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In Vivo Ca^{2+} Imaging of Prefrontal Cortex Astrocytes with Miniscopes

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INTRODUCTION

Astrocytes are highly heterogeneous neuroglial cells in the brain that are fundamental elements in local synaptic plasticity and critical determinants of cognition.[1] While previous research has established that astrocytes play a supportive role in the hippocampus, a brain region critical for spatial working memory (SWM)[2], their role in the medial prefrontal cortex (mPFC), another region important for learning and memory, is not as well characterized. Astrocytes communicate through Ca^{2+} waves, thereby making Ca^{2+} signaling an important measure for astrocytic activity.[3] Using a miniature microscope in freely behaving rats and a delayed spontaneous alternation task, we aim to achieve broader understanding about the dynamics of astrocytic activity and how it affects SWM in the mPFC. Additionally, previous research has suggested during periods of high activity, like working memory, glucose preferentially enters the brain through astrocytes[4] and can enhance working memory.[5]

METHODOLOGY

Virus Injection and Lens Implantation

Animals were anesthetized using isoflurane and placed in a stereotaxic frame. AAV5 pZac2.1 *GfaABC$_1$D*-cytoGCaMP6f (Addgene) was injected at one site (AP: +2.8, ML: +/-0.7, DV: -4.0) in the mPFC (0.5µL, rate: 0.25µL/min). The needle remained in place for 1 min after the injection was complete. After vector delivery, a gradient refractive index (GRIN) lens probe (0.5 mm diameter x 6.1 mm length) was implanted (AP: +2.5; ML: +/-0.5; DV -3.7).

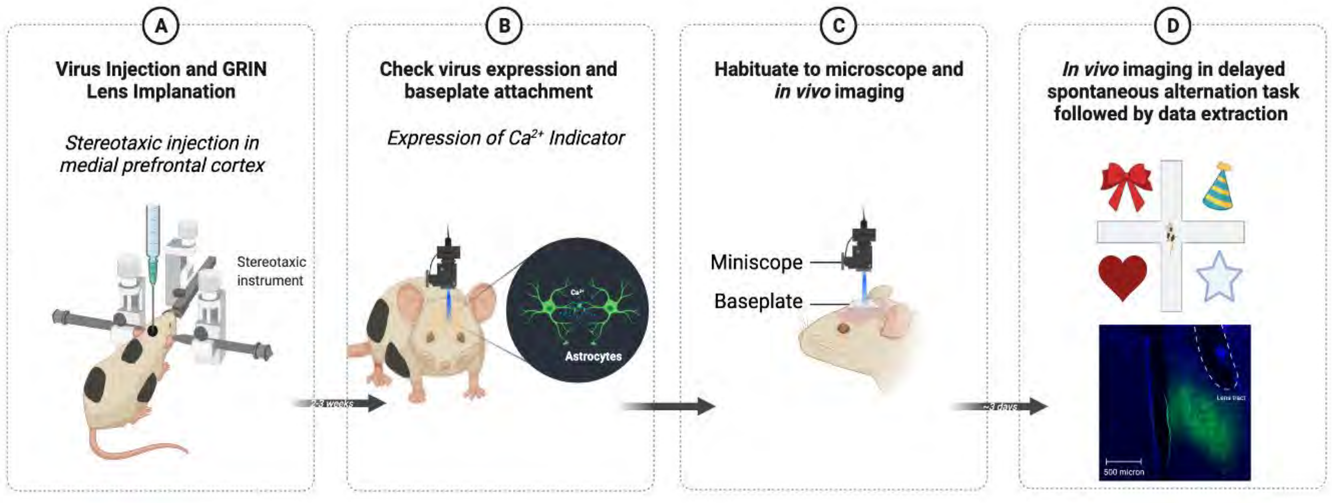

Fig. 1. Timeline for *in vivo* Ca^{2+} imaging. **A)** All female and male Long Evans rats received a virus injection in the mPFC during stereotaxic surgery. **B)** Miniscope was used to check viral expression. **C)** Baseplate attachment surgery performed when astrocytes were visualized. Subjects were habituated with dummy scopes prior to delayed spontaneous alternation task. **D)** Subjects received either saline vehicle or glucose (250 kg/mg) on testing days and were counterbalanced to account for testing bias. Using a 20-minute 4-arm delayed spontaneous alternation task with extra-maze visual cues that were changed each session, SWM was assessed. During performance, Ca^{2+} fluorescent images were acquired with the head-mounted miniature microscope (miniscope.org) at 30 frames per second. Microinjection of AAV5 pZac2.1 *GfaABC$_1$D*-cytoGCaMP6f (green) in mPFC.

RESULTS

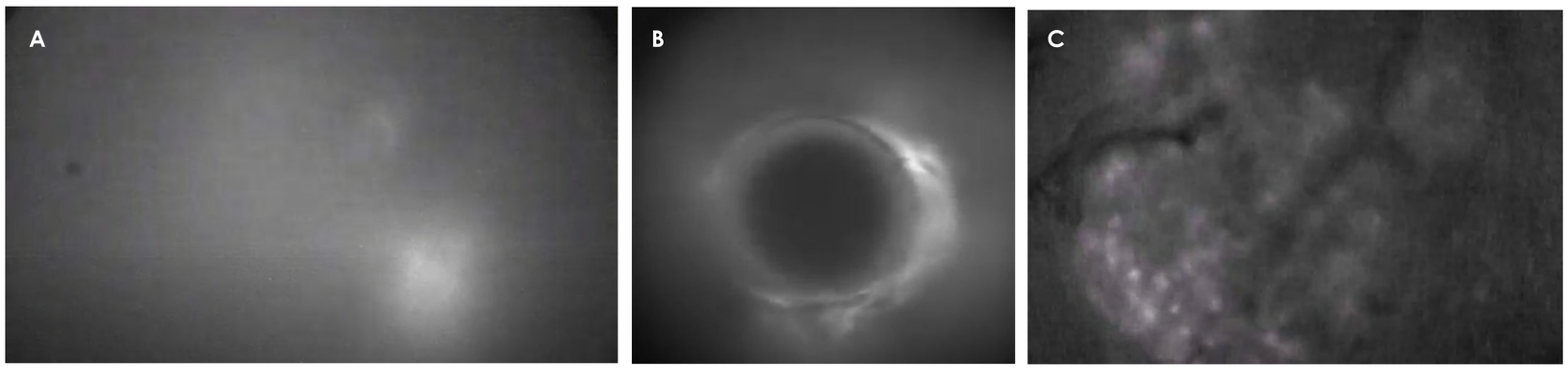

Fig. 2. Snapshots from miniscope video recording during habituating and behavior sessions. A) V3 miniscope video with some expression of astrocytes seen in white fluorescent spots. B) V4 miniscope video with lens visible but no astrocytes seen. C) Clear astrocyte activity during sleep acquired using an Inscopix miniscope (Ingiosi et al., 2020)

Troubleshooting and Issues

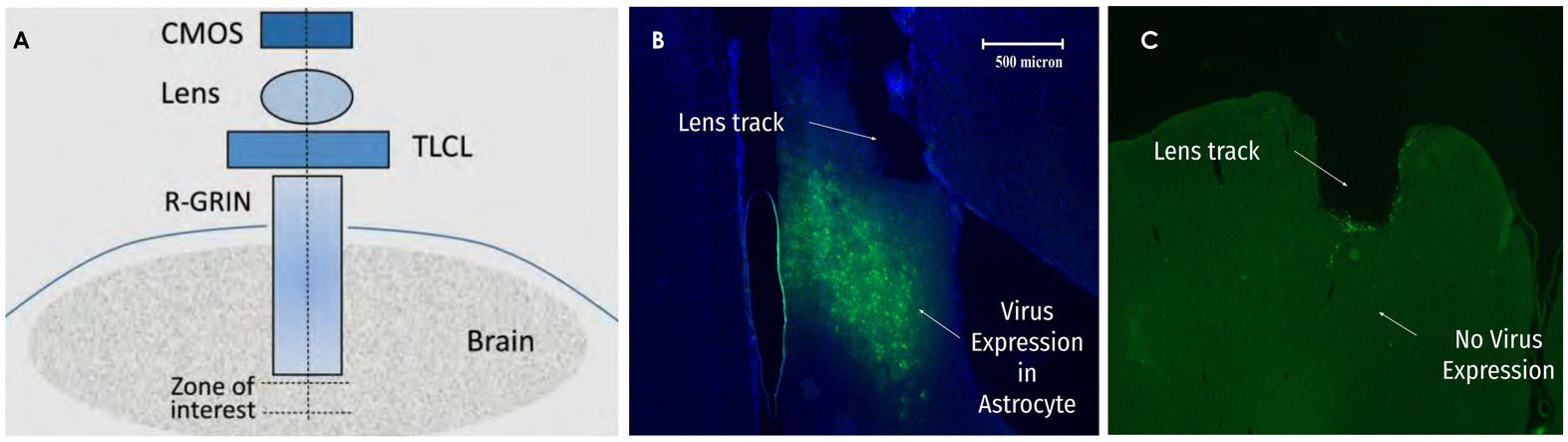

Fig. 3. Possible issues checked during troubleshooting. A) Schematic of lens and miniscope alignment. Miniscope and lens needs to be within working distance of the miniscope (0.7 - 1.1mm). B) Histology photo of PFC stained with DAPI (blue) and GCAMP6f virus expression (green) shows lens placement and successful virus expression. C) Histology of PFC that shows lens placement but no virus expression under the lens.

DISCUSSION & FUTURE DIRECTIONS

- Problems in the image acquisition phase prevented us to move on to behavioral testing
- By examining the lens placement and virus expression in the histology photos, we were able to isolate the virus expression to be the main issue
- New virus would be used and tested, and behavioral data can be obtained

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