Reproductive State and Arginine Vasotocin in the Brains of Female Upland Chorus Frogs

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Introduction

Animal behavior is a diverse product of internal and external factors, mediated by the nervous system. One way in which the nervous system regulates behavior is through neuromodulation, where molecules can alter neural activity by regulating excitability and synaptic transmission within specific brain regions [1]. Arginine vasotocin (vasotocin/AVT) is a neuromodulator implicated in both male and female amphibian social behaviors, but the distribution of vasotocin in the brain is variable across amphibian species and current research on its role in behavior is male-focused. The few studies on female frogs suggest a role of vasotocin in female sexual receptivity and reproductive behavior, however, these effects are also variable across species. Additionally, there is evidence that vasotocin affects sexually-motivated responses to sound in female frogs, and that reproductive condition may be a prerequisite for sexual motivation. The aim of this study is to map vasotocin immunoreactivity in the brain of gravid and non-gravid female Upland chorus frogs (P*seudacris feriarum)* to gain a better understanding of its distribution and possible role in driving the seasonal changes in female receptivity. I hypothesize that there will be differences in the intensity and distribution of vasotocin immunoreactivity between gravid and non-gravid females, particularly in areas associated with social and reproductive behavior.

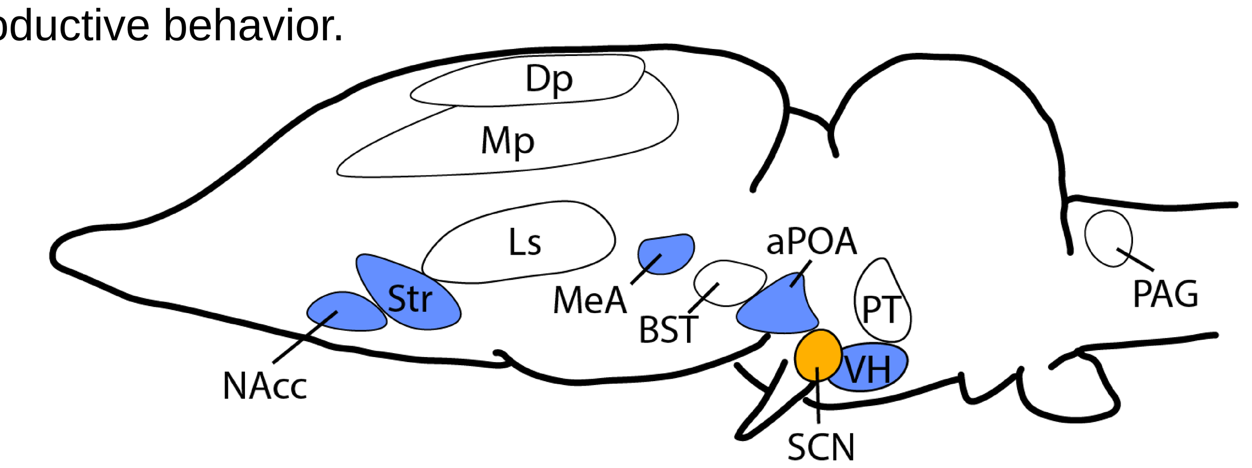

Figure 1. Brain regions targeted for vasotocin imaging. Unhighlighted and blue regions represent all parts of the social decision-making network in amphibians [2], blue regions represent areas of social network where vasotocin molecules have been found in the family treefrog Hylidae (Wilczynski et al. 2017), and yellow regions represent other areas vasotocin has been found in treefrogs that are not a part of the social network [3].

Methods

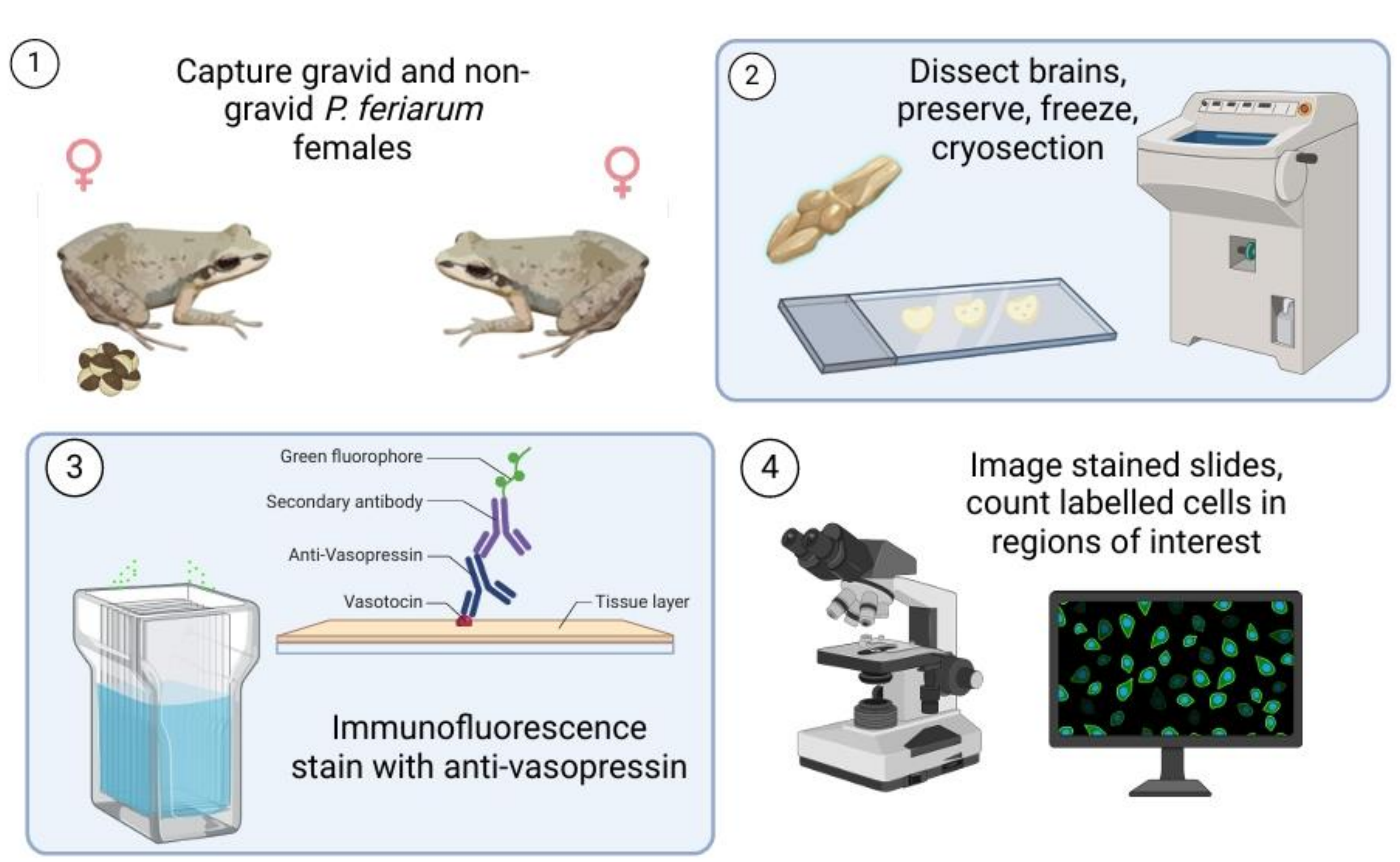

Figure 2. Representation of methods created in BioRender by Carlie Ochoa.

Anticipated Results

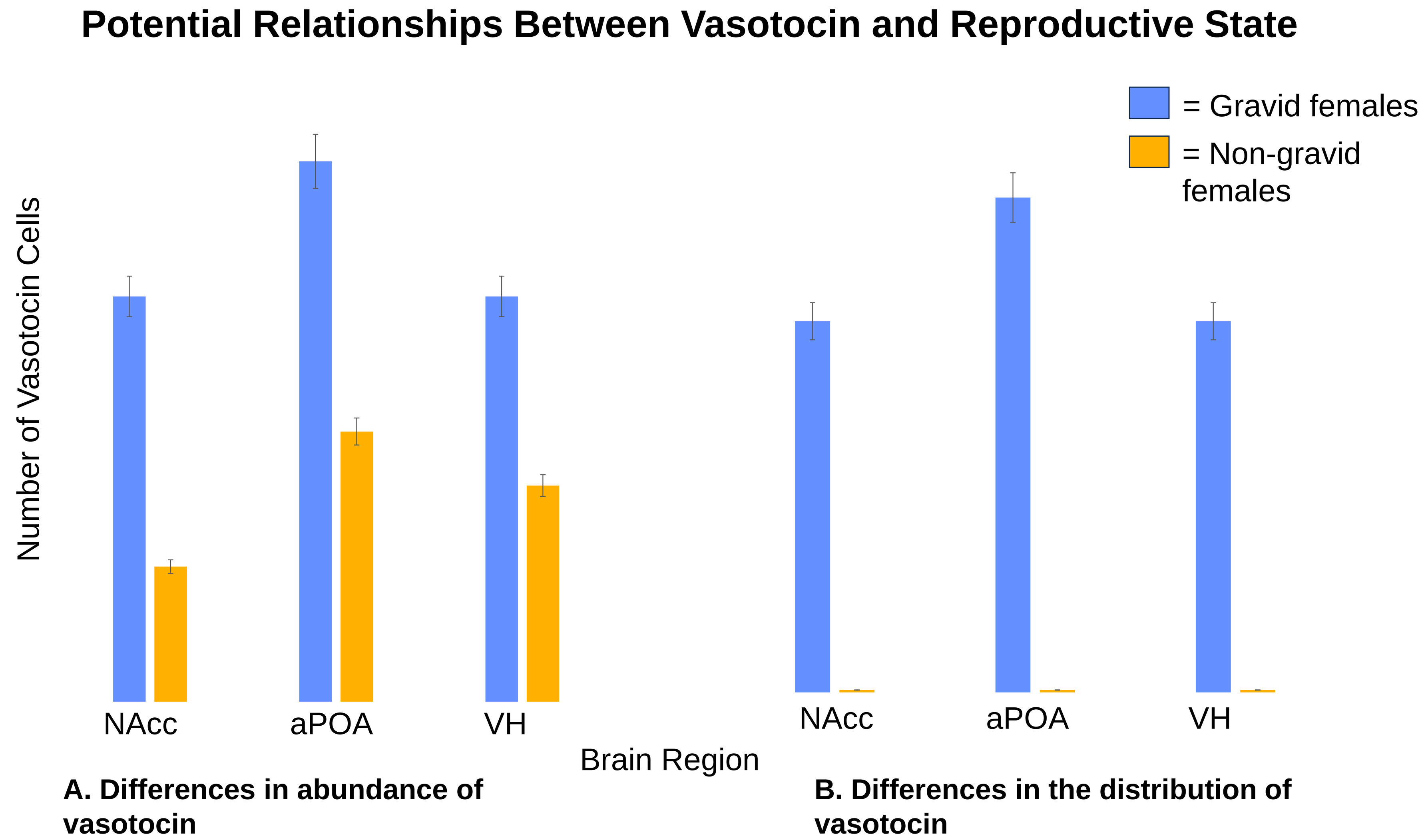

Figure 3. Two different possible outcomes for the relationship between vasotocin and reproductive state. **(A)** Vasotocin may be present in the same brain regions across both groups but at higher levels in gravid females, or **(B)** vasotocin may be present in gravid females but absent in non-gravid females in certain brain regions.

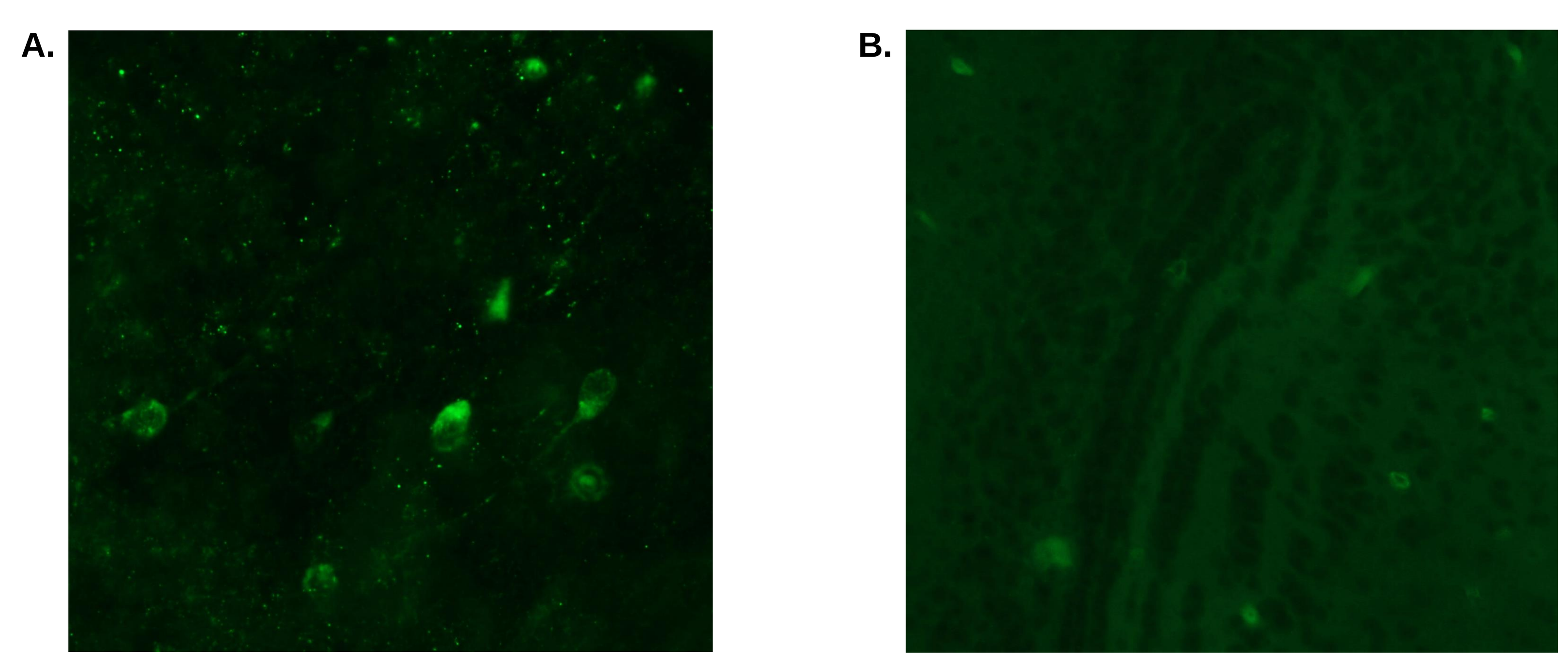

Figure 4. Example images of vasotocin staining. **(A)** Image with cells containing vasotocin. **(B)** Image with no vasotocin cells.

Next Steps

- Currently, I am in the process of imaging the remaining slides. Following this, I will count cells in the relevant brain regions, including the **1) nucleus accumbens (Nacc), 2) bed nucleus of the stria terminalis (BST), 3) anterior preoptic area (aPOA), 4) dorsal pallium (Dp), 5) lateral septum (Ls), 6) medial amygdala (MeA), 7) medial pallium (Mp), 8) striatum (Str), 9) posterior tuberculum (PT), 10) ventral hypothalamus (VH), and suprachiasmatic nucleus (SCN)** (Figure 1).
- After cell counts, I will use an independent t-test for overall cell count differences between the two groups, and if significant differences are found, I will follow up with a Tukey's Honestly Significant Difference (HSD) test determine which brain regions are contributing to these group differences.

Discussion

Female mate choice can drive speciation through shaping male traits and behaviors, but the mechanisms behind female choice are not completely understood. Neuromodulation can drive these behaviors, but effects of neuromodulators can be variable and specific, differing in even closely related species [1]. Vasotocin as a neuromodulator can have varying behavioral effects in amphibians, and injections of vasotocin in female frogs have been documented to increase sexually-motivated receptivity to male advertisement calls [4]. In *P. feriarum,* females are only responsive to male calls when gravid, suggesting reproductive condition is a prerequisite for receptivity to male acoustic signaling. Thus, exploring vasotocin in the brain in different reproductive states could give us insight into why these changes in behavior occur. Additionally, vasotocin has not been documented in the *Pseudacris* genus, so investigating vasotocin in the social decision-making brain regions for a new species can allow for more comparative studies and a better understanding of consistencies or differences among species. Understanding if and how the distribution of vasotocin in the brain changes with reproductive state will further explain its role in female reproduction and aid in explaining the causes of these multifaceted behaviors and the implications neurobiology can have on evolutionary processes.

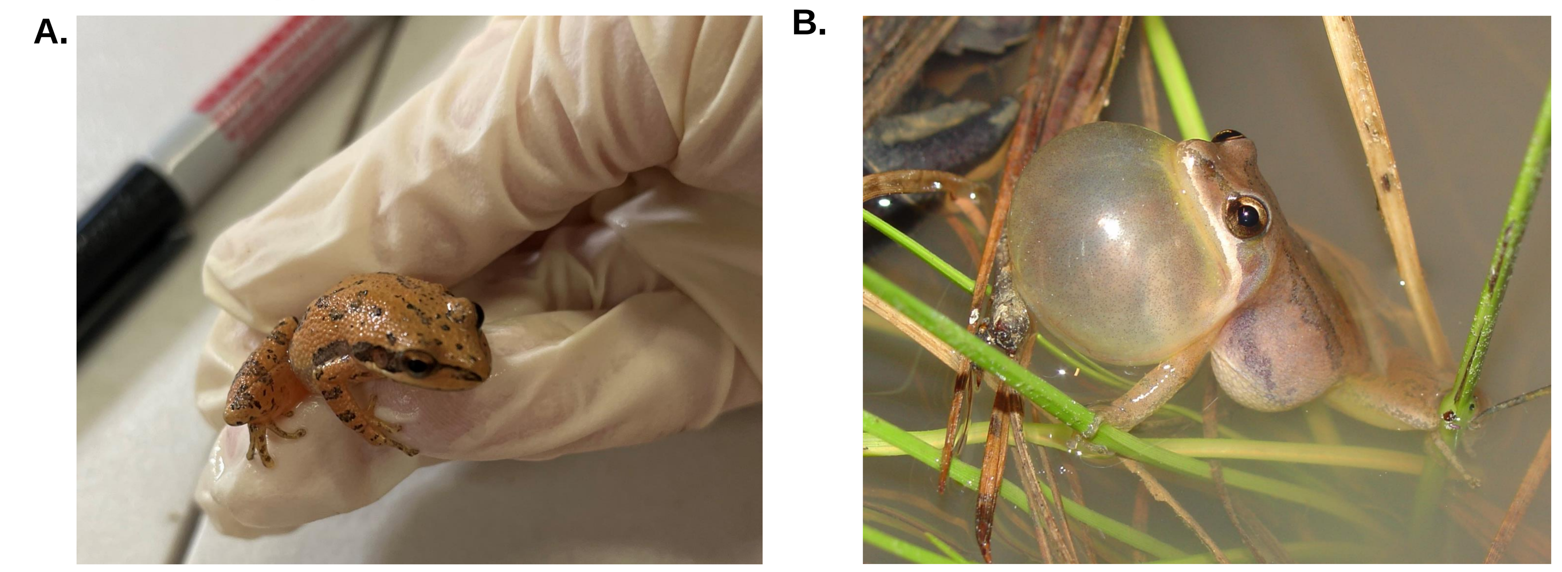

Figure 5. Photos of *Pseudacris feriarum.* **(A)** One of the experimental females; photo taken by Kaya Simmons. **(B)** *P. feriarum* in the wild; photo taken by Aubrey Heupel.

Acknowledgements

I would like to thank Carlie Ochoa for all the guidance and support throughout this project and its development. I would also like to thank Dr. Lemmon for her support for this project and role as a mentor. Lastly, I would like to thank Dr. Emily Duval and Dr. Elizabeth Hammock for serving on my committee for this honors project.

References

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[2] O'Connell, L. A., & Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science*, *336*(6085), 1154-1157.

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