Investigating a Neural Circuit for Female Reproductive Behavior using Viral Tools in Songbirds

Elena Cruz-Adames COL 2022
Marc F. Schmidt, College of Arts and Sciences, Department of Biology

Introduction

A. There are analogous female reproductive displays and brain regions within female mammals and birds (PAG = ICo + DM).

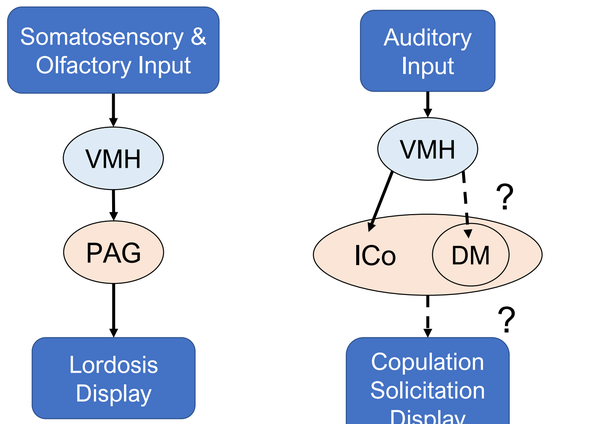


Fig. 1 The hypothalamus is a region in the brain that controls a variety of basic life functions including reproduction. The **ventromedial hypothalamus (VMH)** has been shown to have an important role in female sexual behavior in rodents and birds.

Methods

- Employ a series of anterograde and retrograde tracers including BDA and AAV9-GFP to outline the connectivity of the VMH and DM.
- Injected brainstem nucleus RAm bilaterally in 8 birds using stereotaxic coordinates and electrophysiology.
- Animals were perfused with 4% paraformaldehyde (PFA).
- Brains were removed and then put into a 30% sucrose solution until ready for sectioning.
- Coronal sections (40µm) were prepared with a sliding microtome and collected in PBS.
- Sections were mounted onto slides for either Nissl stain or were stained with Hoechst and then coverslipped with Fluoro-Gel and imaged with a dissecting microscope.

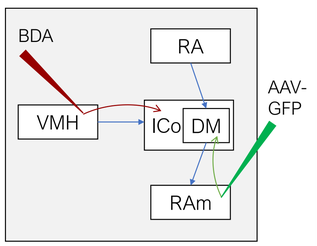


Fig. 2 Plan for injections of anterograde and retrograde tracers.

Results

B. Finding RAm using stereotaxic coordinates and recording of brainstem.

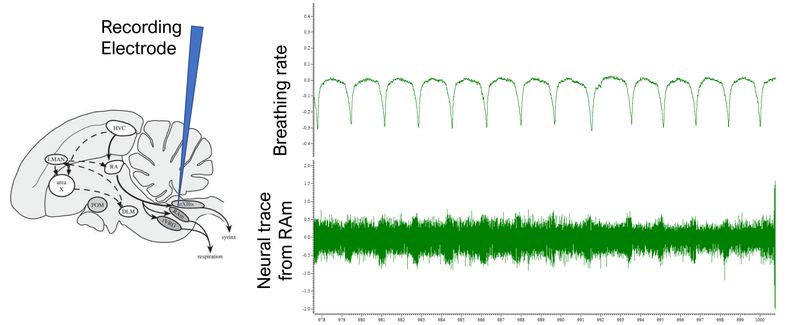
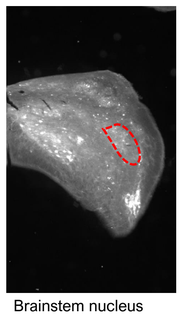
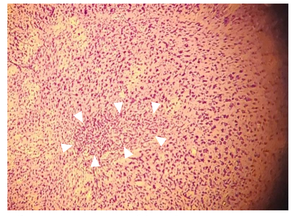


Fig. 3 Diagram of songbird brain on left. Using stereotaxic coordinates (Lateral: 1.1mm, Posterior: 4.0mm), we recorded neural activity using a tungsten electrode in the brainstem to locate RAm as it is linked with respiratory (expiratory) activity. On the right are recordings of the breathing rate and neural trace that were collected during a surgery for bilateral RAm injections.

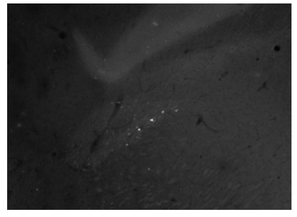
C. Identifying and visualizing areas of interest with Cresyl Violet (Nissl) stain and fluorescence.



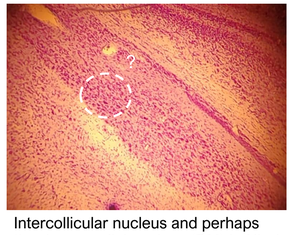
Brainstem nucleus RAm outlined in red



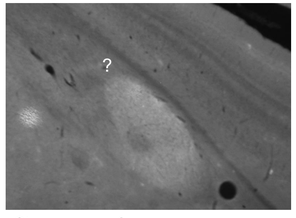
Robust nucleus of the Arcopallium (RA) in Nissl



Backlabelled cells in RA



Intercollicular nucleus and perhaps the dorsomedial nucleus (DM) in Nissl



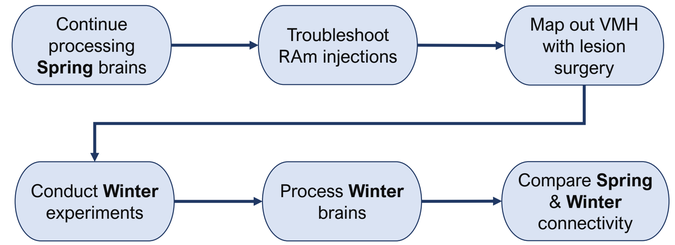
Still searching for backlabelled cells in DM in fluorescence

Fig. 4 Coronal sections of RAm, RA, and DM. Brainstem nucleus RAm is our injection target. RA projects to DM and RAm. DM also projects to RAm. We expect to see backlabelled cell bodies in areas RA and DM with immunofluorescence. All images are oriented with the left being towards the midline (medial) and right is lateral.

Fall Timeline

D. Planned experiments for independent study.

- We know that when females are out of breeding season, they will not produce copulation solicitation displays because they are not receptive.
- Therefore, we did our first set of experiments in late spring/early summer so that the brains reflected breeding season conditions. Our next set of experiments will be done in the fall so that the brains reflect out of season conditions.



E. Hormone levels may enable plasticity in pathway for CSD.

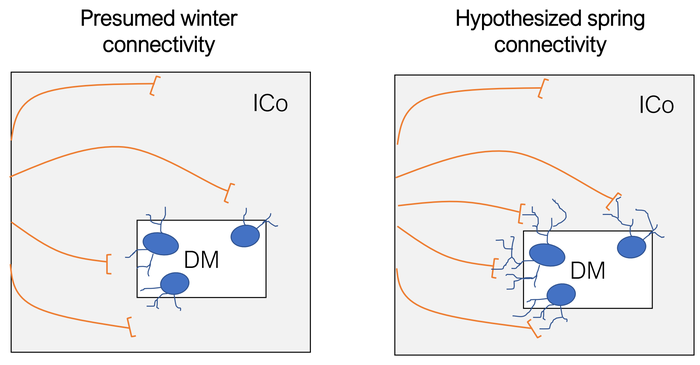


Fig. 5 Presumed winter connectivity and hypothesized spring connectivity. The VMH projects to the ICo region surrounding the DM nucleus. Large grey square is ICo, white rectangle is DM, DM cells and dendrites are in blue, and VMH axons are in orange.

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Contact

Presenter: Elena Cruz-Adames
University of Pennsylvania
eca705@sas.upenn.edu

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