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High-frequency oscillations and seizure-like discharges in the entorhinal cortex of the in vitro isolated guinea pig brain

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ABSTRACT

We analyzed the patterns of seizure-like activity and associated high-frequency oscillations (HFOs) induced by the K^+ channel blocker 4-aminopyridine (4AP, 50 μM) or the $GABA_A$ receptor antagonist bicuculline methiodide (BMI, 50 μM) in the in vitro isolated guinea pig brain preparation. Extracellular field recordings were obtained from the medial entorhinal cortex (EC) using glass pipettes or silicon probes; 4AP or BMI were applied through the basilar artery. Ripples (80–200 Hz) or fast ripples (250–500 Hz) occurred at higher rates shortly before ictal events induced by 4AP or BMI, respectively. In addition, during the ictal period, ripples were mostly associated with 4AP-induced ictal events whereas fast ripples predominated during ictal discharges induced by BMI. Finally, ripples occurred at higher rates during the clonic phase of 4AP-induced ictal events compared to the tonic phase, while higher rates of fast ripples characterized the clonic phase in both 4AP- and BMI-induced ictal discharges. These differences in HFO occurrence presumably reflect the diverse action of these two convulsants on $GABA_A$ receptor signaling.

1. Introduction

Recent studies have shown the occurrence of high-frequency oscillations (HFOs, ripples: 80–200 Hz, fast ripples: 250–500 Hz) in the EEG of patients presenting with mesial temporal lobe epilepsy and in animal models mimicking this neurological disorder (Jacobs et al., 2012; Jefferys et al., 2012). HFOs have been reported to increase in occurrence during the transition from interictal to ictal activity in EEG recordings obtained from humans (Zijlmans et al., 2011; Weiss et al., 2016) as well as from acute and chronic epileptic rodents (Bragin et al., 2005; Lévesque et al., 2012; Salami et al., 2015). In addition, specific seizure onset patterns – such as the low-voltage fast or the hypersynchronous patterns – are associated with an increased occurrence of distinctive types of HFOs; for instance, in pilocarpine-treated epileptic animals low-voltage fast onset seizures are characterized by increased ripple rates while fast ripples are the predominant HFOs in hypersynchronous onset seizures (Lévesque et al., 2012).

An increase in HFOs also occurs during the pre-ictal and/or ictal periods in in vitro models of epileptiform synchronization. Khosravani et al. (2005) found that the power spectral amplitude of HFOs increases in the hippocampus during the transition from pre-ictal to ictal state in brain slices bathed with low-Mg^{2+} medium; in addition, increased rates of HFOs have been identified during seizure-like events (SLEs) induced in the piriform and entorhinal cortices of extended brain slices treated with the K^+ channel blocker 4-aminopyridine (4AP) (Panuccio et al., 2012; Hamidi et al., 2014). Using optogenetic techniques in the 4AP model, we also found that ripples characterise low-voltage fast-onset SLEs triggered by the stimulation of interneurons whereas fast ripples are associated to hypersynchronous-onset SLEs induced by principal cell stimulation (Shiri et al., 2016).

SLEs are also recorded in the isolated guinea pig brain maintained in vitro. Specifically, SLEs with electrographic features resembling low-voltage fast onset seizures can be recorded from several limbic areas including the entorhinal cortex (EC) following arterial application of either 4AP or low concentrations of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) (Gnatkovsky et al., 2008; Uva et al., 2015). To date, however, it is unknown whether and how HFOs occur in this in vitro model of ictogenesis, which is a close-to-in vivo preparation preserving the tridimensional extension of neuronal circuits. Therefore, we investigated here the occurrence of HFOs during SLEs generated by the medial EC in the isolated guinea pig brain preparation following 4AP or BMI treatment.

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2. Materials and methods

2.1. Animals and brain preparation

The number of animals used in this study was minimized according to the international guidelines on the ethical use of animals (European Communities Council Directive of 24 November 1986, 86/109/EEC). The experimental protocol was reviewed and approved by Committee on Animal Care and Use and by the Ethics Committee of the Fondazione Istituto Neurologico.

Brains of 24 female Hartley guinea-pigs (150–200 g, Charles River, Italy) were isolated and maintained in vitro according to the standard procedure described elsewhere (de Curtis et al., 1998). Animals were anesthetized with sodium thiopental (125 mg/Kg i.p., Farmotal, Pharmacia, Italy) and trans-cardially perfused with a cold (10 °C), carboxygenated (95% O_2/5% CO_2) solution containing 126 mM NaCl, 3 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, 15 mM glucose, 2.1 mM HEPES and 3% dextran M.W. 70000 (pH adjusted at 7.1 with HCl). After decapitation, brains were transferred into a recording chamber. A cannula was inserted in the basilar artery and brain perfusion with the solution mentioned above (7 ml/min, pH 7.3, 15 °C) was restored via a peristaltic pump (Minipulse 3, Gilson, France). The temperature of both the perfusate and the chamber was slowly (0.2 °C/min) raised to 32 °C by a temperature controller (PTC 10, NPI, Germany), before starting the electrophysiological experiment.

Extracellular recordings were performed in the medial entorhinal cortex (EC) using 0.9 M NaCl-filled glass pipettes (5–10 MΩ resistance) or 16-chanel linear silicon probes (50 or 100 μm contact separation; Neuronexus, Ann Arbor, MI, USA) positioned under visual control with a stereoscopic microscope. Responses evoked by electrical stimulation of the lateral olfactory tract through an iso-

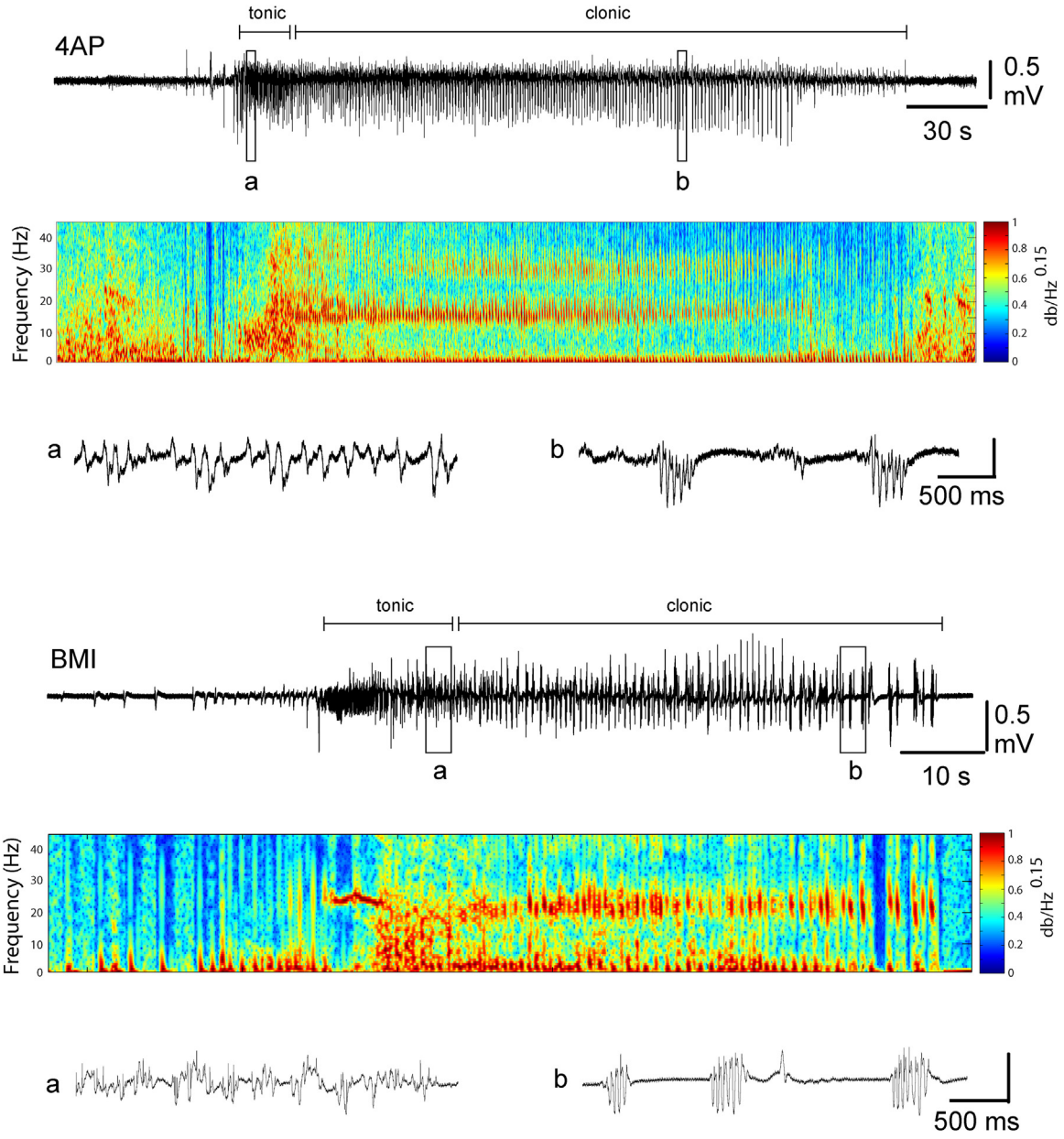

Fig. 1. Representative SLEs induced by arterial application of 4AP (A) (50 μM; 4-min) or BMI (B) (50 μM; 3-min). Corresponding spectrograms are reported below seizure-like events. On bottom of each panel illustrative portions of tonic and clonic phases are shown at an expanded time scale.