Identifying neuronal phenotypes of chameleon brains with the use of immunohistochemical techniques: A chemoarchitecture study
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Introduction

Biodiversity hotspots provide useful information about rare species and their evolutionary relationships. In this study, we sought to understand the neuroanatomy of rare and poorly understood reptiles from the Eastern Afrotropical biodiversity hotspot of central Africa. We characterized expression of neuropeptides in chameleon species collected from this area in order to rescue precious data about their neuroanatomy before they go extinct.

We recently published an initial chemoarchitecture study with preliminary data from these animals (Figure 1). Our objective was to extend our previous study by characterizing additional locations of peptide expression in the brains of these species.

Chameleon brain tissue was sectioned and immunohistochemically stained for tyrosine hydroxylase (TH) and neuropeptide Y (NPY) neuropeptides that play a role in homeostatic functions. The results of this study will allow us to investigate evolutionary differences and build a foundation for future anatomical and functional investigations of these rare specimens.

Materials and Methods

Tissue Sectioning. For this study, brain tissue was obtained from Agama (cf. finchi), T. johnstonii, R. kerstenii, and R. boulengeri as described by Hughes et al. (2016). Brains were mounted onto the freezing stage of a sliding microtome, cut into 30 µm-thick sections and collected in phosphate-buffered saline.

Immunohistochemistry (IHC). Sections were reacted with primary and secondary antibodies as listed in Table 1. Sections were then coverslipped using buffered glycerol. Antibody specificity issues were addressed by applying multiple control tests during the immunohistochemistry runs (data not shown).

Nissl Staining. Sections were mounted on gelatin-coated slides, dehydrated in ethanols, dehydrated in xylene, stained in 0.5% thionin, and differentiated in 0.4% acetic acid. Slides were coverslipped with DPX.

Wide Field Epifluorescence Imaging. Tissue was examined with a Zeiss M2 Axioimager equipped with an X-Y-Z motorized stage and filter sets appropriate for the fluorophores we used. The microscope was connected to a cooled EXi Blue camera driven by Velocity Software (Perkin Elmer Corporation) installed on a Macintosh Pro computer.

Table 1. Reagents used for immunohistochemistry

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Antigen/Conjugate</th>
<th>Host</th>
<th>Type</th>
<th>Source</th>
<th>Dilution</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>NPY</td>
<td>Sh</td>
<td>polyclonal IgG</td>
<td>Immunostar</td>
<td>1:4,000</td>
<td>15 h, 4°C</td>
</tr>
<tr>
<td>Secondary</td>
<td>anti-NPY IgG</td>
<td>Dn</td>
<td>Cy3-conjugated</td>
<td>Jackson</td>
<td>1:500</td>
<td>5 h, RT</td>
</tr>
</tbody>
</table>

Results

1. Initial cytoarchitectural characterization of the T. jacksonii brain

Figure 2. Rostral (A) to caudal (C) drawings of coronal hemi-sections of the chameleon brain (Bennis et al., 1994). (D) Nissl staining of T. jacksonii.

Table 2. Distribution of NPY throughout four different chameleon species

<table>
<thead>
<tr>
<th>Species</th>
<th>NPY Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agama</td>
<td>NPY in thalamus, hypothalamus, and mesencephalon</td>
</tr>
<tr>
<td>Trioceros johnstonii</td>
<td>NPY in thalamus, hypothalamus, and mesencephalon</td>
</tr>
<tr>
<td>Rieppeleon kerstenii</td>
<td>NPY in thalamus, hypothalamus, and mesencephalon</td>
</tr>
<tr>
<td>Rhampholeon boulengeri</td>
<td>NPY in thalamus, hypothalamus, and mesencephalon</td>
</tr>
</tbody>
</table>

Conclusions

Studying rare and endangered species before they go extinct is crucial to understanding their unique characteristics. This study is significant because it provides novel information about rare reptiles that can be used to understand their evolutionary relationships and unique neuroanatomical features.

This is an extended analysis of the distribution of NPY throughout four different chameleon species. Our results remain consistent with previous studies on the distribution of NPY (Bennis et al., 2001). In chameleons, NPY has been implicated in neuroendocrinological mechanisms which regulate skin coloration. It also plays a role in the visual system; fibers were found to be prominent in visual centers of the hypothalamus and thalamus, consistent with results from Bennis and colleagues.

Further characterization of the cytoarchitectures of the chameleon brain will provide a better toolset for neuroanatomical studies. It will also provide insight on the comparative distribution of NPY, facilitating the design and execution of future in vivo studies.

Specificity issues were encountered with the TH antibody that are currently being addressed. Further experimentation will include characterization of this as well as various other peptides.

References


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