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Review Article

Piriform cortex ictogenicity *in vitro*

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A B S T R A C T

The piriform cortex is recognized to play critical roles in focal ictogenesis, both in animal models and in humans. We review here the contribution of *in vitro* studies performed on rodent brain tissue that were aimed at understanding the ictogenic properties of the piriform cortex and the contiguous olfactory areas. During *in vitro* experiments, epileptiform events can be easily generated in the piriform area by diverse pro-convulsive drugs (4-aminopyridine, bicuculline, picrotoxin) or by electrical stimulation. Simultaneous intracellular and field potential recordings performed on *in vitro* preparations, which include brain slices of rats and mice and the isolated brains of guinea pigs, demonstrated that both the piriform cortex proper and the endopiriform nucleus (also considered part of the piriform area) generate interictal spikes, high-frequency oscillations and seizure-like activities that mimic focal discharges. These findings were confirmed both by optical recordings of intrinsic signals coupled with brain activity and by fast imaging of optical signals generated by voltage-sensitive dyes. Overall, these studies demonstrated that epileptiform discharges effectively propagate from the piriform structures to the limbic regions, supporting the conditions for secondarily generalized ictogenesis.

1. Introduction

The piriform cortex (PC) is a three-layered paleocortex implicated in the olfactory sensory system in mammals. It is considered a primary olfactory sensory region and retains a diffuse and broad associative connectivity with its main afferent input region, the olfactory bulb (OB). The PC is broadly connected with several other olfactory and limbic areas, such as the olfactory tubercle, the anterior olfactory nucleus and the lateral entorhinal cortex as well as with subcortical areas (for review see Loescher and Ebert, 1996; Neville and Haberly, 2004; Vismer et al., 2015). In relation to such extended associative connectivity, a recent study (Meissner-Bernard et al., 2019) has confirmed that the PC is not just involved in odor perception, but represent a critical component for olfactory memory traces (for review see Wilson and Sullivan, 2011). It is also worth to emphasize that the PC conveys direct sensory information to the limbic-hippocampal system without any interposed synaptic station in subcortical and thalamic nuclei, as it is observed for all other sensory inputs.

The PC is subdivided in a rostral and a caudal portion that mainly differ in the proportion of afferent/associative fiber content in the superficial plexiform layer I. The anterior piriform cortex (APC) extends between the OBs and the surface point where the lateral olfactory tract (LOT, which is formed by OB mitral cell axons) disappears as a defined structure at the ventral cortical surface (arrowhead in Fig. 1A). LOT fibers fan out in both the APC and the more caudal posterior piriform cortex (PPC), the latter being characterized by a thicker intra-PC associative fiber layer (see below). In the most anterior portion of the PC a deeper region termed the endopiriform nucleus has been identified (EPN; Fig. 1B).

Because of its relatively simple arrangement, at least when compared to the six-layered neocortex and in virtue of its extensive rostral-to-caudal homogeneous and highly laminar organization, the PC was one of the most studied cortical regions in early *in vitro* brain slice studies (Yamamoto and McIlwain, 1966; Harvey et al., 1974; Scholfield, 1978). As reviewed in the initial part of our review, these experiments have set the basis for defining the functional connectivity of the PC. In addition, we will address here the ability of PC networks to generate epileptiform patterns under different experimental conditions that include the 4-aminopyridine (4AP) model of epileptiform synchronization.

Abbreviations: 4AP, 4-aminopyridine; APC, Anterior Piriform Cortex; EPN, Endopiriform Nucleus; EC, Entorhinal Cortex; HFOs, High-Frequency Oscillations; LOT, Lateral Olfactory Tract; OB, Olfactory Bulbs; PC, Piriform Cortex; PPC, Posterior Piriform Cortex; SLEs, Seizure-Like Events

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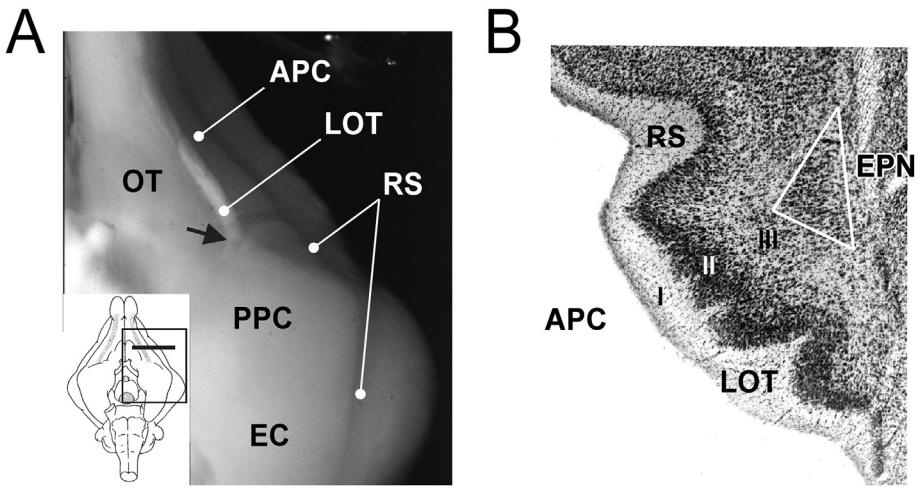

Fig. 1. A. Picture of the ventral view of the olfactory area in the *in vitro* isolated guinea pig brain. *APC* = anterior piriform cortex; *OT* = olfactory tubercle; *LOT* = lateral olfactory tract; *PPC* = posterior piriform cortex; *EC* = entorhinal cortex; *RS* = rhinal sulcus. B. Thionine-stained coronal section of the guinea pig brain that illustrates the positions of the *APC*, the *LOT* and the *RS*. The rostro-caudal level of the coronal slice is illustrated as a thick line in the drawing in A. The endopiriform nucleus (*EPN*) is outlined by the white triangle. Layers I, II and III of the APC are marked.

2. Organization of the piriform cortex networks

The anatomy of the mammalian PC and its intrinsic and extrinsic connections have been extensively analyzed in the 1970s and 80s (for review see Krettek and Price, 1977; Luskin and Price, 1983a,b; Haberly, 2001; Neville and Haberly, 2004). As detailed elsewhere in this Special Issue, the PC is organized with a plexiform layer I, which is virtually without neurons except some sparse GABAergic interneurons (Suzuki and Bekkers, 2012; Large et al., 2016), and two cellular layers II and III, with higher and lower neuron density, respectively (Fig. 1B). Moreover, the layer I of the PC is subdivided in layer Ia, in which LOT fibers formed by the axons of OB mitral cells are running, and layer Ib that consists of intra-PC associative cortico-cortical fibers. Layer Ia is thicker in the APC, and associative fibers forming layer Ib are more abundant in the PPC (Price, 1973; Haberly and Price, 1978; Neville and Haberly, 2004). Cellular layers II and III contain pyramidal neurons that extend a principal dendrite toward the plexiform layer, where a dense net of laminarly arranged excitatory synapses are formed by terminals of afferent (layer Ia) and associative fibers (layer Ib). Both layers II and III contain pyramidal and multipolar neurons and different subpopulations of GABAergic inhibitory interneurons (Neville and Haberly, 2004; Suzuki and Bekkers, 2011). The EPN is formed by spiny multipolar neurons interconnected with the more superficial neurons in layers II and III (Behan and Haberly, 1999).

On the basis of this anatomical organization, the seminal studies of Lewis Haberly and colleagues have set the functional groundwork of the intrinsic PC networks. This work began with series of neurophysiological experiments performed *in vivo* in the opossum and in the rat brain; these studies utilized a lateral approach to record with extracellular electrodes the field potentials generated by the PC networks (Haberly, 1973; Rodriguez and Haberly, 1989; Ketchum and Haberly, 1993; for review see Neville and Haberly, 2004). These studies were then followed by intracellular recordings performed both *in vivo* and *in vitro* from rat brain slices, thus further detailing the contribution of individual subpopulations in the superficial and deep layers neurons (including the EPN) to the patterns of networks activity generated in the PC (Haberly and Bower, 1984; Hoffman and Haberly, 1989, 1991; Tseng and Haberly, 1989a,b). The results obtained from these experiments complemented with previous reports focusing on the characterization of synaptic potentials generated by PC neurons (Scholfield, 1978; Constanti et al., 1980).

More recent *in vitro* studies confirmed the extensive connectivity of pyramidal PC neurons that forms a large associative excitatory network (Franks et al., 2011). Moreover, intra-PC GABAergic networks responsible for feedback and feedforward inhibition of PC pyramidal cells have been described (Suzuki and Bekkers, 2011, 2012; Large et al., 2016). Besides the neurophysiological methods applied to study epileptiform events in the PC, newly developed techniques, such as chemogenetic and optogenetic procedures, demonstrated to be helpful tools to improve the identification of the neuronal elements and the synaptic network critical for seizure initiation and propagation. With these methods it is possible to achieve inhibitory or excitatory control of the neuronal activity with an increased temporal and spatial resolution and a cell-type and projection-pathway specificity. Optogenetic activation of GABAergic interneurons *in vitro* demonstrated a rostral to caudal difference of inhibitory circuits within the APC, characterized by strong inhibition of principal neurons by caudal stimulation sites, whereas interneurons are strongly inhibited by rostral sites (Large et al., 2018). Opto- and chemo-genetics provide a new vitality to the study of the epileptiform activity in the PC and the entire limbic area (for review see Tønnesen et al., 2009 and Forcelli, 2017).

In vitro brain slice studies were further integrated by experiments performed on the isolated guinea pig brain preparation maintained *in vitro* by arterial perfusion (de Curtis et al., 1991; de Curtis et al., 2016). In this preparation the connectivity within and between olfactory and limbic areas is preserved, and it can be analyzed without the technical limitations that occur both in the *in vitro* brain slice preparations or during *in vivo* experiments. The *in vitro* isolated guinea pig brain was crucial to define with both neurophysiological and imaging techniques i) the intrinsic connectivity patterns of the APC and PPC (Biella and de Curtis, 1995), ii) the interactions between the PC and the entorhinal and the perirhinal cortices (Biella and de Curtis, 2000; Biella et al., 2003) and iii) the connectivity patterns between the PC and other olfactory regions (Uva et al., 2006; Carriero et al., 2009). As discussed in the next section, experiments performed in rodent brain slices and in the isolated guinea pig brain were thoroughly utilized to characterize the intracellular and multisite extracellular correlates of interictal and ictal epileptiform discharges.

3. Epileptiform synchronization in the piriform cortex *in vitro*

The PC has been proposed to have a crucial role in the generation of acute convulsive seizures induced by the localized injection of proconvulsive agents in the EPN (the so-called *area tempestas,* as described by Piredda and Gale, 1985). These findings were recently revitalized by imaging studies that have demonstrated structural abnormalities within the PC and in adjacent structures in patients with human focal epilepsies (Laufs et al., 2011; Centeno et al., 2011; Vaughan and Jackson, 2014); it was proposed that the involvement of this region in the epileptogenic network of focal epilepsies. The PC and the EPN have been also identified as key structures for epileptogenesis in the kindling model of epilepsy (Loescher and Ebert, 1996; Morimoto et al., 2004).

Interestingly, the PC and the EPN have been shown to be damaged during convulsive *status epilepticus* induced by chemoconvulsant treatments such as pilocarpine or kainic acid; these experimental procedures lead to a chronic epileptic condition that reproduces some of the crucial features of human temporal lobe epilepsy (Mello and Covolan, 1996; Morimoto et al., 2004; Curia et al., 2008; Levesque and Avoli, 2013).

Epileptiform activity can be reproduced in *in vitro PC* preparations by acute pharmacological manipulations. However, as described below (Section 3) and with the exception of the 4AP model of epileptiform synchronization, prolonged seizure-like events (SLEs) are not commonly recorded *in vitro*. Hence, many of the experiments performed *in vitro* have addressed the network activity contributing to interictal-like events. Several investigators have reported that interictal epileptiform activity can be induced *in vitro* in the PC by electrical stimulation (Pelletier and Carlen, 1996; Demir et al., 1999a), by pharmacological manipulations with different convulsive drugs (Galvan et al., 1982; Demir et al., 2001) or by altering the ionic composition of the perfusing medium (Hoffman and Haberly, 1989; Demir et al., 1999b). Epileptiform events, which were characterized by large excitatory synaptic potentials dependent on the activation of NMDA and AMPA subtypes of ionotropic glutamate receptors (Hoffman and Haberly, 1993; Hoffman and Haberly, 1991), were identified in early studies, in which solutions with low magnesium and high potassium were employed to perfuse APC and PPC slices (Hoffman and Haberly, 1989; Hoffman and Haberly, 1993).

Multiple site recordings performed in coronal APC slices demonstrated that the interictal epileptiform potentials (spikes at times followed by brief afterdischarges) evoked by LOT stimulation initiated in the EPN and in layer III, and then secondarily propagated to layer II (Hoffman and Haberly, 1993). SLEs were not observed in these experiments even when low concentration of the $GABA_A$ receptor antagonist, bicuculline methiodide (2.5 μM), was added to the perfusate (Hoffman and Haberly, 1993). Interestingly, interictal epileptiform potentials (but not SLEs) were also observed in coronal PC slices that included the EPN obtained from epileptic rats previously kindled with a standard stimulation protocol (Haberly and Sutula, 1992). Population epileptiform potentials, which were dependent on AMPA receptors, were also observed when brain slices were bathed in a high-potassium and low-magnesium solutions, and they were abolished by local application of cobalt to block synaptic transmission by competing with calcium-mediated currents in the EPN (Hoffman and Haberly, 1996).

Imaging of PC activity propagation induced by perfusing PC slices with voltage sensitive dyes confirmed the initiation of epileptiform interictal-like spikes in the EPN; in coronal rat slices bathed in low-chloride solutions spikes generated in the EPN propagated to the most superficial PC layers (Demir et al., 1998; Demir et al., 1999b). The same group of researchers also performed experiments on longitudinal brain slices that included both APC and PPC and the rostro-caudal association fiber system that connects these two regions; they reported that interictal epileptiform discharges still originated in the EPN as well as that large amplitude interictal spike discharges propagated within superficial layers along intrinsic associative fiber system (Demir et al., 2001). The results obtained on longitudinal PC slices are similar to the *in vivo* findings originally obtained by Haberly and Sutula (1992) who employed current-source density analysis and intracellular recordings in the PC of kindled rats.

The competitive $GABA_A$-receptor antagonist (bicuculline) and the non-competitive blocker of $GABA_A$ receptor-associated chloride channels (picrotoxin) were also used to induce epileptiform discharges in PC coronal slices (Demir et al., 1998) and in the PC in the *in vitro* guinea pig isolated brain (de Curtis et al., 1994; Federico et al., 1994; Federico and MacVicar, 1996; Forti et al., 1997). Current source density analysis of field potential laminar profiles demonstrated that interictal epileptiform activity evoked by LOT stimulation was characterized by large amplitude population spikes that showed maximal amplitude in layers II (de Curtis et al., 1994). Also, spontaneous interictal spikes (not induced by LOT stimulation) were associated to bursting discharges of pyramidal and multipolar neurons of layers II and III of the APC (but not of the PPC) that generated delayed giant synaptic potentials in superficial layer Ib, where intra-PC associative synapses are laminarly concentrated (Forti et al., 1997; de Curtis et al., 1999). As for the low-magnesium and the low-chloride *in vitro* models, also $GABA_A$-receptor blockers did not induce SLEs in the PC. This is not a unique feature of the PC, since it has been observed in other cortical structures maintained *in vitro* during treatment with $GABA_A$ receptor blockers (Rigas and Castro-Alamancos, 2004).

In the *in vitro* isolated guinea pig brain, $GABA_A$ receptor antagonists induced a prominent interictal spiking and only occasionally generated SLE. Simultaneous recordings in the PC and in limbic structures such as the entorhinal cortex (EC) and hippocampus of the isolated guinea pig brain have demonstrated two different and independent patterns induced by arterial perfusion of 50 μM bicuculline. Namely, periodic interictal spiking was commonly observed in the PC, while SLEs could be identified in the limbic areas (Librizzi and de Curtis, 2003; Uva et al., 2005; see Fig. 3A). Focal SLEs occurring in the hippocampus-EC lasted > 30 s and were characterized by a low-voltage fast activity at 20–30 Hz followed by large amplitude spiking that gradually evolved in periodic bursting (Uva et al., 2005; Boido et al., 2014). It should be mentioned, however, that in one third of these experiments SLEs occurring in the hippocampus/EC areas were associated with periodic interictal spikes occurring in the PC at 0.1–0.2 Hz; when active interictal spiking was not present in the PC, SLEs initiating in the limbic cortices could secondarily propagate rostrally to entrain olfactory cortices (Librizzi and de Curtis, 2003).

SLE spread from the EC to the PPC and to the periamygdaloid cortex was also described with intrinsic optical signal imaging in the isolated guinea pig brain following sustained stimulation of the EC during arterial (systemic) perfusion of 20 μM bicuculline (Federico and MacVicar, 1996). Interestingly, interictal-like events initiated in the APC of the isolated guinea pig brain by local application of pro-convulsive drugs propagate to the PPC and to the entorhinal cortex and hippocampus of both hemispheres (de Curtis et al., 1994; Uva et al., 2005). In conclusion, these studies demonstrate that several experimental procedures including high-frequency electrical stimulation, antagonism of the $GABA_A$ receptors, or manipulation of the ionic composition of the bathing medium, can make the PC, and in particular the EPN, able to generate interictal spikes that could effectively propagate to the limbic regions. Interestingly, these experimental manipulations failed in eliciting SLEs in the PC, while inducing them in limbic areas such as the EC.

4. Interictal and ictal discharges induced by 4-aminopyridine in the piriform cortex *in vitro*

Unlike $GABA_A$-receptor antagonists, the potassium channel blocker 4AP promotes SLEs along with interictal discharges in the PC (and in other limbic cortices). This compound, which is commonly used to reproduce SLEs in several *in vitro* preparations (Galvan et al., 1982; Avoli, 1990; Avoli et al., 1993; Avoli et al., 1996; reviewed by Avoli and de Curtis, 2011), presumably causes an increase in transmitter release at both excitatory and inhibitory terminals, thus potentiating synaptic strength (Buckle and Haas, 1982; Perreault and Avoli, 1991). Pioneering studies performed on guinea pig PC slices have indeed revealed that prolonged perfusion of 10 μM 4AP depolarized PC neurons and promoted the generation of SLEs (Galvan et al., 1982).

As illustrated in Fig. 2A, these results were recently confirmed by employing field potential recordings from the PC in coronal or sagittal rat brain slices (Panuccio et al., 2012). In both type of brain slices, interictal and SLEs with similar duration and frequency of occurrence were induced by bath application of 4AP. Moreover, SLEs were readily abolished by the NMDA receptor antagonist CPP while interictal activity continued but was greatly reduced in the absence of ionotropic

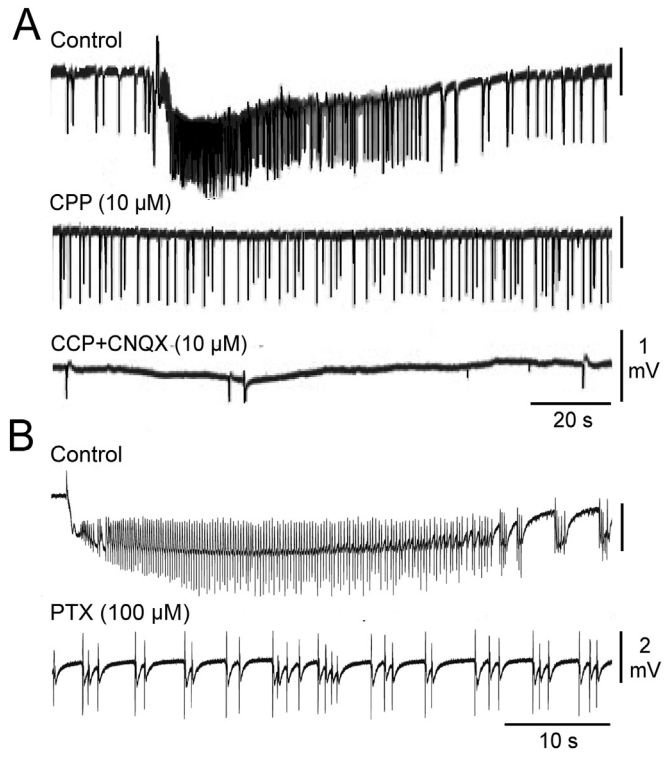

Fig. 2. Pharmacological characteristics of the epileptiform activities induced by 4AP in the rat PC slices. A. SLEs induced by slice perfusion with 50 μM 4AP are abolished by the NMDA receptor antagonist CPP; note that interictal spikes are not affected and also that interictal-like events continue during subsequent application of the non-NMDA receptor antagonists CNQX, though at reduced frequency and with lower amplitude. B. SLEs in PC slices are abolished by the $GABA_A$ receptor antagonist picrotoxin, a pharmacological procedure that makes recurrent interictal discharges occur. Data were obtained during the experiments published by Panuccio et al. (2012).

glutamatergic transmission (Fig. 2A). Finally, 4AP-induced SLEs were abolished by the $GABA_A$ receptor antagonist picrotoxin, a pharmacological procedure that disclosed a pattern of recurrent interictal activity (Fig. 2B). Overall, the pattern and pharmacological features of the SLEs and interictal spikes recorded in the study by Panuccio et al. (2012) resembles what seen in brain slices of the juvenile hippocampus as well as of several mature cortical structures (Avoli and de Curtis, 2011).

SLEs in sagittal PC slices initiated mostly in the APC, whereas interictal activity did not have any preferential site of origin (Panuccio et al., 2012). High frequency oscillations (HFOs, see below) at 80–200 Hz were detected mainly at the beginning of the SLE in both PPC and APC (Hamidi et al., 2014; see paragraph 4). N-Methyl-D-aspartate (NMDA) receptor antagonism did not altered SLEs, whereas in the absence of ionotropic glutamatergic transmission SLEs in the PC were blocked or decreased in amplitude and duration (Carriero et al., 2010).

A SLE pattern different from what described above was observed in the olfactory cortices when arterial perfusion of 50 μM 4AP was performed in the *in vitro* isolated guinea pig brain. In this preparation, simultaneous recordings in APC, PPC, olfactory tubercle, medial EC and hippocampus during a single 4AP perfusion (Carriero et al., 2010; Uva et al., 2013; Fig. 3B) induced parallel and coexisting SLEs characterized by completely different patterns and periodicity in olfactory and limbic regions (Uva et al., 2013; Uva et al., 2017). As extensively reviewed over the last 10 years (Avoli and de Curtis, 2011; de Curtis and Avoli, 2016; Avoli et al., 2016), limbic SLEs induced by 4AP were characterized by a low-voltage fast activity in the *beta-gamma* range at onset; this onset pattern mimics what is commonly observed in humans when depth electrodes are utilized during long-term intracranial monitoring performed to identify the epileptogenic zone in patients with drug-resistant focal epilepsies candidate to epilepsy surgery (de Curtis and Gnatkovsky, 2009; Gnatkovsky et al., 2019; see also Vaughan and Jackson, 2014). Unlike limbic cortex, SLEs induced by 4AP in the guinea pig PC was characterized by fast activity at high frequencies (20–60 Hz) superimposed to a slow extracellular potential shift (Carriero et al., 2010; Uva et al., 2013; Uva et al., 2017). In the olfactory cortices (including the PC), SLEs showed a shorter duration compared to EC SLEs (< 1 min), recurred at higher rate (every 0.5–3 min) and were independent from SLEs in limbic regions (Fig. 3B). Intracellular recordings obtained from the APC revealed that the onset of seizure correlates with a gradual depolarization with action potential firing of superficial layer II neurons, with no involvement at onset of deep layer neurons. During the ictal event, neuronal firing was abolished for 10–30 s in all neurons and gradually restored and synchronized before seizure termination (Uva et al., 2013, 2017).

The site of initiation of the 4AP-induced SLE recorded in the PC of the isolated guinea pig brain was identified within the plexiform layer I formed by unmyelinated afferent and associative fibers that form synaptic terminations on the apical dendrites of layer II-III principal cells. Further analysis demonstrated that at SLE onset, a negative, large and long-lasting potential was recorded in layer I with a 16-channel linear silicon probe inserted across layers in the PC (Uva et al., 2017). The layer I current sink identified by current-source density analysis of the laminar PC field profiles during SLE was associated with a fast-rising increase of extracellular K^+ in the cortical surface, presumably due to 4AP-driven increased synaptic activity. We hypothesized that the K^+ increase was favored by K^+ release by very active unmyelinated layer I fibers. The K^+ diffuses to the deeper PC layers, where it depolarizes principal neurons in layers II-III during the late part of the SLE and promotes a further increase of the extracellular K^+ sustained by the spiking activity of these neurons and by the activation of postsynaptic $GABA_A$ receptors (see for review: Di Cristo et al., 2018). Interestingly, evoked responses during the 4AP-induced SLE were abolished in the PC, suggesting that synaptic activity and the concomitant K^+ rise are important to trigger the SLE, whereas the progression of the SLE is independent of synchronous synaptic transmission (Uva et al., 2017). This unusual SLE feature resembles the pattern observed in patients with extra-temporal pharmacoresistant focal lobe epilepsy, defined as P-type seizure pattern (Uva et al., 2017; Gnatkovsky et al., 2019).

5. High frequency oscillations occurring in the PC during epileptiform activity

The analysis of EEG recordings obtained from epileptic patients and animals *in vivo*, as well as from brain slices recorded *in vitro*, recently revealed the occurrence of high-frequency oscillations (HFOs) that are closely related to the paroxysmal activity generated from the epileptic brain tissue (Jefferys et al., 2012; Jiruska et al., 2017; Lévesque et al., 2018). HFOs are extracted by amplifying the appropriately filtered EEG signal (which *in vivo* is usually obtained with intracerebral recording electrodes), and they have been categorised into two groups, based on their frequency content. Ripples that comprise events between 80 and 200 Hz (Fig. 4A) and fast ripples that comprise events between 250 and 500 Hz (Fig. 4B; Bragin et al., 1999). In epileptic patients and animal models, HFOs are observed in association with interictal and ictal activity, but can also occur alone (Jefferys et al., 2012; Jiruska et al., 2017; Lévesque et al., 2018). HFOs are useful in clinical practice to localize the seizure onset zone in focal epileptic disorders, which is fundamental for performing successful surgical interventions in patients with pharmacoresistant focal epilepsy; HFO removal during surgery, indeed, is highly predictive of a positive outcome (Zijlmans et al., 2012). Several studies have proposed that HFOs may represent better biomarkers than interictal spikes for identifying seizure onset zones in these patients (Jacobs et al., 2008, 2012; Staba, 2012).

In *in vitro* preparations, HFOs are mainly associated to epileptiform

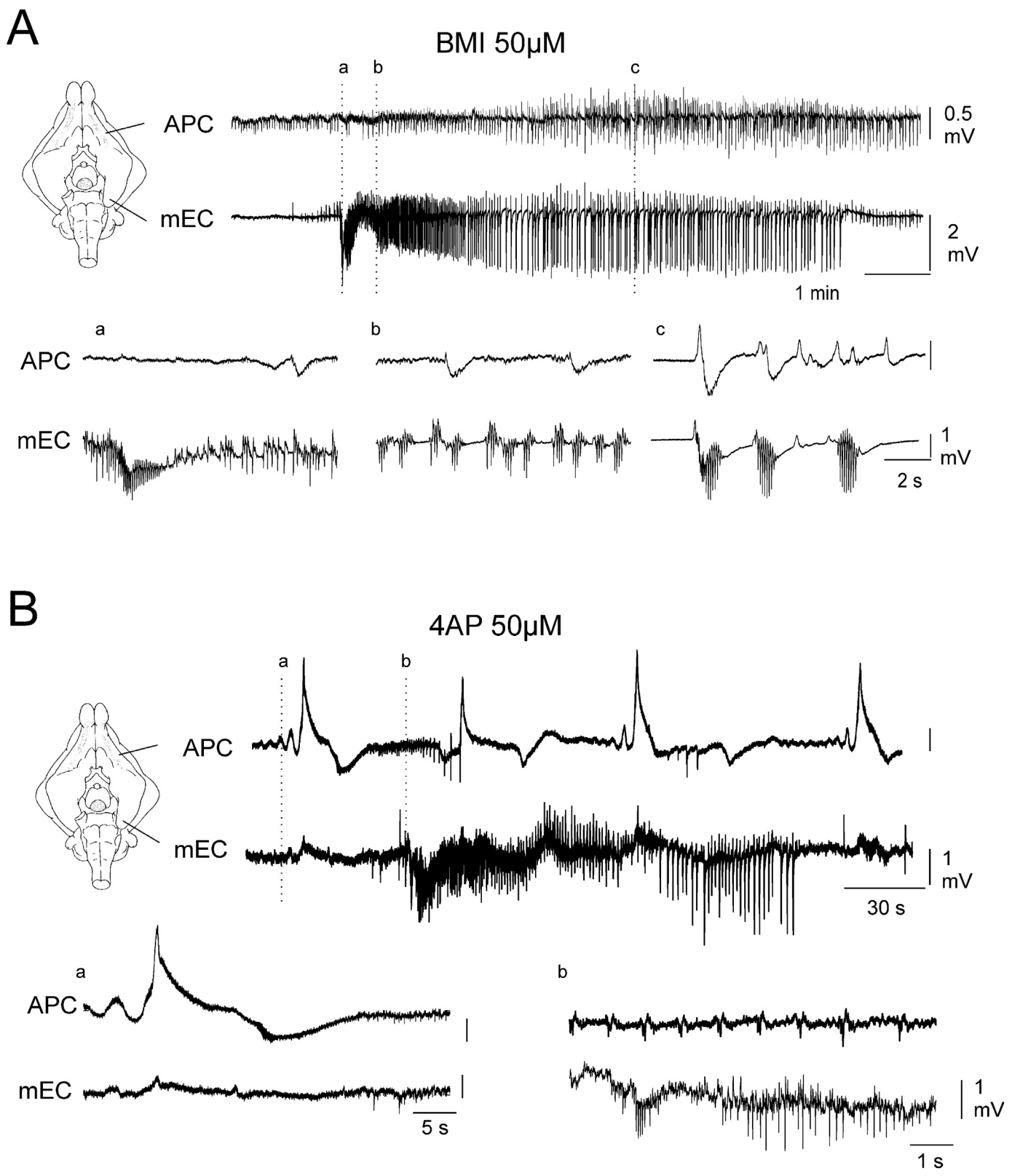

Fig. 3. Different seizure-like events (SLEs) induced by bicuculline and 4-aminopyridine in the *in vitro* isolated guinea pig brain preparation. Simultaneous recordings were performed in the anterior piriform cortex (APC) and in the medial entorhinal cortex (mEC in the left schemes). A. Arterial perfusion of 50 μM bicuculline methiodide for 3 min induces periodic interictal spiking in the PC and SLEs characterized by fast activity at onset (a) in the mEC. PC spikes and mEC SLEs were independent. PC interictal spiking may synchronized with bursting potential observed during the late phase of the mEC SLE (c). B. Arterial perfusion of 50 μM 4AP elicited independent and distinct SLEs in the PC and in the mEC. MEC SLEs were longer than PC SLEs and were characterized by fast activity at onset (b). PC SLEs were brief (< 1 min) and correlated with fast activity superimposed to a large amplitude plateau potential (a).

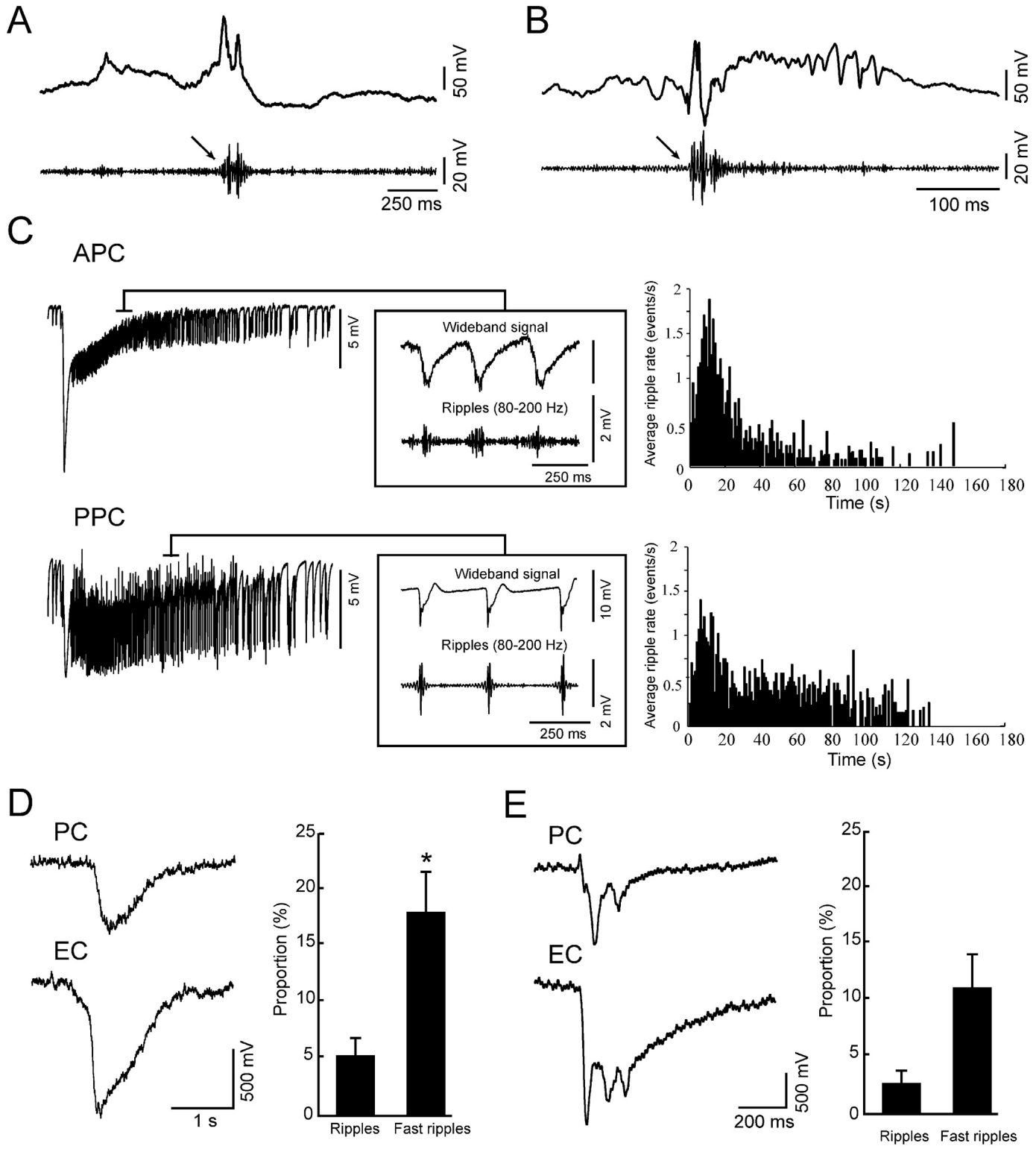

Fig. 4. High-frequency oscillations during epileptiform discharges. A. Example of a ripple associated to an interictal spike in a pilocarpine-treated animal. The top trace shows the wideband signal (1–500 Hz) whereas the bottom trace shows the signal filtered between 80 and 200 Hz. The arrow points to the detected ripple. B: Example of a fast ripple associated to an interictal spike in a pilocarpine-treated animal. The top trace shows the wideband signal (1–500 Hz) whereas the bottom trace shows the signal filtered between 250 and 500 Hz. The arrow points to the detected fast ripple. C: Recordings from a rat brain slice that includes the PC, showing a representative example of a 50 μM 4AP-induced ictal discharge in the anterior (APC) and posterior regions (PPC). The average distribution of ripples during ictal events is shown (n = 4 slices, 22 ictal events). Note that ripples tend to occur at the onset of 4AP-induced ictal discharges. D: Example of a 4AP-induced slow interictal discharge recorded in the piriform cortex of a rat brain slice. Note that a significanlty higher proportion of slow interictal discharges are associated to fast ripples compared to ripples ($p < .01$). E: Example of a polyspike interictal discharge. No significant difference is observed between the proportion of polyspike discharges associated to ripples or fast ripples. Data were obtained from experiments published in Hamidi et al., 2014; Lévesque et al., 2015 and Panuccio et al., 2012.

activity and rarely occur alone (Panuccio et al., 2012; Hamidi et al., 2014). It has been proposed that ripples mirror summated Cl^- dependent, inhibitory postsynaptic potentials mainly generated by the soma of pyramidal cells in response to GABA released from interneurons, suggesting that they mainly rest on GABAergic transmission, and specifically on $GABA_A$ receptor signaling (Jefferys et al., 2012; Jiruska et al., 2017; Lévesque et al., 2018). Fast ripples would instead mirror the uncontrolled "in-phase" or "out-of-phase" firing of principal cells due to a collapse of perisomatic inhibition (Jefferys et al., 2012; Jiruska et al., 2017; Lévesque et al., 2018).

In the anterior and posterior regions of the rat PC maintained under 4AP *in vitro*, HFOs occur during low-voltage fast onset ictal discharges, mainly at their onset (Panuccio et al., 2012; Hamidi et al., 2014). HFOs are mostly characterized by ripples (Fig. 4C), with a virtual absence of fast ripples, which is similar to what is observed in other regions of the temporal lobe *in vivo* (Lévesque et al., 2018), and supports the hypothesis that PC ictogenesis in the 4AP model does not rely on principal cell synchronization (Avoli and de Curtis, 2011). HFOs are also related to 4AP-induced interictal spikes in the rat PC (Hamidi et al., 2014; Shiri et al., 2015), but the occurrence of ripples and fast ripples is modulated by interictal activity patterns. Indeed, slow interictal discharges occurring synchronously between PC and EC are mostly associated to fast ripples (Fig. 4D), whereas synchronous polyspike interictal discharges show similar rates of ripples and fast ripples (Fig. 4E). It should be emphasized that removing the connections between PC and EC did not change the occurrence of ripples and fast ripples during epileptiform activity (Hamidi et al., 2014).

6. Conclusions

Epileptiform discharges are observed in the PC maintained *in vitro* during appropriate stimulation and after perfusion of pro-epileptic agents. *In vitro* data confirm that both the EPN and the PC are required to promote and sustain epileptiform discharges. These *in vitro* findings are in agreement with the work of Karen Gale, who initially described the EPN as a crucial ictogenic site (Piredda and Gale, 1985) and later included also PC superficial layers II and III into the so-called *area tempestas* (Maggio and Gale, 1989; Wardas et al., 1990). Local applications of pro-convulsive drugs in this area were responsible for the generation of bilateral seizure discharges that involved limbic cortices and thalamic nuclei (Halonen et al., 1994; Cassidy and Gale, 1998).

These findings are corroborated by the *in vitro* demonstration that epileptiform activity locally generated in the PC effectively propagates to the limbic regions, therefore suggesting that PC discharges do not remain confined within the olfactory region and spread out to entrain larger brain areas. Human studies strongly suggest that the PC plays a facilitating and amplifying role in human focal ictogenesis, thus influencing the development of pathological networks (see other chapters in this Special Issue; Vaughan and Jackson, 2014). Future therapeutic strategies that aim to control seizure spread and propagation by opto- and chemogenetic inactivation of the *area tempestas* could be useful for controlling the epileptiform activity, as demonstrated in the hippocampal CA1/CA3 and PC pyramidal neurons (Tønnesen et al., 2009); this may open the door to new therapeutic horizons for the cure of drug-resistant focal seizures.

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