NEUROSCIENCE
RESEARCH ARTICLE

A. Mikroulis et al. / Neuroscience 369 (2018) 386–398

Immature Status Epilepticus: *In Vitro* Models Reveal Differences in Cholinergic Control and HFO Properties of Adult CA3 Interictal Discharges in Temporal *vs* Septal Hippocampus

Apostolos Mikroulis,[†‡] Christos Panagiotis Lisgaras[†¶] and Caterina Psarropoulou[*]

Laboratory of Animal & Human Physiology, Dept. Biological Applications and Technology, Faculty of Health Sciences, University of Ioannina, 45110 Ioannina, Greece

Abstract—We have earlier demonstrated that a Status Epilepticus (SE) during CNS development has long-lasting effects on cholinergic neurotransmission, detectable *in vitro* and *in vivo*. In this work, we aimed to localize changes in temporal (T) vs septal (S) hippocampus and to correlate adult CA3 interictal epileptiform discharge (IED) frequency changes to those of Ripples (R) and Fast Ripples (FR) of the High-Frequency Oscillations (HFOs). Spontaneous IEDs were induced by bathing slices in Mg^{2+}-free ACSF or in 4-Aminopyridine (4-AP, 50 μM) and data were analyzed separately for each model. IED frequencies were similar in same origin normal (N) slices across models, but differed in SE slices, being lower in Mg^{2+}-free ACSF than in 4-AP, suggesting a post-SE long-term increase in a K^+ conductance. Rs and FRs detected within IEDs had generally higher power in 4-AP than in Mg^{2+}-free ACSF; FR/R ratio was the highest in T-SE slices in 4-AP and similar in all other slice groups. Carbachol or eserine increased IED rates universally, but had region- and conditioning-specific effects on HFOs, suggesting that IED frequency and HFOs represent possibly independent indices of excitability. The muscarinic antagonist atropine depressed IED rates with increasing effectiveness in S slices post-SE in both models. In conclusion, the long-term effects of an immature SE are region-specific within the hippocampus, affect differently synchronizing components like the IED frequency and HFOs and may shape neurotransmitter effects (ACh) on neuronal networks, thus affecting seizure threshold and information processing, especially in behavioral conditions of rising extracellular ACh levels.

Keywords: seizures, temporal hippocampus, septal hippocampus, muscarinic, interictal, high-frequency oscillations.

INTRODUCTION

Immature sustained and generalized seizures are linked to adult cognitive and behavioral abnormalities by a host of clinical (Camfield and Camfield, 2002; Nolan et al., 2003; Besag, 2004; van Rijckevorsel, 2006) and experimental (Lynch et al., 2000; Rutten et al., 2002; Kubova et al., 2004; Zhang et al., 2004; Cornejo et al., 2007; Holopainen, 2008; Castelhano et al., 2013; Cordova et al., 2013; Zhou et al., 2015) studies. We have earlier shown that an immature Status Epilepticus (SE)-like seizure provokes functional changes detectable in the adult CNS both *in vitro* (Meilleur et al., 2000; Meilleur et al., 2003; Potier et al., 2005; Mikroulis and Psarropoulou, 2012) and *in vivo* (Kouis et al., 2014).

The hippocampus, a formation pivotal in learning and memory has also a low threshold for epileptic discharge generation. Structurally, it is formed by a series of parallel neuronal circuits, which however differ in their connectivity to other brain areas (Andersen, 2007) as well as in their excitability (Papatheodoropoulos et al., 2002; Papatheodoropoulos and Kostopoulos, 2002; Papatheodoropoulos et al., 2005), along its longitudinal axis. The dorsal (septal) hippocampus is primarily involved in cognitive functions (Moser and Moser, 1998) while ventral (temporal) hippocampus is in addition involved in stress, emotion, and motivation (Adhikari et al., 2010; Fanselow and Dong, 2010), having also a

*Corresponding author at: Dept of Biological Applications & Technology, University of Ioannina, Ioannina 45500, Greece. Fax: +30-(26510)-07061.
E-mail addresses: apostolos.mikroulis@med.lu.se (A. Mikroulis), christos.lisgaras@einstein.yu.edu (C. P. Lisgaras), cpsarrop@uoi.gr (C. Psarropoulou).
† Equal contribution.
‡ Present address: Experimental Epilepsy Group, Wallenberg Neuroscience Center, BMC, Sölvegatan 17, 221 84 Lund, Sweden.
¶ Present address: Saul R. Korey Department of Neurology, Laboratory of Developmental Epilepsy, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Rose F. Kennedy Center, Room 313, Bronx, NY 10461, Unites States.
Abbreviations: 4-AP, 4-aminopyridine; ACh, acetylcholine; ACSF, artificial cerebrospinal fluid; CCh, carbachol; Ch, cholinergics; FRs, fast ripples; $GABA_A$, γ-aminobutyric acid type A receptor; HFOs, high-frequency oscillations; IEDs, interictal epileptiform discharges; N, normal; NMDA, *N*-methyl-D-aspartate; Rs, ripples; S, septal slice; SE, status epilepticus; T, temporal slice.

https://doi.org/10.1016/j.neuroscience.2017.11.029
0306-4522/

lower threshold for seizure generation (Gilbert et al., 1985; Papatheodoropoulos et al., 2005; Toyoda et al., 2013). We therefore sought to determine in this work whether adult septal and temporal hippocampal networks would be affected differently by an early-life SE-like seizure.

Interictal discharges, recorded from epileptic brain between seizures, are not accompanied by behavioral manifestations, but recent evidence suggests that they may correspond to a cognitive impairment (Binnie, 2003; Pressler et al., 2005; Holmes and Lenck-Santini, 2006; Khan et al., 2010; Glennon et al., 2016). Their role in precipitating seizures remains controversial (Staley et al., 2005; Avoli et al., 2013). Interestingly, they have also been recorded in a wide range of behavioral and psychiatric disorders, including attention deficit, anxiety disorders and psychoses (reviewed in (Barkmeier and Loeb, 2009)), suggesting that their role may be wider than previously thought and accordingly, their study may have implications surpassing epilepsy research. Interictal-like epileptiform discharges (IEDs) *in vitro* can be generated by omitting Mg^{2+} from the perfusion medium (Mg^{2+}-free ACSF) or by adding 4-Aminopyridine (4-AP). The former increases excitability by permitting the endogenous glutamate-induced activation of NMDA receptors (Coan and Collingridge, 1987) and the latter by blocking the I_A K^+ current and by increasing transmitter release (Perreault and Avoli, 1991). Using either, we have sought to uncover whether the detection of any long-term effects of immature SE depends on the generation mechanism of the adult IEDs. In this background, we have examined the role of endogenous acetylcholine (ACh) in controlling IEDs, following our earlier findings (Meilleur et al., 2000; Meilleur et al., 2003; Potier et al., 2005; Mikroulis and Psarropoulou, 2012; Kouis et al., 2014).

Finally, IEDs contain High-Frequency Oscillations (HFOs) in the Ripple (80–200 Hz, R) and Fast Ripple (200–600 Hz, FR) ranges, both of which are implicated in seizure onset (Bragin et al., 2002; Ibarz et al., 2010; Wu et al., 2010; Jefferys et al., 2012; Staba, 2012; Xu et al., 2016). We have therefore sought to determine whether Rs and FRs would be affected by any of the above-mentioned factors, i.e. animal treatment (N or SE), slice origin (T or S), IED generating mechanism (Mg^{2+}-free ACSF or 4-AP), addition of cholinergics, and moreover whether any such changes would correspond to IED frequency changes in any specific pattern.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried on 104 Sprague–Dawley rats. Animals were housed at the University of Ioannina Animal Facility; they had free access to pellet food and water and were exposed to a 12-h light/dark cycle. Animal treatment and experimental procedures were conducted in accordance to the Directive of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (2010/63/EE) and approved by the Prefectural (Epirus) Animal Care and Use Committee (EL33-BIO01). Throughout this protocol every care was taken to minimize suffering and the number of animals used.

Seizures

A total of 71 postnatal day 20 (P20) juvenile rats were injected i.p. with the $GABA_A$ channel blocker pentylenetetrazole (Ramanjaneyulu and Ticku, 1984; Huang et al., 2001) (70–90 mg/kg dissolved in 0.9% NaCl). The starting dose was 70 mg/kg and whenever this dose failed to produce a generalized seizure up to 2 increments of 10 mg/kg each were administered in 30-min intervals reaching a cumulative dose of 80 or 90 mg/kg respectively. Convulsive behavior was monitored visually for 4 h and was matched against the Racine scale (Racine, 1972). Mortality rate was ~20%, while 20% of surviving rats displayed only minor convulsive behavior and were not subsequently used. Rats displaying a ≥20 min sustained generalized seizure, hereafter referred to as SE, were used for electrophysiology ($n = 40$). Animals from these same litters injected i.p. with identical saline volume ($n = 64$), hereafter referred to as normal or N, were used as controls.

Slice preparation and extracellular electrophysiological recordings

Hippocampal slices were prepared from adult animals aged 3 months (average, range of 2–6 months). Animals were decapitated under deep isoflurane anesthesia, the brain was quickly removed and the hippocampi were separated. Three to 6 temporal or septal 500-μm-thick transverse slices were cut from each hippocampus using a Vibratome (Series 1000, PELCO 101). Throughout the preparation process the tissue was hydrated or submerged in cool (4 °C), oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, glucose 10, at pH 7.4 (all reagents were purchased from Sigma). Slices were then placed in two independent submersion or Haas-type interface chambers, were perfused with heated (32 ± 1 °C) oxygenated ACSF with no added Mg^{2+} (Mg^{2+}-free ACSF) or alternatively with ACSF containing 50 μM 4-AP and were allowed to equilibrate for at least 1 h before recording started. The volume of each submersion channel was 0.28 ml and the perfusion rate was ~1–2 ml/min.

Extracellular recording electrodes made with borosilicate glass and filled with 4 M NaCl were placed in the CA3 hippocampal pyramidal layer of a total of 216 slices, 133 temporal (T) and 83 septal (S). Signals were amplified (AxoClamp 2B or 900A, Axon Instruments/Molecular Devices), stored in a PC using Axoscope software (Molecular Devices) and/or recorded in paper (DASH II MT, AstroMed) and rates of IED recurrence or frequency (Hz) were measured manually. After recording stability was ensured (recording periods of >20 min), control frequency was calculated from a 3- to 5-min period obtained immediately before the onset of drug perfusion. Each agent was perfused for 10 min, during which, the maximal frequency change observed