Experimental Neurology 271 (2015) 319–328

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

# Entorhinal cortex lesions result in adenosine-sensitive high frequency oscillations in the hippocampus

Franco Ortiz [a,b], Rafael Gutiérrez [a,*]

[a] Department of Pharmacobiology, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 14330, México
[b] Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F. 04510, México

ARTICLE INFO

*Article history:*
Received 2 February 2015
Received in revised form 19 May 2015
Accepted 7 June 2015
Available online 10 June 2015

*Keywords:*
Entorhinal cortex
CA3
Dentate gyrus
Ripples
Fast ripples
High frequency oscillations
Cortical trauma
Adenosine

ABSTRACT

Entorhinal cortex (EC) projections to the hippocampus run along the perforant path and activate the hippocampal area CA3 and the dentate gyrus (DG), which, in turn, drives CA3. Because cortical trauma damages the source of inputs to the hippocampus, we hypothesize that such an event can be reflected in immediate alterations of the hippocampal oscillatory activity. We here explore whether acute, localized disruption of EC–EC connectivity is involved in the generation or modulation of high frequency oscillations (HFOs) in the hippocampus. We conducted *in vitro* electrophysiological recordings in CA3 and DG of combined EC–hippocampal transversal slices prepared from intact brains and from brains with a spatially defined, transversal cut of the EC made *in situ*, 2 h before *in vitro* recordings commenced. We also determined if pharmacological manipulations of the adenosine system modulated the fast oscillatory activity. EC–hippocampal slices prepared from brains, in which a transversal lesion of the EC was uni- or bilaterally conducted *in situ*, *displayed* spontaneous epileptiform events with superimposed ripples (150–250 Hz) and fast ripples (>250 Hz), whereas those obtained from non-lesioned brains *did not have* spontaneous HFOs. However, in the latter, high frequency stimulation applied to the perforant path produced ripple activity in area CA3. Spontaneous fast ripples were prevented by conducting the slicing procedure and incubating the slices both in a $Na^+$-free medium and in a low $Ca^{++}$-high $Mg^{++}$ medium for an hour before recording commenced, under normal $Na^+$ concentration. Activation of $A_1$, but not $A_2$, receptors produced a strong inhibition of the incidence and spectral power of fast ripples but did not change their intrinsic frequency. Our data show that the disruption of EC-to-EC connections can immediately disinhibit hippocampal CA3 area to generate HFOs on top of epileptiform events, probably constituting an irritating focus long before overt epileptic activity can be detected behaviorally. Therefore, the activation of the adenosinergic system can possibly be regarded as an immediate intervention strategy to avoid epileptogenesis.



## 1. Introduction

Traumatic brain injury (TBI) and its consequences have been difficult to address experimentally, mainly because TBI patients often present a variety of lesions that differ in severity and distribution. Thus, animal models of TBI may not replicate this span of lesions. On the other hand, the latter can be designed to directly address the effects of specific lesions in order to rule out their involvement in a more complex and realistic case. Although TBI usually refers to a damage affecting the brain function resulting from non-penetrating loading of the contact and non-contact type (O'Connor et al., 2011), indentation and damage to very restricted cortical areas (for a review on military TBI see Risdall and Menon, 2011) resulting in deafferentation may shed light into their functional interactions in health and disease. One of the consequences of TBI is post-traumatic epilepsy, although its development in human patients can take weeks or years. In clear contrast to human cases, where the worst case scenario is a concussion that leads to tissue damage with diffuse axonal injury and intracranial hemorrhage, animal models can replicate the causes of the injury (Frey et al., 2009) as well as produce controlled, more severe "axonal injury", like the deafferentation of entorhinal cortex (EC)-to-EC connections that we here study.

High frequency oscillations (ripples and fast ripples) constitute events reflecting high synchrony physiological patterns in the nervous system (Sullivan et al., 2011). Fast ripples (>250 Hz) were considered a biomarker of epileptic activity in the hippocampus and entorhinal cortex, signaling its point of origin (Bragin et al., 1999), however they possess distinct characteristics with respect to events in the ripple frequency range (up to 250 Hz; Sullivan et al., 2011). Although initiation of interictal and ictal activity in the hippocampus can be independent of the EC (Dzhala and Staley, 2003), in combined EC–hippocampal slices, seizure-like activity is generated primarily in the entorhinal and perirhinal cortices and from there it propagates to hippocampal areas

* Corresponding author at: Department of Pharmacobiology, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Calzada de los Tenorios No. 235, México D.F. 14330, México.
*E-mail address:* rafagut@cinvestav.mx (R. Gutiérrez).

http://dx.doi.org/10.1016/j.expneurol.2015.06.009
0014-4886/

CA1 and CA3 (Jones and Heinemann, 1988; de Guzman et al., 2004; Avoli et al., 2002; Avoli and de Curtis, 2011). Moreover, experimental and human studies in temporal lobe epilepsy have shown neuronal cell loss in layer III of the EC (Du et al., 1993, 1995), which may facilitate reentrant activation of the hippocampal–EC loop *via* disinhibition of local networks in CA1 (Empson and Heinemann, 1995). Thus, hippocampal lesions themselves are not a prerequisite for HFOs to appear (Jiruska et al., 2010). Because the dentate gyrus (DG) regulates the flow of normal and pathological information between the EC and the hippocampus proper (Buzsáki et al., 1983; Heinemann et al., 1992; Krook-Magnuson et al., 2015), post-traumatic alterations can be reflected in the DG-to-CA3 synapse (Santhakumarm et al., 2003; Heinemann et al., 1992). Furthermore, it has been shown that in a model of TBI, where an injury is provoked by fluid percussion over the parietal cortex, hilar neurons of both hippocampi die as fast as 4 h after the concussion, possibly leading to hyperexcitability in the DG (Lowenstein et al., 1992). Thus, changes in excitability and network interactions in the cortex may precede the involvement of the hippocampus during the development of temporal lobe epilepsy (de Curtis and Paré, 2004; Lowenstein et al., 1992). For example, long range projecting GABAergic cells from the EC to the hippocampus (Germroth et al., 1989; Melzer et al., 2012) may be tonically suppressing the expression of HFOs of the hippocampus.

We here present data supporting the proposal that EC lesions disrupt a tonic inhibitory control of the EC on the hippocampal circuitry responsible for generating epileptiform events containing ripple and fast ripple activity and that by disrupting EC-to-EC projections, the hippocampal circuitry is functionally reorganized along its axis. With field recordings of EC-hippocampal slices *in vitro* we here show that epileptiform events with fast ripple activity appeared after conducting a uni- or bilateral transversal lesion of the EC *in situ*, in adult rats. By contrast, slices obtained from non-lesioned rats did not have spontaneous fast ripples, but could be *originated* after 4–6 trains of an LTP-induction stimulation protocol over the perforant path *in vitro*. High frequency oscillations could be suppressed by adenosine $A_1$ receptors ($A_1$Rs) but not by adenosine $A_{2A}$ receptors ($A_{2A}$Rs). Our results suggest that the oscillatory activity of the hippocampus can be immediately altered by EC-to-EC disruption, thus, displaying epilepsy-related fast ripples probably through a functional disinhibition (Jiruska et al., 2010).

## 2. Materials and methods

The Ethics Committee for Animal Research of our institution approved all experimental procedures, which were performed in adherence to the "Guide for the Care and Use of Laboratory Animals" (NIH 8th edition 2011). We used transversal, combined EC–hippocampus slices (400 μm) obtained from adult (230 g) Wistar rats (Gutiérrez, 2000). Some brains (see Results) were subjected to a uni- or bilateral lesion of the EC (Fig. 1A) *in situ*, before being taken out of the skull for the preparation of the slices. Also, to evoke HFOs in combined EC–hippocampal slices from intact brains, we stimulated the perforant path with a repeated LTP-inducing stimulation protocol (Gutiérrez, 2002), hereafter called high frequency stimulation (HFS). To deliver this HFS, we used a bipolar electrode placed over the perforant path to provide trains of 0.1 ms pulses at 100 Hz, with a duration of 1 s, and an intertrain interval of 1 min delivered every 15 min without varying the stimulus intensity, until sharp waves with an amplitude of at least 0.2 mV were apparent (see results in Fig. 2C) (Gutiérrez, 2002; Behrens et al., 2005). Stimuli were provided with a Grass S11 stimulator through a photoelectric stimulus-isolating unit (PSIU 6; Grass Technologies, Warwick, RI) connected to a bipolar (tip separation 50 μm), glass-insulated platinum wire (25 μm) electrode. The current intensity, at which the trains were delivered, was determined for each experiment with an input–output curve; the stimulation current was fixed at the value that evoked 60% of the maximal field response under normal ACSF perfusion.

The EC was uni- or bilaterally cut with microsurgery scissors (500 μm tip; WPI, cat. 501777) after rapid removal of the skull and chilling the brain with several drops of ACSF at 4 °C, *in situ*. The cut on each hemisphere was performed at the following coordinates taken from the adult rat brain atlas (Paxinos and Watson, 1997): AP, from −5.3 to −6.8 (from Bregma); L, from the surface of the brain to 6; H, between 4 and 6, while the brain was still embedded in the skull. Performing the cuts *in situ* allowed us to maintain the stiffness of the brain, which warranted its steady position while cutting, and thereby, the repeatability of the lesions.

The brains were next removed, submerged in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 124; KCl, 3; $NaH_2PO_4$, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; and glucose 10; pH 7.35 and cut with a Vibroslicer (Leica VT1200) in transversal slices of 400 μm thickness. A group of rats (see Results) were deeply anesthetized (Pentobarbital, 50 mg/kg$^{-1}$, i.p.) and intracardially perfused with the following solution (in mM): sucrose, 210; KCl, 2.8; $MgSO_4$ 2; $NaH_2PO_4$, 1.25; $NaHCO_3$, 26; glucose, 10; $CaCl_2$, 1; $MgCl_2$, 1; at 4 °C; the brains were removed and slices were obtained while the tissue was submerged in 4 °C sucrose-containing ACSF.

Slices of both groups of rats (*i.e.*, intracardially perfused or not) were kept submerged either in the recording, normal ACSF or in a low-$Ca^{++}$, high $Mg^{++}$-containing solution with the following composition (in mM): NaCl, 125; KCl, 2.5; $NaH_2PO_4$, 1.25; $MgCl_2$, 4; $CaCl_2$, 1; $NaHCO_3$, 25; glucose, 10; ascorbic acid, 0.4; pH 7.35, at room temperature at

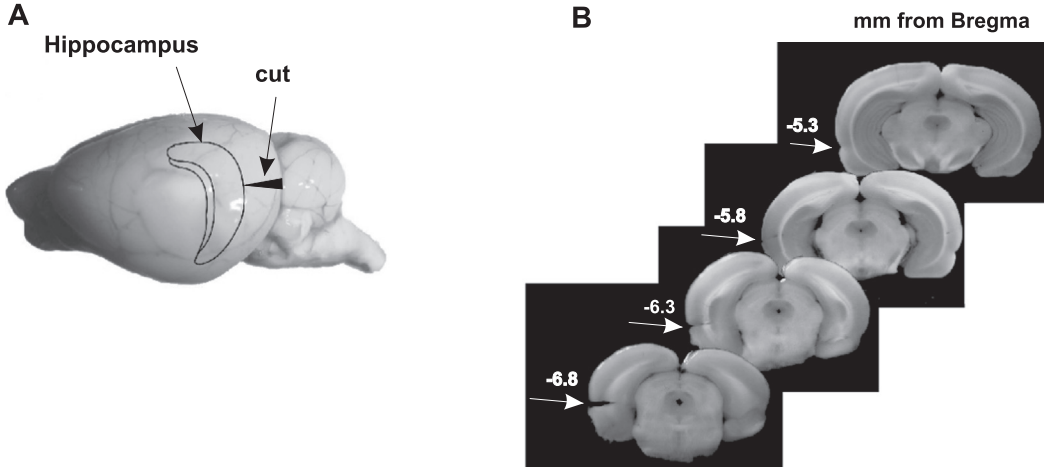


**Fig. 1.** Lesion of the entorhinal cortex. A) Photograph of a rat brain with a schematic view of the hippocampus and the location where the entorhinal cortex was cut. B) Photographs of a series of coronal brain slices prepared in an antero-posterior direction indicating the extent of the cut (arrows).