Immunohistochemical and cytoarchitectonic characterization hypocretin 1/orexin a and neuropeptide Y in chameleon species *Trioceros jacksonii*

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Introduction

The Jackson's chameleon, *Trioceros jacksonii*, is native to the highlands of central Kenya. Though well known for their advanced visual abilities, the brain of the chameleon and other reptiles of the Chihuahuan Desert are not well studied.

In the mammalian brain, hypocretin 1/orexin a (H/O) has been found to regulate arousal, wakefulness, and appetite while neuropeptide Y (NPY) plays an important role in homeostatic processes. Spatial arrangement of NPY in the reptilian brain has been found to be similar to the mammalian brain, implying equivalent functions.

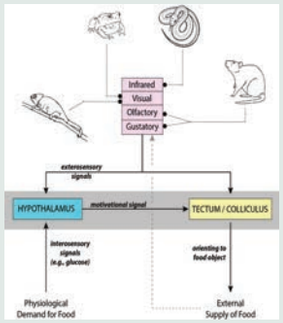


Figure 1. The relationship between the hypothalamus and tectum, that display the physiological demand for food, may have the ability to inhibit physical orientation to an external supply of food.

The importance of NPY in the chameleon extends to the visual system as well. Since NPY and H/O have an interactive importance particularly in feeding, we aim to create a more complete analysis of the T. Jacksonii brain by focusing on the optic tectum and hypothalamus.

This project is only the initial step of a larger investigation. We hope to learn about brain cytoarchiture and cell characterization across various organisms in order to identify better tools for the study of human neuroanatomy.

The objective of the present study was to identify the spatial arrangement of NPY and H/O in addition to characterizaing the cytoarchitecture of the chameleon brain.

Materials and Methods

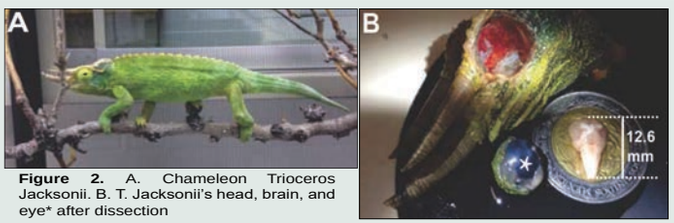


Figure 2. A. Chameleon Trioceros Jacksonii. B. T. Jacksonii's head, brain, and eye* after dissection

Immunohistochemistry (IHC). Fixed frozen T. jacksonii chameleon brain was cut (20 µm-thick) and collected in phosphate-buffered saline. Sections were reacted with primary and secondary antibodies as listed in Table 1. Sections were then coverslipped using buffered glycerol. Specificity issues were addressed by applying multiple control tests during the immunohistochemistry runs (data not shown).

Nissl. 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 Retiga Blue camera driven by Volocity Software (Perkin-Elmer Corporation) installed on a

Table 1. Reagents used for immunohistochemistry

Reagent	Antigen/Conjugate	Host	Type	Source	Dilution	Incubation
Primary	Neuropeptide Y	Rb	polyclonal IgG	ImmunoStar	1:1,000	15 h, 4°C
	Hypocreti 1/Orexin A	Gt	polyclonal IgG	Santa Cruz	1:5,000	15 h, 4°C
Secondary	anti-rabbit IgG	Dk	Cy3-conjugated	Jackson	1:500	4 h, RT
	anti-goat IgG	Dk	Alexa 488-conjugated	Jackson	1:500	4 h, RT

Results

1 NPY is found throughout the extent of the *T. jacksonii* brain

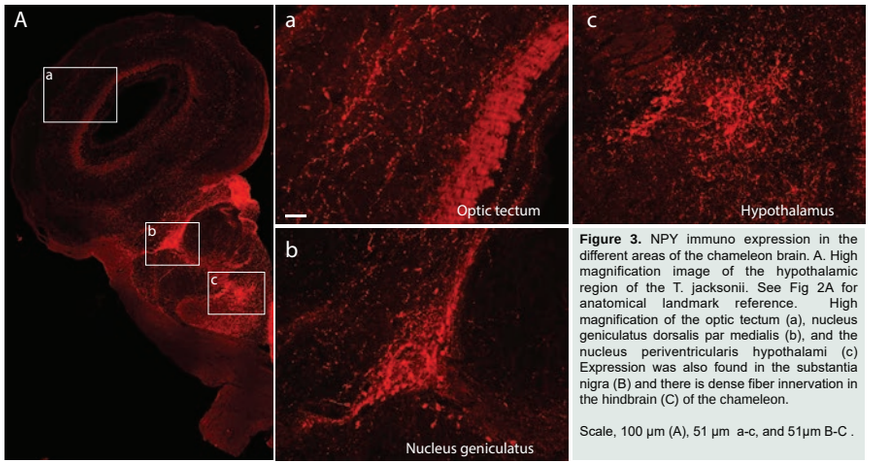


Figure 3. NPY immuno expression in the different areas of the chameleon brain. A. High magnification image of the hypothalamic region of the T. jacksonii. See Fig 2A for anatomical landmark reference. High magnification of the optic tectum (a), nucleus geniculatus dorsalis par medialis (b), and the nucleus periventricularis hypothalami (c) Expression was also found in the substantia nigra (B) and there is dense fiber innervation in the hindbrain (C) of the chameleon.

Scale, 100 µm (A), 51 µm a-c, and 51µm B-C .

2 Initial cytoarchitectonic characterization of the *T. jacksonii* brain

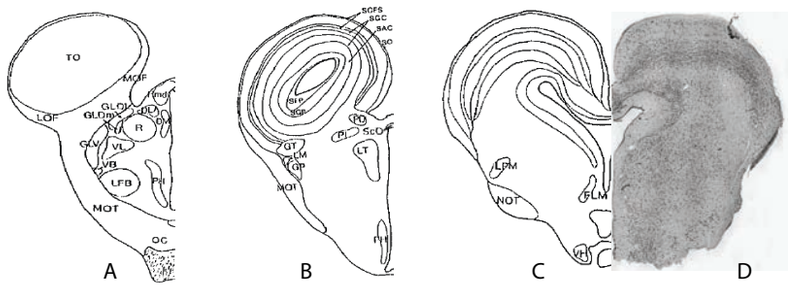


Figure 4. Rostral (A) to caudal (C) drawings of coronal hemi-sections of the chameleon brain (Bennis et al. 1994) and (D) Nissl staining of T. jacksonii. Abbreviations: including the nucleus dorsolateralis anterior (DM), fasciculus longitudinalis medialis (FLM), nuclues geniculatus lateralis dorsalis pars lateralis (GLDl), nucleus geniculatus lateralis dorsalis pars medialis (GLDm), nucleus geniculatus lateralis ventrialis (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), optic chiasm (OC), nucleus posterodorsalis (PD), nucleus periventricularis hypothalami (PH), nucleus pretactalis (PI), nuclues roudus (R), stratum album centrale (SGC), stratum griseum et fibrosum superficiale (SGFS). stratum griseum periventriculare (SGP), stratum opticum (SO), suprachiasmatic optic fascicle (SOF), tetum opticum (TO), nucleus ventrobasalis (VB), nucleus ventralis hypothalami (VH), nucleus ventrolateralis pars dorsalis (VL), nucleus ventromedialis thamali (VM).

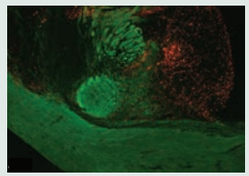
Conclusions

Characterization of NPY and H/O in the chameleon brain is important in order to determine their role in homeostatic processes. Also, it is necessary to investigate the neuroanatomy of T. Jacksonii because it will facilitate the design and execution of future in vivo studies.

Our results confirm the analysis made by Bennis et al. (2000) on the Chameleo chameleon, we found NPY containing cells in the midbrain, including the geniculatus lateralis pars dorsalis, perventricularis hypothalami, substantia nigra and optic tectum. Additionally, we found robustly labeled fibers in two distinct regions of the hindbrain; additional analysis is required to determine their location. Specificity issues were encountered with H/O antibody that are currently being addressed.

The present study is the first attempt to create a cytoarchitectonic and immunohistochemical characterization of NPY and H/O in the chameleon brain. Further characterization is currently being done to ultimately create a chameleon brain atlas.

Figure 5. Initial attempt of H/O characterization in T. Jacksonii brain. This tissue is from the same staining used in the present study as depicted in Figure 3. H/O antibody was found to be non-specific. Techniques are now being implemented to perfect the staining.



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