Vasopressin receptor expression in green anole (*Anolis carolinesis*) brains in relation to season (breeding versus non-breeding) and sex (male versus female)

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INTRODUCTION

The social behavior neural network contains a variety of signaling neuropeptides, such as vasopressin (VP), and their receptors.[1-3] Previous studies in rodents and songbirds have demonstrated that there are differences in neural vasopressin receptor (VPR) expression based on sex (male vs. female) and season (breeding vs. non-breeding), although the various results are inconclusive and sometimes contradictory. VPRs are G protein-coupled receptors consisting of several subtypes; In mammals, the V1aR subtype is the subtype most closely associated with social behavior regulation, and it is that subtype that is examined here.[4] Understanding differences in receptor expression can help us to understand behavioral differences across sexes and seasons. Using green anoles (*Anolis carolinesis*), we here compare males and females, from breeding and non-breeding states, for amount of V1aR expression across pertinent brain regions. Based on previous research, **we predict that breeding males will have generally higher V1aR expression than females and non-breeding males. We also predict no difference in V1aR expression among females between seasons.**

METHODS

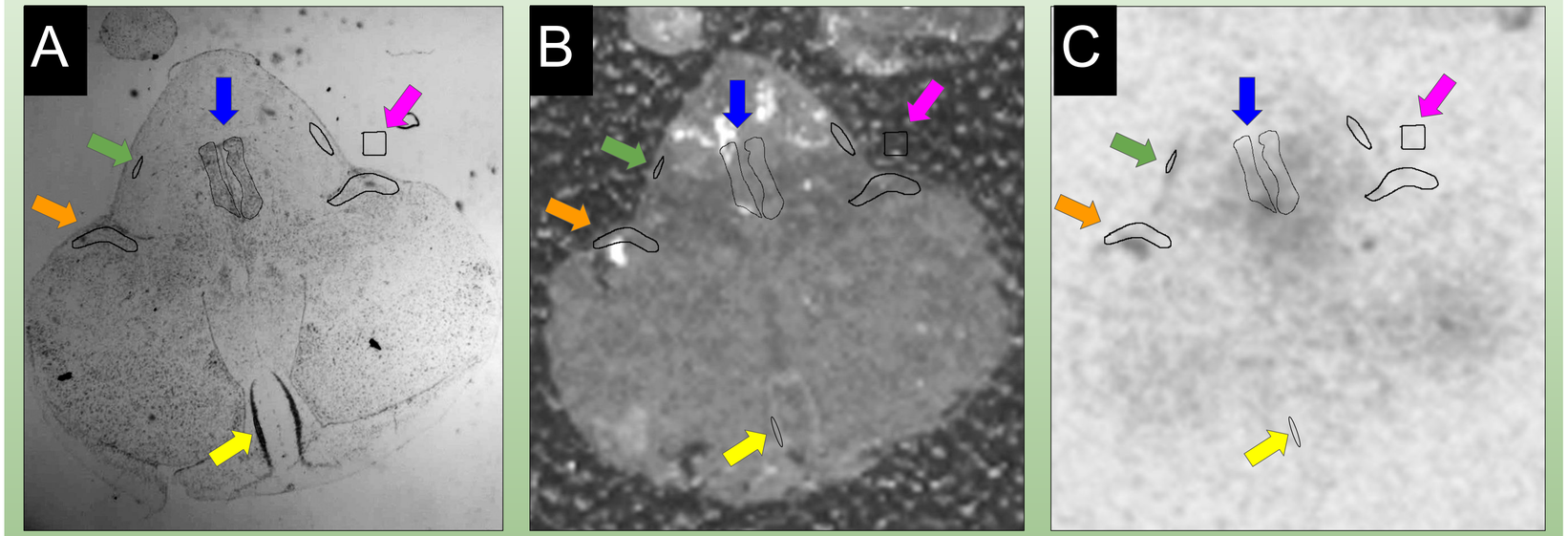

Figure 1: (A) Nissl stain outlines of the brain regions (blue arrow = preoptic area (POA), orange arrow = medial amygdala (Amy), green arrow = fiber control region, pink arrow = background, yellow arrow = cortex, and ventromedial hypothalamus (VMH, not shown). (B) Underlying scan of radioactive-ligand-treated tissue. (C) Autoradiography: dark patches on developed film indicate presence of radioactive ligand binding in that area.

METHODS CONT.

1. Subjects

We compared 10 breeding-season (05/2016, 6 males and 4 females) and 12 non-breeding-season (11/2016, 6 males and 6 females) subjects.

2. Brain Processing

Brains were dissected, fresh-frozen, and sectioned at 20 µm and thaw-mounted into four series. One series underwent Nissl staining and brightfield photomicrographs were captured (Fig. 1A). A second series underwent ligand binding using an I-125 radioactively labeled V1aR antagonist. Scans of these brain sections were obtained (Fig. 1B), and the sections were also used to expose film (Fig. 1C).

3. Brain Section Alignment and Outline

Adobe Photoshop CS6 was used for digital analysis. Scans of the slides for a brain series were overlaid on top of their respective autoradiography image. Each Nissl stain brain section image (from an adjacent series) was aligned as best as possible with its corresponding brain section. Average pixel brightness was obtained on the autoradiography layer for three target brain regions (POA, Amy, VMH), two control brain regions (fiber and cortex), and a background region of the slide where brain was not located. A standard was present on each film, and it values were used to convert pixel brightness to decay counts per minute (cpm). Radiation measures were controlled in three ways: by subtracting fiber, cortex, or background levels.

4. Statistical Analysis

A two-way ANOVA was run to compare radiation measures (cpm) across sex, season, and the interaction of sexXseason for each brain region.

RESULTS

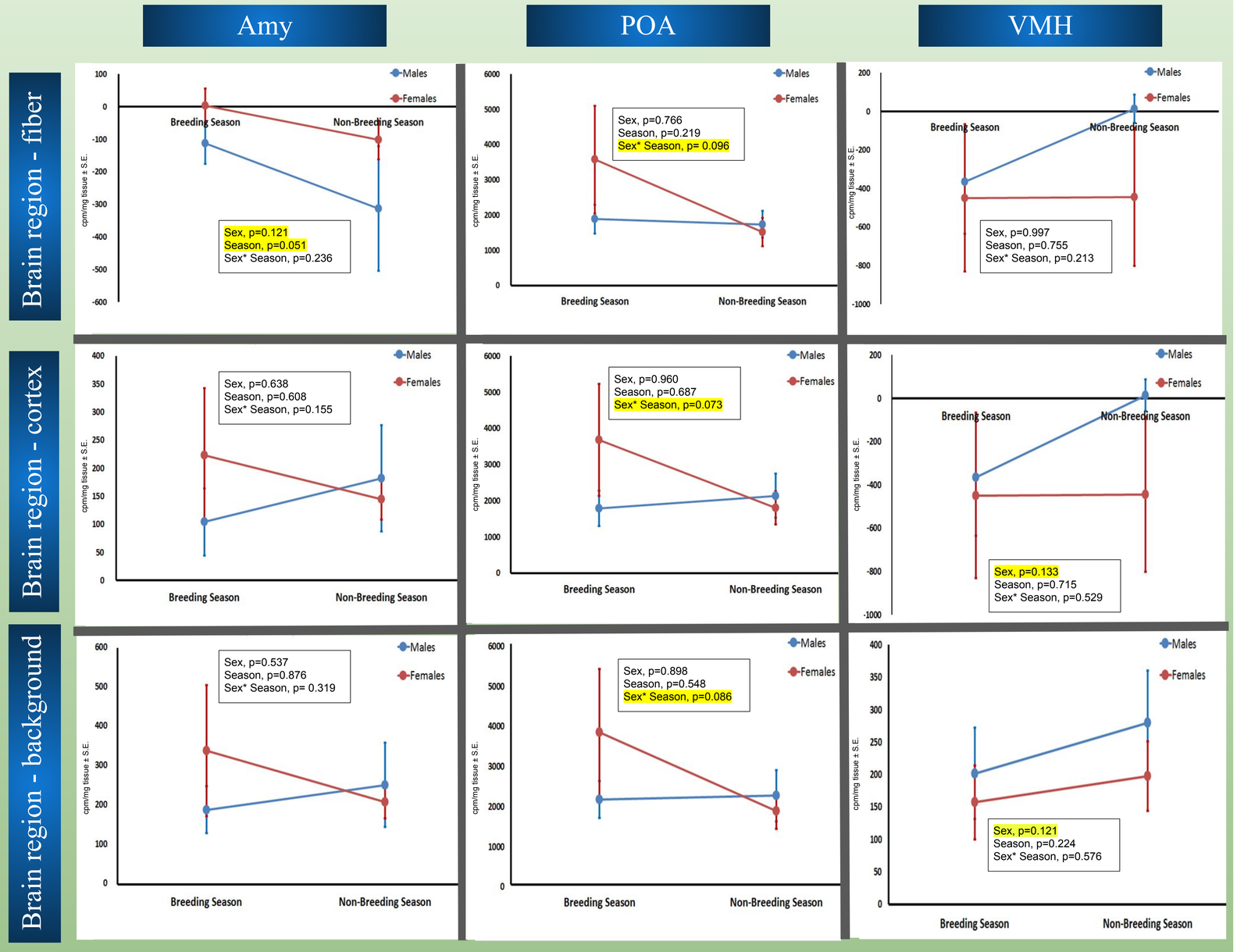

Figure 2: Results from a two-way ANOVA analysis indicates that there were no significant differences in vasopressin receptor expression for sex (male versus female), season (breeding versus non-breeding), or the interaction between sex and season for any of the targeted brain regions (Amy, POA, VMH), regardless of the control used. However, we did see strong trends in some cases (highlighted in yellow).

DISCUSSION AND CONCLUSION

- This pilot study was run on a subset of brains from a larger set of breeding and non-breeding animals. Therefore, although our results found no significant results, the trends are promising enough that we will run the full set of subjects for analysis.
- Our trends partly match our predictions, which are based on previous findings in mammals and birds[5-7] and show higher V1aR levels for the POA and VMH in males than in females. However, at least one other study finds generally higher V1aR levels in females than males[8].
  - As predicted, we saw a trend for more V1aR signal in the VMH of males than females.
  - Counter to predictions, we saw more V1aR signal in the POA of breeding females than in males and non-breeding females.
- Fiber may not be best control, since fiber values were variable across brain sections. Cortex and background subtractions result in similar data patterns.
- This study is novel as we know of no other studies of sex and seasonal differences in VP receptors in reptiles.
- In a future study, we hope to examine VP receptor expression in anole lizards that vary in behavioral boldness within sexual and aggressive interactions; we hypothesize that receptor expression patterns may underlie these differences in social boldness.

LITERATURE CITED

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