Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

The primate seahorse rhythm

L.M.G. Campos[a,*], Roelf J. Cruz-Rizzolo[b], L. Pinato[a]

[a]Department of Speech-Language and Hearing Therapy, São Paulo State University, Marilia, SP, Brazil
[b]Department of Basic Sciences, São Paulo State University, Araçatuba, SP, Brazil

ARTICLE INFO

Article history:
Accepted 31 March 2015
Available online 8 April 2015

Keywords:
Hippocampus
Learning and memory
PER2
Calcium binding proteins
Circadian cycle

ABSTRACT

The main Zeitgeber, the day–night cycle, synchronizes the central oscillator which determines behaviors rhythms as sleep–wake behavior, body temperature, the regulation of hormone secretion, and the acquisition and processing of memory. Thus, actions such as acquisition, consolidation, and retrieval performed in the hippocampus are modulated by the circadian system and show a varied dependence on light and dark. To investigate changes in the hippocampus' cellular mechanism invoked by the day and night in a diurnal primate, this study analyzed the expression of PER2 and the calcium binding proteins (CaBPs) calbindin, calretinin and parvalbumin in the hippocampus of *Sapajus apella*, a diurnal primate, at two different time points, one during the day and one during the dark phase. The PER2 protein expression peaked at night in the antiphase described for the suprachiasmatic nucleus (SCN) of the same primate, indicating that hippocampal cells can present independent rhythmicity. This hippocampal rhythm was similar to that presented by diurnal but not nocturnal rodents. The CaBPs immunoreactivity also showed day/night variations in the cell number and in the cell morphology. Our findings provide evidence for the claim that the circadian regulation in the hippocampus may involve rhythms of PER2 and CaBPs expression that may contribute to the adaptation of this species in events and activities relevant to the respective periods.

1. Introduction

In primates, the hippocampus (named after its resemblance to the *seahorse*, from the Greek *hippos* meaning "horse" and *kampos* meaning "sea monster") (Squire and Zola-Morgan, 1991) presents subdivisions such as the dentate gyrus (DG) and the *Cornu Ammonis* (CA) divided into CA1, CA2 and CA3 with varied cell morphology, connectivity and electrophysiological properties (Igarashi et al., 2014a; Mishkin, 1978; Paxinos and Watson, 1998; Swanson et al., 1987). These subdivisions are arranged in layers or *strata*, arranged in the DG from the innermost to the outermost layers: the polymorphic, granular (*stratum granulare*) and molecular (*stratum moleculare*) layers and, in the CA, the *stratum moleculare*, *stratum lacunosum*, *stratum radiatum*, *stratum lucidum*, pyramidal layer, *stratum oriens* and *alveus* (Altman et al., 1973; Paxinos and Watson, 1998).

Physiologically, its functions are related to the neuronal circuitries involved in the processes of learning and memory, including spatial navigation, olfactory-spatial associative memory, and motivated or emotional behaviors (Bliss and Collingridge, 1993; Eichenbaum, 1999; Igarashi et al., 2014b;

*Correspondence to: Departamento de Fonoaudiologia, UNESP—Av. Vicente Ferreira, 1278, Marília 17515-901, SP, Brazil.
E-mail address: leilacampos@usp.br (L.M.G. Campos).

http://dx.doi.org/10.1016/j.brainres.2015.03.054
0006-8993/

Milenkovic et al., 2013; Moser and Moser, 1998; Squire, 2007). Furthermore, it may participate in a variety of autonomic, neuroendocrine, defensive, ingestion-related and reproductive control systems (Bannerman et al., 2004; Kjelstrup et al., 2008).

Many of these features are interlinked and are responsive to environmental changes (Hauber and Bareiss, 2001; Martin-Fairey and Nunez, 2014; Smarr et al., 2014). The light/dark cycle is the most striking of the environmental variations that are able to exert a potent synchronizer role in the biological rhythms (Moore-Ede et al., 1982; Mrosovsky and Salmon, 1987; Rea, 1998).

When the interactions between organisms and their environments are considered, it is necessary to understand the morphology and physiology of the structures that enable replies to the challenges inherent to the physical nature of environmental variations.

Briefly, the circadian biological rhythms are controlled by a circadian timing system where the cells of the hypothalamic suprachiasmatic nucleus (SCN) express autonomous rhythmic gene expressions that have their individual circadian phase synchronized by inter-neuronal communication. Cell-autonomous circadian oscillation is driven by a negative feedback loop of transcription regulation of the so-called clock genes, where CRY/PER heterodimers act as the transcriptional repressors of their own genes (Reppert and Weaver, 2001).

The integrated rhythmic SCN activity works as a pacemaker for the circadian clocks of non-SCN cells, each of which also rhythmically express clock genes (Duncan et al., 2013). This is reflected in behaviors such as sleep–wake behavior, body temperature, the regulation of hormone secretion, and the acquisition and processing of memory (Chaudhury and Colwell, 2002; Gerstner et al., 2009; Nesca and Koulack, 1994; Ruby et al., 2008, 2013).

Different models in rodents and humans have highlighted remarkable changes in cognitive functions, memory acquisition and recall across the day as well as the circadian modulation of neurogenesis, synaptic remodeling, intracellular cascades, and the epigenetic regulation of gene expression (Hauber and Bareiss, 2001; Martin-Fairey and Nunez, 2014; Smarr et al., 2014). Furthermore, functions such as processing and learning in the hippocampal memory have been described as important to integrate the circadian information of independent oscillators (Garren et al., 2013; Maury and Queinnec, 1992; Nesca and Koulack, 1994; Smarr et al., 2014). Some of these models have shown evidence of the rhythmic expression of clock genes in the hippocampus (Duncan et al., 2013; Lamont et al., 2005; Otalora et al., 2013; Verwey et al., 2007). In rodents was demonstrated rhythms in *Per*2 expression in light/dark (LD) or constant darkness (DD) cycles and that these rhythms are autonomous as they are present in isolated hippocampal slices maintained in culture (Wang et al., 2009).

However, the specific means by which the circadian rhythm affects the functioning of the hippocampus are not yet fully clarified. The PER2 protein is one of the key proteins involved in this molecular timing system, and it is known to present variations in its expression in this and other brain areas (Chaudhury et al., 2008; Lamont et al., 2005; Verwey et al., 2007; Zhu et al., 2014).

The calcium binding proteins (CaBPs) calbindin (CB), calretinin (CR) and parvalbumin (PV) partially operate as buffers, decreasing the concentration of cytoplasmic Ca^{2+}in neurons (Baimbridge et al., 1992; Camp and Wijesinghe, 2009; Hof et al., 1999). Thus, changes in the expression of CaBPs may be correlated with the neurochemical, morphological and functional answers provided by the cells (Baimbridge et al., 1982; Baimbridge and Miller, 1982; Gulyás et al., 1991).

This previous knowledge leads one to question whether these molecules could be responsive to environmental changes in order to adapt the functioning of the hippocampus in the most favorable period to their functions in each species.

Traditionally, this issue has been evaluated in rodents kept in artificial light cycles, but analyzing this issue in a primate model exposed to a natural light cycle during the day and the night would add relevant information to body of knowledge in this area.

Thus, to investigate if hippocampus neurons regulate their protein expression in a regular circadian manner in a diurnal primate, this study analyzed the expression of the PER2 protein and of CaBPs CB, CR and PV in the hippocampus of the *Sapajus apella* in two different time points, one during the day and one during the dark phase.

2. Results

The hippocampus appeared as a collection of neurons agglomerated in different identifiable layers above the entorhinal cortex in the medial temporal lobe (Fig. 1).

The PER2 protein was expressed in a high density of neurons strongly stained in the three layers of hippocampus at night, unlike during the day (Fig. 2). In the polymorphic layer, 92.0±6.8 PER2-IR cells were visualized in the day and 237.0±21.4 at night. In the granular layer, an increase in the PER2-IR O.D. was shown at night (169.2±1.5) compared to the day (54.8±5.3). In the pyramidal layer, 126.0±19.7 PER2-IR cells were visualized in the day and 248.7±25.5 at night (Fig. 2K–M).

The CaBPs also showed day/night variations in the IR intensity, the number of IR cells, and the cells' morphology. Furthermore, PV-IR, CB-IR and CR-IR neurons showed different patterns of distribution between the layers analyzed, as described below.

2.1. *Granular layer*

A low number of strongly stained PV-IR neurons was found in this layer, with a higher number found at night (7.6±1.3 day; 22.2±4.2 night, $p=0.005$) (Fig. 3M). These neurons at night expressed PV-IR showing long fibers that reached the molecular layer of the DG (Fig. 6H), unlike the morphological pattern shown in the day (Fig. 6A). On the other hand, the most of CB-IR neurons in this layer showed morphological features very common in interneurons in both periods. These neurons exhibited an oval form and staining in a short part of dendrites projected in the direction of the molecular layer without a strongly marked long extension (Fig. 3E and H). The CB-IR neurons exhibited a temporal pattern similar to that