# Glutamatergic Pedunculopontine Nucleus (PPN) Projections as Mediators of Locomotion

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## Introduction

- The pedunculopontine nucleus (PPN) in the midbrain has previously been identified as a mesencephalic locomotor region (MLR) (Esposito & Arber 2016).
- Glutamatergic projections to the PPN from the basal ganglia, and subsequent descending pathways, are implicated in locomotion initiation and speed (Roseberry et al. 2016).
- We hypothesize that there may be ascending projections from the PPN to the ventromedial thalamus (VM) that also influence locomotor behavior. This study aims to identify those pathways and understand their roles in locomotion state and speed.

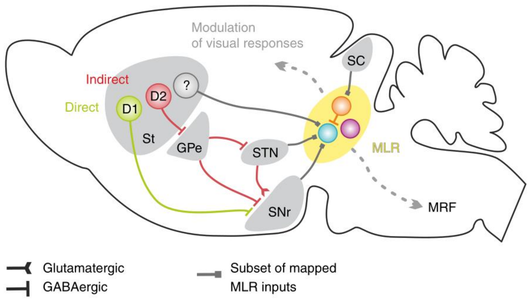



**Figure 1: Identified VM to MLR pathways**
(Esposito and Arber, 2016 CB)

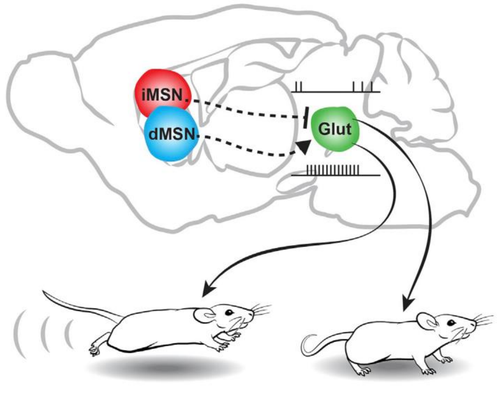



**Figure 2: Glutamatergic projections influence locomotion speed**
(Roseberry et al., 2016 Cell)

## Methods

- Inject Adeno-Associated Virus (AAV) into VM or PPN of 8-week-old vGluT-2-Cre mice, followed by cannula insertion into either region

| | Soma | Terminal |
|---|---|---|
| Stimulation | Experiment. 1 (n = 2):<br>• AAVrg-Flex-Flpo injection to VM, AAV8-fDIO-ChRger2-mScarlet3 injection to PPN<br>• Cannula insertion to PPN | Experiment 2 (n = 2):<br>• AAV8-DIO-ChRger2-mScarlet3 injection to PPN<br>• Cannula insertion to VM |

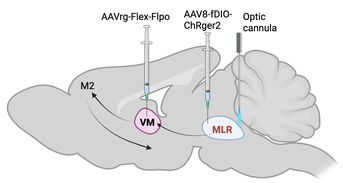



**Figure 3: Surgical Schematic for Experiment 1**

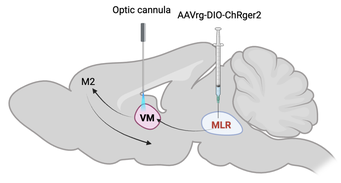



**Figure 4: Surgical Schematic for Experiment 2**

- 3 weeks post-surgery: optogenetic experiments examining initiation and speed of locomotion

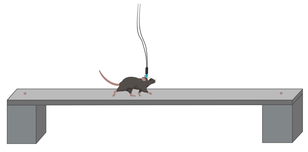


**Figure 5: Linear track setup for locomotion speed experimentation**

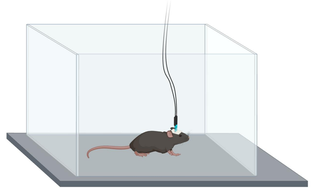
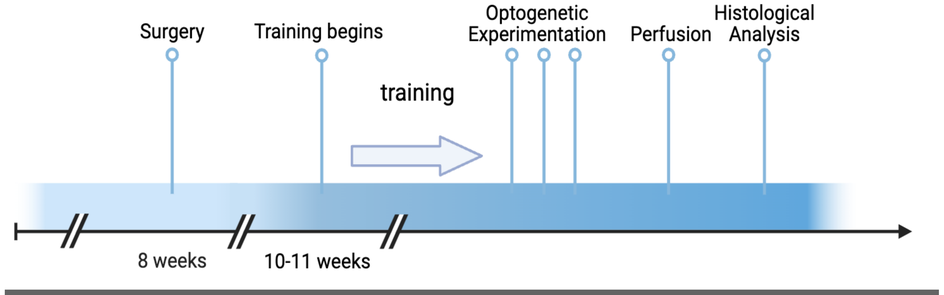


**Figure 6: Open field setup for locomotion initiation experimentation**

- Perfusion, cryosection, and confirm opsin expression and cannula tip via immunohistochemistry staining




## Results

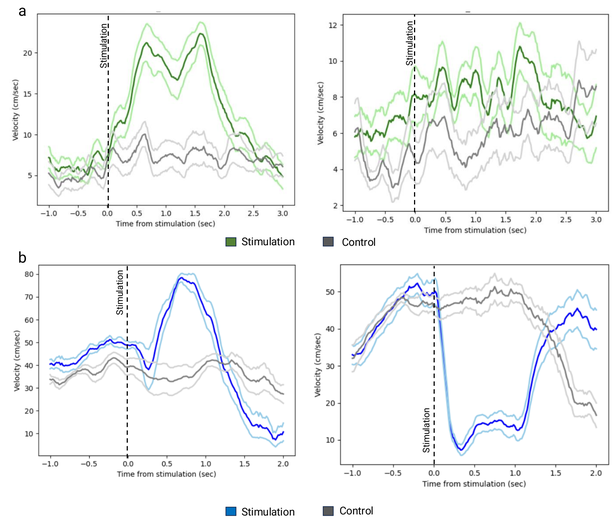



**Figure 7: Velocity of locomotion following optogenetic stimulation of the PPN under Experiment 1 conditions.** (a) Mean velocity across >10 Open Field trials with 1.5mW of optic stimulation for 1.5 second(s). Lighter shading indicates standard error. (b) Mean velocity across >10 Linear Track trials with 1.5mW of optic stimulation for 1.0 second(s). Lighter shading indicates standard error.

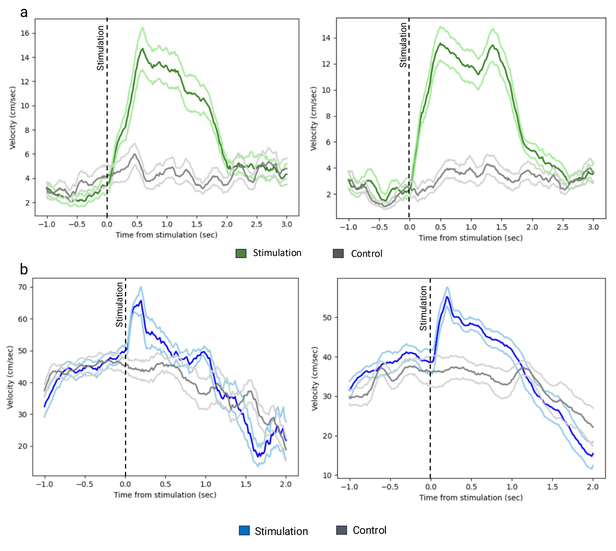



**Figure 8: Velocity of locomotion following optogenetic stimulation of the VM under Experiment 2 conditions.** (a) Mean velocity across >10 Open Field trials with 10.0mW of optic stimulation for 1.5 second(s). Lighter shading indicates standard error. (b) Mean velocity across >10 Linear Track trials with 3.0mW of optic stimulation for 1.0 second(s). Lighter shading indicates standard error.

## Discussion

- Our results support a role for VM–PPN pathways in regulating locomotion initiation and velocity. Optogenetic stimulation of both the soma and axon terminals induced locomotor activity and increased velocity in three of four mice.
- Interestingly, in Experiment 1, PPN soma stimulation produced an initial decrease in velocity followed by a sharp increase, suggesting the presence of modulatory or inhibitory projections that transiently suppress locomotion.
- Histological verification of opsin expression and cannula placement is ongoing and necessary to confirm target specificity. One mouse in Experiment 1 exhibited an opposite behavioral response, potentially due to mistargeted injection or cannula misplacement.

## Acknowledgements

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