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Topographic specializations of catecholaminergic cells and ganglion cells and distribution of calcium binding proteins in the crepuscular rock cavy (*Kerodon rupestris*) retina

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ABSTRACT

The rock cavy (*Kerodon rupestris*) is a crepuscular Hystricomorpha rodent that has been used in comparative analysis of retinal targets, but its retinal organization remains to be investigated. In order to better characterize its visual system, the present study analyzed neurochemical features related to the topographic organization of catecholaminergic cells and ganglion cells, as well the distribution of calcium-binding proteins in the outer and inner retina. Retinal sections and/or wholemounts were processed using tyrosine hydroxylase (TH), GABA, calbindin, parvalbumin and calretinin immunohistochemistry or Nissl staining. Two types of TH-immunoreactive (TH-IR) cells were found which differ in soma size, dendritic arborization, intensity of TH immunoreactivity and stratification pattern in the inner plexiform layer. The topographic distribution of all TH-IR cells defines a visual streak along the horizontal meridian in the superior retina. The ganglion cells are also distributed in a visual streak and the visual acuity estimated considering their peak density is 4.13 cycles/degree. A subset of TH-IR cells express GABA or calbindin. Calretinin is abundant in most of retinal layers and coexists with calbindin in horizontal cells. Parvalbumin is less abundant and expressed by presumed amacrine cells in the INL and some ganglion cells in the GCL. The topographic distribution of TH-IR cells and ganglion cells in the rock cavy retina indicate a suitable adaptation for using a broad extension of its inferior visual field in aspects that involve resolution, adjustment to ambient light intensity and movement detection without specialized eye movements.

1. Introduction

The rock cavy (*Kerodon rupestris*, order Rodentia, family Caviidae) is a typical Brazilian species that inhabits the semi-arid northeast region, ranging from the state of Piauí to the north of the state of Minas Gerais (Cabrera, 1961; Lacher, 1981). It belongs to the suborder Hystricomorpha, which comprises species with a great variety of phenotypes, body sizes and diel activity patterns. Due to this diversity in morphology and ecology, many hystricomorpha rodents have been used as model species in investigations of the visual system, including the diurnal agouti (*Dasyprocta aguti*) (Silveira et al., 1989; Picanço-Diniz et al., 1991; Rocha et al., 2009) and degu (*Octodon degus*) (Chávez et al., 2003; Jacobs et al., 2003; Vega-Zuniga et al., 2013), the crepuscular chinchilla (*Chinchilla lanigera*) (Detwiler, 1949; Müller et al., 2010; Lima et al., 2010), capybara (*Hydrochaerus hydrochaeris*) (Silveira et al., 1989) and guinea pig (*Cavia porcellus*) (Jacobs and Deegan, 1994; Peichl and Gonzalez-Soriano, 1994; Parry and Bowmaker, 2002) as well as the nocturnal spotted paca (*Cuniculus paca*) (Silveira et al., 1989) and the moon-toothed degu *Octodon lunatus* (Vega-Zuniga et al., 2013).

Behavioral observations in field have seen that the rock cavy

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emerges to forage both day and night, but most of the activity occurs during the day, with peaks of activity at dawn and dusk (Lacher, 1981). In line with these observations, an investigation performed under controlled laboratory conditions showed that this species was active throughout the 24-h day, with peaks during sunrise and sunset, featuring a predominantly crepuscular behavior (Sousa and Menezes, 2006). A recent study on the anatomical features of the rock cavy eye also indicated that its optic design favors light sensitivity at the expense of a reduced resolution, which is compatible with vision under mesopic conditions (Oliveira et al., 2014). Other studies have shown that this rodent has a retino-hypothalamic projection that is anatomically similar to that described for diurnal or nocturnal mammals (Nascimento-Junior et al., 2010b), but peculiarities were found with regard to the presence or distribution of vasoactive intestinal peptide (VIP), substance P, galanin and vasopressine (VP) positive neurons in the suprachiasmatic nucleus (SCN) (Nascimento-Junior et al., 2010b) when compared to other mammals. Moreover, the absence of encephalin-positive neurons or terminals was also noticed in the rock cavy intergeniculate leaflet (Nascimento-Junior et al., 2010b), contrasting with what has been described in the diurnal *Octodon degus* (Goel and Lee, 1996), for example. Analysis of other visual pathways has shown that the rock cavy has direct retinal projections to the thalamic paraventricular (Nascimento-Junior et al., 2008) and mediodorsal (Nascimento-Junior et al., 2010a) nuclei, which are involved in circadian rhythms and the modulation of visual recognition, respectively. Moreover, retinal fibers in the rock cavy lie in the caudal Zona Incerta (Morais et al., 2014) and such projection is in part distinct from that found in the rat and hamster (Power et al., 2001; Youngstrom et al., 1991) or in the diurnal Nile grass rat *Arvicanthis niloticus* (Gaillard et al., 2013). These findings suggest functional specializations in the rock cavy retinal targets which have motivated our group to investigate topographic and neurochemical features related to its retinal organization.

In a mammalian retina, the dopaminergic system is considered fundamental to the light/dark adaptation process and contrast gain in environments under different lighting conditions (Witkovsky, 2004; Jackson et al., 2012; Yang et al., 2013). This catecholaminergic system also contributes to the circadian rhythmicity, either by the presence of core circadian clock genes or by interactions with intrinsically photosensitive ganglion cells containing melanopsin (Witkovsky et al., 2003; Vugler et al., 2007; Pozdeyev et al., 2008; Korshunov et al., 2017). In a retinal dopamine-depleted mouse model, significant disruptions were observed in high-resolution, specific deficits in light responses, contrast sensitivity, acuity and circadian rhythms (Jackson et al., 2012).

Taking into account that from a comparative point of view, interspecific variation in mammalian visual capabilities can occur as the result of an interplay between phylogenetic history, visual anatomy, ecology and diel activity (Veilleux and Kirk, 2014), we investigated which types of retinal specializations are defined by the topographic distribution of catecholaminergic amacrine cells and ganglion cells in the rock cavy. In addition, we also examined neurochemical features related to the coexistence of GABA or calcium binding proteins in these cells or in some other retinal neurons bearing in mind the importance of these bioactive substances for modulating the activity of retinal circuits. Thus, the selective expression of a specific calcium binding protein is useful for further knowledge on functional features of catecholaminergic or other retinal cells in the rock cavy. Recent evidence has indicated that the activation of ganglion cells containing parvalbumin *per se* can improve direction- and orientation-selectivity responses in the visual cortex of mice (Duan et al., 2017).

2. Materials and methods

2.1. Animals and tissue preparation

Eyes were obtained from 8 young adult animals (6 females and 2 males) from the municipality of Jucurutu in the northeast region of the state of Rio Grande do Norte, Brazil. The capture of young adult animals with age superior to 2 months according to their body weight (Lacher, 1981), was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, SISBIO #22403-1). The animals were caught in the dry season of the Brazilian northeast (from January to March and September to December) and were exposed to the corresponding environmental light, temperature and air humidity conditions. Maximum care was taken to avoid inflicting pain and suffering on the animals during the experimental procedures using the criteria established and approved by the Ethics Committee for Experimental Animal Care of the Federal University of Rio Grande do Norte (UFRN), #0152009, and in accordance with the National Research Council of the National Academy that was published in “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research”. All the animals used in this study were sacrificed at a light phase of the day, around 4 pm. Previously, they had been submitted to dark adaptation for 3 h in order to facilitate the removal of the dark pigment epithelium attached to the outer surface of the retina (Ullmann et al., 2012). Then, they were pre-anesthetized with 5 mg/kg of both tramadol hydrochloride (Cristalia, Brazil) and xylazine (Agener União, Brazil) and their states were maintained with isoflurane (BioChimico, Brazil) and oxygen. Under deep anesthesia, the animals were perfused transcardially with 300 ml of a 0.9% saline solution in 0.1 M phosphate buffer, pH 7.4 (PB), containing heparin (Parinex, Hipolabor, 2 ml/1000 ml of saline solution), followed by 700 ml of fixative solution, consisting of a 4% paraformaldehyde (PA) in PB.

After perfusion, the eyes were oriented with a dorsal and temporal incision while still in the head. After enucleation, the eyecups were immersed in buffered PA 4% (Sigma-Aldrich) for ~2 h followed by immersion in PB. For wholemount preparations, the right retinas of 8 specimens were dissected and kept in PB at 4 °C. To obtain the vertical sections, the left retinas from 4 specimens were cryoprotected with 30% sucrose in PB and were sectioned at 20 µm using a cryostat (Leica CM 1900, Germany). The sections were collected on gelatin-coated slides and stored at −20 °C until use.

2.2. Wholemount preparation, nissl staining and immunohistochemistry

For analysis of neurons in the ganglion cell layer, retinal wholemounts of the right eyes of 2 female rock cavys were processed using Nissl staining according to protocol described in Stone (1981). Pigment epithelium that was tightly adhered to the retina was bleached with a solution of 3% hydrogen peroxide (H_2O_2) in phosphate buffered saline (PBS) for 24 h (Coimbra et al., 2009). After this procedure, the retinas were rinsed with PBS and treated with hyaluronidase (510 UI/ml) in PBS for 30 min at 37 °C to assist in the removal of the vitreous humor. Nissl staining was done using cresyl violet following the protocol previously described by Oliveira et al. (2006).

Immunohistochemical analysis of tyrosine hydroxilase (TH) positive cells in retinal wholemounts was carried out on the right eyes of 6 animals (2 males and 4 females). In one, (animal number 4), both eyes were analyzed. The wholemounts were submitted to tissue antigenic retrieval by immersion in a 0.2 M borate buffer, pH = 9 at 60 °C for 1 h, following Borner et al. (2011). Then, they were rinsed 3 times in PBS for 5 min each time. The inactivation of endogenous peroxidase was obtained using 10% methanol + 3% H_2O_2 in PBS for 15 min, followed by three rinses in PBS (5 min each) and one wash in PBS + 5% Triton X-100 for 30 min (Coimbra et al., 2012). Following three rinses in PBS (5 min each), retinal wholemounts were incubated in 10% normal goat serum (NGS) in PBS + 0.3% Triton X-100 (PBSTX) for 2 h and subsequently in a solution containing rabbit polyclonal anti-TH primary antibody (1:500, Millipore) and 1% NGS for 5 days at 4 °C. After several rinses in PBS, the retinal wholemounts were incubated in the goat-anti-rabbit biotinylated secondary antibody (1:1000, Jackson Immuno Research Lab) for 3 days at 4 °C and then in an avidin-biotin-peroxidase complex (1:200, ABC kit Vector) for 3 days at 4 °C. After being rinsed,