Validation of custom antibodies against androgen receptors alpha and beta in *Astatotilapia burtoni*

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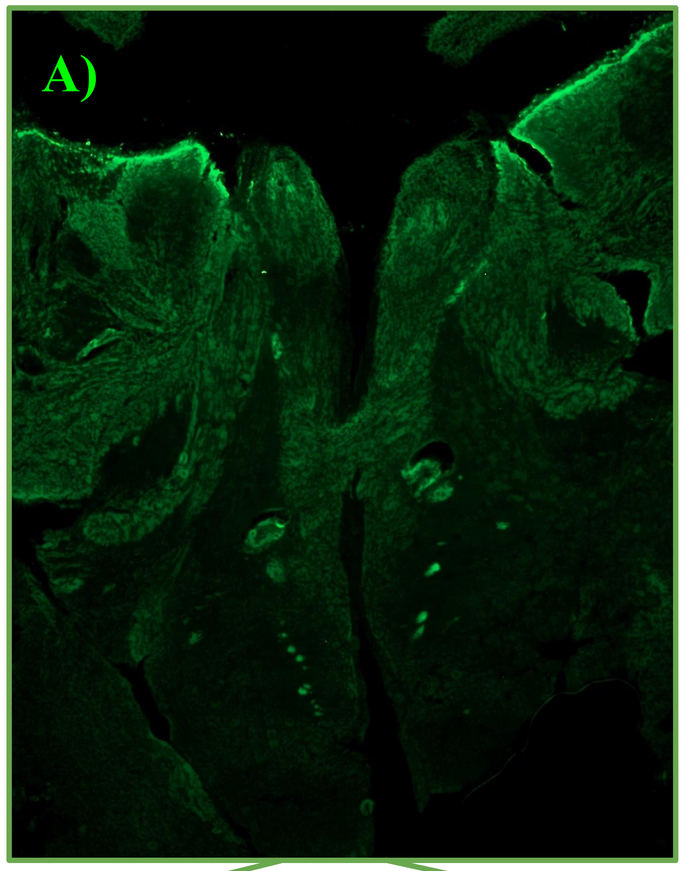
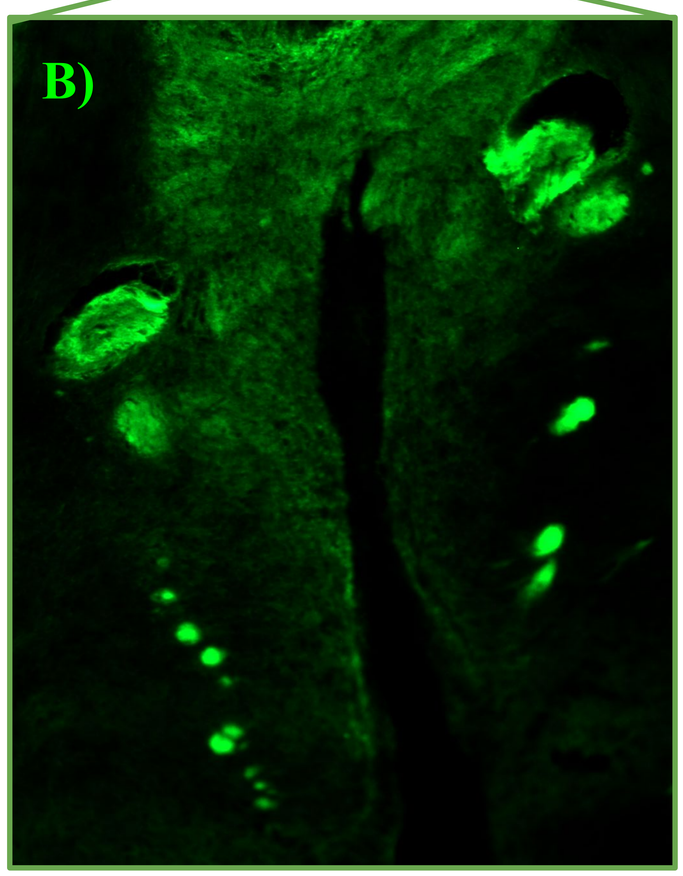
Introduction

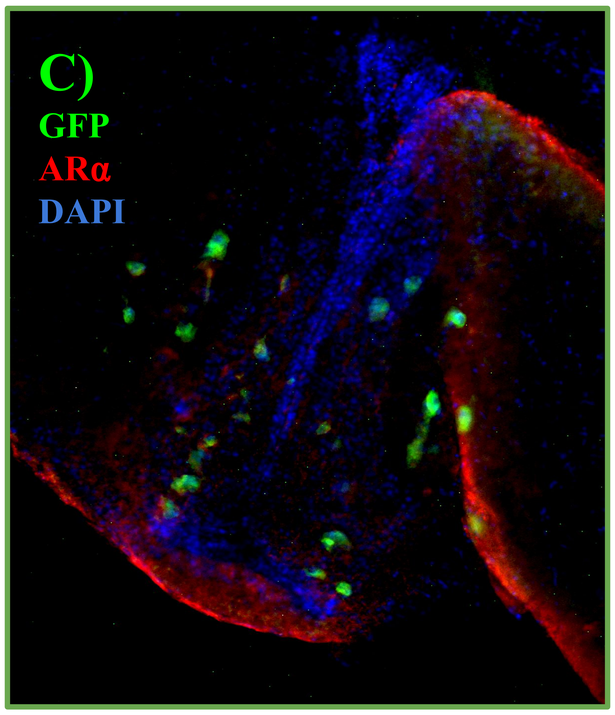
- *A. burtoni* are unique as they have two paralogs of androgen receptors (AR), ARα and ARβ, whereas most species have only one
- Previous research shows that androgen signaling affects the morphology and function of Gonadotropin neuron releasing hormone (GnRH) neurons, which regulate reproductive hormone levels and modulate physiology and behavior. However, it is unclear how androgen signaling exerts these effects.
- Goal: To determine the spatial relationship of the two androgen receptors and GnRH-1 neurons; whether androgen receptors are located in GnRH-1 neurons in the *A. burtoni* brain

Methods

1) Fish were euthanized via. cold water immersion for 5 minutes, followed by dissection and brain extraction
2) Extracted brains were stored in paraformaldehyde x 2 hours then sucrose until embedding in Neg50
3) Brains were sectioned at 30µm thickness with cryostat
4) Immunohistochemistry (IHC) was performed using AR alpha & beta chicken primary antibody (1:1,000 or 1:1,000; Aves labs) overnight at room temperature & 488 goat anti chicken secondary antibody for one hour
5) Sections were imaged underneath Nikon Eclipse 80i microscope

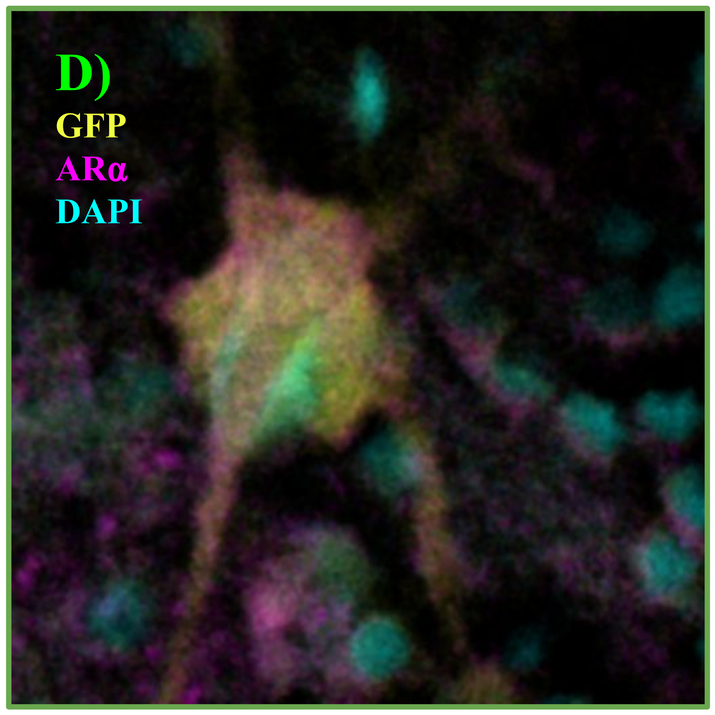






Figures

A) Figure 1: Pre-optic area (POA) of ARα in a male wild-type fish, 4x
B) Figure 2: POA of ARα in a male wild-type fish, 20x
C) Figure 3: Colocalization of ARα (red), GFP-labeled GnRH (green) with a DAPI background (blue) in the rostral POA of a GG+ female, 20x
D) Figure 4: Colocalization of ARα (magenta) and GFP-labeled GnRH (yellow) with a DAPI background (cyan) in the rostral POA of a GG+ female, 20x, taken on a confocal microscope



Results

- A 1:500 primary antibody ratio was necessary as the fluorescent signal was not clearly visible at 1:1000
- ARα appears in the POA in a locations similar to that of the known GnRH-neurons
- Whilst it has been assumed that AR is a nuclear protein, the irregular and non-circular shape of the stain suggests otherwise
- ARβ in the tectum is reminiscent of neuropil structures or hypothalamic projections
- Female transgenic fish that possess a green fluorescent protein tag on their GnRH gene (GG+fish) suggest colocalization of GnRH neurons in relation to ARα

Further Research

- Investigate colocalization in male GG+ fish, including ARα and ARβ
- Stain ARβ with synapsin to investigate the type of structure ARβ resides in and where, if not nuclear, in the cell it resides

Acknowledgements

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