What brain regions are responsible for conspecific mate recognition?

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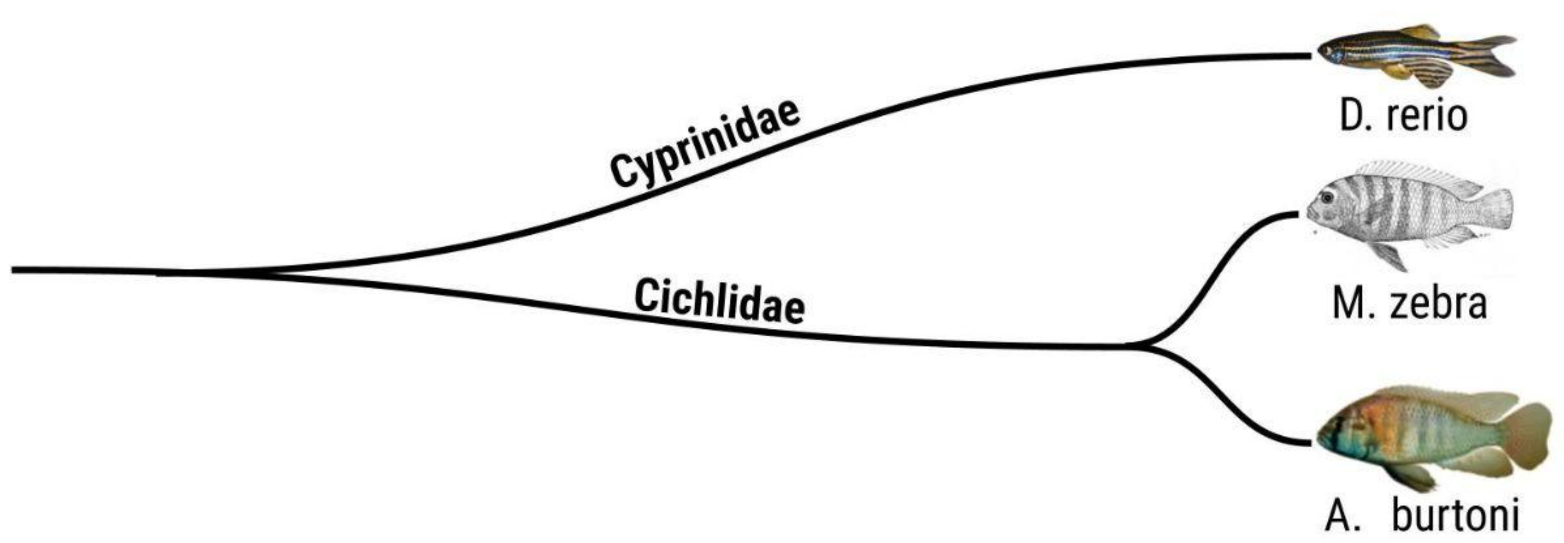
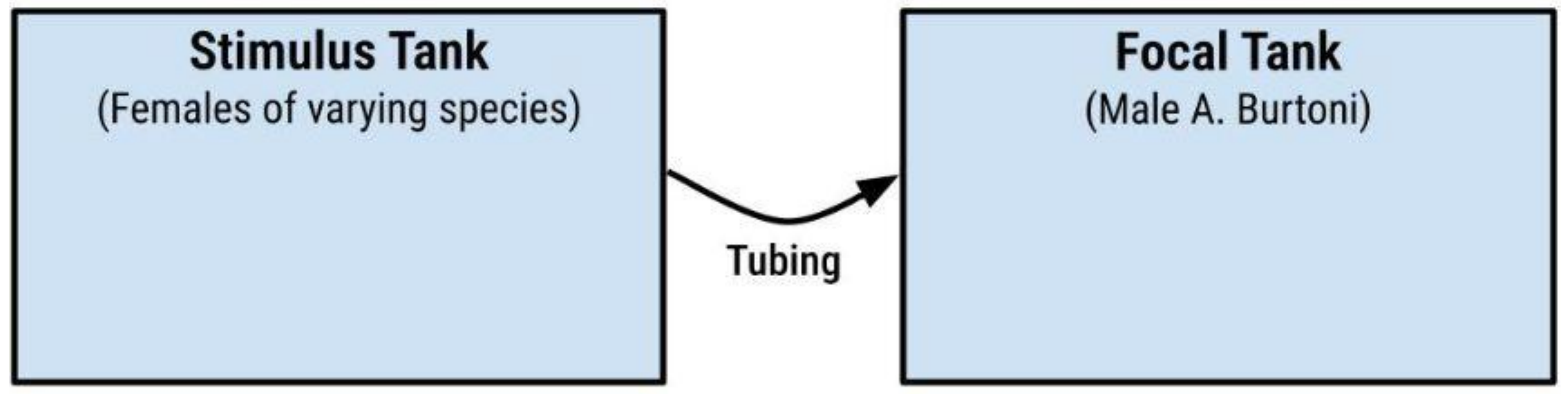
Background

- *Astatotilapia burtoni* is a species of cichlid fish from East Africa. Cichlids are remarkable for having evolved into thousands of species all in the same area and yet continuously mate with their own species. They have also evolved unique social behavior like mouthbrooding.
- Pheromones are an important part of cichlid social behavior. Fertile females release prostaglandin F2α (PGF2α) related pheromones that males need to detect in order to successfully mate (Li et al., 2024). Female pheromones also prevent males from mouthbrooding.
- Pheromones are important for cichlid behavior, but it is not known what brain regions in the male brain respond to female pheromones. Some clues can come from work in zebrafish (Yabuki et al., 2016).
- I hypothesize that male brains will have different levels of preoptic area activation dependent on odor species. In the future, once quantification has been done I would predict that the conspecific species reaction would be the highest.



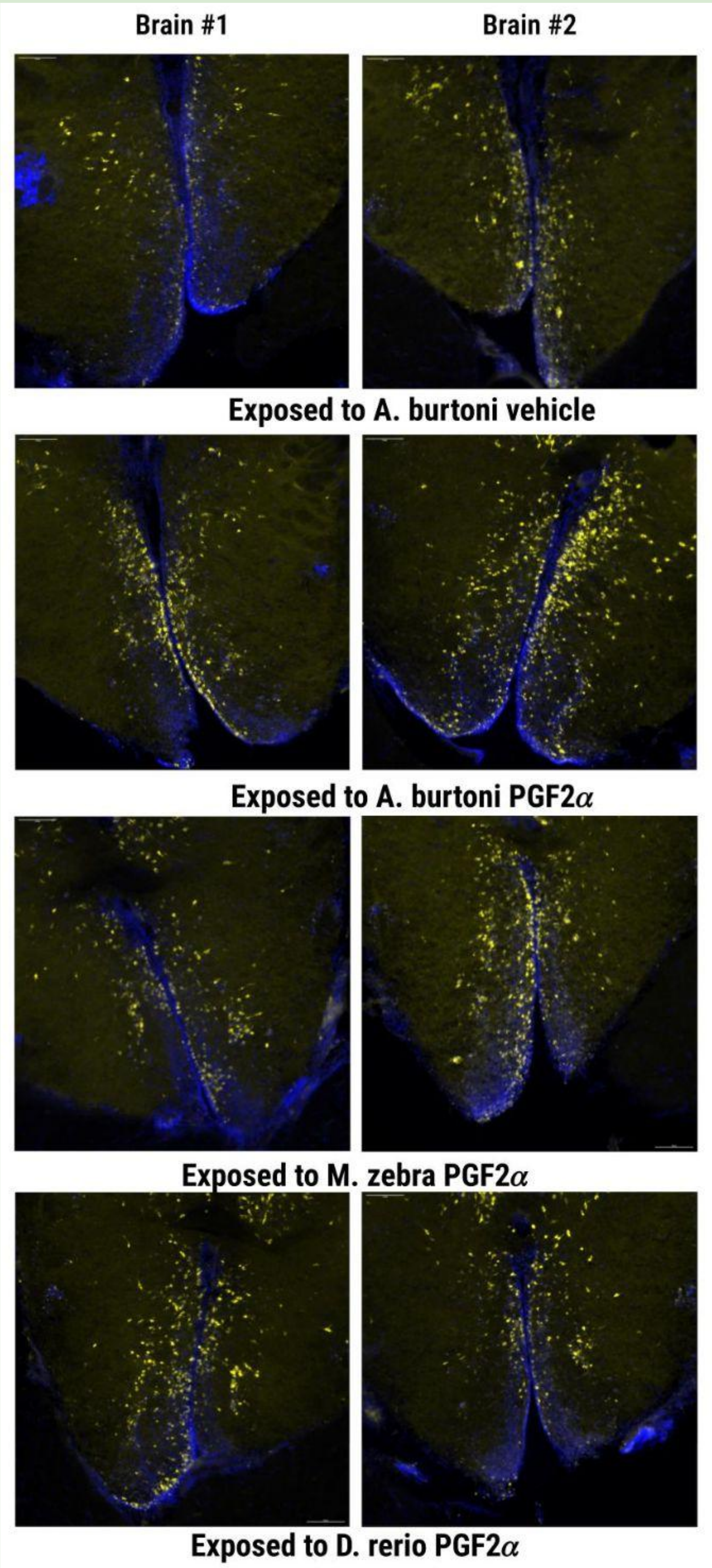
Methods

- In total 9 brains were collected from A. burtoni adult males 30 minutes after being exposed to the scent of female fish
- The exposure groups were n=2 vehicle injected A. Burtoni, and the following injected with PGF2A n=3 M. Zebra, n=2 A. Burtoni, and n=2 D rerio
- Males were exposed to the scent of the stimulus fish in a covered tank without seeing them
- Brains were fixed in 4% PFA sectioned at 30 microns and stained using immunohistochemistry targeting pS6 (activation marker) with DAPI counterstain
- Slides were imaged using wide-field fluorescence microscopy

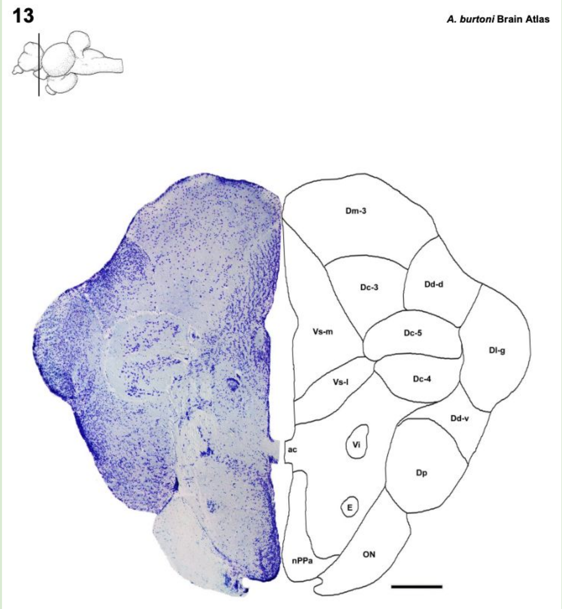


Results

The activity marker pS6 is strongly expressed in the POA.



pS6 (yellow) stain shows cells recently activated in the POA region. DAPI (blue) stain shows the nucleus of each cell.



Preoptic Area (POA)
Region of the hypothalamus that contains portions of the neural system responsible for olfaction and pheromone signaling.

Quantification of images is ongoing and will test for significant differences in pS6 cell count and intensity.

- Quantify cell number and fluorescence intensity in Preoptic area using imageJ to determine significance of staining.
- Other regions of interest include the telencephalon, optic tectum, hypothalamus, cerebellum, and olfactory epithelium (Yabuki, 2016).

Interpretations

- **The males exposed to PGF2a A. Burtoni females appear to have more activation than that of the vehicle, and other species.**

Comparison	Expectation	Interpretation
PGF2α A. burtoni female vs. vehicle A. burtoni female	Greater activation of POA in PGF2α exposed male than vehicle exposed male	POA has pheromone signaling receptors
PGF2α A. burtoni female vs. PGF2α D. rerio female	Greater activation in brain of A. burtoni exposed male than D. rerio exposed male	A. burtoni can detect their own species through pheromone signaling
PGF2α M. zebra female vs. PGF2α D. rerio female	Greater activation in brain of M. zebra exposed male than D. rerio exposed male	A. burtoni can detect similar cichlid species compared to zebrafish

Future Directions

- The next step in this project is to quantify the cell number and fluorescence intensity of the POA and other regions of interest.
- This experiment lays the groundwork to recreate a larger trial with a bigger sample size that can determine if A. burtoni are able to detect species through pheromone signaling. This would be of large significance given the unknown mechanism through which cichlids in lake Tanganyika do conspecific mating while surrounded by thousands of fish of very similar species.
- It would be interesting to see the difference in activation and pheromone detection within mutant fish like TRPC2 lines that that have altered olfactory receptors.
- Potential for exploration into other regions in order to determine if the POA is responsible for species differentiation or if other parts of the brain also play a role.

Acknowledgements

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