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 Afromontane 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.

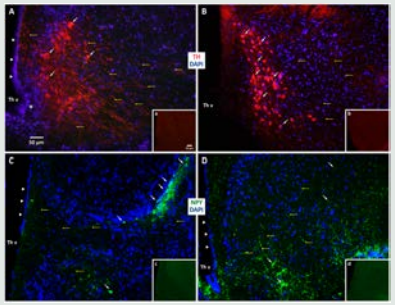

Figure 1. Preliminary data collected from target species (Hughes et al., 2016). Images show fluorescent stain of TH (red), NPY (green), and DAPI (purple) in four species collected from the Eastern Afromontane biodiversity hotspot.

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 ethanol, defatted 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 Volocity Software (Perkin-Elmer Corporation) installed on a Macintosh Pro computer.

Table 1. Reagents used for immunohistochemistry

Reagent	Antigen/Conjugate	Host	Type	Source	Dilution	Incubation
Primary	NPY	Sh	polyclonal IgG	ImmunoStar	1:4,000	15 h, 4°C
Secondary	anti-sheep IgG	Dk	Cy3-conjugated	Jackson	1:500	5 h, RT

Results

1 Initial cytoarchitectural characterization of the *T. jacksonii* brain

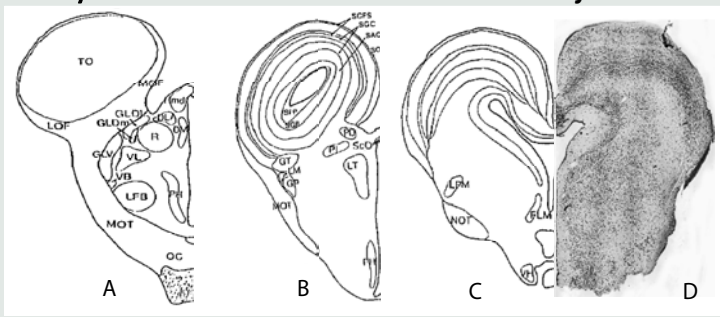

Figure 2. Rostral (**A**) to caudal (**C**) drawings of coronal hemi-sections of the chameleon brain (Bennis et al., 1994). (**D**) Nissl staining of *T. jacksonii*.

Abbreviations: including the nucleus dorsolateralis anterior (**DM**), fasciculus longitudinalis medialis (**FLM**), nuclues geniculatus lateralis dorsalis pars lateralis (**GLDl**), nucleus geniculatus lateralis dorsalis pars medialis (**GLDm**), nucleus geniculatus lateralis ventralis (**GLV**), nucleus geniculatus pretectalis (**GP**), nucleus griseus tectalis (**GT**), nucleus habenularis dorsomedialis (**Hmd**), lateral forebrain bundle (**LFB**), nucleus lentiform mesencephali (**LPM**), lateral optic fascicle (**LOF**), nucleus lentiformis thalami (**LT**), medial optic fascicle (**MOF**), marginal optic tract (**MOT**), nucleus opticus tegmenti (**NOT**), tectum opticum (**TO**), nucleus ventrobasalis (**VB**), nucleus ventralis hypothalami (**VH**), nucleus ventrolateralis pars dorsalis (**VL**), nucleus ventromedialis thalami (**VM**).

2 Distribution of NPY throughout four different chameleon species

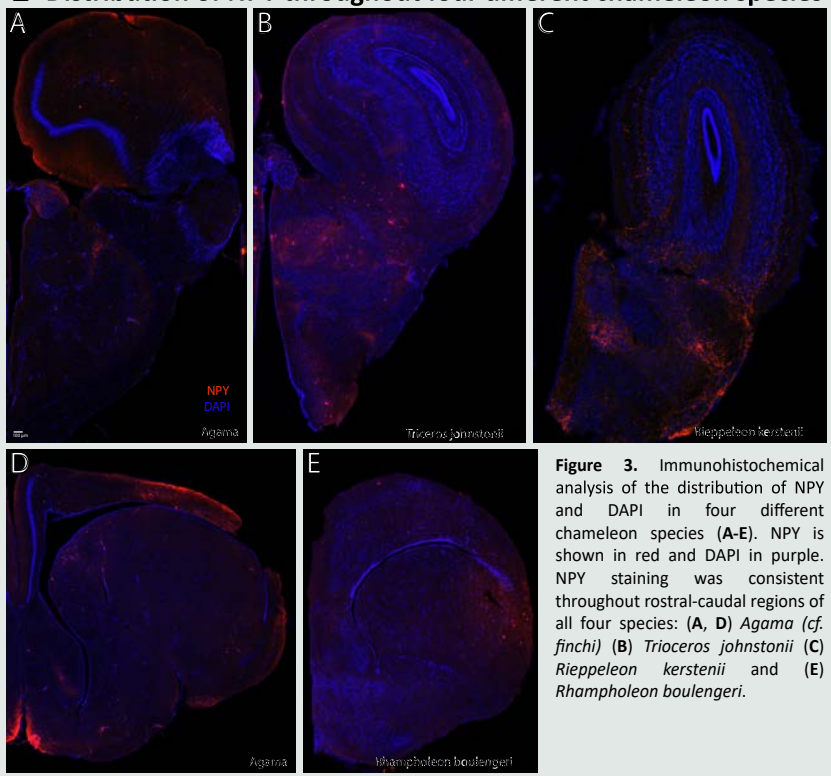

Figure 3. Immunohistochemical analysis of the distribution of NPY and DAPI in four different chameleon species (**A-E**). NPY is shown in red and DAPI in purple. NPY staining was consistent throughout rostral-caudal regions of all four species: (**A**, **D**) *Agama (cf. finchi)* (**B**) *Trioceros johnstonii* (**C**) *Rieppeleon kerstenii* and (**E**) *Rhampholeon boulengeri*.

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 cytoarchitecture 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.

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