Journal of Chemical Neuroanatomy 77 (2016) 100–109

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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

# Nuclear organization of the substantia nigra, ventral tegmental area and retrorubral field of the common marmoset (*Callithrix jacchus*): A cytoarchitectonic and TH-immunohistochemistry study

José R.L.P. Cavalcanti[a,b,*], André L.B. Pontes[b], Felipe P. Fiuza[b], Kayo D.A. Silva[b], Fausto P. Guzen[a,b], Eudes E.S. Lucena[a,b], Expedito S. Nascimento-Júnior[c], Judney C. Cavalcante[c], Miriam S.M.O. Costa[c], Rovena C.G.J. Engelberth[b], Jeferson S. Cavalcante[b,**]

[a] Department of Biomedical Sciences, Laboratory of Experimental Neurology, Health Science Center, University of State of Rio Grande do Norte, Mossoró, RN, Brazil
[b] Department of Physiology, Laboratory of Neurochemical Studies, Biosciences Center, Federal University of Rio Grande do Norte, Natal, RN, Brazil
[c] Department of Morphology, Laboratory of Neuroanatomy, Biosciences Center, Federal University of Rio Grande do Norte, Natal, RN, Brazil

ARTICLE INFO

*Article history:*
Received 15 December 2015
Received in revised form 6 May 2016
Accepted 7 May 2016
Available online 9 June 2016

*Keywords:*
*Callithrix jacchus*
Retrorubral field
Substantia nigra pars compacta
Tyrosine-hydroxylase
Ventral tegmental area

ABSTRACT

It is widely known that the catecholamine group is formed by dopamine, noradrenaline and adrenaline. Its synthesis is regulated by the enzyme called tyrosine hydroxylase. 3-hydroxytyramine/dopamine (DA) is a precursor of noradrenaline and adrenaline synthesis and acts as a neurotransmitter in the central nervous system. The three main nuclei, being the retrorubral field (A8 group), the substantia nigra *pars compacta* (A9 group) and the ventral tegmental area (A10 group), are arranged in the die-mesencephalic portion and are involved in three complex circuitries – the mesostriatal, mesolimbic and mesocortical pathways. These pathways are involved in behavioral manifestations, motricity, learning, reward and also in pathological conditions such as Parkinson's disease and schizophrenia. The aim of this study was to perform a morphological analysis of the A8, A9 and A10 groups in the common marmoset (*Callithrix jacchus* – a neotropical primate), whose morphological and functional characteristics support its suitability for use in biomedical research. Coronal sections of the marmoset brain were submitted to Nissl staining and TH-immunohistochemistry. The morphology of the neurons made it possible to subdivide the A10 group into seven distinct regions: interfascicular nucleus, raphe rostral linear nucleus and raphe caudal linear nucleus in the middle line; paranigral and parainterfascicular nucleus in the middle zone; the rostral portion of the ventral tegmental area nucleus and parabrachial pigmented nucleus located in the dorsolateral portion of the mesencephalic tegmentum. The A9 group was divided into four regions: substantia nigra *compacta* dorsal and ventral tiers; substantia nigra *compacta* lateral and medial clusters. No subdivisions were made for the A8 group. These results reveal that A8, A9 and A10 are phylogenetically stable across species. As such, further studies concerning such divisions are necessary in order to evaluate the occurrence of subdivisions that express DA in other primate species, with the aim of characterizing its functional relevance.



*Abbreviations:* 3N, oculomotor nucleus; 3v, 3rd ventricle; Aq, cerebral aqueduct; Cli, caudal linear nucleus of the raphe; cp, cerebral peduncle; fr, fasciculus retroflexus; IF, interfascicular nucleus; IP, interpeduncular nucleus; mcp, middle cerebellar peduncle; ml, medial lemniscus; MN, mammilary nucleus; PAG, periaqueductal gray; PBP, parabrachial pigmented nucleus; PIF, parainterfascicular nucleus; PN, paranigral nucleus; RLi, rostral linear nucleus; RN, red nucleus; RRF/A8, retrorubral field; SN/A9, substantia nigra *pars compacta* (nuclear complex); SNCD, substantia nigra dorsal tier; SNCL, substantia nigra lateral cluster; SNCM, substantia nigra medial cluster; SNCV, substantia nigra ventral tier; SNR, substantia nigra reticulate; STh, subthalamic nucleus; SuM, supramammilary nucleus; VTA/A10, ventral tegmental area (nuclear complex); VTAR, ventral tegmental area rostral part; xscp, decussation of superior cerebelar peduncle; ZI, zona incerta.

* Corresponding author at: Department of Biomedical Sciences, Laboratory of Experimental Neurology, Health Science Center, State University of Rio Grande do Norte, 59607-360, Mossoró RN, Brazil.
** Corresponding author.
*E-mail addresses:* rodolfolopes@uern.br, joserodolfolopes@uol.com.br (J.R.L.P. Cavalcanti).

http://dx.doi.org/10.1016/j.jchemneu.2016.05.010
0891-0618/

## 1. Introduction

Although some recent research in neuroscience points to modern possibilities of interventions to solve old problems, fundamental characteristics of encephalic nuclei and the formation of neural circuits remain to be clarified (Lammel et al., 2014). Studies focusing on nuclei that express dopamine fall under this category, exploring its morphological and functional aspects, with discussions on models of neurodegenerative diseases and also therapeutic possibilities.

Close to fifty years ago, Arvid Carlsson and others developed studies that generated new ways to study neuroscience. They attested that the akinetic effects caused by reserpine (a drug whose action is related to catecholamine depletion) could be reversed by intravenous injection of 3,4-dihydroxyphenylalanine (DOPA), as it is a precursor of dopamine, as well as noradrenaline and adrenaline. In light of this finding, they suggested that the reversal of the effects caused by reserpine was specifically related to increasing levels of dopamine and not noradrenaline (Carlsson et al., 1957). Later, they concluded that 3-hidroxityramine/dopamine (DA) acted as a neurotransmitter in the central nervous system, in addition to its role as a precursor of noradrenaline and adrenaline synthesis (Carlsson et al., 1958; Bertler and Rosengren, 1959). As such, DA is a monoamine included in the catecholamine group and is a major neurotransmitter in the modulation of brain function, playing a crucial role in the adaptation of animal behavior throughout evolution (Smeets and González, 2000; Jones and Pilowski, 2002; Yamamoto and Vernier, 2011).

It is known that the catecholamine groups A8-A17 use DA as a neurotransmitter (Dahlström and Fuxe, 1964; Björklund and Dunnett, 2007a), because the neurons of these groups express tyrosine hydroxylase (TH), but not dopamine beta-hydroxylase, the active enzyme in the conversion step for noradrenaline/adrenaline. Thus, they are typically considered dopaminergic groups (Björklund and Dunnett, 2007b). Within ten mapped nuclei, A8, A9 and A10 consist of larger nuclei and are coincident with the retrorubral field (RRF), the substantia nigra *pars compacta* (SNC), and the ventral tegmental area (VTA), respectively (Björklund and Dunnett, 2007b). They are involved in three circuitries called mesostriatal, mesolimbic and mesocortical (Dahlström and Fuxe, 1964; German and Manaye, 1993; François et al., 1999; Smith and Kieval, 2000; Björklund and Dunnett, 2007b), related to motor control, motivation, cognition, reinforcement learning and some neurological/psychiatric disorders, such as Parkinson's disease and schizophrenia (Chudasama and Robbins, 2004; Nicola et al., 2005; Fields et al., 2007; De Araujo et al., 2010; Freire and Santos, 2010; Cohen et al., 2012).

Despite these nuclei being located in the ventral midbrain, their ontogeny remains a matter of discussion. They are often genuinely called mesencephalic, since these neurons are generated from the midbrain floor plate during embryonic development and give rise to the three groups mentioned above (Ono et al., 2007; Hegarty et al., 2013). However, non-midbrain structures appear as potential candidates for precursors of dopaminergic cells of the nuclei in question. Studies from the TH expression in the nervous system of embryos have identified the presence of dopaminergic cells in the basal regions of prosomers (P1-3), within the isthmus and the midbrain, suggesting that the A8, A9 and A10 cores are dien-mesencephalic (Marín et al., 2005). Nevertheless, these neuronal clusters can be parceled into cytoarchitectonic and chemoarchitectonic grounds, as described in mice (Fu et al., 2012).

Considering the functional relevance and morphological aspects above-mentioned, it is necessary to expand studies on these structures, including species that are more similar to humans. The marmoset (*Callithrix jacchus*) is a new world primate, derived from the Amazon basin, belonging to the Callithricidae family, of the Callitrichinae subfamily. It is a small neotropical primate, measuring about 30 cm in body and with a 17 cm tail, which gives the animal balance. Marmoset weight ranges from 230 to 420 g, and this relatively small size and social organization in small family groups facilitates its captivity. The marmoset becomes sexually mature at fifteen months, and is then considered an adult animal until it reaches eight years old (Abbot et al., 2003).

The marmoset has been adopted as a model for studies of a number of neurological disorders such as Parkinson's disease (Gnanalingham et al., 1993; Santana et al., 2014), Huntington's disease (Kendall et al., 1998), Alzheimer's disease (McLean et al., 2000), stroke (Bihel et al., 2010), multiple sclerosis (Genain and Hauser, 1997), and spinal cord injury (Iwanami et al., 2005), as well as in basic neuroscience research (Pinato et al., 2007; Cavalcante et al., 2011; Lima et al., 2012; Sousa et al., 2013).

This study aims to describe the morphological aspects of A8, A9 and A10 dopaminergic nuclei of the marmoset by TH-immunohistochemistry, in order to provide a foundation for future research on hodological and functional aspects of these neuronal groups in this species, and thereby broadening the basis for understanding evolutionary processes associated with the nuclear organization of this neuronal system.

## 2. Materials and methods

### 2.1. Animals and perfusion

Six young adult male marmosets were used in the study, aged between 4 and 6 years old, from the primatology center of the Federal University of Rio Grande do Norte were used. Approval for the experiments was obtained from the local Animal Experimentation Ethics Committee (Protocol 014/2014) in compliance with National Institute of Health (NIH) guidelines. All efforts were made to minimize the number of animals and their suffering.

Individuals were housed for a short adaptation period in $3.00 \times 2.00 \times 2.60$ m masonry cages consisting of four wire screen walls, ceramic tile ceilings with a natural soil floor, along with creeping vegetation and rocks to simulate their natural habitat. The animals were exposed to environmental temperature, air humidity and light, and had unlimited access to food and water. Each individual was pre-anesthetized with 5 mg/kg intramuscular injections of tramadol chloridrate and xylazine chloridrate, and maintained with isofluoran and 100% oxygen. Upon deep anesthesia, they were perfused using a cannula positioned into the ascending aorta, connected to a peristaltic pump (Cole-Parmer). After cutting the right auricula, we infused 400 ml of 0.9% saline solution in 0.1 M pH 7.4 phosphate buffer containing 5000 IU/ml heparin (Parinex, Hipolabor, Sabará, MG, Brazil, 2 ml/1000 ml of saline solution) for approximately five minutes. Next, 700 ml of 4% paraformaldehyde in a 0.1 M pH 7.4 phosphate buffer was administered.

### 2.2. Tissue processing

The skulls were removed after perfusion to expose the dorsal surface of the encephalon, which was then sectioned into 3 blocks by way of two coronal sections: one at the bregma level and the other at the lambda level. Finally, the brains were removed from the skull (Fig. 1), stored for four hours in a 30% sucrose and 4% paraformaldehyde in 0.1 M pH 7.4 phosphate buffer solution. After post-fixation, the brains were stored for 24–48 h in a 30% sucrose solution in 0.1 M pH 7.4 phosphate buffer and then sectioned by dry ice freezing onto a sliding microtome, obtaining 30 μm coronal sections. The sections were sequentially collected into 6 compartments for all cases, each containing one of every 6 sections, thereby