Journal of Neuroscience Methods 260 (2016) 83–90

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

The *in vitro* isolated whole guinea pig brain as a model to study epileptiform activity patterns

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HIGHLIGHTS

- The *in vitro* isolated brain retains the connectivity between remote brain areas.
- Acute epileptiform activities can be induced by simple pharmacological manipulations.
- Epileptiform patterns determinants can be identified in a close-to-*in vivo* condition.

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 23 March 2015
Accepted 24 March 2015
Available online 2 April 2015

Keywords:
Ictogenesis
Interictal
In vitro guinea pig brain
Seizures

ABSTRACT

Background: Research on ictogenesis is based on the study of activity between seizures and during seizures in animal models of epilepsy (chronic condition) or in *in vitro* slices obtained from naïve non-epileptic brains after treatment with pro-convulsive drugs, manipulations of the extracellular medium and specific stimulation protocols.
New method: The *in vitro* isolated guinea pig brain retains the functional connectivity between brain structures and maintains interactions between neuronal, glial and vascular compartments. It is a close-to-*in vivo* preparation that offers experimental advantages not achieved with the use of other experimental models. Neurophysiological and imaging techniques can be utilized in this preparation to study brain activity during and between seizures induced by pharmacological or functional manipulations.
Results: Cellular and network determinants of interictal and ictal discharges that reproduce abnormal patterns observed in human focal epilepsies and the associated changes in extracellular ion and blood–brain permeability can be identified and analyzed in the isolated guinea pig brain.
Comparison with existing methods: Ictal and interictal patterns recorded in *in vitro* slices may show substantial differences from seizure activity recorded *in vivo* due to slicing procedure itself. The isolated guinea pig brain maintained *in vitro* by arterial perfusion combines the typical facilitated access of *in vitro* preparations, that are difficult to approach during *in vivo* experiments, with the preservation of larger neuronal networks.
Conclusions: The *in vitro* whole isolated guinea pig brain preparation offers an unique experimental model to study systemic and neurovascular changes during ictogenesis.

Published by Elsevier B.V.

Contents

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http://dx.doi.org/10.1016/j.jneumeth.2015.03.026
0165-0270/Published by Elsevier B.V.

Epilepsy is a comorbidity of diseases due to diverse pathogenic factors that result in paroxysmal changes of brain activity. Both systemic or focal alterations may contribute to set a chronic epileptic condition characterized by abnormal excitability that underlies the spontaneous generation of seizures. Several pathogenic factors (genetic, metabolic, degenerative, *etc.*) activate an epileptogenic process that may involve different neuronal, glial and neurovascular brain compartments. The processes that lead to a chronic epileptic condition are the target of research on *epileptogenesis*. The analysis of the mechanisms that generate a seizure is the study of *ictogenesis*.

Research on ictogenesis is based on the study of activity between seizures and during seizures in animal models of epilepsy (chronic condition) and seizures. In the latter group of models, interictal and seizure discharges are studied in normal non-epileptic brains following acute pharmacological and electrical manipulations that promote seizures. *In vitro* brain slice preparations have been largely utilized since early 1980s to study ictogenesis. Seizures are most frequently analyzed on *in vitro* slices obtained from naïve non-epileptic brains after treatment with pro-convulsive drugs, manipulations of the extracellular medium and specific stimulation protocols (see Pitkänen et al., 2006). Similar treatments are also utilized to induce seizure activity on *in vitro* slices from epileptic animals, since brain slices do not generate spontaneous seizure activity, but may produce interictal events such as spikes or other interictal patterns.

Ictal events and interictal patterns recorded in slices *in vitro* may show substantial differences from seizure activity recorded *in vivo* (see de Curtis and Avanzini, 2001; de Curtis and Gnatkovsky, 2009). These differences are likely due to the reduction of local networks preserved within a slice and to the alteration of the neuro-glio-vascular interactions associated with the slicing procedure. To overcome slice tissue limitations, more complex *in vitro* models have been developed, that combine the typical facilitated access of *in vitro* preparations with the preservation of larger neuronal networks. The isolated guinea pig brain maintained *in vitro* by arterial perfusion represents one of these preparations (de Curtis et al., 1991, 1998a, 2012; Muhlethaler et al., 1993). In the *in vitro* isolated whole brain (IWB) of the guinea pig, the intrinsic connectivity within and between diencephalic and telencephalic structures of both hemispheres is maintained (Uva and de Curtis, 2005). Moreover, the vascular system, the blood–brain barrier and their interaction with the brain parenchyma is functionally preserved (de Curtis et al., 1998a; Librizzi et al., 2001, 2012). The study of ictogenesis in the IWB has the same limitation of other *in vitro* preparation with respect to the ability to generate spontaneous seizures. Pro-epileptic drugs and high-frequency stimulations must be employed, indeed, to obtain seizure activity in the IWB. Seizure patterns in the IWB closely resemble seizures recorded *in vivo* in rodents models of epilepsy and in humans (see below). In the last 20 years of experimental work on the guinea pig IWB, we mainly focused on the characterization of system physiology of the olfactory and limbic systems. In these regions, we reproduced interictal and seizure patterns identified in human epilepsies to study cellular activity, network interactions, extracellular ion changes and neurovascular alterations responsible for ictogenesis.

1. Recording brain activity in the IWB

The most frequently explored areas in the IWB are the olfactory and limbic regions: activity have been recorded in the olfactory bulb (OB; Uva et al., 2006; Carriero et al., 2009), in the piriform cortex (PC; Biella and de Curtis, 1995, 2000; Biella et al., 1996), in the amygdala (Carriero et al., 2009), in the entorhinal and perirhinal cortex (EC and PRC; Biella et al., 2000, 2002; Gnatkovsky et al., 2004) and in the hippocampal region such as the CA1 region, the dentate gyrus (DG) and the subiculum (Boido et al., 2014; Uva and de Curtis, 2003, 2005). These regions can be easily reached in the IWB when the brain is positioned in the recording chamber with its ventral surface exposed (Fig. 1). This represents an advantage compared to the *in vivo* condition, in which cortical structures positioned ventrally at the base of the skull are difficult to access. In the IWB, the position of recording and stimulating electrodes is defined under direct visual control with a stereomicroscope and can be rapidly modified during the experiment. For deep structures, evoked field potentials are utilized to drive the electrodes to the targets. This offers a virtually unlimited accessibility to stimulate and record the ventral brain and the related depth nuclei/structures. The viability of recorded areas can be evaluated in the IWB by monitoring evoked potentials along multi-synaptic pathways following electrical stimulation of the lateral olfactory tract (Fig. 1) or any other brain area. Another advantage of the IWB compared to *in vivo* conditions, is the mechanical stability of the recordings, granted by the absence of respiration and pulsation artifacts (see de Curtis et al., 1991, 1998a; Muhlethaler et al., 1993).

The possibility to perform simultaneous field potential (FP) recordings from different regions on both hemispheres makes the IWB the ideal preparation to study propagation of both normal and epileptiform activity in anatomically connected brain regions. FPs are recorded with different types of extracellular electrodes: glass micropipettes filled with 0.9% NaCl; tungsten or stainless steel wires with variable tip diameter, and multichannel electrodes with diverse arrangements of recording sites. Multi-site recordings performed in different cortical areas with glass micropipettes during seizure activity induced pharmacologically by arterial perfusion of 50 μM bicuculline methiodide (BMI) are illustrated in Fig. 2. In this acute model of ictogenesis, simultaneous extracellular recordings performed in the PC, in the lateral and medial EC, in different subfields of the hippocampal formation (CA1 and DG) demonstrate that seizure-like events are generated in EC-hippocampus (Librizzi and de Curtis, 2003; Uva et al., 2005). We also demonstrated that, as in chronic models of temporal lobe epilepsy (Bragin et al., 1999) and in human mesial temporal lobe epilepsy (Bragin et al., 2005), in this acute model of temporal lobe seizures two different patterns of seizure initiation, defined as low-voltage fast activity (FA) and hypersynchronous activity (HSA) were observed (Boido et al., 2014). In this study, simultaneous extracellular FP recordings performed from ventral hippocampal and parahippocampal subregions demonstrated that both patterns are locally generated within the limbic area. Medial-EC, CA1 and subiculum are involved in all phases in both FA- and HSA-onset seizures. Moreover, we showed that the DG has an important role in the maintenance and development of the seizure, but is inactive at the onset of FA seizures. Surprisingly, pre-subiculum and para-subiculum provide minimal participation to seizure activity, despite the documented anatomical connections with the EC and the hippocampal formation.

To analyze local activations in a laminated structure (*i.e.*, cortical regions) 16-ch linear silicon probes (50 or 100 μm contact separation; Neuronexus, Ann Arbor, MI, USA) are inserted perpendicular to cortical lamination in the structures of interest (Boido et al., 2014; Uva and de Curtis, 2005; Uva et al., 2005). FPs laminar profiles are recorded with silicon probes and are then analyzed by applying current-source-density (CSD) analysis (de Curtis et al., 1994; Biella and de Curtis, 1995), which is a useful method to distinguish locally generated components from far field components passively propagated in the tissue, and to characterize spatial and temporal arrangement of locally generated responses. Electrolytic lesions performed at the end of the experiments are used to reconstruct the position of the silicon probes tracks on histological sections. The use of multichannel silicon probes to perform laminar