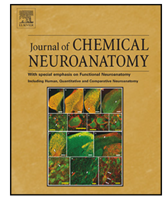
Journal of Chemical Neuroanatomy 57–58 (2014) 42–53

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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



Efferent projections of the suprachiasmatic nucleus based on the distribution of vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) immunoreactive fibers in the hypothalamus of *Sapajus apella*

L.M.G. Campos [a,*], R.J. Cruz-Rizzolo [c], Ii-Sei Watanabe [a], L. Pinato [b,1], M.I. Nogueira [a,1]

[a] Department of Anatomy, Institute of Biomedical Science, University of São Paulo, SP, Brazil
[b] Department of Speech Language and Hearing Therapy, São Paulo State University, Marília, SP, Brazil
[c] Department of Fundamental Sciences, São Paulo State University, Araçatuba, SP, Brazil

ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form 8 March 2014
Accepted 9 March 2014
Available online 13 April 2014

Keywords:
Circadian rhythms
Neuroanatomy
Primate
Anticipatory behavior

ABSTRACT

The suprachiasmatic nucleus (SCN), which is considered to be the master circadian clock in mammals, establishes biological rhythms of approximately 24 h that several organs exhibit. One aspect relevant to the study of the neurofunctional features of biological rhythmicity is the identification of communication pathways between the SCN and other brain areas. As a result, SCN efferent projections have been investigated in several species, including rodents and a few primates. The fibers originating from the two main intrinsic fiber subpopulations, one producing vasoactive intestinal peptide (VIP) and the other producing arginine vasopressin (AVP), exhibit morphological traits that distinguish them from fibers that originate from other brain areas. This distinction provides a parameter to study SCN efferent projections. In this study, we mapped VIP (VIP-ir) and AVP (AVP-ir) immunoreactive (ir) fibers and endings in the hypothalamus of the primate *Sapajus apella* via immunohistochemical and morphologic study. Regarding the fiber distribution pattern, AVP-ir and VIP-ir fibers were identified in regions of the tuberal hypothalamic area, retrochiasmatic area, lateral hypothalamic area, and anterior hypothalamic area. VIP-ir and AVP-ir fibers coexisted in several hypothalamic areas; however, AVP-ir fibers were predominant over VIP-ir fibers in the posterior hypothalamus and medial periventricular area. This distribution pattern and the receiving hypothalamic areas of the VIP-ir and AVP-ir fibers, which shared similar morphological features with those found in SCN, were similar to the patterns observed in diurnal and nocturnal animals. This finding supports the conservative nature of this feature among different species. Morphometric analysis of SCN intrinsic neurons indicated homogeneity in the size of VIP-ir neurons in the SCN ventral portion and heterogeneity in the size of two subpopulations of AVP-ir neurons in the SCN dorsal portion. The distribution of fibers and morphometric features of these neuronal populations are described and compared with those of other species in the present study.

© 2014 Elsevier B.V. All rights reserved.

Abbreviations: AHA, anterior hypothalamic area; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DMH, dorsomedial hypothalamic nucleus; DMSO, dimethyl sulfoxide; Ir, immunoreactive; LH, lateral hypothalamus; LPOA, lateral preoptic area; LZ, lateral zone; ME, median eminence; MZ, medial zone; MPOA, medial preoptic area; NeuN, neuronal nuclei protein; OPT, optic tract; OX, optic chiasm; PVN, paraventricular hypothalamic nucleus; PZ, periventricular zone; RCA, retrochiasmatic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; SOr, retrochiasmatic part of the optic nucleus; SOX, supraoptic decussation; SPVZ, subparaventricular zone; STC, circadian timing system; SVN, subventricular nucleus; VIP, vasoactive intestinal peptide; VMH, ventromedial hypothalamic nucleus; AVP, arginine vasopressin.

* Corresponding author. Tel.: +55 11981840870.
E-mail address: leilacampos@usp.br (L.M.G. Campos).

[1] These authors are joint senior authors.

http://dx.doi.org/10.1016/j.jchemneu.2014.03.004
0891-0618/© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Most species exhibit biological, physiological, endocrine, and behavioral rhythms that are influenced by daily or seasonal variations in temperature, humidity, and photic periods (Moore-Ede et al., 1982; Reppert and Weaver, 2002). Some of these phenomena, known as the circadian rhythms, exhibit a 24-h oscillation period, and the fact that they occur continuously under constant environmental conditions suggests the existence of an endogenous temporal oscillation system (Moore-Ede et al., 1982; Reppert and Weaver, 2002).

The suprachiasmatic nucleus (SCN) is considered to be the master pacemaker of the endogenous temporal oscillation system (Moore-Ede et al., 1982). The circadian function of the SCN is due to the autonomous oscillatory capacity of its cells, which in turn, arises from transcription and translation feedback loops and results in the rhythmic expression of "clock genes" (Reppert and Weaver, 2001).

The localization of the SCN quite consistent among mammals. The SCN appears as a number of small neuron agglomerates in the periventricular region of the anterior hypothalamus, which is adjacent to the third ventricle and immediately dorsal to the optic chiasm (OX). Nevertheless, the SCN exhibits interspecific variations in its three-dimensional shape, volume, density, organization, and cell chemical identity and size (Cassone et al., 1988; Lydic et al., 1982; Pinato et al., 2007; Rocha et al., 2014).

The endogenous rhythm generated in the SCN is synchronized to the external environment by several neural input pathways and endocrine secretory events (Cipolla-Neto et al., 1988), while output pathways allow the SCN to synchronize secondary oscillators, such as the dorsomedial hypothalamic nucleus (DMH), substantia nigra, and cerebellum (Gooley et al., 2006; Mordel et al., 2013).

The internal neurochemical composition of the SCN is rather complex, as it includes populations of neurons, fibers, and endings that produce and/or release several neuroactive substances. The SCN composition may exhibit interspecific variation among rodents, and mainly between rodents and primates (Buijs, 1997; Cavalcante et al., 2002; Moore, 1993; Pinato et al., 2007; Rocha et al., 2014).

Among the main neuroactive substances in SCN neurons are vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP). In rodents, the differential array of these neuropeptides determines the division of the SCN into a ventrolateral portion ("core"), which is neurochemically characterized by the presence of VIP-immunoreactive (VIP-ir) neurons, and a dorsomedial portion ("shell"), which is mainly composed of AVP-immunoreactive (AVP-ir) neurons (Abrahamson and Moore, 2001; Cassone et al., 1988; Ramanathan et al., 2006; Ueda et al., 1983; Reghunandanan and Reghunandanan, 2006; Watts and Swanson, 1987a).

These portions differ further in their functions and efferent patterns. VIP-ir neurons are mainly involved in the mechanism underlying the synchronization of circadian rhythms to external events and project intrinsically to the SCN dorsomedial portion and extrinsically to various hypothalamic areas (Dai et al., 1997; Jacomy et al., 1999; Moore, 2013). In turn, the AVP-ir neurons present in the SCN dorsomedial portion are involved in the generation of circadian rhythms and project to areas such as the subparaventricular zone (SPVZ), paraventricular hypothalamic nucleus (PVN), dorsomedial hypothalamic nucleus (DMH), and ventromedial hypothalamic nucleus (VMH). Conversely, VIP-ir fibers diffusely innervate the anterior aspect of the medial preoptic area (POA) and the central and medial aspects of the anteroventral hypothalamic area (AVH) (Dai et al., 1997). Together, the hypothalamic areas receiving VIP-ir or AVP-ir fibers are considered to be receiving areas of the SCN (Abrahamson and Moore, 2001; Acosta-Galvan et al., 2011; Buijs, 1997; Cassone et al., 1988; Dai et al., 1997).

Because anterograde tracer injection is a difficult procedure to conduct in primates, studies that combine tracer injection and immunohistochemical techniques have shown that the SCN efferent pathways could be mapped via analysis of AVP and VIP immunoreactivity because the morphologic features of the VIP-ir and AVP-ir SCN efferent fibers are different from the fibers originating in other nuclei (Buijs, 1997; Leak and Moore, 2001; Watts and Swanson, 1987b).

The interspecific variation of the components of the circadian timing system (STC), and the opportunity to investigate this system in various models suggests that it is relevant to examine the STC in other species, particularly primates. Studies conducted with nonhuman primates are of great value to understand the structural and functional organization of the primate brain. As such, some species, including *Sapajus apella*, have evolved several behavioral and anatomical characteristics that are similar to those of humans. *S. apella* exhibits diurnal and social habits, altricial young, intense maternal care, complex fine motor skills, and well-developed frontal lobes (Dum and Strick, 2005; Fragaszy and Adams-Curtis, 1991; Ross et al., 2010). Our group has been working with the circadian timing system of this diurnal primate and with the *Callithrix jacchus* in the last decade. We have described the SCN intrinsic organization and afferent connections (Pinato et al., 2007, 2009; Cavalcante et al., 2011; Rocha et al., 2014) and presently would like to demonstrate a pattern of potential efferent projections to complement the anatomical description of our primate model. In the present study, AVP-ir and VIP-ir fiber mapping in the hypothalamus of the primate *S. apella* allowed for the characterization of the SCN receiving areas, which may be directly related to SCN functions.

2. Materials and methods

In the present study, the brains of 3 adult male tufted capuchin monkeys (*S. apella*) who weighed 2–3 kg and were obtained from the Center of Tufted Capuchin Monkey Procreation of the São Paulo State University (UNESP) in Araçatuba, SP, Brazil were used. The animals were kept in individual cages under natural light and were fed a controlled diet consisting of eggs, fruit, granulated ration with protein, and dried corn; water was provided ad libitum. The procedures involving animal use were compliant with the "*Guidelines for the care and use of mammals in neuroscience and behavioral research* (2003)" and were approved by the local ethics committee (protocol no. 133).

Animals were anesthetized at approximately 11:00 h, and transcardial perfusion of 1 L of 0.9% saline solution was subsequently applied, followed by 2 L of 4% paraformaldehyde in 0.1 M sodium acetate buffer (pH 6.0, 4 °C) and 2 L of 4% paraformaldehyde in 0.1 M sodium borate buffer (pH 9.5, 4 °C). Brains were divided into two blocks and placed in a cryoprotectant composed of borate buffer (pH 9.0), 10% glycerol (Labsynth, Brazil) and 2% dimethyl sulfoxide (DMSO – Sigma–Aldrich, USA) over five days, followed by immersion in a similar solution with 20% glycerol. After cryoprotection, the brain blocks were cryosectioned into 30-μm-thick sections using a cryostat (Leica CM 1850, Microsystems AG, Germany) and stored as 10 different stepwise series in an anti-freeze solution of 240 ml of ethylene glycol (Labsynth, Brazil), 200 ml of 0.1 M phosphate buffer (pH 7.4), 200 ml of water, and 120 g of sucrose (Labsynth, Brazil) until the time of immunohistochemical processing or Nissl staining.

2.1. Immunoperoxidase

Brain sections were processed using immunohistochemical techniques for VIP, AVP, and neuronal nuclei protein (NeuN). Sections were washed using a solution TBS-TX buffer composed of Tris (Amresco, USA), NaCl (Labsynth, Brazil), Triton X-100 (Amresco, USA), and water (0.05 M TBS-TX) at pH 7.6. Next, the sections were incubated for 48 h at 4 °C in 0.05 M TBS-TX buffer; 2% normal serum (Vector Laboratories, USA); and anti-VIP (rabbit, 1:250, Abcam), anti-AVP (rabbit, 1:5000, Merck Millipore Corporation, Germany), or anti-NeuN (mouse, 1:1000, Merck Millipore Corporation, Germany) primary antibodies. The sections were washed with 0.05 M TBS-TX and incubated in a solution including 2% normal serum and biotinylated secondary antibody specific for the primary antibody species for two hours. The sections were then washed with 0.05 M TBS-TX, incubated in a solution containing avidin-biotin complex (Vector Laboratories, USA) for two hours, and washed with Tris–HCl buffer (pH 7.6). Labeling was developed using 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma–Aldrich, USA) as a chromogen. Next, the sections were mounted on gelatin-coated slides and dehydrated; coverslips were placed on the slides using DPX as the mounting medium (Sigma–Aldrich, USA).

Download English Version:

https://daneshyari.com/en/article/1988838

Download Persian Version:

https://daneshyari.com/article/1988838

Daneshyari.com