Exploring the Neural Mechanisms of Hibernation in Arctic Ground Squirrels Through AAV Gene Delivery

Authors & Affiliations

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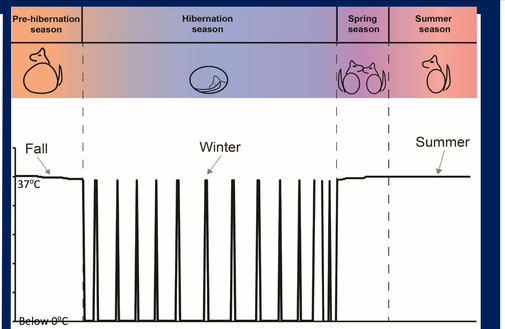
Introduction and Background

- Arctic ground squirrels (AGS) hibernate for up to 8 months a year[1].
- Virally deliverable gene constructs would be valuable tools for uncovering the neural mechanisms regulating hibernation.
- The tropism of an Adeno-Associated Virus (AAV) serotype is highly dependent on both species and target cell type[2].
- To determine which serotypes best transduce cells in the AGS brain, we injected four AAV serotypes encoding the fluorescent reporters tdTomato and eGFP under the CAG promoter into the frontal cortex and right lateral ventricle.



Adapted from Frare and Drew, 2017

Objective

To qualitatively assess the tropism of AAV1, AAV2, AAV8, and AAV9 by co-localizing viral reporters GFP (AAV2 and AAV8) and tdTomato (AAV1 and AAV9) with immunofluorescently labeled biomarkers for neurons (NeuN+), astrocytes (GFAP+), microglia (IBA1+), and tanycytes (Vimentin+).

Methods

Three female AGS were anesthetized, prepared, and secured in a stereotaxic frame. After skull leveling (≤0.1 mm DV variance between bregma and lambda), craniotomies were performed and a cannula/Hamilton syringe delivered 10 µL pAAV1-CAG-tdTomato + pAAV2-CAG-GFP into the right lateral ventricle (10 min). Additional injections (1 µL, 1 mm depth, 1 min) were made into the right (AAV1/2) and left (AAV9/8) frontal cortex. Viral titers were 6×10^{13} vg/mL. After 4 weeks, animals were perfused with saline followed by 4% PFA (PBS). Brains were fixed (24 h), stored in PBS + 0.02% sodium azide, cryoprotected in a sucrose gradient (5–30%), embedded in OCT, frozen (<−60 °C), sectioned (40 µm), and stored until staining. Sections were washed, permeabilized (0.5% Triton X-100), blocked (10% NDS), and incubated overnight (4 °C) with primary antibodies (NeuN, GFAP, IBA1, Vimentin), followed by AlexaFluor 647 secondary (2 h), Hoechst counterstain, and mounting. Images (4×–40×) were acquired via fluorescence microscopy and 40× images were deconvolved in FIJI using theoretical PSFs and the Richardson–Lucy algorithm (15 iterations).

Discussion

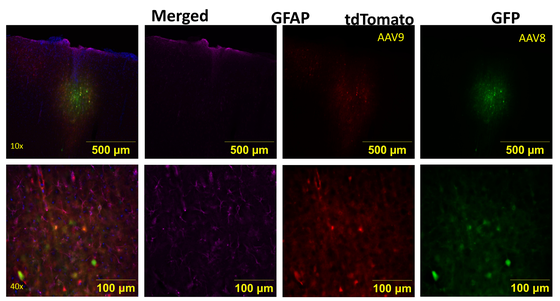
All four AAV serotypes (1, 2, 8, 9) strongly transduced cortical and hippocampal neurons, supporting their use as neuronal vectors in AGS, but none transduced cortical astrocytes—unlike rodent and primate models. Astrocyte targeting was region-specific, with AAV1 labeling hippocampal CA1 astrocytes but not cortex. AAV1 and AAV2 transduced the lateral ventricle lining, with AAV2 broadly targeting α/β tanycytes and AAV1 favoring α-tanycytes. Microglial transduction was minimal and inconclusive. 2D imaging limited accurate quantification, indicating the need for 3D analysis. Future work will use z-stack imaging with CellPose for objective co-localization, expand IHC sampling, assess AAV8/9 microglial tropism, normalize GFP/tdTomato signals via antibody labeling, and apply cell-specific promoters (e.g., hSyn, Rax) to improve targeting.

This work was funded by NASA Grants 80NSSC20M0070, NNX13AB28A, 22-22EPSCoR-0018, COBRE Grant P20GM130443 and INBRE Grant P20GM103395.

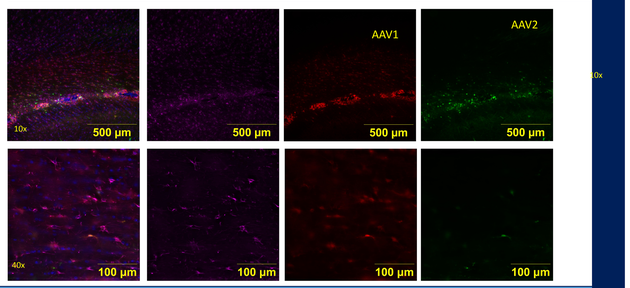
Special thanks to the Center for Transformative Research in Metabolism (TRiM), the Health & Metabolism Research (HaMR) Core, Jim Janoso, Maria Silverthorn, and Chris Terzi.

Astrocytes

Left Frontal Cortex
AAV8 and **AAV9** both fail to transduce cortical astrocytes. Interestingly, **AAV1** and **AAV2** also failed to transduce cortical astrocytes. However, tdTomato + astrocytes were found in the stratum oriens of the CA1 layer of the hippocampus.

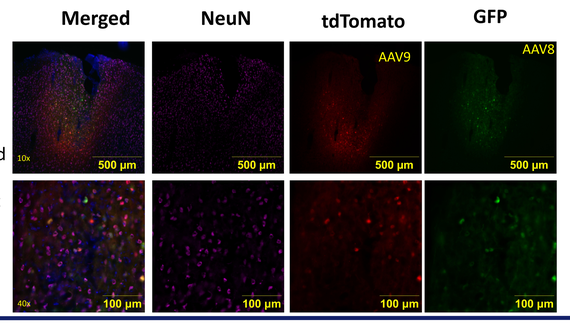


Hippocampal CA1
While neither GFP nor tdTomato were found in astrocytes in the cortex, **AAV1** strongly transduced astrocytes in the CA1 layer. **AAV2** may transduce these astrocytes as well, but a z-stack 3D analysis is needed for an objective measure of cell types transduced.

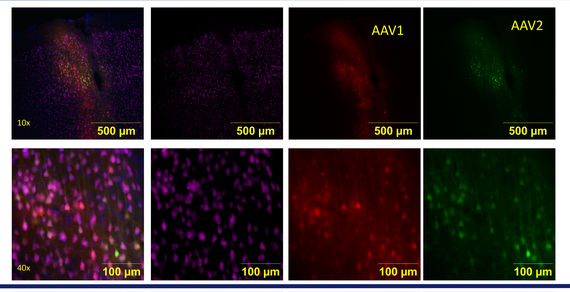


Neurons

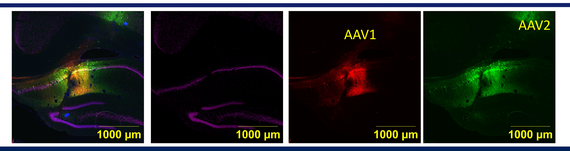
Left Frontal Cortex
Both **AAV8** and **AAV9** transduce neurons to a similar degree. In all 40x images, blurring due to 40µm sections and widefield strongly obscured images. Deconvolution vastly improved clarity, but 2D quantification from a 40µm section with CellPose[5] gave dubious results (not shown).



Right Frontal Cortex
AAV1 and **AAV2** also expressed well in neurons. **AAV1** seemed to transduce a larger area of neurons, but this may be due tdTomato being ~6x brighter than GFP[6].

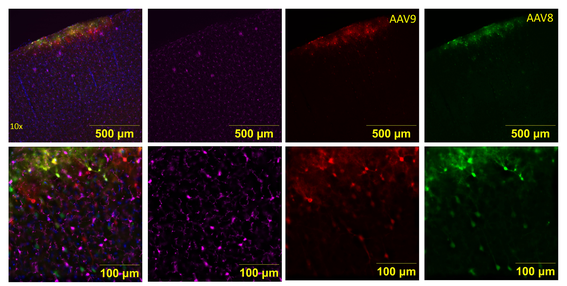


Hippocampal CA1
AAV1 and AAV2 are seen in the CA1 layer of the hippocampus.

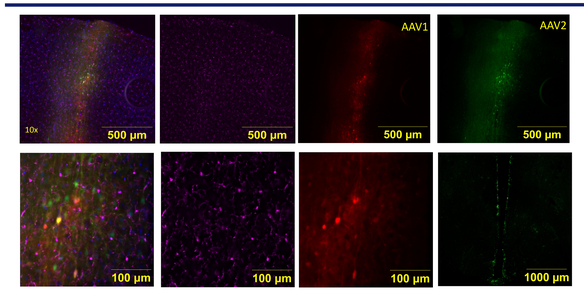


Microglia

Left Frontal Cortex
AAV8 and **AAV9** may not transduce cortical microglia. A z-stack 3D analysis of the site of injection in the left frontal cortex needs to be performed in order to determine the tropism of **AAV8** and **AAV9** in the AGS as no AGS sections that had IHC for IBA1 included the true injection site.

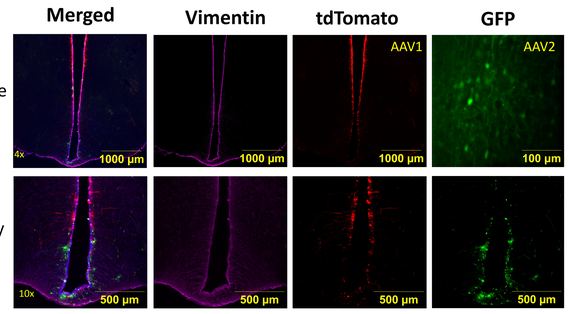


Right Frontal Cortex
AAV1, but not **AAV2** may transduce cortical microglia at a low rate. However, it is possible that the sparse co-localization seen is from microglia interacting with neurons. Qualitative assessment of hippocampal tissue stained for IBA1 yielded similar insights.



Tanycytes

Third Ventricle
AAV1 and **AAV2** transduce the lining of the third ventricle and median eminence. Interestingly, **AAV2** seemed to transduce both α and β tanycytes more effectively than **AAV1**.



Median Eminence
α (top rows) and β (bottom rows) tanycytes line the median eminence and are identified by single processes. Both AAV1 and AAV2 transduced α and β tanycytes, with AAV1 showing α specificity, while AAV2 showed broader transduction, including neurons and astrocytes.