (Pumain, Menini, Heinemann, Louvel, & Silva-Barrat, 1985; Timofeev & Steriade, 2004). Nevertheless, several studies have shown that these seizure-like discharges are abolished by specific neurotransmitter receptor antagonists.

3. INTERACTIONS BETWEEN LIMBIC AREAS

Limbic structures display a high level of feedforward as well as feedback connectivity (McIntyre & Schwartzkroin, 2008). Temporal lobectomy, first achieved by Horsley in 1886, was progressively refined based on anatomopathological examination (Falconer, 1974; Margerison & Corsellis, 1966) and intracranial stereo-EEG evaluation (Lieb, Engel, Brown, Gevins, & Crandall, 1981; Talairach et al., 1974). Nowadays, anterior mesial temporal resection remains the most common procedure for MTL surgery (Vives, Lee, Doyle, & Spencer, 2008). Pathogenicity of medial temporal structures was progressively recognized and resection evolved in that sense; however, precise interictal and ictal interactions of limbic structures in human remains poorly understood, partly due to the constraints in terms of spatial sampling during depth EEG studies (Spencer & Spencer, 1994). Developed at the Montreal Neurological Institute, intraoperative electrocorticography helped in identifying the pathological nature of the amygdalo-hippocampus complex, eventually leading to the selective amygdalo-hippocampectomy procedure as an attempt to reduce functional postoperative deficit (Olivier, 2010; Wieser & Ya argil, 1982). However, tractography studies using diffusion-tensor imaging suggest that the main neocorticolimbic fiber tracts may still be affected in selective approach, underlying the role played by neocorticolimbic connectivity in MTL epileptogenicity (Colnat-Coulbois et al., 2010). Another crucial aspect of limbic connectivity with regard to epileptogenicity and seizure spread concerns the interhemispheric connections through anterior and posterior commissures. The classical view of a vestigial anterior commissure (Gloor, Salanova, Olivier, & Quesney, 1993) has been somewhat challenged by recent DTI studies, showing an anterior interamygdalian commissural connectivity (Colnat-Coulbois et al., 2010). Despite this evidence, the question of whether different areas of the limbic system interact during epileptiform synchronization and, above all, how they interact remains unclear.

Over a decade ago, we reported that during application of 4AP or medium containing low Mg^{2+} , cutting the Schaffer collaterals in mouse brain slices comprising interconnected hippocampus and entorhinal cortex abolishes the propagation of CA3-driven interictal discharges to the entorhinal cortex and discloses slow interictal spikes and seizure-like events in the latter structure (Fig. 4.3B; Barbarosie & Avoli, 1997; Barbarosie, Louvel, Kurcewicz, & Avoli, 2000). Therefore these findings—which have later been replicated in rat brain slices by extending the analysis to other limbic areas such as the perirhinal cortex, the amygdala, or the insular cortex—indicate that CA3 output activity controls rather than

reinforces the expression of ictal epileptiform activity in the entorhinal cortex and other para-hippocampal areas (Avoli & de Curtis, 2011).

Although a definitive answer to what determines the control exerted by hippocampal output activity on parahippocampal ictogenesis remains to be found, experimental evidence suggests that a main player relates to the ability of CA3-driven interictal spikes to hinder the transient elevations in extracellular $[K^+]$ that occur during the slow interictal events and may be instrumental for ictal discharge onset (Fig. 4.3B). Therefore, CA3-driven interictal events should insure that GABA release during the slow interictal discharge is downregulated, thus controlling the associated elevations in extracellular $[K^+]$.

This hypothesis is further supported by experiments in which para-hippocampal structures such as the EC, the amygdala, or the insular cortex are stimulated at frequencies similar to those of CA3-driven interictal spikes (i.e., at 0.5–1.0 Hz; Fig. 4.3C; Avoli & de Curtis, 2011; Avoli, de Curtis, et al., 2013). Clinical studies have indeed shown that low-frequency stimulation—delivered through transcranial magnetic or deep-brain electrical procedures—can reduce seizures in epileptic patients not responding to conventional antiepileptic therapy (Tergau, Naumann, Paulus, & Steinhoff, 1999; Theodore & Fisher, 2004; Vonck, Boon, Achten, De Reuck, & Caemaert, 2002).

The ability of hippocampal outputs to control ictogenesis may also be relevant in MTLE, a disorder that is associated with CA3 and CA1 subfield damage (Gloor, 1997); according to this view, a decrease in hippocampal output activity secondary to cell damage may facilitate the generation of ictal discharges in parahippocampal structures (Barbarosie & Avoli, 1997, D'Antuono et al., 2002, Panuccio et al., 2010). The sclerotic hippocampus presents with almost complete loss of CA1 neurons (the Sommer sector), especially at the subicular boundary, as well as disappearance of hilar cells in the dentate gyrus (endfolium sclerosis). Neuronal cell loss is also found in the medial EC (especially in layer III) and in the amygdala. Recent evidence obtained from studies performed in animal models mimicking MTLE suggests that the pattern of neuronal damage found in Ammon's horn sclerosis cuts off the normal physiological route of hippocampal network activity (the trisynaptic hippocampal pathway), thus reinforcing the activity of direct connections between EC–CA1 (when spared) and EC–subiculum (D'Antuono et al., 2002, Panuccio et al., 2010). These findings are challenging the traditional view that has emphasized the role played by mossy fiber sprouting in the dentate gyrus in epileptogenesis.

4. SEIZURE-ONSET TYPES

4.1. Human background

Depth electrode recordings in MTLE patients have opened a new window to the analysis of seizure-onset patterns. With surface electrodes, it is difficult to perform a detailed analysis of seizure onsets because signals originating from deep regions are attenuated before reaching the scalp and noise introduced by muscle artifacts further compounds the problem. This induces low reliability between independent observers when trying to identify regions of seizure onset and when classifying seizures based on their onset EEG pattern (Spencer et al., 1985). However, the use of depth electrodes implanted in temporal and extratemporal

regions of MTL patients has led to the identification of two main seizure-onset types (Bragin, Wilson, Fields, Fried and Engel, 2005; Ogren et al., 2009; Spencer et al., 1992; Velasco et al., 2000). HYP seizures, which are characterized by rhythmic high-amplitude spikes followed by fast activity in the 10–20 Hz frequency range, mainly originate from hippocampal regions and rarely spread to areas adjacent or contralateral to the seizure-onset zone (Velasco et al., 2000); in addition, they are often associated with mesial temporal lobe sclerosis (Spanedda, Cendes, & Gotman, 1997; Spencer et al., 1992; Townsend, 1991).

Further anatomopathological and magnetic resonance imaging studies have shown that patients presenting with HYP seizures have extensive cell loss in the dentate gyrus and in all hippocampal areas except CA2 and presubiculum (Spencer et al., 1992; Velasco et al., 2000). The second type of seizure onset, termed LVF, is characterized by the occurrence of a positive- or negative-going spike that is followed by the appearance of low-amplitude, high-frequency activity. Compared to HYP seizures, the site of origin of LVF seizures is more diffuse and often extrahippocampal; moreover, these seizures spread more rapidly and more widely to regions ipsilateral and contralateral to the side of onset (Engel et al., 1990; Spencer et al., 1992; Townsend, 1991; Velasco et al., 2000). Patients with LVF seizures also show more extensive and diffuse neuronal loss with bilateral hippocampal atrophy associated with cell loss in the CA2/CA4 regions (Ogren et al., 2009; Velasco et al., 2000). Thus, taken together, the differences between the two seizure-onset types indicate that they may arise through different mechanisms as well as through the predominant involvement of specific types of neurons (i.e., glutamatergic and inhibitory cells).

4.2. Experimental evidence *in vitro*

LVF and HYP seizures can be reproduced in *in vitro* brain slice preparations. As shown in Fig. 4.4A, during application of 4AP, EC networks commonly generate LVF seizures (panel a); however, in few cases, HYP can occur (panel b; Avoli, Panuccio, et al., 2013).

Interestingly, in combined brain slices comprising the EC and the perirhinal cortex, both seizure-onset types can co-occur: LVF seizures are usually recorded in the EC while HYP seizures predominate in the perirhinal area (Köhling et al., unpublished results; Fig. 4.4B). Indeed, HYP seizures could reflect the synchronous firing of principal (glutamatergic) cells, whereas LVF seizures could be related to an inhibitory process with enhancement of interneuronal activity that leads to synchronous GABA_A receptor-mediated signaling at the seizure onset. Results supporting this hypothesis also come from *in vitro* studies showing that in the human epileptic tissue recorded *in vitro*, preictal discharges of HYP-like ictal events induced by low Mg²⁺ are not suppressed by GABA_A receptor antagonists (Huberfeld et al., 2011). Moreover, ripples, with the virtual absence of fast ripples, were shown to be related to LVF ictal events induced by 4AP *in vitro* in the piriform cortex (Panuccio et al., 2012) and in the EC (Avoli, Panuccio, et al., 2013).

We have also reported that in the 4AP model of *in vitro* limbic epileptiform synchronization both EC and perirhinal networks can be seizure-onset zone but with a clear predominance of the latter structure (De Guzman, D'Antuono, & Avoli, 2004). Finally, it has been shown that ictal discharges in combined rat hippocampus–EC slices bathed with Mg²⁺-free medium initiate and propagate in variable ways with about 35% of these events starting in

hippocampal areas, 25% in the EC, and the remaining 40% of unclear origin (Schulte-Weß S. & Köhling R., unpublished observations; Fig. 4.4C).

4.3. Chronic models of MTLE

Results obtained to date in animals models of MTLE support clinical findings on seizure-onset types. Bragin, Azizyan, Almajano, Wilson and Engel (2005) reported that an SE induced by the local administration of kainic acid in the CA3 region of the hippocampus is followed by the occurrence of both HYP and LVF seizures, within a week after the injection. In the same study, recordings with depth electrodes in hippocampal and para-hippocampal regions demonstrated that HYP seizures mostly originated from hippocampal structures ipsilateral to the injection of kainic acid. These seizures also occurred in most cases when the animal was sleeping or immobile and they were rarely associated with behavioral symptoms, suggesting that they presumably remained focal. On the other hand, the seizure-onset zones of LVF seizures were located in both hippocampus and EC and they frequently propagated to other brain structures. Interestingly, during HYP seizures, a transition to an LVF pattern was associated with propagation of seizures to other brain regions outside of the hippocampus. Similar results have been obtained with the pilocarpine model of MTLE, since HYP seizures mostly originate from the CA3 region of the hippocampus whereas the seizure-onset zones of LVF seizures are located in both the hippocampus and the entorhinal cortex or are located outside of the hippocampus (Lévesque, Salami, Gotman, Avoli, 2012).

As shown in Fig. 4.5A, the analysis of HFOs occurring during the preictal and ictal periods has revealed distinct patterns of HFO occurrence: HYP seizures were mostly associated with fast ripple occurrence, whereas fast ripples occurred rarely during LVF seizures (Bragin, Azizyan, et al., 2005; Lévesque et al., 2012). On the other hand, LVF seizures were mainly associated with an increase of activity in the beta–gamma range (15–20 Hz; Bragin, Azizyan, et al., 2005) or in ripple occurrence (Lévesque et al., 2012). These increases in occurrence of fast ripples during HYP seizures and ripples during LVF seizures are mostly occurring in seizure-onset zones, suggesting that they could reflect distinct mechanisms of ictogenesis (Lévesque et al., 2012).

5. MODULATION OF EPILEPTIFORM SYNCHRONIZATION BY NEUROSTEROIDS

Neurosteroids are a class of compounds that can modulate neuronal excitability at the level of ion channels and membrane receptors (Akk et al., 2009; Mellon & Griffin, 2002). They are endogenously synthesized, mainly in glial cells and principal neurons, from circulating steroidal precursors but exert no known genomic effects (Agís-Balboa et al., 2006; Reddy, 2010). There are three main classes: the pregnane, androstane, and sulfated neurosteroids (Reddy, 2010). The former two exert mainly inhibitory effects and the later, excitatory (Majewska, 1992; Reddy, 2010). More specifically, pregnane neurosteroids are positive allosteric modulators of GABA_A receptor function and have been shown to provide some anti-ictogenic effect (Reddy & Rogawski, 2012).

Allotetrahydrodeoxycorticosterone (THDOC) and allopregnanolone are examples of pregnane neurosteroids that can be found endogenously at nanomolar concentrations (Reddy, 2011). By increasing the mean open time of GABA_A receptor channels, THDOC effectively potentiates both phasic and tonic currents (Reddy, 2011; Wohlfarth, Bianchi, & Macdonald, 2002); however, it is worth noting that the δ subunit of the GABA_A receptor confers a greater transduction of the neurosteroid signal upon binding of THDOC (Wohlfarth et al., 2002). GABA_A receptors containing the δ subunit are mainly found extrasynaptically and are more resistant to desensitization but have a low affinity to GABA, even at saturating concentrations (Glykys & Mody, 2007; Wohlfarth et al., 2002). In animal models of MTLE, subunits of GABA_A receptors undergo alterations that render them less sensitive to neurosteroid modulation (Mtchedlishvili, Bertram, & Kapur, 2001). In the epileptic rat, a downregulation of the δ subunit as well as an upregulation of the $\alpha 4$ subunit have been reported; these changes have been proposed to contribute to increased excitability (Peng, Huang, Stell, Mody, & Houser, 2004; Smith et al., 1998). THDOC can enhance the tonic current generated by these GABA_A receptors in a way that increasing GABA alone cannot (Wohlfarth et al., 2002).

By modulating the efficacy of GABA_A receptor function, THDOC acts as a broad spectrum anticonvulsant, enhancing the inhibitory tone of the brain (Reddy, 2004; Rupprecht, Hauser, Trapp, & Holsboer, 1996). THDOC protects against seizures induced by pilocarpine, kindling, and GABA_A receptor antagonists in animal models of epilepsy *in vivo* (Reddy, 2011). Furthermore, *in vitro* studies performed in rat piriform cortex and hippocampus show that inhibitory neurosteroids can produce a concentration-dependent suppression of epileptiform activity in 4AP and picrotoxin-treated slices along with a corresponding reduction in the occurrence of HFOs (Herrington, Lévesque, & Avoli, 2013; Salazar, Tapia, & Rogawski, 2003; Fig. 4.5B). Following pilocarpine treatment in rats, *in vivo* studies have shown that the induction of neurosteroid synthesis can potentially delay the development of spontaneously occurring seizures following SE (Biagini et al., 2006). Neurosteroids play an important role in regulating neural excitability and can potentially contribute to epileptogenesis and ictogenesis, a phenomenon that has also been identified in humans.

Patients with MTLE can experience both sexual and reproductive dysfunction. In men, these changes are associated with low levels of testosterone, which may be attributed to the suppression of the hypothalamic–pituitary–gonadal axis by limbic seizures (Reddy & Rogawski, 2012). Following surgical removal of the epileptic foci, the levels of serum androgens normalize. Additionally, catamenial seizure exacerbations affect up to 70% of women at a child-bearing age with epilepsy. In these patients, seizures occur in cyclical patterns associated with specific phases of the menstrual cycle, which can be related to the withdrawal of the anticonvulsant effects of neurosteroids (Reddy & Rogawski, 2012).

6. CONCLUSIONS

We have reviewed the neuronal processes leading to epileptiform synchronization under acute pharmacological manipulations as well as in brains made chronically epileptic by experimental procedures such as the pilocarpine-induced SE. These data underscore the importance to distinguish two main patterns of seizure onsets: HYP and LVF seizures. These

different types of seizures are associated with specific increases in ripple and fast ripple rates during both preictal period and seizure activity itself. Perhaps and more important, HYP and LVF seizures may mirror and thus result from a decrease and an increase of GABA_A receptor signaling, respectively. In this respect, and with potential implications for the mechanisms underlying epileptogenesis, we anticipate that neurosteroids may play an important modulatory action on limbic networks excitability.

In the context of MTLE, LVF and HYP seizures could reflect distinct patterns of up- or downregulation of specific interneuron types perhaps resulting from the neuronal loss that characterizes mesial temporal sclerosis. We anticipate that further experiments will provide definitive information regarding the mechanisms underlying seizure initiation in MTLE both in animal models and in humans, thus leading to specific pharmacological treatments as well as to the development of seizure prediction tools.

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ABBREVIATIONS

4AP	4-aminopyridine
HFOs	high-frequency oscillations
HYP	hypersynchronous onset
IPSPs	inhibitory postsynaptic potentials
LVF	low-voltage fast onset

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Figure 4.1.

Interictal (asterisks) and ictal (dotted lines) epileptiform discharges recorded with depth EEG electrodes from a patient with MTLE (A), from a pilocarpine-treated epileptic rat (B), and with an extracellular microelectrode from the EC area in a rat brain slice treated with 4AP (C). *EEG recordings shown in (A) were kindly provided by Drs. P. Perucca, F. Dubeau, and J. Gotman at the Montreal Neurological Institute & Hospital.*

Figure 4.2.

(A) Intracellular (K-acetate-filled microelectrode, Cell) and field potential (Field) recordings obtained in a neocortical slice during application of medium containing the GABA_A receptor antagonist bicuculline methiodide. (B) Intracellular (K-acetate-filled microelectrode, Cell) and field potential (Field) recordings obtained from the CA3 subfield during 4AP application. Note that the intracellular counterpart of the “fast” interictal discharges recorded from a CA3 pyramidal cell consists of depolarizations that trigger bursts of fast action potentials, while the counterpart of the “slow” interictal spike is characterized by a slow depolarization with a single fast action potential. (C) Ectopic spikes (arrow) occur during the slow depolarization associated with the slow interictal discharge recorded during 4AP application; intracellular recording was obtained with a K-acetate-filled microelectrode. (D) Simultaneous field potential and extracellular [K⁺] recordings of the glutamatergic-independent interictal spike generated by CA3 neuronal networks during concomitant application of 4AP and glutamatergic antagonists; note that the extracellular [K⁺] increases up to approximately 5.0 mM from a resting concentration of 3.2 mM shortly after the negative peak of the field potential. (E) Interictal activity recorded with depth electrodes from the EC, CA3, and amygdala in a pilocarpine-treated animal during the chronic epileptic period. The expanded traces show in detail the two types of interictal spikes.

Figure 4.3.

(A) Field and intracellular (Cell) recordings obtained from a mouse EC neuron during application of Mg^{2+} -free medium. Both interictal (asterisks) and ictal (dotted line) discharges occurred during this experimental procedure. (B) Changes induced by Schaffer collateral cut on the epileptiform activity recorded from the EC in a combined mouse brain slice. Under control conditions, fast CA3-driven interictal activity is generated by EC neuronal networks; however, cutting the Schaffer collateral prevents CA3-driven interictal activity to propagate to the EC and uncovers slow interictal events along with ictal discharge in this area. Note that the slow interictal spike (asterisk) is associated with an increase in extracellular $[\text{K}^+]$ that is smaller than what occurring in coincidence with the initial spike leading to ictal activity (arrow). Note also the much larger elevation in extracellular $[\text{K}^+]$ occurring during the overt ictal discharge. (C) Ictal discharges recorded *in vitro* from the EC during application of 4AP do not occur during local electrical stimulation implemented at 0.5 Hz. Note also that ictal activity reappears following termination of the stimulating protocol.

Figure 4.4.

(A) LVF and HYP ictal discharges recorded *in vitro* from the EC in two different experiments during application of 4AP. Asterisks highlight the interictal-like events that precede the beginning of the ictal activity. (B) Field potential recordings obtained in a rat brain slice during application of 4AP show that EC and perirhinal cortex (PC) can generate at the same time LVF and HYP seizures, respectively. (C) Ictal activity in an EC–hippocampus brain slice during application of Mg^{2+} -free medium can initiate in different areas with variable propagation. Schematic diagram of propagation directions are shown in (a) where arrows represent direction (arrowheads) and percentages (arrow width) of propagation directions. Data used for this analysis rest on simultaneous field recordings obtained from areas CA1, CA3, DG of the hippocampus and EC in 51 combined rat brain slices (b) Percentages of propagation directions corresponding to arrow widths shown in (a). Note that 35% of the ictal discharges arose in hippocampus and propagated to EC, 24.5% of them started in EC and propagated to hippocampal areas, with latencies of 60–175 ms, while 40.5% of them had latencies of less than 5 ms, which hindered the identification of any definite initiation site. From: Schulte-Wess, S. *Iktogenese und Propagation epileptischer Aktivität im entorhinalen Cortex und Hippocampus*. Dissertation, Muenster, 2007**.

Figure 4.5.

(A) EEG recordings of spontaneous seizures obtained from the CA3 region of the hippocampus in a pilocarpine-treated rat. Panel (a) shows an example of an LVF seizure in the CA3 region of the hippocampus. Note the occurrence of a single positive-going spike at the onset of the seizure, followed by activity in the 5–20 Hz range. The analysis of HFOs shows that high rates of ripples compared to fast ripples are associated with this type of seizure onset ($n = 18$ LVF seizures). Panel (b) shows an example of a HYP seizure recorded in the same animal in the CA3 region of the hippocampus ($n = 21$ HYP seizures). Note the occurrence of multiple ictal spikes followed by activity in the 10–20 Hz range. Compared to LVF seizures, HYP seizures are mostly associated with the occurrence of fast ripples. (B) Ictal events recorded with an extracellular micro-electrode from the piriform cortex during 4AP application show a concentration-dependent decrease in duration with increasing concentrations of THDOC (a and b). Note in (c) that THDOC reduces HFO occurrence to a greater extent than it reduces duration of ictal events.