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The subiculum and its role in focal epileptic disorders

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Abstract: The subicular complex (hereafter referred as subiculum), which is reciprocally connected with the hippocampus and rhinal cortices, exerts a major control on hippocampal outputs. Over the last three decades, several studies have revealed that the subiculum plays a pivotal role in learning and memory but also in pathological conditions such as mesial temporal lobe epilepsy (MTLE). Indeed, subicular networks actively contribute to seizure generation and this structure is relatively spared from the cell loss encountered in this focal epileptic disorder. In this review, we will address: (i) the functional properties of subicular principal cells under normal and pathological conditions; (ii) the subiculum role in sustaining seizures in *in vivo* models of MTLE and in *in vitro* models of epileptiform synchronization; (iii) its presumptive role in human MTLE; and (iv) evidence underscoring the relationship between subiculum and antiepileptic drug effects. The studies reviewed here reinforce the view that the subiculum represents a limbic area with relevant, as yet unexplored, roles in focal epilepsy.

Keywords: focal epileptic disorders; limbic system; mesial temporal lobe epilepsy; subiculum; temporal lobe.

Introduction

Neuroanatomical studies have established that the subicular complex, which includes prosubiculum, subiculum, presubiculum, parasubiculum and postsubiculum (Ding 2013; O'Mara 2005; O'Mara et al. 2001; Witter et al. 2000), represents a major synaptic relay station in the

hippocampal formation that interconnects cortical and subcortical structures belonging and thus constituting the so called limbic system (Gloor 1997; Lopes da Silva et al. 1990; Naber and Witter 1998; Naber et al. 2001; Van Hoesen et al. 1979; Witter et al. 1990) (Figures 1 and 2). It is well established that the limbic system is involved in the processes of learning and memory (Buzsáki 2015; Gloor 1997; Oliva et al. 2016; Sharp and Green 1994; Sun et al. 2019; Torromino et al. 2019). Like the hippocampus, the subicular complex, hereafter referred for sake of simplicity as subiculum, is constituted of a three-layered allocortex (also known as heterogenetic cortex) that is composed of a molecular layer, a pyramidal cell layer, and a polymorphic fiber layer. While, the molecular layer is contiguous with the strata lacunosum-moleculare and radiatum of the CA1 subfield, the cell layer contains large pyramidal neurons, which are, however, less densely “packed” than those in the CA1 subfield, along with several subtypes of interneurons that release the inhibitory transmitter GABA (Figure 1) (Ding 2013; O'Mara 2005; O'Mara et al. 2001; Witter et al. 2000).

Several studies (see for review O'Mara 2005) have reported that the subiculum plays a fundamental role in elaborating the information occurring in limbic networks during cognitive functions. Accordingly, *in vivo* recordings performed in freely moving animals have firmly demonstrated that principal (i.e., pyramidal) neurons in the subiculum exhibit “location specific” firing patterns suggesting a contribution of this region to spatial learning (Sharp 2006; Sharp and Green 1994; Sun et al. 2019). For instance, studies performed by Taube (Taube 1995; Taube et al. 1990) have shown that cells in the dorsal presubiculum discharge as a function of the animal's head direction in its environment, independently of the animal's location and/or behavior. Similarly, other studies have reported location specific firing of cells in the subiculum (Barnes et al. 1990; Brotons-Mas et al. 2010; Lever et al. 2009; Sharp 2006, 1999). Interest in the properties and function of the subicular complex also comes from evidence obtained from patients presenting with Alzheimer's disorders that have shown a preferential localization of beta amyloid plaques in this structure (Davies et al. 1988; Miller et al. 1987).

According to their firing pattern, subicular principal (glutamatergic) pyramidal neurons have been divided into

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Figure 1: Schematic drawing of the localization of hippocampal and parahippocampal areas in a rat brain slice.

CA, cornu ammonis; DG, dentate gyrus; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex; PC, perirhinal cortex. Scale bar, 500 μ m. Modified from the study of de Guzman et al. (2006).

Figure 2: Schematic drawing of the anatomical connections between different areas of the temporal lobe.

Note the strategic position of the subiculum, which act as a major relay station that bi-directionally interconnects cortical and subcortical structures.

bursting and non-bursting cells. Burst firing is known to occur under physiological conditions and it is believed to increase the reliability of excitatory synaptic connections thus contributing to synaptic plasticity (Cooper et al. 2005, 2003; Lisman 1997; Simonnet and Brecht 2019). However, evidence obtained over the last few decades regarding the presence and presumptive role of neuronal bursting in pathophysiological conditions, such as epileptic disorders, cannot be overlooked. Accordingly, experimental studies

have identified action potential bursting as the hallmark of interictal discharges generated by cortical networks in models of focal epilepsy in both *in vivo* (Ayala et al. 1973; Matsumoto and Marsan 1964; Prince 1969) and *in vitro* preparations (e.g., (Gutnick et al. 1982; Johnston et al. 1986; Perreault and Avoli 1992, 1991; Schwartzkroin and Prince 1978; Wong and Prince 1978).

As emphasized by Gloor in the book titled *The Temporal Lobe and Limbic System* (1997) in which the role played by temporal lobe areas in focal epileptic disorders was also addressed, it was known since the late 1890s that the subiculum remains relatively spared in the cell loss that characterizes Ammon's horn sclerosis (*cf.* (Bratz 1889). Such sclerosis (i.e., neuronal cell loss with consequent reorganization of limbic networks leading to functional changes) represents the main pathological hallmark in patients presenting with mesial temporal lobe epilepsy (MTLE) (Engel 1996; Gloor 1997). This evidence was still valid when Stafstrom (2005) reviewed in his short but comprehensive paper the role of the subiculum in focal epileptic disorders. Fifteen years later, we have acquired more information on the functional characteristics of subicular networks and on the role of this limbic structure in focal epileptic disorders. Moreover, some studies have challenged the notion that neurons in the subiculum are not damaged in patients and animal models of MTLE (Alonso-Nanclares et al. 2011; Fabo et al. 2008; Furlinger et al. 2001; Miyata et al. 2013; Wozny et al. 2005). Therefore, we considered both timely and worth to address in this review the contribution and role played by the subiculum in the pathophysiological processes that lead to epileptic conditions, such as MTLE, in humans and in experimental animal models both in *in vitro* and in *in vivo* preparations. Specifically, we will address here: (i) the electrophysiological properties of subicular principal neurons along with their response to cholinergic (atropine sensitive) activation; (ii) the functional changes that occur in animal models of focal epileptic disorders both in *in vivo* and *in vitro* preparations; (iii) some specific features that characterize the activity of the subiculum in human focal epileptic disorders, and in particular MTLE; and finally (iv) the response of the subiculum to antiepileptic drugs and the potential role played by this structure in pharmacoresistance.

Functional and morphological properties of principal cells in the subiculum

Up to 1993, little was known on the fundamental mechanisms regulating the functional properties of principal cells

in the subiculum. As illustrated in Figure 3 (panels Aa and B), in that year, four papers originating from independent laboratories reported that a large proportion of neurons in the subiculum are prone to generate burst of action potentials in response to intracellular pulses of depolarizing current (Mason 1993; Mattia et al. 1993; Stewart and Wong 1993; Taube 1993). As further discussed below, such bursting was also observed at the end of hyperpolarizing current commands suggesting its contribution to rebound excitation (Figure 3Ab) (*cf.*, Menendez de la Prida et al. 2003). This evidence has been confirmed and further elaborated in several studies that have appeared in the following years (Fiske et al. 2020; Joksimovic et al. 2017; Jung et al. 2001; Mattia et al. 1997a, 1997b; Menendez de la Prida 2003; Menendez de la Prida et al. 2003). To note that most of these studies focused on the subiculum proper; however, Menendez de la Prida et al. (2003) also recorded neurons in the rat presubiculum and parasubiculum, and found that bursting cells were also present in these limbic areas but not in the presubiculum superficial layers.

Some further, relevant, characteristics of the intrinsic bursting generated by principal neurons in the subiculum are shown in Figure 3B–D; these findings were originally reported by (Mattia et al. 1997a, 1993). First, it was found that the strength of the bursting response during depolarizing commands depends on the resting membrane potential (RMP); thus, bringing the membrane to a value 10 mV more hyperpolarized than RMP, with steady current injection, could cause a weaker burst of action potentials (Figure 3B). Second, as illustrated in Figure 3C, the burst of action potentials was surprisingly turned into a regular firing mode by depolarizing the neurons with steady current injection (see also Mason 1993; Menendez de la Prida et al. 2003; Stewart and Wong 1993); this behavior closely resembled what has been reported with the low-threshold Ca^{2+} current in thalamocortical neurons (Llinás and Steriade 2006; McCormick 1992; Steriade and Llinás 1988). Third, prolonging the depolarizing intracellular current injection usually made the neuron generate, after the initial burst response, regular action potential firing (Figure 3D) (Mattia et al. 1997b) (but see also the data reported later by Menendez de la Prida (2003), who demonstrated the ability of some subicular cells to generate recurrent bursts of action potentials that persist during long-lasting depolarizing commands). To note that Menendez de la Prida et al. (2002, 2003), performing a detailed morphological analysis of the soma shape of subicular cells, have found that bursting neurons were larger in size when compared to regularly firing neurons. These investigators also distinguished bursting neurons as

Figure 3: Electrophysiological properties of bursting in neurons in the subiculum.

A to C panels show bursts of action potential firing generated by a subicular neuron during intracellular injection of depolarizing current pulses (**Aa**) and upon termination of a hyperpolarizing pulse (**Ab**), so called rebound excitation. In **B**, note the effects on the burst response by hyperpolarizing the membrane potential, with steady current injection, by 10 mV; such procedure reveals a depolarizing afterpotential (*) while reducing the post-burst AHP (arrow). In **C**, the intrinsic burst response is abolished by depolarizing the RMP to -51 mV. **D**: Repetitive firing induced by prolonged, intracellular depolarizing pulses; note that the initial burst is followed by action potential regular firing throughout the pulse when the intensity of the depolarizing current pulse is increased. **E**: Ionic mechanisms underlying the bursting responses. Under control conditions, brief depolarizing and hyperpolarizing current pulses evoke action potentials bursts (inset in the right top, represents the synaptic responses to stimuli delivered in the CA1-alveus at two different intensities); during bath application of Ca^{2+} channel blockers (Co^{2+} and Cd^{2+} , 2 and 1 mM, respectively), action potential bursts are still evoked while the synaptic responses are abolished; however, the slow depolarization and the superimposed action potential burst are abolished by successive application of the Na^{+} channel blocker tetrodotoxin (TTX). **F**: Voltage responses generated by a subicular neuron to injections of depolarizing and hyperpolarizing current pulses during bath application of TTX (1 μM) and TEA (10 mM) and after further addition of Co^{2+} and Cd^{2+} (2 and 1 mM, respectively). Pulses of intracellular current injected under both experimental conditions were from ± 0.1 nA to ± 1 nA by increments of ± 0.1 nA; arrows point to the “on” and “off” of the intracellular current pulses. Note that high threshold spikes occur in the presence of TTX and TEA and that they are blocked by Co^{2+} and Cd^{2+} suggesting that they reflected high threshold Ca^{+} current. RMP = approx. -70 mV. Note that arrows point to the “on” and “off” of the intracellular current pulse while numbers on the center of each trace give the amount of current injected (nA). Panels **A** to **E** are modified from Mattia et al. (1993), while panel **F** is modified from Mattia et al. (1997a).

weak and strong bursters, and found that strong bursters had pyramid-shaped soma while weak bursters presented with both pyramidal and non-pyramidal oval shape (Menendez de la Prida et al. 2003, 2002).

As further discussed in Section 4, local GABAergic inhibition and in particular the component due to the activation of GABA_A receptors (Menendez de la Prida 2006, 2003), is crucial in regulating the gating function exerted by the subiculum on hippocampal output activity (Benini and Avoli 2005) as well as in controlling the recurrent network activity that is presumably generated within the subicular network by interconnected principal cells (Harris and Stewart 2001; Harris et al. 2001; Witter 2006). It should also be noted that Panuccio et al. (2012) have recorded phasic and tonic inhibitory currents from principal cells and interneurons of the rat subiculum; it was found in this study that interneurons generate spontaneous IPSCs that occur less frequently and exhibit smaller charge transfer when compared to those recorded from principal cells; in addition, it was shown that subicular bursting cells are constrained by greater tonic inhibitory currents than those generating regular firing or inhibitory interneurons (Panuccio et al. 2012).

Regular and bursting principal cells are anatomically distributed according to specific patterns along the proximal-distal and dorso-ventral axis of the rodent subiculum, and the latter corresponds to the anteroposterior axis in monkeys and humans (Strange et al. 2014). Bursting cells in rats appear to be located at more distal locations (close to presubiculum) (Jarsky et al. 2008; Staff et al. 2000) whereas regular spiking cells can be found located at more proximal sites (close to CA1) (Greene and Totterdell 1997). Considering the dorso-ventral axis, regular spiking cells are located on the superficial layers whereas bursting cells are usually observed in deep layers (Greene and Totterdell 1997). A more recent study by Kim and Spruston (2012) has also demonstrated that the majority (~80%) of regular spiking cells that are located in proximal regions project to the lateral amygdala, lateral entorhinal cortex, nucleus accumbens and the orbitofrontal cortex; in contrast, most (~80%) bursting cells situated in the distal regions of the subiculum project to the presubiculum, medial entorhinal cortex, retrosplenial cortex and ventromedial hypothalamus. Simonnet and Brecht (2019) also proposed that bursting cells can be distinguished as sparsely (80%) and dominantly (20%) bursting cells, depending on their average bursting rate; in this study, sparsely bursting cells appeared to have a low bursting rate (0.8 Hz) while dominantly bursting cells were characterized by a higher (4.3 Hz) rate. In addition, Simonnet and Brecht (2019) reported that both cell types are uniformly distributed

along the proximodistal axis. These results, therefore, challenge the notion that bursting cells are positioned only at proximal sites (Cembrowski et al. 2018).

It is important to note that the classification of cells into regular or bursting cells is based on criteria that are mostly dependent on firing patterns and that are highly different between laboratories. Fiske et al. (2020), therefore, recently used double and triple patch recordings to classify subicular pyramidal cells using multivariate methods based on multiple parameters not exclusively linked to firing patterns. They reported the existence of two subpopulations of subicular principal cells (type 1 and type 2) that differed in firing rates, dendritic arborizations and network connections. In addition, they showed that under pharmacological blockade of GABA_A and GABA_B receptor-mediated transmission, and in the absence of extrasubicular excitation, these two types of subicular principal cells can generate epileptiform activity due to their high degree of interconnectivity. These results were similar to what was previously obtained by (Böhm et al. 2015), who provided the first description of the functional connectivity of local subicular circuits with multiple recordings. However, Fiske et al. (2020) found one type of connection between subicular principal cells (between type 2 and type 1 cells or between bursting and non-bursting cells) that was not observed in the study of Böhm et al. (2015). Such level of heterogeneity in subicular principal cells was also reported in the study performed by Cembrowski et al. (2018), who found the existence of eight subclasses of principal cells in the subiculum based on single-cell RNA sequencing. Moreover, these populations of cells projected to different downstream structures and expressed different intracellular protein products (Cembrowski et al. 2018).

Ionic mechanisms underlying action potential burst firing

The original findings reported by Mattia et al. (1994) indicated that voltage-dependent Na⁺ currents may play a major role in the generation of the action potential bursts recorded from subicular principal cells. As shown in Figure 3E, this conclusion rested on the persistence of the ‘intrinsic’ bursting response during application of Ca²⁺ blockers and their subsequent elimination by application of the voltage-dependent Na⁺ channel blocker tetrodotoxin (TTX). Similar results were later obtained by Menendez de la Prida et al. (2003). However, opposite views were proposed in two independent studies carried out by Stewart and Wong (1993) and by Taube (1993);

these investigators concluded that voltage-dependent Ca^{2+} currents were responsible for the bursting, since TTX-resistant spikes could be recorded in bursting, but not in regular-spiking neurons. Jung et al. (2001) have later shown that action potential bursting in subicular principal cells is driven by a Ca^{2+} tail current. In addition, pharmacological, molecular and genetic findings have revealed – similar to what identified in thalamocortical neurons (Llinás and Steriade 2006; McCormick 1992; Steriade and Llinás 1988) – a prominent role of low-voltage-activated T-type Ca^{2+} channels in the burst firing generated by subicular principal as well as by inhibitory cells (Joksimovic et al. 2017). Presumably, and in agreement with what proposed by Menendez de la Prida (2003) and by Stafstrom (2005), both voltage-gated Ca^{2+} and Na^{+} components can be present concomitantly in subicular bursting cells and thus these two ionic mechanisms are not incompatible. We agree with such view but we need to remark that the findings obtained by Mattia et al. (1994, 1997a, 1997b) firmly demonstrated that: (i) the generation of action potential bursting under control conditions is mainly dependent on a voltage-gated, Na^{+} current (Mattia et al. 1997a, 1994), which is persistent as shown in several studies performed in principle cells of the neocortex (Connors et al. 1982; Stafstrom et al. 1985, 1984) and entorhinal cortex, while (ii) high threshold Ca^{2+} spikes can be disclosed only when K^{+} channels are pharmacologically blocked (Figure 3F); such characteristic is common to several types of cortical neurons (Connors et al. 1982; D’Antuono et al. 2001a; Wong and Prince 1978).

As already mentioned, action potential bursting is also generated by subicular principal cells at the end of a hyperpolarizing current command suggesting a potential role in rebound excitation. As illustrated in Fig. 4A, the generation of this ‘anodal break’ bursting response is dependent upon the duration of the preceding hyperpolarizing current pulse indicating that a hyperpolarization of the RMP is instrumental in causing the rebound depolarization. The inward rectification in the hyperpolarizing direction (also called anomalous rectification, Spain et al. 1987) was abolished in subicular principal cells by extracellular application of Cs^{+} (Figure 4B), a pharmacological property that has been identified in a variety of cortical (Halliwell and Adams 1982; Maccaferri et al. 1993; Spain et al. 1987; Stafstrom et al. 1984) and subcortical (Kamondi and Reiner 1991; McCormick and Pape 1990; Yarom and Llinás 1987) neurons, in which it has been attributed to the activation of a mixed Na^{+} - K^{+} current, variably referred as I_Q , I_H , I_F or I_{AR} . To note that evidence for the presence in subicular principal cells of inward

Figure 4: Electrophysiological properties of bursting in neurons in the subiculum.

A: Hyperpolarizing current pulses of increasing duration cause a rebound depolarizing response at the current break. **B:** After extracellular application of Cs^{+} the inward rectification during hyperpolarizing current pulses (presumably due to an I_H) is blocked; note that Cs^{+} brings the RMP from -61 to -65 mV. **C:** Subthreshold voltage-dependent oscillations are generated by bursting subicular neurons; note that these subthreshold oscillations of the membrane potential develop with increasing levels of depolarization as well as that clustered firing with interposed oscillations occurs in this experiment at -55 mV. **D:** Samples of subthreshold oscillations recorded under control conditions and during application of ionotropic excitatory amino acid and GABA_A receptor antagonists; note that the oscillations are not influenced by these pharmacological procedures. **E:** Samples obtained under control conditions and during application the Na^{+} -channel blocker TTX; note that the subthreshold oscillations are abolished by TTX. Panels A and B are modified from the study by Mattia et al. (1997a) while panels C to E are modified from the study by Mattia et al. (1997b).

rectification in the hyperpolarizing direction, was also found by Menendez de la Prida (2003). In addition, Stewart and Wong (1993) have reported a similar sag along with an ‘anodal break’ bursting response subicular neurons, and they remarked that these bursting responses could not be observed when recording from non-bursting neurons.

Subicular bursting cells can also generate persistent, subthreshold voltage-gated oscillations at a frequency of approx. 5 Hz during steady depolarization of the RMP to values that are less negative than -60 mV. Moreover, further depolarization of these subicular neurons discloses

clusters of single action potentials with a periodicity of 0.9–2 Hz (Figure 4C) (Mattia et al. 1997b; but also see Figure 6 in Menendez de la Prida et al. 2003). The generation of these membrane oscillations was not prevented by concomitant application of ionotropic glutamatergic and GABA_A receptor antagonists (Figure 4D), as well as by Ca²⁺-channel blockers, or Cs⁺ (not illustrated); however, these oscillations were readily abolished by the Na⁺-channel blocker TTX (Figure 4E). Similar subthreshold intrinsic oscillations have also been ascribed to the activation of a persistent, voltage-gated Na⁺ current in hippocampal (Leung and Yim 1991), entorhinal (Alonso and Llinás 1989; Klink and Alonso 1993) and neocortical (Nuñez et al. 1992; Silva et al. 1991) principal cells. Moreover, a similar spike-clustering phenomenon has been reported in stellate cells of the entorhinal cortex (Alonso and Klink 1993).

I_{CAN} and theta-like oscillation during atropine-sensitive cholinergic activation

The excitability and plasticity of subicular neurons is controlled by several neuromodulators (Cooper et al. 2003; Palacios-Filardo and Mellor 2019; Wozny et al. 2008). However, a mechanism that may be rather relevant for epileptiform synchronization rests on the activation of muscarinic receptors by acetylcholine. Accordingly, acute pilocarpine-induced seizures in animals depend on the activation of muscarinic receptors (Turski et al. 1984, 1983), chemical kindling by activation of muscarinic receptors induces evoked and spontaneous seizures (Wasterlain and Jonec 1983), and muscarinic cholinergic receptors are down-regulated in the animal electroshock and kindling models (Dasheiff and McNamara 1980; Dasheiff et al. 1982). It should also be mentioned that over seven decades ago, Tower and McEachern (1949) reported that acetylcholine was present in the cerebrospinal fluid of 77% of epileptic patients compared to 15% of non-epileptic patients.

In vitro, the cholinergic agonist carbachol (CCh) induces powerful excitatory effects that include the well-known decrease of K⁺ mediated conductances (including the so-called M current) (Brown and Adams 1980; Krnjević 1993) as well as an increase of the inward rectification in the hyperpolarizing direction (also cf. Colino and Halliwell 1993) (Figure 5A). It is well established that K⁺ currents underlie after-hyperpolarizations in neurons; however, and perhaps more important, application of CCh discloses, in subicular bursting cells, depolarizing plateau potentials in response to brief pulses of depolarizing current

(Kawasaki et al. 1999) (Figure 5B–D). As in other brain structures such as the CA1 subfield of the hippocampus (Colino and Halliwell 1993; Fraser and MacVicar 1996) or the entorhinal cortex (Klink and Alonso 1997a, 1997b), these depolarizing plateau potentials are known to be contributed by a Ca²⁺-dependent, non-selective cationic conductance (also called I_{CAN}). In principle cells of the subiculum (as well as in other cortical neurons), these plateau potentials are voltage-dependent since their magnitude depends on the intensity and/or duration of the triggering depolarizing pulse (Figure 5B) and is influenced by injecting hyperpolarizing commands – which would repolarize the membrane potential – immediately after the depolarizing pulse (Figure 5C). To note that the original experiments shown in Figure 5 (Kawasaki et al. 1999) were performed in the presence of ionotropic glutamatergic receptor antagonists; however, their voltage-sensitivity suggests that under control conditions (i.e., with operative synaptic transmission), both plateau potential occurrence and size should be modulated and perhaps triggered by ongoing depolarizing and hyperpolarizing synaptic inputs within the subicular networks.

As shown in Figure 5D, CCh-induced plateau potentials are caused in subicular bursting neurons by the activation of muscarinic receptors since atropine application abolished them (but this pharmacological procedure did not influence the initial action potential burst), whereas the nicotinic receptor antagonist D-tubocurarine failed in doing so (not shown) (Kawasaki et al. 1999). To note, once more, that CCh-induced plateau potentials did occur in the presence of ionotropic excitatory amino acid receptor antagonists and that their generation was not influenced by further application of GABA_A and GABA_B receptor antagonists (Kawasaki et al. 1999). Therefore, the CCh-induced plateau potentials represent intrinsic neuronal events that are not actively contributed by glutamatergic excitatory or GABAergic inhibitory synaptic currents. Long-lasting, post-stimulus depolarizations also occur in CA3 pyramidal cells (Caeser et al. 1993), olfactory cortex neurons (Constanti et al. 1993) and neocortical neurons (Andrade 1991; Schwindt et al. 1988) during cholinergic activation. In all these studies, the plateau potentials or the slow post-stimulus depolarizations were shown to be dependent on muscarinic receptor activation.

Oscillations at approx. 5 Hz do also occur during the plateau potentials generated by subicular bursting neurons during activation of muscarinic receptors (Figure 5C, top trace) (Kawasaki et al. 1999). This aspect has been further investigated by D'Antuono et al. (2001b), who found that application of CCh induces subicular network oscillations (intra-oscillatory

Figure 5: Effects induced by carbachol (CCh) on the intrinsic excitability and bursting responses of subicular neurons.

A: Under control conditions a depolarizing current pulse (0.3 nA) induces a burst of action potentials that is terminated by a burst-afterhyperpolarization (arrow), while hyperpolarizing pulses elicit membrane responses characterized by time-dependent sags followed by rebound depolarizations (asterisk). Application of CCh (50 μ M) induces an increase in membrane input resistance (but note that the RMP is kept at control values with steady hyperpolarizing current injection). Note also that in the presence of CCh, an action potential burst is still triggered by the 0.3 nA depolarizing pulse while the burst-afterhyperpolarization (arrow in the control sample) is decreased and replaced by two action potentials. Note that the termination of the depolarizing pulse is followed by a slow depolarization (double arrow-heads). **B:** Plateau potentials increase in duration and amplitude when the triggering pulse is increased in duration. **C:** Injection of a brief (duration = 20 ms) hyperpolarizing pulse of increasing amplitude immediately after the initial depolarizing command makes the plateau potential decrease in duration and eventually disappear. Note the occurrence of membrane oscillatory events at around 3 Hz during the plateau potential shown in the upper trace. **D:** Bath application of atropine abolishes the plateau potentials triggered in this subicular bursting cell by brief pulses of intracellular depolarizing current (0.8 nA). Panels A–D is modified from the study by Kawasaki et al. (1999).

frequency at approx. 8 Hz) that are recorded simultaneously from both intracellular and extracellular microelectrodes. It is well known that CCh induces field potential oscillations that have frequencies similar to the physiologic theta rhythm (i.e., 5–12 Hz) (Bland 1986; Buzsáki 2002; Dickson et al. 2000; Lévesque et al. 2017) as well as that, *in vivo*, physiologic theta oscillatory activity modulates synaptic plasticity related to sensorimotor integration, spatial navigation and learning (Buzsáki and Moser 2013; Düzel et al. 2010; Hasselmo and Stern 2014; Huerta and Lisman 1993). However, it has also been proposed that the electrographic features of the oscillations induced by CCh *in vitro* may more closely resemble epileptiform activity than *in vivo*

Figure 6: Effects induced by antagonists of excitatory and inhibitory transmission on the oscillatory activity generated by subicular neurons during application of CCh.

A: Intracellular and field recordings obtained from the subiculum in a brain slice preparation during application of CCh (control) and after application of the non-NMDA receptor antagonist 6-cyano-7-nitro-quinoxaline-2, 3-dione (CNQX); note that under control conditions, asynchronous oscillations (arrows) can precede the network oscillations that are evident in the field trace; note also that during CNQX application field potential oscillations disappear but the intracellular rhythmic depolarizations (arrows) continue to occur. The RMP of this cell was –65 mV but was set to –58 mV to facilitate the occurrence of the intrinsic rhythmic membrane oscillations. **B:** Lack of effects induced by GABA_A (BMI) and GABA_B (CGP 35348) receptor antagonists on the recurrent bursting induced by intracellular current injection during application of CCh and ionotropic excitatory amino acid receptor antagonists (CNQX and CPP). **C:** The recurrent bursting induced by CCh during blockade of GABAergic and ionotropic glutamatergic receptor antagonists is not induced by a depolarizing command during application of the muscarinic receptor antagonist atropine; under this pharmacological procedure, tonic firing is generated even during sustained depolarization of the membrane achieved by injecting large amplitude of depolarizing current. The traces shown below each intracellular recording represent the current monitor. Panels A–C are modified from the study by D’Antuono et al. (2001a).

physiological theta oscillations (Cataldi et al. 2011; Lévesque and Avoli 2018; Williams and Kauer 1997). Therefore, the oscillatory activity induced by CCh in subicular networks *in vitro* may represent a prototype of epileptiform synchronization.

As shown in Figure 6A (arrows in the intracellular recording trace), D'Antuono et al. (2001b) found that during CCh application, a small (from -65 to -58 mV), steady depolarization of the RMP induces membrane oscillations in the intracellularly recorded subicular principal cell as well as that these intrinsic oscillations appear to lead to synchronous network oscillatory activity that was observed in the field potential (extracellular) recording. Interestingly, these field oscillations but not the intrinsic oscillatory activity were abolished by the non-NMDA receptor antagonist CNQX. In addition, during CCh application, subicular cells could generate intrinsic burst oscillations within a 2–7 Hz range when depolarized with steady current injection in the presence of antagonists of ionotropic glutamatergic and of GABA_A and GABA_B receptors (Figure 6B). However, these 'intrinsic' oscillations were readily abolished by Ca²⁺ channel blockers, intracellular Ca²⁺ chelation, or replacement of extracellular Na⁺ while they persisted during intracellular recordings that were obtained with microelectrodes containing QX-314, which is a blocker of voltage-gated Na⁺ channels (not illustrated). Finally, these intrinsic oscillations, like the depolarizing plateau potentials, were promptly abolished by the muscarinic receptor antagonist atropine (Figure 6C).

Overall, these data demonstrate a voltage-dependent propensity of subicular principal cells to generate oscillatory activity that is associated with action potential bursts during muscarinic receptor activation. Moreover, their pharmacological properties suggest the involvement of I_{CAN} in such intrinsic oscillatory bursting behavior that is then integrated and processed within the neuronal subicular networks mainly via non-NMDA receptor-mediated excitatory synaptic transmission. As recently reviewed by us (Lévesque and Avoli 2018), a similar muscarinic-dependent mechanism could presumably contribute to the CCh-induced oscillations that have been recorded from several types of cortical networks *in vitro*.

Subicular functions in animal models of MTLE

In vivo evidence

The active role played by the subiculum in MTLE has been reported in several animal studies using chemoconvulsants

such as pilocarpine or kainic acid (KA). Pilocarpine is a cholinergic muscarinic agonist that when administered systemically in rodents, induces a *status epilepticus* (SE) that is followed by a latent period during which no seizures are observed and then by recurrent, spontaneous non-convulsive and convulsive seizures (Curia et al. 2008; Turski et al. 1989, 1983). KA, which is a cyclic analog of L-glutamate and an agonist of the ionotropic kainate receptor, when administered either topically in the brain or systemically in animals, also induces an SE that is followed by a latent period and then by occurrence of spontaneous focal seizures that can become secondarily generalized (Ben-Ari and Lagowska 1978; Ben-Ari et al. 1979; Cavalheiro et al. 1982; Nadler 1979). Indeed, the pilocarpine and KA models reproduce both the histopathological alterations (i.e., the Ammon's horn sclerosis) and the spontaneous, recurrent focal seizures that are observed in patients presenting with MTLE (Curia et al. 2008; Lévesque and Avoli 2013).

Expression of FosB/ Δ FosB, a protein that is elevated by long-term drug abuse and chronic stress (Nestler 2008) and that accumulates in the nuclei of neurons participating to seizure activity (Morris et al. 2000), is increased in the rat subiculum 24 h after pilocarpine-induced SE, and it remains elevated up to seven days later (Biagini et al. 2005). These data, therefore, suggest a robust activation of this limbic structure during the initial SE as well as during the latent period that leads to the occurrence of spontaneous seizures in this animal model of MTLE. Lévesque et al. (2012) have also reported that within two weeks after a pilocarpine-induced SE, spontaneous seizures, which are characterised at their onset by periodic multiple pre-ictal spikes and thus defined as hypersynchronous-onset seizures, most often initiate in the hippocampus CA3 subfield and in subiculum (Figure 7A). High rates of fast ripples (250–500 Hz), which have been established to reflect pathological network activity in seizure onset zones (Jefferys et al. 2012; Jiruska et al. 2017), were also observed in these regions at the onset of and during hypersynchronous-onset seizures within the first 20 (Figure 7B) as well as 50 days after SE (Figure 7C) (Behr et al. 2017). Finally, Toyoda et al. (2013) have reported that in the pilocarpine model of MTLE, ictal activity onset mainly occurs in the hippocampus and subiculum up to 26 weeks after the pilocarpine-induced SE. The subiculum can thus represent a potential and preferential target for therapeutic strategies that are aimed at preventing seizure generation (see also Section 5).

Using tetrode-wire recordings in awake pilocarpine-treated epileptic rats, Toyoda et al. (2015) have specifically analysed the contribution of subicular interneurons and principal cells to the initiation of focal seizures (i.e., ictogenesis). They reported that interneurons

Figure 7: Seizures and phase-amplitude coupling in the subiculum of pilocarpine-treated animals.

A: Representative recording of a spontaneous seizure with a hypersynchronous-onset pattern in a pilocarpine-treated animal within the first 2 weeks after SE. Note the multiple periodic pre-ictal spikes at seizure onset (asterisks) in subiculum and CA3. Arrowhead indicates seizure onset in CA3. These multiple pre-ictal spikes were often observed in these two regions before hypersynchronous-onset seizures. **B:** Rates of HFOs (ripples and fast ripples) 40 s before hypersynchronous-onset seizures in the subiculum and CA3 region ($n = 196$ seizures, 8 animals) within the first 20 days after SE. Note that in both regions rates of fast ripples (250–500 Hz) significantly increased before seizure onset and during seizures. **C:** Similar results were observed between day 27–53 after status epilepticus; fast ripples occurred at significantly higher rates in the subiculum and CA3 region before and during hypersynchronous-onset seizures ($n = 25$ seizures, 7 animals) ($**p < 0.01$). **D:** Relationship between phase-amplitude coupling strengths and the number of seizures per day in epileptic pilocarpine-treated animals. In both subiculum and CA3, higher coupling strength between slow (0.18–4 Hz) and fast (80–250 Hz) oscillations was associated with higher number of seizures per day in subiculum and CA3. (NEC = non-epileptic controls). Panels A and B are modified from the study by Lévesque et al. (2012); panel C derives from the study by Behr et al. (2017); panel D comes from the study by Samiee et al. (2018).

recorded in the subiculum of these epileptic animals display the largest and earliest increase in firing activity before seizure onset. Moreover, the subiculum shows the greatest increase in field theta activity before the start of focal seizures, which may indicate that spontaneous subicular theta oscillations, which are presumably supported

by interneuron firing (Amilhon et al. 2015; Klausberger et al. 2005, 2003), represent a predominating factor for the increased interneuron activity in this region. According to (Toyoda et al. 2015), similar finding could not be identified in other limbic regions such as the hippocampus. However, experiments performed in Karen Moxon's laboratory have shown that putative interneurons in the CA3 subfield of pilocarpine-treated rats fire in synchrony with local field oscillations at theta and gamma frequencies “beginning minutes before the onset” of seizure activity (Grasse et al. 2013; Karunakaran et al. 2016). (Fujita et al. 2014) also shown in pilocarpine-treated animals that subicular principal cells did not increase their firing rates during the theta oscillatory activity recorded before seizure onset.

Samiee et al. (2018) have also analysed the relationship between the phase of slow oscillations and the amplitude of faster rhythms in control animals and in pilocarpine-treated rats using phase-amplitude coupling (PAC). PAC is a type of cross-frequency coupling in which the phase of slow oscillations modulates the amplitude of faster rhythms (Tort et al. 2010). It was found in this study that the strength of PAC coupling between slow (0.18–4 Hz) and fast (80–250 Hz) oscillations is stronger in CA3 and subiculum in pilocarpine-treated animals compared to controls (Samiee et al. 2018). Moreover, PAC indices in these two regions showed a positive correlation with seizure occurrence since strong PAC was associated to higher seizure rates over time within two weeks after SE (Figure 7D). Changes in PAC occurring in the CA3 hippocampal subfield and subiculum, which are often seizure onset zones in the pilocarpine model (Lévesque et al. 2012; Toyoda et al. 2013), could therefore reflect time-windows during which neuronal networks undergo substantial changes in neuronal network connectivity and excitability.

The development of chemogenetics, which rely on the modification of an endogenous receptor or the production of a modified receptor that responds to previously unrecognized molecule (Roth 2016), was used by Wang et al. (2017) to investigate whether activation of subicular GABAergic cells could have anti-ictogenic properties in *Vgat::hM3Dq* mice in an animal model of MTLE caused by intra-hippocampal injection of KA. These investigators reported that systemic administration of Clozapine N-oxide, a procedure that depolarizes (and thus excites) subicular GABAergic neurons in these transgenic mice, during the latent period significantly decreased the number of convulsive seizures up to three months after SE. However, when Clozapine N-oxide was administered during the chronic period, it prolonged the duration of seizures. Therefore, activation of GABAergic neurons in the subiculum early after SE can presumably restrain the spreading of focal ictal activity from the onset zone thus

preventing their generalisation (Wang et al. 2017). In contrast, this chemogenetic procedure facilitates ictogenesis when performed during the chronic period.

In vitro evidence

Experiments performed in combined hippocampus-entorhinal cortex brain slices obtained from control rats during bath application of the K^+ channel blocker and convulsive drug 4-aminopyridine have demonstrated that the subiculum plays a powerful gating role on hippocampal output activity. This function mainly depends on GABA_A receptor-mediated inhibition and it controls hippocampal-parahippocampal interactions that are known to be involved in the generation of limbic seizures (Benini and Avoli 2005). As illustrated in Figure 8A, field potential recordings obtained in these extended brain slices during application of 4-aminopyridine, revealed a pattern of fast interictal-like events that were evident in the hippocampal CA3 area, became of small amplitude in the subiculum, and failed to propagate to the entorhinal cortex where slow interictal and ictal epileptiform events could occur (Avoli et al. 1996; Barbarosie and Avoli 1997). However, antagonizing GABA_A receptors with picrotoxin made CA3-driven interictal activity spread to the entorhinal cortex, a phenomenon that corresponded to potentiation of the subicular network activity, an effect that was assessed with field potential recordings (Figure 8A). This change in excitability was characterized in subicular principal cells – which were recorded intracellularly – by the progressive disappearance of hyperpolarizing IPSP that, interestingly, appeared to be driven by the CA3 spikes under control conditions (Figure 8B, arrows in the expanded panels). The progressive changes induced by picrotoxin on the subicular IPSPs and their relation with the ability of CA3-driven interictal spikes to propagate to the entorhinal cortex are clearly shown in the experiment illustrated in Figure 8B (Benini and Avoli 2005). The function of GABA_A signaling in the subiculum was further addressed by Benini and Avoli (2005) by employing isolated subicular minislices. As illustrated in Figure 8C, low-amplitude synchronous events could be recorded in the presence of 4-aminopyridine in all areas of the minislice while subsequent addition of picrotoxin induced recurrent epileptiform discharges that were characterized intracellularly by typical paroxysmal depolarizing shifts (*cf.*, Schwartzkroin and Prince 1978). In Figure 8D, the intracellular activity corresponding to the synchronous events recorded in the presence of 4-aminopyridine only is illustrated at different membrane potentials; this experimental procedure revealed a reversal potential of the

Figure 8: Role of inhibition in the gating function of the subiculum.

A: Effects of the GABA_A receptor antagonist picrotoxin on the epileptiform activity induced by 4-aminopyridine (4AP); extracellular recordings were simultaneously obtained from the entorhinal cortex (EC), subiculum (Sub) and the CA3 subfield. Note under control conditions, the CA3-driven interictal discharges do not propagate to the EC (left lower inset) while after potentiation of the subicular network activity by picrotoxin (arrow), CA3-driven interictal events do propagate to the EC. Inserts show the position of the recording microelectrodes in the brain slice and an expanded interictal event occurring during application of 4AP only.

B: Simultaneous field (EC, CA3) and intracellular (subiculum, RMP = -60 mV) recordings during control (4AP) conditions show that this subicular cell is silent at RMP, that postsynaptic potentials coincide with the CA3 discharges interictal discharges (arrows in the expanded samples), and that a large amplitude inhibitory postsynaptic potential (IPSP) (asterisk) occurs when the interictal spike in CA3 is followed by a small amplitude event (*cf.*, Perreault and Avoli (1992). Monitoring the evolution of the intracellular activity recorded from the subicular neuron since the initial application of picrotoxin (early) until the complete synchronization between hippocampal and parahippocampal structures (late) shows IPSP disappearance. Insets below traces demonstrate expansions of selected events. **C:** Changes induced by antagonizing GABA_A receptors on the activity recorded simultaneously with field and intracellular recordings in the isolated subiculum during 4AP application; as shown in the inset, field recordings could be obtained from the proximal (prox), middle (mid) and distal portions of the subiculum; note that in the presence of picrotoxin there is increased field activity with robust intracellular bursting.

D: Intracellular recording of the 4AP-induced events at different RMPs, obtained by injection of intracellular current, reveal reversal potentials of the hyperpolarizing components at -73 and -76 mV. Note that in C and D action potentials were truncated. Panels A–D are modified from the study by Benini and Avoli (2005).

hyperpolarizing component of these events at membrane potentials between -73 and -76 mV suggesting the involvement of Cl^- and bicarbonate currents (*cf.*, Lamsa and Kaila 1997).

Knopp et al. (2005) have employed the pilocarpine model of MTLE to analyze both the electrophysiological and histological features of subicular neurons in non-epileptic control (NEC) and epileptic rodent brains. They found in these experiments that burst-spiking principal cells outnumbered regular firing neurons by about two to one in NEC brain slices and that this ratio ‘practically’ reversed in the epileptic tissue; as discussed below, these changes in bursting propensity were however not confirmed in a subsequent study published by de Guzman et al. (2006) and Knopp et al. (2005) also discovered enhanced network excitability, which included spontaneous rhythmic activity, polysynaptic responses, and all-or-none evoked bursts of action potentials, in the epileptic subiculum. In addition, they identified in the epileptic tissue a principal cell loss of about 30% along with reduced arborization and spine density in the proximal part of the apical dendrites suggesting a partial de-afferentation of the subiculum from the CA1 area (Knopp et al. 2005). In a subsequent study (Knopp et al. 2008), they identified a significant loss of parvalbumin- and calretinin-immunoreactive interneurons in the subiculum of pilocarpine-treated animals. These immune-histochemical findings were mirrored by a decrease in evoked inhibitory post-synaptic currents along with a low rate in occurrence of miniature inhibitory post-synaptic currents. Therefore, the results obtained by Knopp et al. (2008) suggest that pre-synaptic GABAergic inputs to subicular principal cells, and thus their inhibitory control, are decreased in epileptic animals.

Similar experiments were also carried by de Guzman et al. (2006), who studied the intrinsic and synaptic responses generated by subicular neurons following activation of CA1 and entorhinal cortex inputs as well as the function of inhibition by employing brain slices that were obtained by NEC and pilocarpine-treated epileptic rats. In these experiments, intracellular injection of pulses of depolarizing current induced a similar incidence of bursting and regular firing principal cells in the subiculum of brain slices obtained from NEC and pilocarpine-treated animals. However, as illustrated in Figure 9A, subicular neurons recorded in brain slices from these epileptic rats generated hyperexcitable responses when activated by inputs originating from the CA1 area; these responses included epileptiform bursts that were associated with a late depolarization that increased in amplitude during steady injection of hyperpolarizing current (Figure 9A, panel b in the pilocarpine-treated section). By computing input–output

curves of the postsynaptic responses generated by these subicular neurons prior to the appearance of action potential(s), de Guzman et al. (2006) found that the strength of the CA1 stimuli that were required to induce a half amplitude of the maximal response, was significantly higher in NEC than in pilocarpine-treated epileptic neurons (Figure 9B).

These investigators also reported that subicular neurons in the epileptic tissue responded to entorhinal cortex inputs by generating multiphasic postsynaptic oscillatory event that could last up to 500 ms (Figure 10A) as well as that subicular neurons in the epileptic tissue generated spontaneous postsynaptic potentials that were characterized by higher frequencies and more depolarized reversal potential than those recorded in NEC brain slices (Figure 10B) (de Guzman et al. 2006). As shown in Figure 10C, de Guzman et al. (2006) also found that

Figure 9: Changes in subicular neuron excitability in pilocarpine-treated epileptic rats

A: Electrical stimulation of CA1 networks reveals hyperexcitability within the epileptic subiculum. In NEC tissue, CA1 single-shock stimulation elicits a sequence of depolarizing–hyperpolarizing postsynaptic responses at RMP (-71 mV). Hyperpolarization of this neuron to -77 , -81 , and -88 mV produces stimulus-induced depolarizing postsynaptic events, while electrical stimulation elicits a single action potential when the cell is depolarized to -66 mV. In subicular neurons from pilocarpine-treated epileptic rats two types of response can be obtained. In the neuron shown in panel **a**, similar stimuli elicit action potential bursting at -65 mV while a depolarizing postsynaptic response is seen at RMP as well as at further hyperpolarized potentials. In another subicular neuron (panel **b**) stimulation induces all-or-none bursting activity that disappears only when the membrane is hyperpolarized to -92 mV.

B: Input–output curves of the postsynaptic responses generated prior to the appearance of action potential(s), in NEC (open dots; $n = 5$ experiments) and pilocarpine-treated epileptic tissue (black dots; $n = 6$). Boltzman sigmoidal parameters were used to fit the current response relationship. Stimulus strength able to evoke the half amplitude of response were significantly different. Panels A and B are modified from the study by de Guzman et al. (2006).

pharmacologically isolated, GABA_A receptor-mediated inhibitory postsynaptic potentials had more positive reversal potentials and a reduction in peak conductance in principle cells of the subiculum that were recorded in the pilocarpine-treated, epileptic tissue when compared with results obtained from NEC subicular neurons. In addition, these electrophysiological data correlated in the epileptic rat subiculum with reduced levels of mRNA expression and immunoreactivity of the cotransporter KCC2 as well as with increased synaptophysin (a putative marker of sprouting) immunoreactivity and decreased number of parvalbumin-positive cells (de Guzman et al. 2006). As already mentioned, Knopp et al. (2008) had previously identified a significant decrease of parvalbumin- and calretinin-immunoreactive interneurons in the subiculum of pilocarpine-treated epileptic animals. Therefore, these data – which were obtained in a rodent model of MTLE – indicate that although cell loss in the

subiculum has not been considered as a pathogenic factor in human MTLE (see Gloor 1997), the vulnerability of subicular GABAergic interneurons may cause an input-specific disturbance of the inhibitory system in this limbic structure.

Evidence obtained *in vitro* by analyzing the field activity recorded from brain slices obtained from pilocarpine-treated epileptic and NEC rodents, during application of 4-aminopyridine, also indicate that the function of the subiculum in gating epileptiform synchronization within the hippocampus-entorhinal cortex circuit is modified in this model of MTLE. First, D'Antuono et al. (2002) reported that entorhinal cortex-driven ictal-like discharges occur throughout the experiment (up to 6 h) in pilocarpine-treated slices while they disappear in NEC slices within 2 h of 4AP application (*cf.* Barbarosie and Avoli 1997). Second, they found that these ictal events could spread to the CA1/subicular

Figure 10: Changes in subicular neuron excitability in pilocarpine-treated epileptic rats

A: In **a**, Single-shock stimulation of entorhinal cortex layer III induces hyperexcitable responses in the pilocarpine-treated subiculum. Note the monophasic postsynaptic response in the NEC experiment and the multiphasic postsynaptic activity in the pilocarpine treated tissue. In **b**, plot of the response duration recorded from neurons recorded in slices obtained from NEC ($n = 13$) and pilocarpine-treated rats ($n = 13$). **B:** In **a**, spontaneous postsynaptic potentials (PSPs), exhibiting excitatory and inhibitory components, occur at higher frequency in subicular neurons from pilocarpine-treated rats as compared to those from NEC animals. Note the changes in polarity induced on both types of cells by hyperpolarizing the membrane potential. In **b** and **c**, plots of the frequency of the spontaneous PSPs and of their reversal potential in NEC and pilocarpine-treated rats. Both values are significantly different. Data were obtained from 12 NEC and 10 pilocarpine-treated subicular neurons. **C:** The pilocarpine treated subiculum exhibits a more positive reversal potential of the pharmacologically isolated GABA_A receptor-mediated IPSP. In **a**, intracellular recording obtained from NEC and pilocarpine-treated subicular neurons during single-shock stimulation in the presence of glutamatergic antagonists. In **b**, mean values of the reversal potential of the pharmacologically isolated IPSPs are significantly more positive in pilocarpine-treated tissue compared to NEC. Note also that a significant reduction in the IPSP peak conductance occurs in the pilocarpine-treated subiculum. Panels A–C is modified from the study by de Guzman et al. (2006).

area via the temporoammonic path (*cf.*, Soltesz and Jones 1995) in epileptic brain slices while they propagate via the trisynaptic hippocampal circuit (*cf.*, Paré et al. 1992) in those that were obtained from NEC animals; such conclusion is supported by the field potential depth profile analysis of the ictal discharges recorded in the subiculum of NEC and pilocarpine-treated mice (Figure 11A–C). Functionally, this change in modality of propagation should allow the ictal activity that initiates in the entorhinal cortex to short-circuit the trisynaptic hippocampal circuit, thus mono-synaptically activating subicular and CA1 neurons. According to the conclusions made by (D’Antuono et al. 2002) this change in propagation modality should ensure a high-fidelity synaptic transfer of ictal discharges between the entorhinal cortex and the hippocampus proper (Table 1).

Further experiments performed in rat pilocarpine-treated brain slices have confirmed that ictal-like discharges induced in the epileptic tissue by bath application

of 4-aminopyridine are reinforced by interactions between entorhinal cortex and subicular networks (Panuccio et al. 2010). As shown in Figure 11D and E, surgical disconnection of the entorhinal cortex from the subiculum reproduced the effects induced by cutting the Schaffer collaterals in NEC slices (Barbarosie and Avoli 1997) while this experimental procedure reduced the duration of ictal-like discharges, which continued to propagate to the hippocampus through the dentate gyrus – in slices obtained from pilocarpine-treated rats. Therefore, these results confirm a functional switch from the trisynaptic to the temporoammonic route (Witter et al. 1989) in sustaining ictal-like synchronization in epileptic limbic networks. As discussed in Section 4.2, the involvement of the subiculum in ictogenesis is supported by the ability of this region to generate spontaneous rhythmic discharges in human epileptic tissue (Cohen et al. 2002; Wozny et al. 2003). It should also be mentioned that Le Duigou et al. (2008) have recorded spontaneous interictal field potentials, in the absence of any pharmacological

Figure 11: Field potential activity generated *in vitro* by brain slices obtained from control and pilocarpine-treated epileptic rats.

A to C: Depth profile characteristics of the field potentials associated with the ictal discharges recorded in the CA1/subicular area of NEC and pilocarpine-treated slices. In **A**, schematic representation of the experimental procedure and field potential recordings obtained at different depths in NEC and pilocarpine-treated slices. In **B** and **C**, depth distribution of the amplitudes of the fast events (population spikes) and of the DC shifts associated with the ictal discharges in NEC (black dots) and pilocarpine-treated slices (unfilled squares). Note that the amplitudes of both fast transients and DC shifts in pilocarpine-treated slices attain maximal values at depths that are greater than those identified in NEC slices. **D:** Slice schematic illustrating the extension of the knife cut performed to lesion the connections between entorhinal cortex (EC) and subiculum (Sub) (dashed line). Note that the epileptiform activity induced by 4-aminopyridine changes following the cut in both NEC and pilocarpine-treated slices and that interictal activity no longer propagates to the entorhinal cortex in both cases. However, in NEC slices this procedure discloses ictal-like activity spreading to the subiculum, whereas in pilocarpine-treated it causes a decrease in ictal-like discharge duration. **E:** Quantification of the effects induced on the ictal-like activity induced by 4-aminopyridine by cutting the connections between entorhinal cortex and subiculum in NEC and pilocarpine-treated slices. Panels A–C is modified from the study by D’Antuono et al. (2002) while D and E are from the study by Panuccio et al. (2010).

Table 1: Summary of findings on subicular function in animal models of MTLE.

<i>In vivo</i> evidence	References
Spontaneous seizures in the pilocarpine model of MTLE most often initiate in CA3 and subiculum	Lévesque et al. 2012; Toyoda et al. 2013
High rates of FRs, which are biomarkers of pathological activity, occur in CA3 and subiculum in the pilocarpine model of MTLE	Behr et al. 2017; Lévesque et al. 2012
Subicular interneurons show the largest activity increase before seizure onset in the pilocarpine model of MTLE	Toyoda et al. 2015
High PAC between slow (0.8–1.4 Hz) and fast (80–250 Hz) oscillations occurs in CA3 and subiculum during interictal periods in the pilocarpine model of MTLE	Samiee et al. 2018
Depolarisation of subicular interneurons with chemogenetics during the latent period decreases the frequency of spontaneous seizures in the kainic acid model of MTLE	Wang et al. 2017
The same procedure performed during the chronic period prolongs seizure duration	Wang et al. 2017
<i>In vivo</i> evidence	References
Subiculum controls hippocampal-parahippocampal interactions through GABAA receptor signaling	Benini and Avoli 2005
Subicular network activity is potentiated by the GABAA receptor antagonist picrotoxin thus making CA3-driven interictal activity spread to the entorhinal cortex	Benini and Avoli 2005
Network activity is enhanced in the subiculum of pilocarpine-treated slices along with interneuron loss; hence, GABAergic inputs to subicular principal cells is decreased in epileptic animals	Knopp et al. 2005, 2008
Subicular neurons in pilocarpine-treated slices respond to entorhinal cortex inputs with multiphasic oscillatory events and generate spontaneous postsynaptic potentials that are characterized by higher frequencies and more depolarized reversal potentials than in controls	De Guzman et al. 2006
Reduced mRNA expression and immunoreactivity of the cotransporter KCC2, increased synaptophysin, and decreased number of parvalbumin-positive cells occurs in the epileptic rat subiculum	De Guzman et al. 2006
In the 4AP model, the subicular role in gating epileptiform activity within hippocampus-entorhinal cortex circuit is modified; this allows ictal activity initiating in the entorhinal cortex to shortcut the tri-synaptic circuit and to activate subicular and CA1 neurons through mono-synaptic connections.	D'Antuono et al. 2001a, 2002; Panuccio et al. 2010

4AP, 4-aminopyridine; FRs, fast ripples (250–500 Hz); MLTE, mesial temporal lobe epilepsy; PAC, phase-amplitude coupling.

treatment, from longitudinal hippocampal slices that were obtained from KA-treated epileptic rats; these investigators found that these interictal events could be initiated in the CA1 and CA3 regions as well as in the subiculum.

Role of the subiculum in human focal epileptic disorders

In vivo evidence

One of the first histological description of the temporal lobe in patients with chronic focal disorders was performed by Bratz (1889), who provided a detailed description of the atrophic hippocampus. He identified neuronal loss in the Ammon's horn along with an extensive neuronal loss in the CA1/CA3 region of the hippocampus while the CA2 subfield, the pre-subiculum, subiculum and the granule cell layer of the dentate gyrus appeared to be preserved. The preservation of the

subiculum still holds true today since subsequent studies have demonstrated that only minor cell loss occurs in this region (Alonso-Nanclares et al. 2011; Fabo et al. 2008; Fisher et al. 1998; Furtinger et al. 2001; Miyata et al. 2013). However, it should be emphasized that epileptic patients without hippocampal sclerosis showed less neuronal damage in the subiculum compared to patients who presented with sclerosis (Furtinger et al. 2001).

The recent development of diffusion magnetic resonance imaging has enabled the quantification of region connectivity and fiber integrity in patients with focal epileptic disorders. Using this method, Rutland et al. (2018) reported that patients presenting with focal epileptic disorders have increased fiber density and increased degree of connectivity in the presubiculum, parasubiculum and subiculum ipsilateral to the seizure onset zone compared to healthy controls. Therefore, these findings further suggest that synaptic reorganization occurs during epileptogenesis. These changes in neuronal circuitry presumably contribute to the pathological synchronization of network

activity within the limbic system and to the transition from interictal to ictal discharges.

Depth electrode recordings in the subiculum of patients presenting with focal epileptic disorders have been performed only in few studies. Staba et al. (2002) found that subicular neurons fired at higher frequencies compared to hippocampal neurons in these epileptic patients. Firing rates of single units in the subiculum and the hippocampus were also higher during slow wave sleep compared to awake states and REM sleep (Staba et al. 2002). Therefore, these findings suggest that signal transmission across neural networks is increased in these regions as well as that such condition should create a low threshold for the initiation and spread of ictal discharges within the limbic system.

A similar active role of the subiculum was also proposed by Fabo et al. (2008), who recorded with depth electrodes both field potential and multiple unit activity in the subiculum of patients presenting with MTLE under general anesthesia. They reported the existence of two types of interictal spikes (type 1 and type 2) with distinct waveform shapes and patterns of multiunit activity. Type 1 interictal spikes were associated with current source density sinks in the pyramidal layer, an increase of multiunit activity during the peak of the initial spike component and oscillations in the high-frequency range (100–200 Hz). In contrast, type 2 interictal spikes were characterized by sources in the somatic region and sinks in the apical dendritic region, an increase of multiunit activity in the somatic layer of the subiculum and oscillations in low frequency ranges (<100 Hz). Both types of interictal spikes were observed by Fabo et al. (2008) in the majority of patients; however, those patients who presented with severe hippocampal sclerosis showed higher rates of type 2 spikes compared to those with mild hippocampal sclerosis. When comparing the synchronicity of interictal spikes recorded from the subiculum and other regions of the temporal lobe, these investigators found that type 1 spikes recorded from the subiculum showed a high degree of synchrony with temporal lobe interictal spikes and that they often preceded them, suggesting that a subicular ‘focus’ could actively contribute to the projection of epileptic activity to other brain regions (Fabo et al. 2008).

***In vitro* evidence**

A striking discovery regarding the functional changes of subicular neurons in epileptic patients was originally reported by Cohen et al. (2002). These investigators found that spontaneous, synchronous and rhythmic activity

could be recorded from subicular networks in brain slices obtained from patients with MTLE. These field events resembled the interictal spikes recorded in the EEG and, interestingly, were blocked by either GABA_A or ionotropic glutamatergic receptor indicating that the neurons involved in this rhythmic activity included both interneurons and a subgroup of pyramidal cells, which generated depolarizing GABAergic synaptic events in “neurons downstream to the sclerotic CA1 region” (Cohen et al. 2002). Similar findings were, shortly after, reported by Wozny et al. (2003, 2005), who recorded spontaneous synchronous events – which were largely contributed by GABA_A receptor-dependent currents – even in brain slices obtained from epileptic patients who did not present with hippocampal sclerosis. It should also be emphasized that earlier *in vitro* studies, which had been performed on brain slices that were obtained from post-surgical human tissue of patients with pharmacoresistant temporal lobe epilepsy, had identified spontaneous sharp waves (Köhling et al. 1998) or rhythmic synchronous activities (Schwartzkroin and Haglund 1986) that were supported by GABA_A receptor-mediated conductances in the neocortex or hippocampus, respectively.

Further studies have shown that changes in Cl[−] homeostasis are involved in the generation of the spontaneous interictal-like spikes that were recorded, and indeed initiated, in the human epileptic subiculum. Huberfeld et al. (2007), employing intracellular recordings, found that in a minority of subicular pyramidal cells these interictal-like spikes were associated to depolarizing GABA_A receptor-mediated postsynaptic events thus indicating perturbed Cl[−] homeostasis. In addition, they demonstrated with *in situ* hybridization that mRNA for KCC2 was absent from approx. 30% of subicular pyramidal cells as well as that neurons that were hyperpolarized during interictal spikes were immunopositive for KCC2 while those generating depolarizations were immunonegative. Overall, these experiments firmly established that decreased KCC2 function can switch GABA_A receptor signaling from inhibitory to excitatory, thus increasing neuronal excitability and, perhaps, contributing to human epileptiform activity (Huberfeld et al. 2007). These data are strikingly similar to the GABA_A receptor-mediated excitation that occurs during early development and results from delayed expression of the KCC2 co-transporter (Ben-Ari et al. 1989; Cherubini et al. 1990; Payne et al. 2003; Rivera et al. 1999). Accordingly, Cohen et al. (Cohen et al. 2002) proposed in their original human subiculum study that “deafferentation may initiate a regressive switch in GABAergic response polarity from hyperpolarizing to depolarizing (Vale and Sanes 2000), perhaps in the most

severely denervated cells”. This view was also tested and proved true by employing a computational model of the subicular network with realistic connectivity in which pyramidal cells incorporated the cotransporter KCC2 and its effects that were exerted on the intracellular/ extracellular concentrations of Cl^- and K^+ (Buchin et al. 2016). Therefore, these studies suggest that molecules acting on this cotransporter may represent useful antiepileptic drugs (*cf.*, Di Cristo et al. 2018; Kaila et al. 2014).

Subicular slices obtained from epileptic patients presenting with MTLE have been also employed to identify the mechanisms involved in the transition to seizure-like activity *in vitro*. In these experiments, Huberfeld et al. (2011) induced ictal-like activity in the subiculum by concomitant pharmacological manipulations and found that the transition to ictal discharge was characterized by pre-ictal spikes that mainly depended on glutamatergic mechanisms and were preceded by pyramidal cell firing, while – as just discussed – interneuron firing preceded interictal spikes that were contributed by both glutamatergic and depolarizing GABAergic conductances. Therefore, these findings suggest that the transition to seizures in the human subiculum, at least under the experimental manipulations used in this *in vitro* study, involves an emergent glutamatergic population activity (Huberfeld et al. 2011).

Regarding the mechanisms that may contribute to hyperexcitability in the human epileptic subiculum, Vreugdenhil et al. (2004) studied with whole-cell recordings the voltage-gated Na^+ currents in pyramidal neurons isolated from the subiculum resected in drug-resistant epileptic patients and in rats. In this study, they could identify a TTX-sensitive, slowly or non-inactivating Na^+ current that displayed density and “relative amplitude contribution”, 3–4 times greater in neurons from drug-resistant epilepsy patients compared to those from rats. Therefore, they have ascribed to voltage-dependent Na^+ currents a role in making seizures occur in epileptic patients (Vreugdenhil et al. 2004). Moreover, since voltage-dependent Na^+ currents contribute to the bursting responses generated by subicular principal cells (see Section 2.1), it is important to mention that Wozny et al. (2005) found in the subiculum of sclerotic and non-sclerotic human tissue similar ratios between bursting and regular firing cells, i.e., 75 and 79%, respectively. These findings were surprising since the same group of investigators reported in the pilocarpine model of MTLE that regular firing neurons predominated over bursters in the subiculum of brain slices that were obtained from pilocarpine-treated epileptic rats (Knopp et al. 2005) (see also Section 3.2).

HFOs have been also analyzed in slices of the subiculum obtained from human hippocampal tissue resected

for treatment of pharmacoresistant epilepsy (Alvarado-Rojas et al. 2015). It was found in this study that ripples (150–250 Hz) occurred spontaneously in extracellular field potentials during interictal discharges (see Cohen et al. 2002) as well as during the pharmacologically-induced pre-ictal discharges preceding ictal activity (see Huberfeld et al. 2011). Notably, ripples occurring in the subiculum during interictal or pre-ictal discharges were associated to different cellular processes: (i) ripples that occurred during interictal discharges were characterized by heterogeneous firing patterns accompanied by rhythmic inhibitory IPSPs in pyramidal cells, which – remarkably – were similar to what was reported for physiological ripples (Ylinen et al. 1995). On the contrary, ripples associated to pre-ictal discharges depended on suprathreshold depolarizations (presumably reflecting glutamatergic EPSPs) that could trigger single cell bursting (Bragin et al. 2011; Ibarz et al. 2010). Therefore, ripples associated to pre-ictal discharges may reflect impaired inhibition and thus mirror dysfunctional synchronization among subicular principal cells.

Subiculum, antiepileptic drugs, and pharmacoresistance

The relationship between the function of the subiculum and its impact on the action of antiepileptic drugs has not, as yet, been extensively investigated. However, two *in vivo* studies – which were performed in the rodent pilocarpine model of MTLE – have shown that pathological network activity in the subiculum is modulated by at least two antiepileptic drugs. Specifically, Lévesque et al. (2015) reported that in epileptic rats treated with a systemic administration of levetiracetam – a second generation anti-epileptic drug that presumably prevents excessive excitatory transmission by controlling the process of pre-synaptic glutamate release (Deshpande and De Lorenzo 2014; Lynch et al. 2004) – rates of seizures and rates of interictal spikes associated with fast ripples are significantly lower in CA3 and subiculum than in untreated controls within two weeks after SE (Figure 12A and B). Similar results were obtained in another study in which lacosamide, an antiepileptic drug that acts on voltage-gated Na^+ channels and presumably facilitates the slow inactivation component of Na^+ channels (Curia et al. 2009; Errington et al. 2008; Niespodziany et al. 2013), was systemically administered to pilocarpine-treated epileptic rats. Similar to what was observed with levetiracetam, seizures as well as fast ripples occurred at lower rates in the hippocampus and subiculum in treated

animals compared to controls (Figure 12C and D) (Behr et al. 2015). These findings, therefore, support the hypothesis that the hyperexcitability of subicular networks following a pilocarpine-induced SE contributes to ictogenesis and epileptogenesis in this model of MTLE (de Guzman et al. 2006; Knopp et al. 2008, p. 2008) as well as that antiepileptic drugs can, at least partially, control these pathological changes. The view that the subiculum represents a target mechanism for anticonvulsant action is in line with what reported by Zhong et al. (2012) who have found in the pilocarpine model of MTLE that 15 min daily epochs of low-frequency stimulation delivered in the rat subiculum at 1 Hz can significantly reduce the occurrence of spontaneous seizures; such an effect was documented up to 35 days after the initial pilocarpine-induced SE.

An *in vitro* study by Kawasaki et al. (1998) has identified some potential targets for the anticonvulsant effects of

topiramate in the rat subiculum. The exact mechanisms of action of topiramate remain unclear to date but data obtained by different researchers suggest that this antiepileptic drug can block voltage-dependent Na^+ and Ca^{2+} currents, inhibit ionotropic glutamatergic transmission and enhance GABA_A receptor signaling (Braga et al. 2009; Kaminski et al. 2004; Qian and Noebels 2003; White et al. 2000, 1997; Zona et al. 1997). As shown in Figure 13A, topiramate induced a steady hyperpolarization (from -59 to -67 mV in this specific experiment) of the membrane potential of subicular principal neurons while depressing the ability of these cells to generate action potential bursts in response to brief (5–150 ms long) depolarizing pulses. These effects were characterized by an increase in the amount of intracellular depolarizing current required for eliciting action potential bursts, and they were also evident when the topiramate-induced steady hyperpolarization was compensated by injecting steady depolarizing current

Figure 12: Effects of antiepileptic drugs on the subiculum of pilocarpine-treated animals.

A: Average seizure rates in pilocarpine-treated controls and in pilocarpine-treated animals in which levetiracetam (LEV) was systemically administered for 14 days. Seizure rates were significantly lower in LEV-treated animals compared to controls ($*p < 0.05$). **B:** Rates of interictal spikes (IIS) with fast ripples (FR) in controls and LEV-treated animals in the subiculum and the CA3 region of the hippocampus. In both regions, rates of interictal spikes with fast ripples were significantly lower in LEV-treated animals compared to controls ($*p < 0.01$). **C:** Average seizure rates in pilocarpine-treated controls and in pilocarpine-treated animals in which lacosamide (LCM) was systemically administered for 14 days. Seizure rates were significantly lower in LCM-treated animals compared to controls ($**p < 0.01$). **D:** Rates of fast ripples associated to interictal spikes in controls and in LCM-treated animals in the subiculum and the CA3 region of the hippocampus. In the subiculum, between day 10 and day 13 after SE, rates of fast ripples associated to interictal spikes were significantly lower in LCM-treated compared to controls ($**p < 0.01$). In CA3, rates of fast ripples associated to interictal spikes were significantly lower in LCM-treated animals compared to controls, only on day 9 after SE. Panels A and B are modified from the study by Lévesque et al. (2015) while D and E are from the study by Behr et al. (2015).

(Figure 13B). In addition, topiramate reduced by approx. 50% the regular action potential firing elicited by prolonged 350–1000 ms depolarizing pulses (Figure 13C). Kawasaki et al. (1998) also reported that the steady hyperpolarization elicited by topiramate in subicular bursting cells was markedly reduced by GABA_A receptor antagonists (not illustrated); this evidence is in line with what reported by (Braga et al. 2009) regarding the effects induced by topiramate in principle neurons of the rat basolateral amygdala *in vitro*. Hence, these findings indicate that the antiepileptic drug topiramate reduces subicular cell excitability by decreasing voltage-gated, Na⁺ electrogenesis and by enhancing GABA_A receptor signaling.

Further experiments (D'Antuono et al. 2007; Palmieri et al. 2000) have investigated whether topiramate and lamotrigine, another antiepileptic drug that is effective in treating patients with focal epileptic disorders (Doose et al. 2003; Gilman 1995), can control epileptiform synchronization and intrinsic depolarizing responses generated *in vitro* by the rat subiculum during activation of

muscarinic receptors caused by bath application of CCh (see Section 2.2). As illustrated in Figure 14A, both topiramate (panel a) and lamotrigine (panel b) decreased in a dose-dependent manner, and eventually abolished, the CCh-induced field oscillations. However, when these antiepileptic drugs were tested on the depolarizing plateau potentials induced by short pulses of depolarizing current in the presence of CCh and ionotropic glutamatergic and GABA receptor antagonists, only topiramate could significantly reduce these intrinsic excitatory responses (Figure 14B) (D'Antuono et al. 2007). These results, therefore, support the view that muscarinic excitation may represent a good, as yet unexplored, target for antiepileptic drug action.

A recent study by Xu et al. (2019) has further established the role of the subiculum in focal seizure disorders by demonstrating that it could play a role in pharmacoresistance. Specifically, they investigated whether modulating the activity of pyramidal neurons in the subiculum could represent a potential target for the control of seizures that are resistant to the standard antiepileptic drug

Figure 13: Effects of the antiepileptic drug topiramate (TPM) on the excitability of subicular neurons *in vitro*.

A and B: Effects induced by topiramate (TPM; 100 and 500 μ M, respectively) on the bursting responses generated by two different subicular neurons. In both cases, TPM hyperpolarizes the RMP and increases the amount of depolarizing current required for eliciting the burst response. Note in in A that the action potential bursts are less frequently triggered upon termination hyperpolarizing commands in the presence of TPM, and in B that the bursting response is decreased even when the RMP is brought to control values. **C:** Effects induced by TPM (500 μ M) on the repetitive action potential firing generated by a subicular bursting neuron during prolonged pulses of depolarizing current (**a**) and plot of the amount of repetitive firing induced by different intensities of intracellular depolarizing current in five subicular neurons under control conditions and during application of TPM (**b**). Note that the RMP during application of TPM was kept at values similar to those seen under control conditions as well as that differences were significant with all current intensities but 0.1 nA. Panels A–C is modified from the study by Kawasaki et al. (1998).

Figure 14: Effects of the antiepileptic drug topiramate (TPM) and lamotrigine (LTG) on the excitability of subicular neurons during carbachol (CCh) treatment.

A: Topiramate (TPM, panel **a**) and lamotrigine (LTG, panel **b**) depress the spontaneous field discharges induced by CCh in the rat subiculum *in vitro*. **B:** Lamotrigine (LTG) does not abolish the depolarizing plateau potential and associated burst firing induced by an intracellular depolarizing pulse during concomitant application of CCh along with excitatory amino acid ionotropic receptor (CNQX and CPP) and GABA receptor (picrotoxin and CGP 35348) antagonists. This response is however markedly reduced by topiramate. Panels A and B are modified from the study by D'Antuono et al. (2007).

phenytoin in a model of MTLE. They showed that optogenetic inhibition of pyramidal subicular neurons can reverse phenytoin resistance whereas activation of these neurons could induce resistance. A similar modulation of pyramidal neurons in the CA1 region of the hippocampus had no effect on phenytoin resistance. To note that the inhibition of subicular pyramidal neuron activity with chemogenetic procedures was also able to reverse phenytoin resistance in animals in which systemic injections of clozapine-N-oxide were performed to inhibit the firing of pyramidal neurons in the subiculum (Xu et al. 2019). According to these authors, pharmacoresistance and subiculum activity could be linked through voltage-dependent Na^+ channel inhibitors, since intrasubicular administration of TTX – which blocked voltage-dependent Na^+ currents and thus action potential firing in subicular neurons – reversed the pharmacoresistance to phenytoin (Xu et al. 2019). Subicular pyramidal neurons may therefore represent a target to control pharmacoresistance in MTLE.

Concluding remarks

In this review, we have focused on the role played by neuronal networks of the subicular complex in focal epileptic disorders such as MTLE, and we have mainly addressed the changes in neuronal excitability occurring under this pathological condition. As shown in Figure 2, the anatomical localization of the subiculum makes this structure control and elaborate hippocampal output activity while receiving inputs from the entorhinal cortex and, through this structure, from several para-hippocampal areas. To note that the subiculum projects also to the hippocampus proper, and specifically to the CA1 subfield. Therefore, in contrast to other brain areas that mainly function as unidirectional input-output relays (e.g., dentate gyrus or CA3 subfield, see Figure 2), the subiculum has a privileged location for modulating bidirectionally the spread of focal epileptic discharges in MTLE and perhaps for initiating them.

In addition, principal (glutamatergic) pyramidal neurons in the subiculum are often capable of generating bursts of action potentials in response to intracellular pulses of depolarizing current. Such bursting propensity, under physiological conditions, may contribute to synaptic plasticity; however, action potential bursting is also a characteristic of epileptiform activity. Unfortunately, inconsistent findings obtained in the studies published to date by analyzing both animal models (see Section 3.2) and patients presenting with MTLE (see Section 4.2), do not allow us to reach any firm conclusion on the exact contribution of the intrinsic burst firing generated by subicular principal cells to focal seizure generation as well as to epileptogenesis in MTLE.

On the contrary, there is firm evidence pointing to anomalies in GABA_A receptor signaling that occur in the subiculum both in human MTLE and in animal models mimicking this disorder. First, it has been shown that subicular slices obtained from MTLE patients generate spontaneous interictal spikes that are associated, in some pyramidal cells, to depolarizing GABA_A receptor-mediated currents suggesting perturbed Cl^- homeostasis (see Section 4.2). A similar shift in the depolarizing direction of GABA_A receptor-mediated postsynaptic potentials has also been identified in principle cells of the subiculum of epileptic rodents (see Section 3.2). Second, the well documented vulnerability of subicular GABA_A ergic interneurons occurring in epileptic rats indicates a network anomaly that should cause an input-specific disturbance of the inhibitory system that may contribute

to hyperexcitability and thus ictogenesis in this limbic structure (see Section 3.2).

We have also review studies that have addressed the impact of antiepileptic strategies on the subiculum (see Section 5). At least two antiepileptic drugs can reduce the occurrence of seizures that initiate in the hippocampus and subiculum in pilocarpine-treated epileptic animals *in vivo*. Moreover, subicular cells respond to several antiepileptic compounds that can reduce voltage-gated Na⁺ currents, enhance GABA_A receptor signaling and control the oscillatory bursting activity induced by CCh *in vitro*. Finally, a recent study has identified a role of the subiculum in pharmacoresistance, specifically that inhibition of subicular pyramidal neuron activity with chemogenetic procedures can reverse phenytoin resistance. In conclusion, although several questions regarding the role of the subiculum in epileptiform synchronization and epileptogenesis remain unanswered, the studies that we have reviewed here, reinforce the view that this structure represents a limbic area that plays fundamental roles in focal epileptic disorders, and in particular in MTLE.

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