Neuroscience 352 (2017) 131–143

PROMINENT DIFFERENCES IN SHARP WAVES, RIPPLES AND COMPLEX SPIKE BURSTS BETWEEN THE DORSAL AND THE VENTRAL RAT HIPPOCAMPUS

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Abstract—Functions of the hippocampus are segregated along its long axis and emerging evidence shows that the local circuitry is specialized accordingly. Sharp waves (SPWs) and ripples are a basic hippocampal network activity implicated in memory processing. Using recordings from the CA1 field of both dorsal (DH) and ventral (VH) rat hippocampal slices we found that SPWs are larger, shorter and occur much more frequently in the VH than in the DH. Clusters of SPWs (i.e. multiple consecutive events grouped in sequences that depend on NMDA receptors) occur with higher probability in the VH and the frequency of occurrence of consecutive intra-cluster events is higher in the VH (∼10 Hz) than in the DH (∼5 Hz). The ripple oscillation displays higher amplitude and frequency in the VH than in DH and the associated multiunit firing peaks at a later phase of the ripple waves in the VH than in the DH. Isolated unit complex spike bursts display a significantly lower number of spikes and longer inter-spike intervals in the VH than in the DH suggesting that the synaptically driven neuronal excitability is lower in the VH. We propose that to some extent these differences result from the relatively higher network excitability of the VH compared with DH. Furthermore, they might reflect specializations that provide the local circuitries of the DH and VH with the required optimal ability for synaptic plasticity and might also suggest that the VH could be a favored site of SPW-Rs initiation.

Key words: hippocampus, dorsoventral, septotemporal axis, sharp wave, ripple oscillation, complex spike burst.

INTRODUCTION

The segregation of functions along the long (dorsoventral or septotemporal) axis of the hippocampus is a continuously expanding field of research (Engin and Treit, 2007; Bast, 2011; Poppenk et al., 2013; Bannerman et al., 2014, Strange et al., 2015). Among the implications of this large-scale functional diversification is the hypothesis that specialization also exists in the local neuronal circuitry along the hippocampus (Small et al., 2011; Strange et al., 2014; Tushev and Schuman, 2016). In fact, growing evidence indicates that there is a specialization of the intrinsic neuronal network between the two opposite hippocampal segments, namely the dorsal (DH) and the ventral (VH) hippocampi, which can be observed at all levels of neuronal organization, including molecular and gene expression profiles (Leonardo et al., 2006; Thompson et al., 2008; Dong et al., 2009; Zoladz et al., 2012; Cembrowski et al., 2016), the properties of pyramidal neurons (Kjelstrup et al., 2008; Dougherty et al., 2012, 2013; Marcelin et al., 2012; Honigsperger et al., 2015; Malik et al., 2016; Babiec et al., 2017; Malik and Johnston, 2017), the molecular composition and the function of neurotransmitter receptors (Sotiriou et al., 2005; Pandis et al., 2006; Gu et al., 2013; McEown and Treit, 2013; Kouvaros and Papatheodoropoulos, 2016a), the synaptic plasticity (Papatheodoropoulos and Kostopoulos, 2000; Maruki et al., 2001; Colgin et al., 2004; Grigoryan et al., 2012; Kenney and Manahan-Vaughan, 2013; Papatheodoropoulos, 2015; Grigoryan and Segal, 2016; Kouvaros and Papatheodoropoulos, 2016b), and the physiological neuronal oscillations (Patel et al., 2013; Long et al., 2015).

The hippocampal network organizes a variety of periodic physiological activities of which the most prominent is the population activity pattern of sharp wave-ripples (SPW-Rs), (Buzsáki, 2015; Colgin, 2016). During SPW-Rs an orchestrated reactivation of assemblies of hippocampal pyramidal cells (also called complex spike cells) occurs that is implicated in synaptic plasticity and memory consolidation (O'Neill et al., 2010; Buzsáki, 2015; Sadowski et al., 2016). One important property of SPW-Rs is that they are an endogenous network activity that is self-organized in the hippocampus independently of its inputs (Buzsaki et al., 1987; Suzuki and Smith, 1988) as well as in isolated hippocampal preparations (Papatheodoropoulos and Kostopoulos, 2002; Wu et al.,

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Abbreviations: DH, dorsal hippocampus; EPSP, excitatory postsynaptic potential; Fv, fiber volley; ICI, intra-cluster interval; IEI, inter-event interval; IEpI, inter-episode interval; MUA, multiunit activity; PS, population spike; SPW-Rs, sharp wave-ripples; SPWs, sharp waves; VH, ventral hippocampus.

http://dx.doi.org/10.1016/j.neuroscience.2017.03.050

2002; Kubota et al., 2003; Maier et al., 2003; Colgin et al., 2004; Papatheodoropoulos, 2010; Aivar et al., 2014); it is therefore considered to be a default activity pattern of the hippocampus, as might also be the theta oscillation (Goutagny et al., 2009). Interestingly, it has been recently shown that the activated neurons during exploration are reactivated during spontaneous SPWs in hippocampal slices *in vitro* (Mizunuma et al., 2014). Considering the recently revealed differences along the long axis of the hippocampus it is of particular importance to study how the isolated local circuits of the two hippocampal segments organize the fundamental default network activity of SPW-Rs.

It is of note that a considerable amount of information regarding the cellular and local network mechanisms that underlie SPW-Rs has recently been accumulated using the preparation of rodent hippocampal slice (Buzsáki, 2015). Using transverse slices from the DH and the VH of adult rats we found remarkable differences in the spontaneous SPW-Rs and the activity of bursting units between the two hippocampal segments; in addition, complex spike bursts are shorter and of lower frequency in the VH than in DH. We propose that these specializations between DH and VH will certainly contribute to the diversification of the way that the incoming information is processed by the circuitries of the two hippocampal segments.

EXPERIMENTAL PROCEDURES

Slice preparation

Slices were prepared from 2–4-month-old Wistar male rats. Animals were housed in the Department of Medicine of the University of Patras under conditions of controlled temperature (21–23 °C) and a 12-h light/dark cycle with free access to food and water. All animal treatment and experimental procedures were conducted in accordance to the Directive Guidelines for the care and use of Laboratory animals of the European Communities Council Directive Guidelines (86/609/EEC) for the care and use of Laboratory animals and approved by the Prefectural (Achaia) Animal Care and Use Committee (No: EL 13BIO04). Accordingly, all measures were taken to minimize animal suffering and to reduce the number of the animals used. The animals were sacrificed under deep anesthesia with diethyl-ether using a guillotine. After the brain was removed from the skull it was placed in cooled (4 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 4, $MgSO_4$ 2, $CaCl_2$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 26 and glucose 10, equilibrated with 95% O_2 and 5% CO_2 gas mixture at a pH 7.4. The two hippocampi were excised free and slices 500–550 μm thick were prepared at an angle perpendicular to the long axis of the structure from the DH and VH using a McIlwain tissue chopper. The slices were immediately transferred on an interface type recording chamber consisting of two independent channels and they were maintained at a constant temperature of 31.5 ± 0.5 °C, continuously humidified with a mixed gas containing 95% O_2 and 5% CO_2 and perfused with standard ACSF at a rate of about 1.5 ml/min. It is noted that although SPW-Rs can be generated along virtually the whole hippocampus of an intact animal (Patel et al., 2013), the slices taken from different sites along the long axis of the structure display noticeably different ability to generate spontaneous SPW-Rs. Thus, the results from previous studies which were, however, based on a limited sample of dorsal slices, have shown that SPW-Rs can be spontaneously generated in ventral but not in dorsal hippocampal slices (Papatheodoropoulos and Kostopoulos, 2002; Colgin et al., 2004; Caliskan et al., 2016). Actually, in our following studies there is a small fraction of dorsal slices that do generate SPW-Rs spontaneously. This could be accounted for by the fact that we use rather thick rat slices compared with most other labs (Aivar et al., 2014; Buzsáki, 2015). Slice thickness appears to be an important factor in determining the generation of spontaneous activity in slices (Wu et al., 2009; Schlingloff et al., 2014). Nevertheless, this should not be the only reason since small CA1 mini-slices can maintain the ability to generate SPW-Rs (Maier et al., 2003; Papatheodoropoulos, 2010). In order to overcome the problem of low likelihood of SPW-Rs emergence in dorsal slices and thus make feasible the comparison between the two hippocampal segments, we systematically monitored a large population of dorsal and ventral slices from a considerable number of rats. We also tried to study dorsal and ventral slices that were obtained from a common population of rats. In general, up to two dorsal and two ventral hippocampal slices from each rat were used in the analysis of various measures. Specifically, we analyzed slices prepared from the tissue extending between 0.5 and about 4.4 mm from the dorsal end and the portion extending between 2.8 and 4.0 mm from the ventral end of the hippocampus. The competitive antagonist of the NMDA receptors 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10 μM; Tocris Cookson Ltd, UK) was used in this study.

Recordings and data processing

Recordings started approximately two hours after the slices were placed in the recording chamber and continued for at least three hours afterward. Field recordings of spontaneous and evoked activity were made from the CA1 field of the hippocampus using carbon fiber electrodes (diameter 7 μm, Kation Scientific, Minneapolis, USA). Electrical stimulation of afferent fibers consisted of pulses (intensity 20–300 μA, duration 0.1 ms, frequency 0.033 Hz) delivered by a bipolar platinum/iridium wire electrode (wire diameter of 25 μm, World Precision Instruments, USA). The potentials were amplified (×500) and filtered at 0.5 Hz–2 kHz using a Neurolog system (Digitimer Limited, UK). The signal was digitized at 5–10 kHz and stored on a computer for off-line analysis using the CED 1401-plus interface and the Signal and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Spontaneous activity

Spontaneous field activity, recorded from the pyramidal cell layer, was categorized as population or network