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Nuclear organization and morphology of cholinergic neurons in the brain of the rock cavy (*Kerodon rupestris*) (Wied, 1820)

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ABSTRACT

The aim of this study was to conduct cytoarchitectonic studies and choline acetyltransferase (ChAT) immunohistochemical analysis to delimit the cholinergic groups in the encephalon of the rock cavy (*Kerodon rupestris*), a crepuscular Caviidae rodent native to the Brazilian Northeast. Three young adult animals were anesthetized and transcardially perfused. The encephala were cut in the coronal plane using a cryostat. We obtained 6 series of 30-μm-thick sections. The sections from one series were subjected to Nissl staining. Those from another series were subjected to immunohistochemistry for the enzyme ChAT, which is used in acetylcholine synthesis, to visualize the different cholinergic neural centers of the rock cavy. The slides were analyzed using a light microscope and the results were documented by description and digital photomicrographs. ChAT-immunoreactive neurons were identified in the telencephalon (nucleus accumbens, caudate-putamen, globus pallidus, entopeduncular nucleus and ventral globus pallidus, olfactory tubercle and islands of Calleja, diagonal band of Broca nucleus, nucleus basalis, and medial septal nucleus), diencephalon (ventrolateral preoptic, hypothalamic ventrolateral, and medial habenular nuclei), and brainstem (parabigeminal, laterodorsal tegmental, and pedunculopontine tegmental nuclei). These findings are discussed through both a functional and phylogenetic perspective.

Abbreviations: 3N, oculomotor nucleus; 3V, third ventricle; A, amygdaloid area; ac, anterior commissure; aca, anterior commissure, ant; Acb, accumbens nucleus; ACh, acetylcholine; acp, anterior commissure, post; AD, anterodorsal thalamic nucleus; AHA, anterior hypothalamic area; APT, anterior pretectal nucleus; Aq, aqueduct; Arc, arcuate hypothalamic nucleus; B, basal nucleus; BIC, nucleus brachium inferior colliculus; bic, brachium inferior colliculus; cc, corpus callosum; ChAT, cholineacetyltransferase; Cl, claustrum; CLi, caudal linear nucleus of the raphe; Co, cortical amygdaloid nucleus; cp, cerebral peduncle; CPu, caudate putamen; csc, commissure superior colliculus; CxA, cortex-amygdala transition; DLG, dorsal lateral geniculate nucleus; DM, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; ec, external capsule; En, endopiriform nucleus; EP, entopeduncular nucleus; f, fornix; fr, fasciculus retroflexus; GP, globus pallidus; hbc, habenular commissure; HDB, diagonal nucleus of Broca, horizontal limb; IC, inferior colliculus; ic, internal capsule; ICj, islands of Calleja; IGL, intergeniculate leaflet; IP, interpeduncular nucleus; LD, laterodorsal thalamic nucleus; LDTg, laterodorsal tegmental nucleus; lfp, longitudinal fasciculus pons; LH, lateral hypothalamic area; LHb, lateral habenular nucleus; lo, lateral olfactory tract; LP, lateral posterior thalamic nucleus; LPO, lateral preoptic area; LV, lateral ventricle; M, mammillary nucleus; Me5, mesencephalic trigeminal nucleus; MG, medial geniculate nucleus; MHb, medial habenular nucleus; ml, medial lemniscos; mlf, medial longitudinal fasciculus; MnPO, median preoptic nucleus; MnR, median raphe nucleus; MPA, medial preoptic area; MS, medial septal nucleus; mt, mammillothalamic tract; och, optic chiasm; opt, optic tract; Pa, paraventricular hypothalamic nucleus; PAG, periaqueductal gray; PBG, parabigeminal nucleus; PF, parafascicular thalamic nucleus; Pir, piriform cortex; PMnR, paramedian raphe nucleus; Pn, pontine nuclei; PT, paratenial thalamic nucleus; PTg, pedunculopontine tegmental nucleus; PV, paraventricular thalamic nucleus; PVP, paraventricular thalamic nucleus, posterior; RCh, retrochiasmatic area; Rt, reticular thalamic nucleus; SC, superior colliculus; SCh, suprachiasmatic nucleus; sm, stria medullaris thalamus; SNC, substantia nigra, compacta; SNR, substantia nigra, reticular; SO, supraoptic nucleus; ST, bed nucleus stria terminalis; st, stria terminalis; STh, subthalamic nucleus; tfp, transverse fibers pons; TT, tenia tecta; Tu, olfactory tubercle; VDB, diagonal nucleus of Broca, vertical limb; VLG, ventral lateral geniculate nucleus; VLH, ventrolateral hypothalamic nucleus; VLPO, ventrolateral preoptic nucleus; VMH, ventromedial hypothalamic nucleus; VP, ventral pallidum; xscp, decussation superior cerebellar peduncle; ZI, zona incerta

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1. Introduction

Acetylcholine (ACh) was the first neurotransmitter that was discovered as the neurotransmitter used by somatic motor and autonomic neurons. It was subsequently identified in several neuronal clusters in the central nervous system, such as interneurons and large projection neurons (Von Bohlen et al., 2006). Neurons that synthesize and release acetylcholine for neurotransmission are referred to as cholinergic neurons (Oda and Nakanishi, 2000).

ACh is a fast-acting, point-to-point neurotransmitter at the neuromuscular junction and in the autonomic ganglia. ACh, however, also appears to act as a neuromodulator in the brain despite its role as the primary excitatory neurotransmitter at the periphery. In the brain, ACh modifies neuronal excitability, alters the presynaptic release of other neurotransmitters, and coordinates the firing of neuronal groups (Picciotto et al., 2012). In this sense, in addition to its role in known motor regulation (Calabresi et al., 2000), central cholinergic modulation participates in functions such as synaptic plasticity (Drever et al., 2011; Giocomo and Hasselmo, 2007; McKay et al., 2007) and neuronal development (Role and Berg, 1996). ACh also participates in the modulation of brain systems such as the mesolimbic dopaminergic system, which is associated with addiction and reward (Omeichenko and Sesack, 2006). It is also involved in the regulation of cortical activity (Hasselmo and Sarter, 2011). Cholinergic signaling may also alter hypothalamic functions, such as thermoregulation (Myers and Waller, 1973), sleep patterns (Steriade, 2004), food intake (Mineur et al., 2011), pancreatic insulin and glucagon release (Ishikawa et al., 1982). Increasing evidence suggests that ACh signaling in a number of brain cells is important for stress response (Mark et al., 1996), and in learning and memory processes (Gais and Born, 2004; Gold, 2003; Hasselmo, 1999, 2006).

In the central nervous system, ACh is found in some populations of cholinergic interneurons that may be found in the prosencephalon and brainstem, as well as so-called large cholinergic neurons in the basal prosencephalon and the mesopontine tegment, which result in large ascending projections. This is in addition to its presence in cholinergic somatic and autonomic motor neurons in the spinal cord and brainstem. The most representative interneurons are those in the striatum. These interneurons interact with the dopaminergic terminals of neurons that project to the striatum from the substantia nigra. There are also cholinergic interneurons sparsely distributed in the cerebral cortex, hippocampus, and olfactory bulb (Von Bohlen et al., 2006). Among the projection neurons, cholinergic groups of neurons in the basal prosencephalon include those in the medial septal nucleus (Ch1), the horizontal and vertical limbs of the diagonal band nucleus (Ch2–Ch3), and the basal nucleus of Meynert (Ch4). These neurons are responsible for large ascending projections and topographically innervate neurons throughout the cerebral cortex, hippocampus, and amygdala. The mesopontine cholinergic neurons are divided into a ventrolateral column (cell group Ch6, or the pedunculopontine nucleus) close to the lateral border of the superior cerebellar peduncle, and a dorsomedial column (cell group Ch5, or the laterodorsal tegmental nucleus), which is a component of the periaqueductal gray located just rostral to the locus coeruleus. Both these nuclei send important descending projections to the pontobulbar reticular formation, vestibular nuclei, locus coeruleus, and several raphe nuclei in addition to providing extensive ascending cholinergic innervation to the thalamus and hypothalamus. It is believed that these projections have a prominent role in regulating the sleep-wake cycle. Ch7 neurons are present in the habenula and project to the interpeduncular nucleus. Finally, Ch8 neurons are located in the parabigeminal nucleus and send projections to the superior colliculus (Mesulam et al., 1983a; Mufson et al., 1986; Von Bohlen et al., 2006).

Cholinergic nuclei are immunohistochemically delimited by ChAT or ACh vesicular transporters in the brain of several species of mammals, such as rat (Armstrong et al., 1983; Ichikawa et al., 1997; Roghani et al., 1998; Schäfer et al., 1998); monotremes (Manger et al., 2002), mole rat (Bhagwandin et al., 2008; Da Silva et al., 2006), bats (Dell et al., 2010; Kruger et al., 2010; Maseko and Manger, 2007; Maseko et al., 2007), porcupine (Limacher et al., 2008), guinea pig (Motts et al., 2008), rock hyrax (Gravett et al., 2009), giraffe (Bux et al., 2010), elephant shrew (Pieters et al., 2010), African pygmy mouse (Kruger et al., 2012), three Afrotherian species (Calvey et al., 2013), Tasmanian devil (Patzke et al., 2014), two species of Euarchontoglires (Calvey et al., 2015a), five species of insectivore (Calvey et al., 2016), the river hippopotamus (Dell et al., 2016), the Goettingen miniature pig (Mahady et al., 2017), non-human primates (Benzing et al., 1993; Calvey et al., 2015b; Kus et al., 2003; Satoh and Fibiger, 1985a,b), and human (Oda and Nakanishi, 2000). Considering the importance of studying neural systems from a comparative evolutionary point of view, it is imperative to extend such studies to the greatest number of species. This is why we chose to undertake this study in the rock cavy.

Fig. 1. Photograph of the rock cavy in captivity.

The rock cavy (*Kerodon rupestris*) (Fig. 1) is classified taxonomically as a representative of the phylum Chordata, class Mammalia, superorder Glires, order Rodentia, suborder Hystricomorpha, family Caviidae, and subfamily Caviinae (Silva Neto, 2000). The suborder Hystricomorpha includes several families with a number of species found in Brazil. In addition to the rock cavy, these species include the agouti (family Dasyproctidae), the paca (family Cuniculidae), and the capybara (Hydrochaeridae). All of these animals are used as experimental models by Brazilian researchers (see for example, Freire et al., 2010; Picanço-Diniz et al., 1991, 2011; Rocha et al., 2009, 2012; Silveira, 1985; Silveira et al., 1989). Phylogenetic studies using a molecular approach have connected the genus Kerodon with the genus Hydrochaeris, which includes the capybara (family Hydrochaeridae) and is closely related to the genus Dolicotis of the subfamily Dolicothinae, whose representative in South America is the Patagonian hare (*Dolichotis patagonum*) (Rowe and Honeycutt, 2002). The rock cavy inhabits the semiarid Caatinga region of the Brazilian Northeast, although it can be found in the Southeast region as far as the state of Minas Gerais. Colonies of rock cavies usually live in cracks and crevices of granitic rocks, which serve as refuge and shelter from predators (Lacher, 1981). This species reaches adulthood at 200 days and can reach up to 50 cm in length and 1 kg in body weight (Roberts et al., 1984).

Behavioral studies conducted in the field have reported that this rodent species emerges to forage throughout both the day and night, but most of the activity occurs during the day, with peaks of activity at dawn and dusk (Carvalho, 1969; Lacher, 1981). In concordance with these observations, an investigation performed under controlled laboratory conditions showed that the rock cavy was active throughout the day, with peaks during sunrise and sunset. The animals were observed to have a predominantly crepuscular behavior (Sousa and Menezes, 2006).

Neuroanatomical studies in the rock cavy were started in our laboratory when this species was adopted as a regional rodent model in circadian rhythm studies. In addition to the characterization of the activity rhythm and other circadian responses (Sousa and Menezes, 2006), the structures controlling circadian rhythmicity–the suprachiasmatic nucleus and the intergeniculate leaflet–have been