Understanding serotonergic regulation of motor functions

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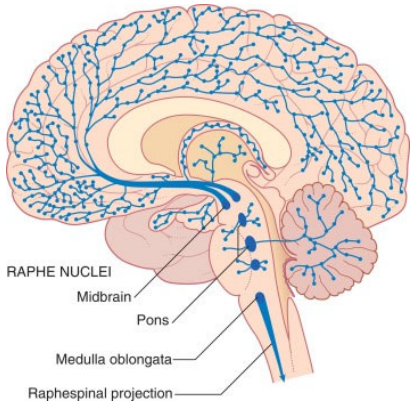
Department of Brain and Cognitive Sciences

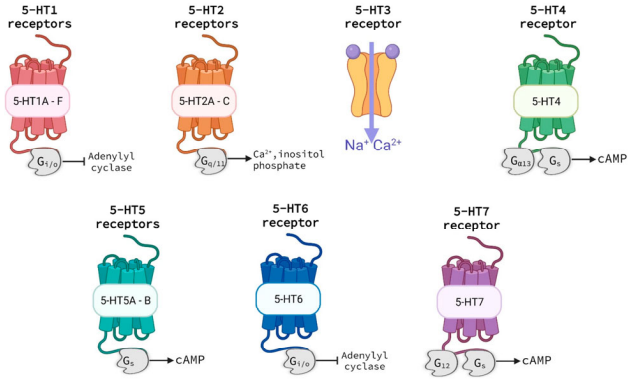
Serotonergic system:

It is a complex system with brain-wide projection, multiple receptors, volume transmission that affects various functions.

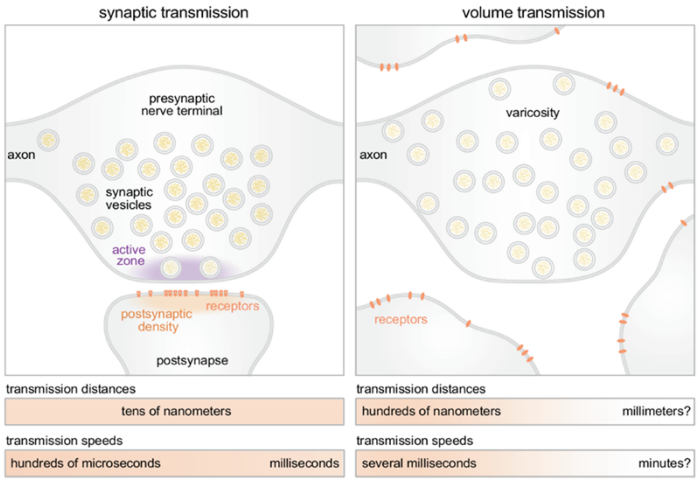
Projections



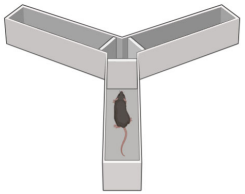
Receptors

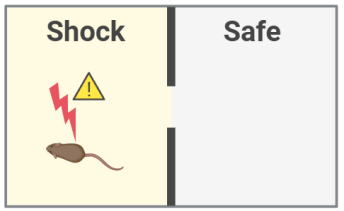


Transmission



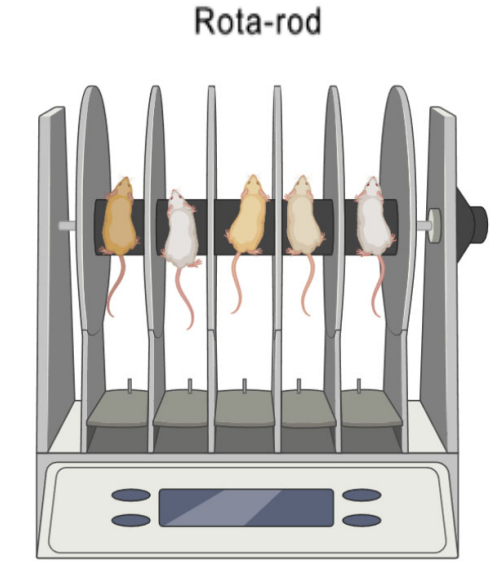
Functions

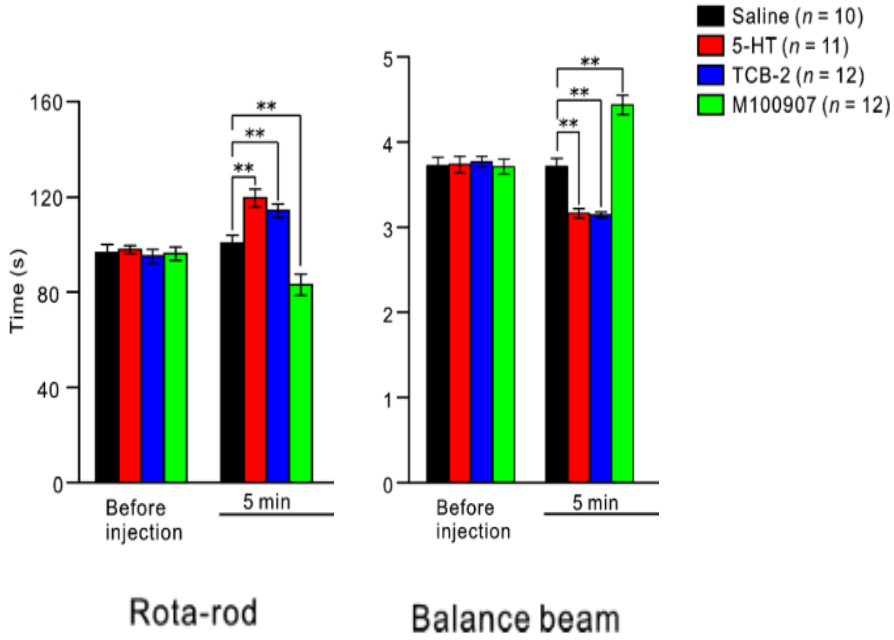




Serotonergic effect on Motor Control:

Serotonin (5-HT) regulates motor ability through Fastigial Nucleus (fDCN)





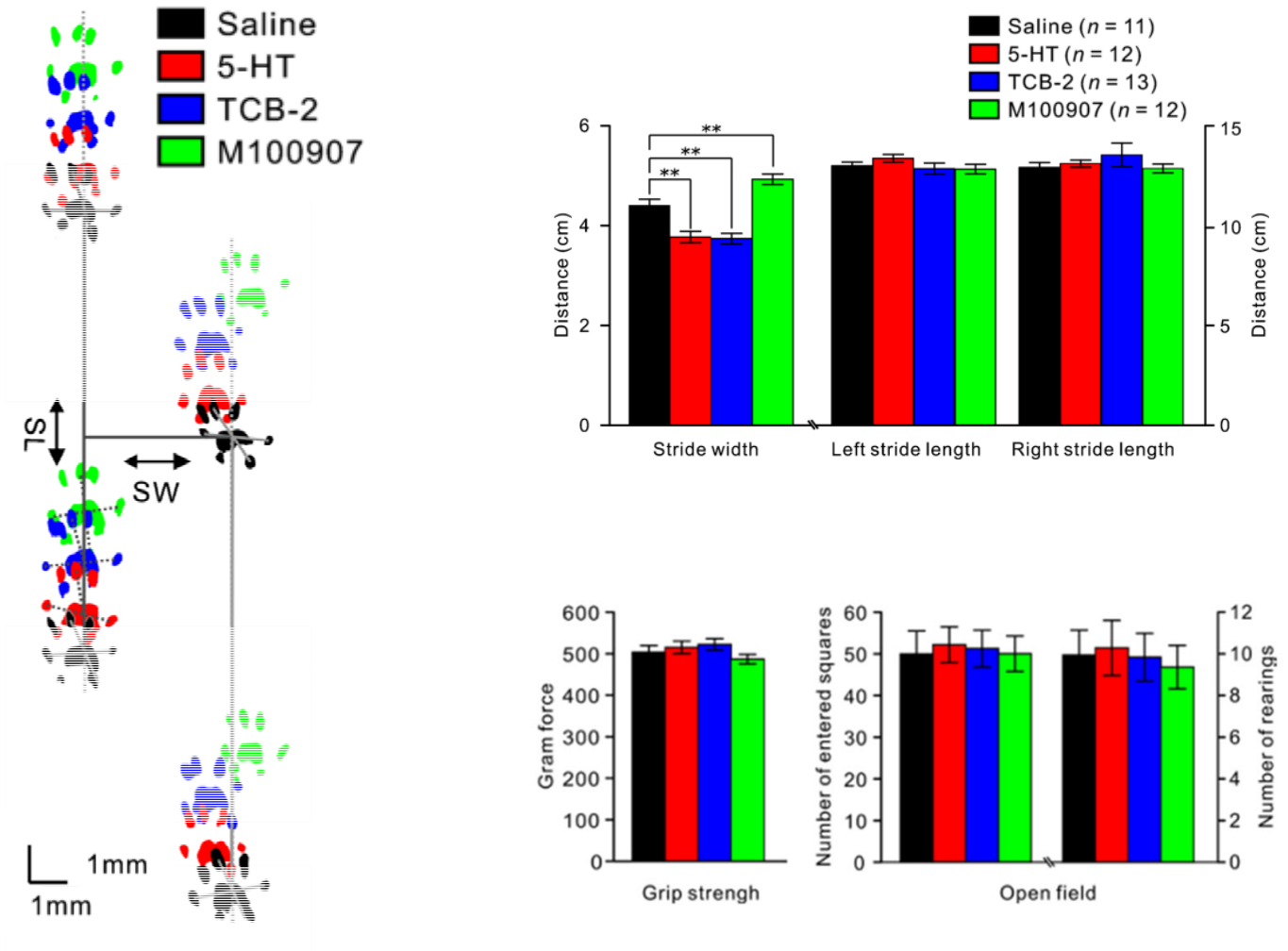
TCB-2: Serotonin 2A receptor Agonist

M100907: Serotonin 2A receptor Antagonist

CZ Zhang et al., 2014

Serotonergic effect on Motor Control:

Serotonin promotes motor performance, especially motor balance and coordination through 2a receptor in the cerebellar fastigial nucleus



Zhang C et al., 2014

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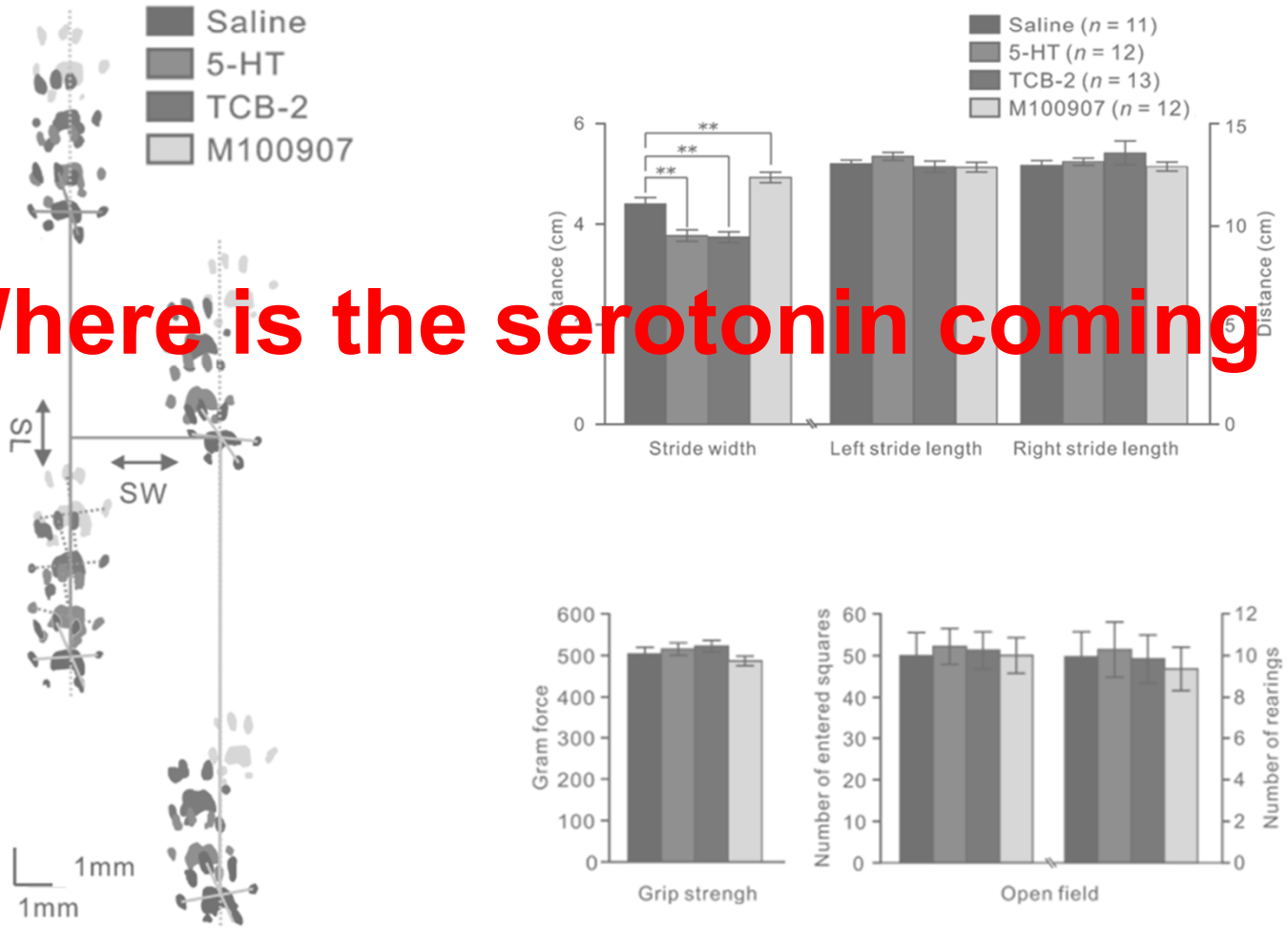


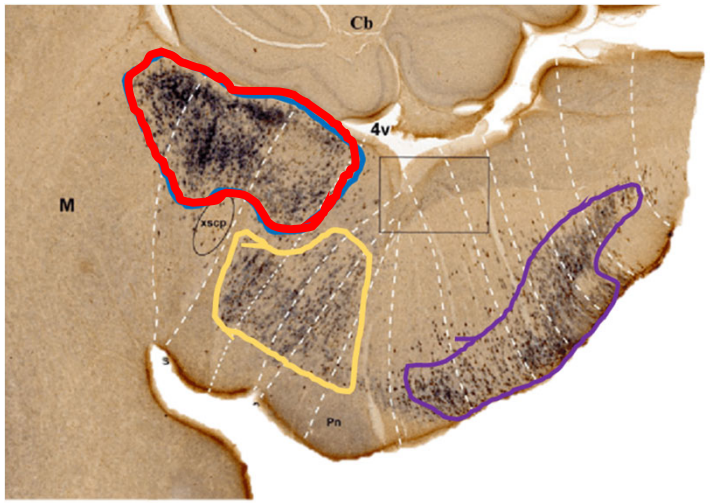
Where is the serotonin coming from?

Zhang C et al., 2014

Serotonergic effect on Motor Control:

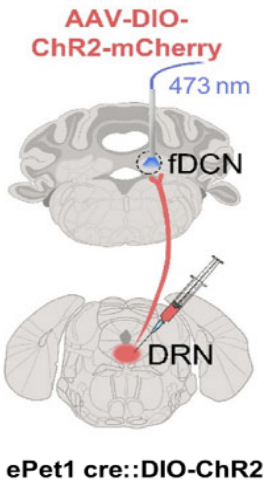
Serotonergic neurons are primarily located in the raphe nuclei, including the dorsal raphe nucleus, median raphe nucleus, and caudal raphe nucleus

Sagittal view of the raphe nucleus

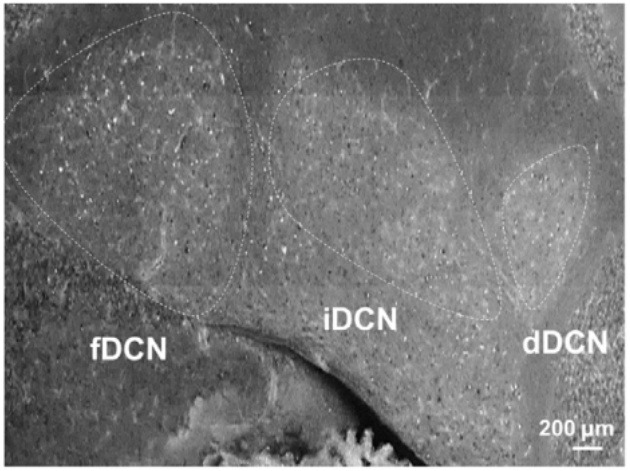


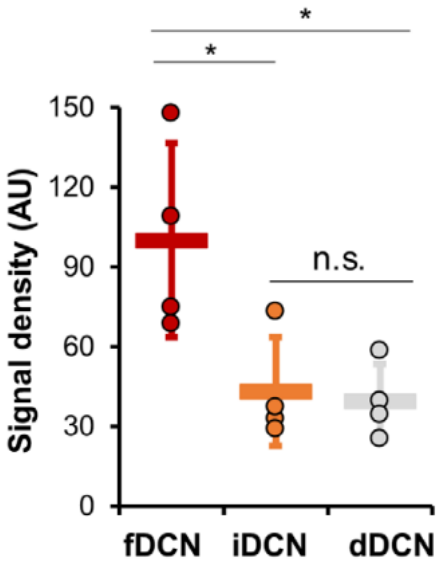
Serotonergic effect on Motor Control:

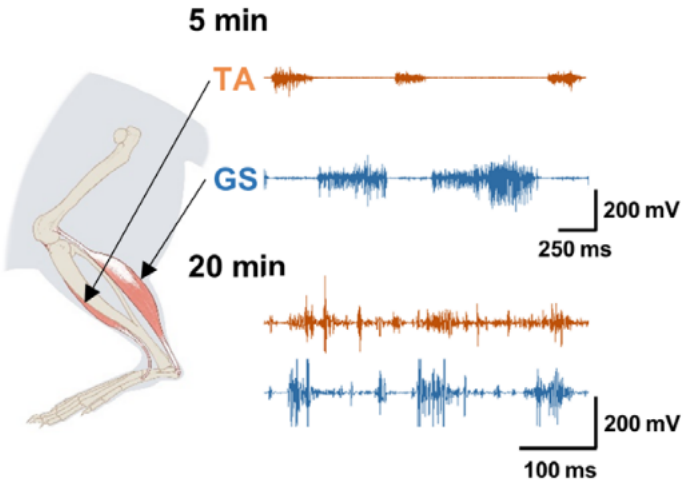
Hyperexcitation of serotonergic neurons at fastigial nucleus induces motor disorder, dystonia



ePet1 cre::DIO-ChR2



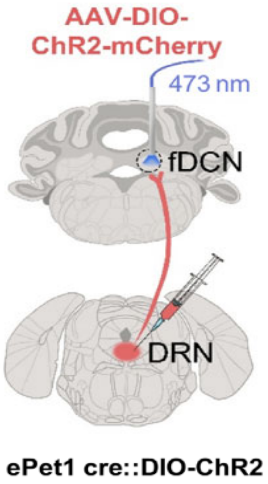




Kim et al., 2021

Serotonergic effect on Motor Control:

Previous studies show DRN serotonergic neurons might regulate motor ability via fastigial nucleus



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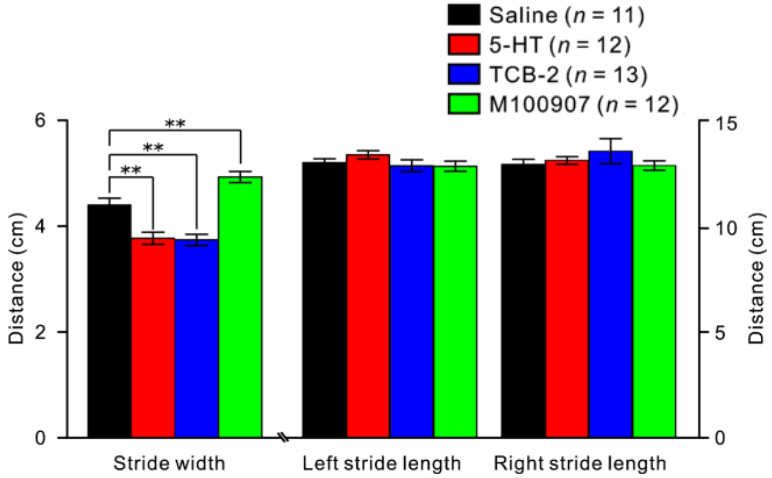

Questions:

1. Could serotonin that regulates motor control from the DRN?
2. What information do these serotonergic neurons encode?

Outline

1. Conducted Experiments
    1. Circuit tracing and cell population
    2. Chemogenetic study
    3. Fiber photometry study
2. Future Experiments

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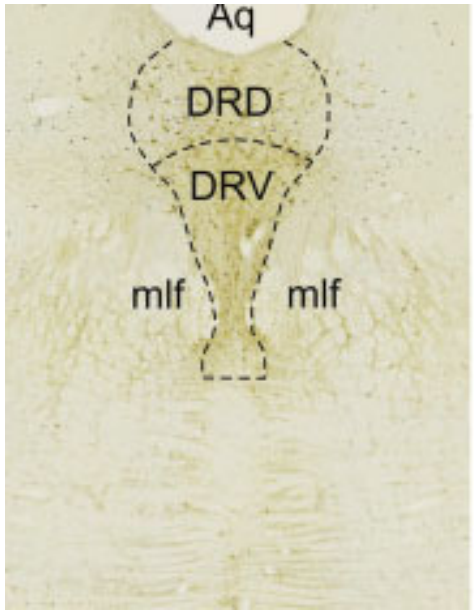
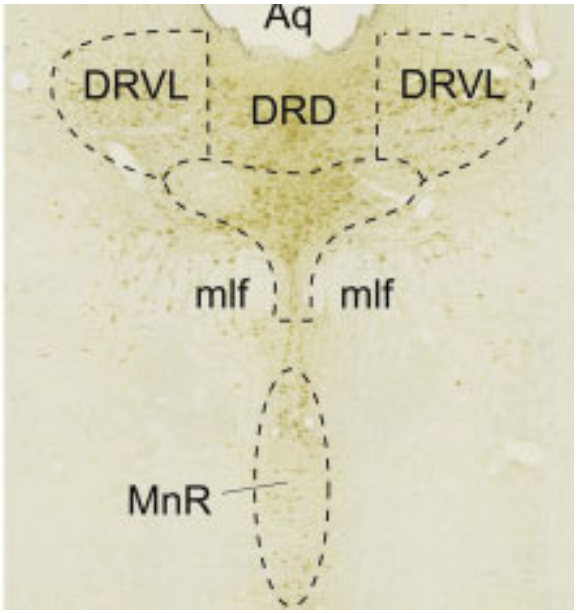
1. FN-projecting DRN population:

DRN can be divided input dorsal (DRD), ventral(DRV), and lateral (DRVL)

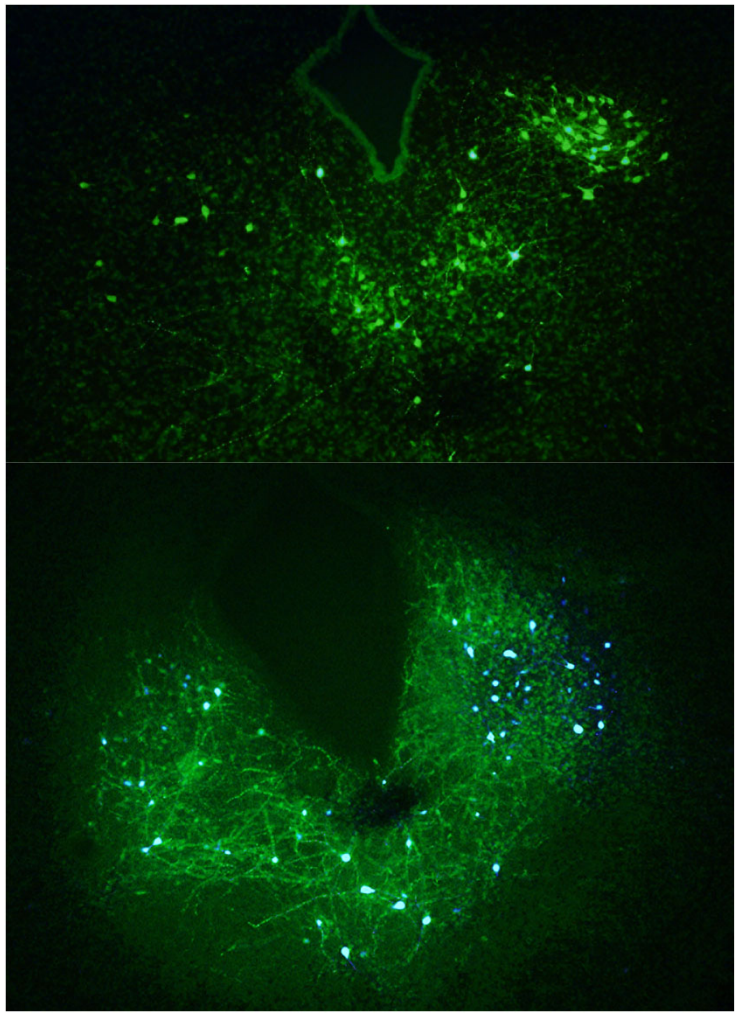


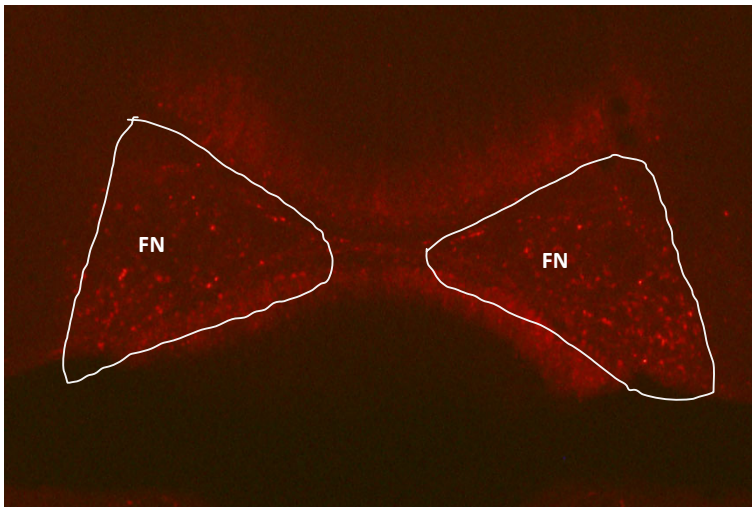


AP: -4.2 -4.6

1. FN-projecting DRN population:

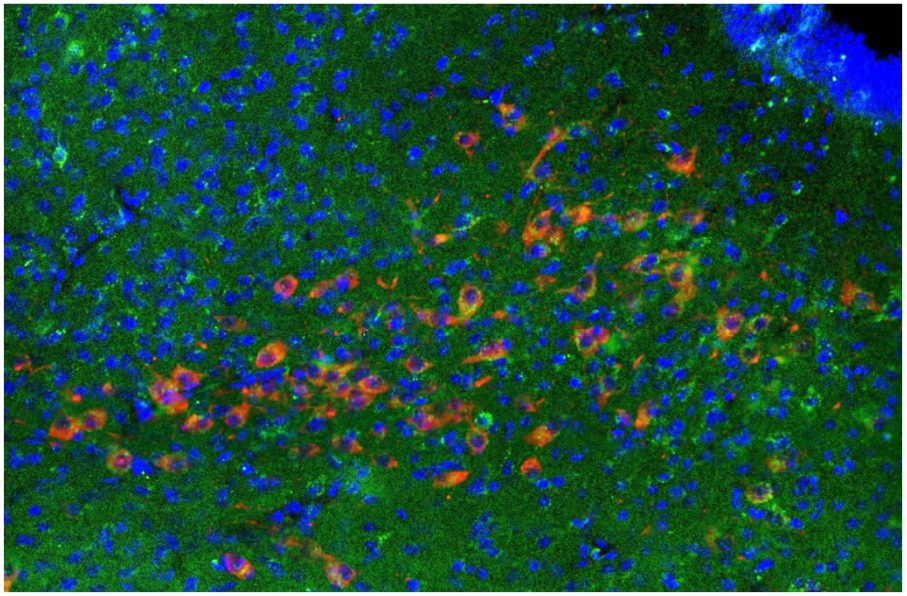
Retrograde experiment showed neurons in the dorsal raphe nucleus project to the fastigial nucleus





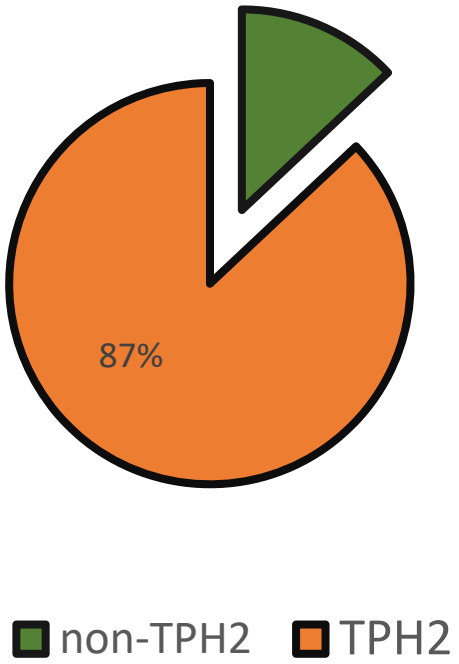
1. FN-projecting DRN population:

Anti-TPH2 staining showed majority of neurons projecting to the fastigial nucleus are serotonergic



Retrogradely labelled

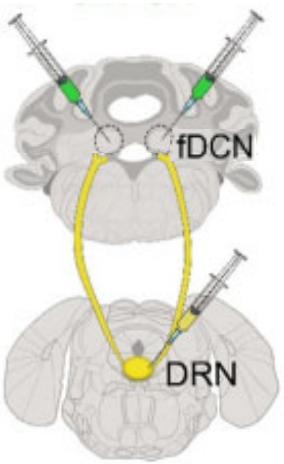
TPH2-positive



DRN-FN Chemogenetic experiments:

Pilot test: Bilateral DRN and FN excitation + Bilateral FN inhibition studies

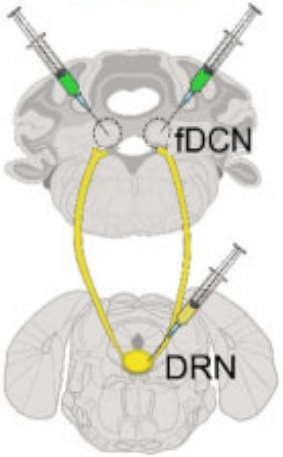
DRN excitation



AAVrg-hSyn-Cre

AAV5-hSyn-DIO-hM3D(Gq)

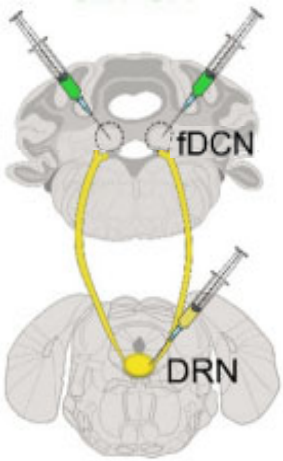
FN excitation



AAV5-hSyn-DIO-hM3D(Gq)

AAV1-hSyn-Cre

FN inhibition

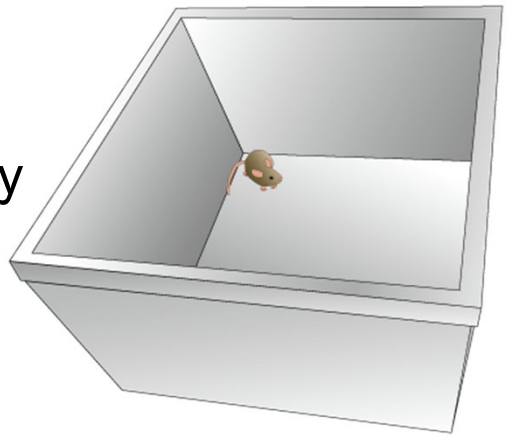


AAV5-hSyn-DIO-hM4D(Gi)

AAV1-hSyn-Cre

21 days Post-surgery

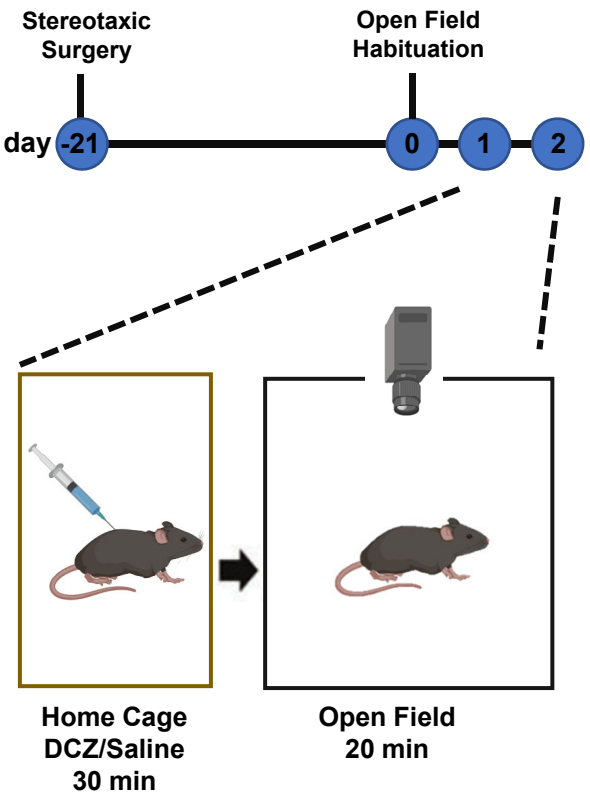
Open Field Test (OFT)

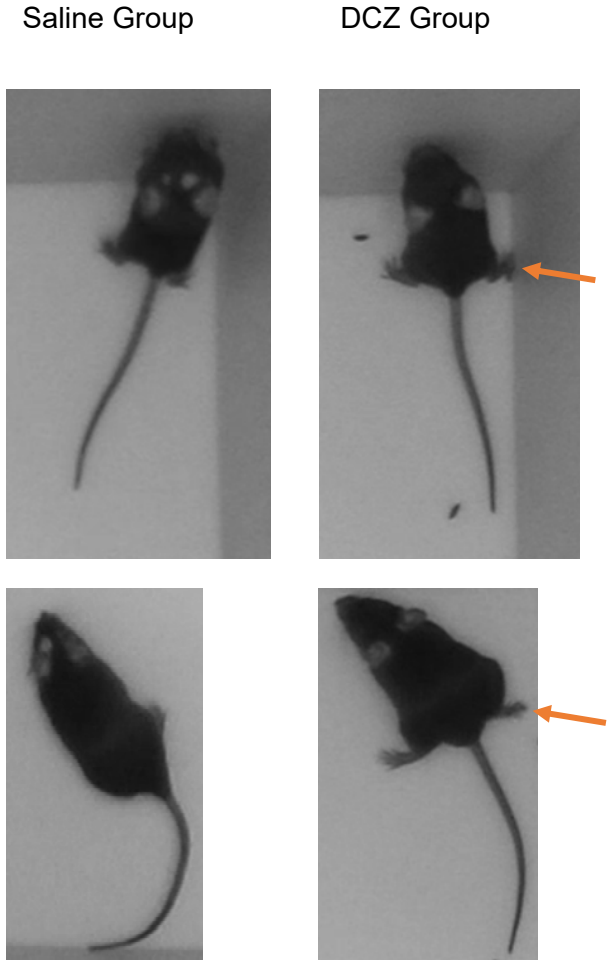


DCZ: 0.2mg/kg in 0.9% saline;
pre-experiment 30min IP injection

DRN-FN Chemogenetic experiments:

Pilot test: Bilateral DRN excitation

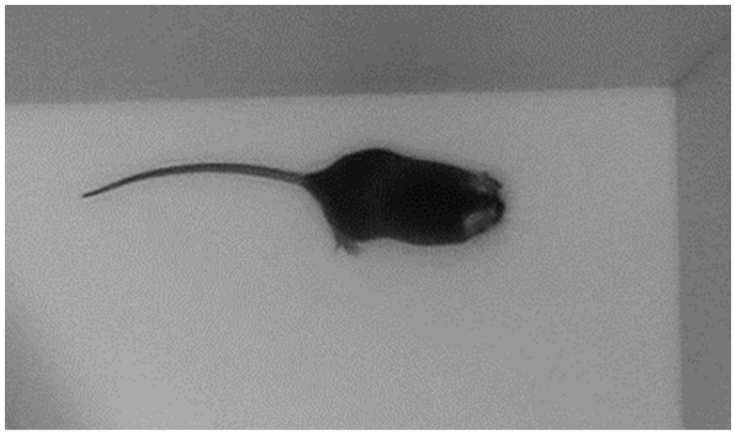




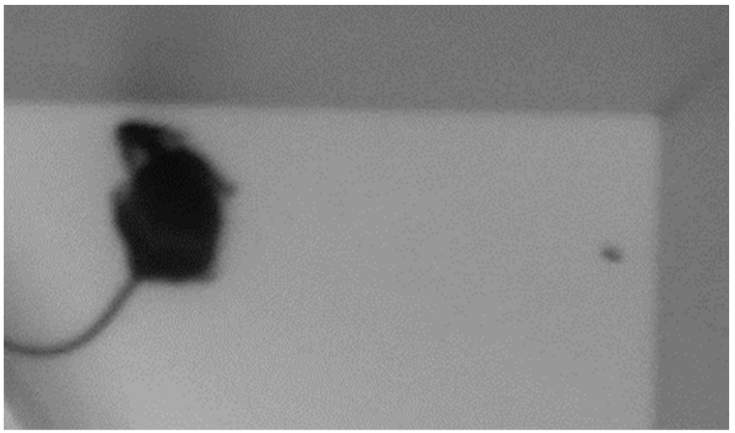
DRN-FN Chemogenetic experiments:

Pilot test: Bilateral DRN excitation

Saline Group



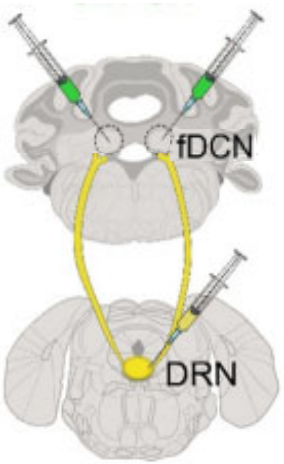
DCZ Group



DRN-FN Chemogenetic experiments:

Pilot test: Bilateral DRN and FN excitation + Bilateral FN inhibition studies

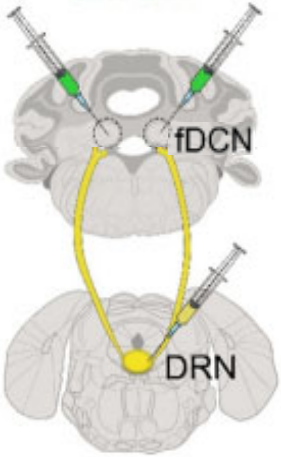
DRN excitation



AAVrg-hSyn-Cre

AAV5-hSyn-DIO-hM3D(Gq)

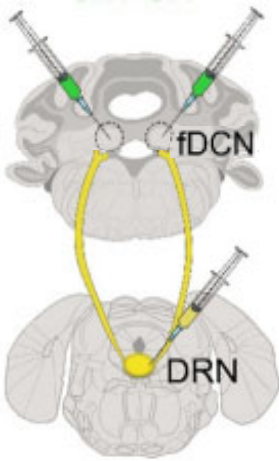
FN excitation



AAV5-hSyn-DIO-hM3D(Gq)

AAV1-hSyn-Cre

FN inhibition



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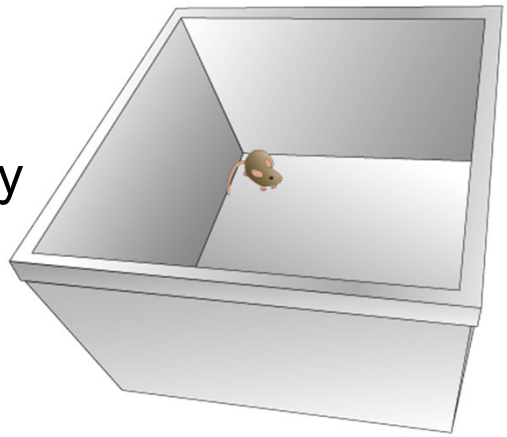

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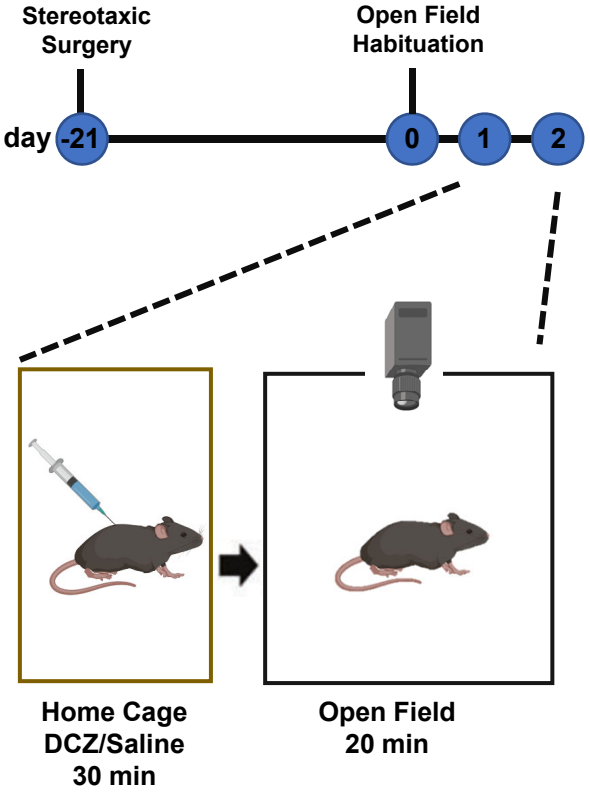
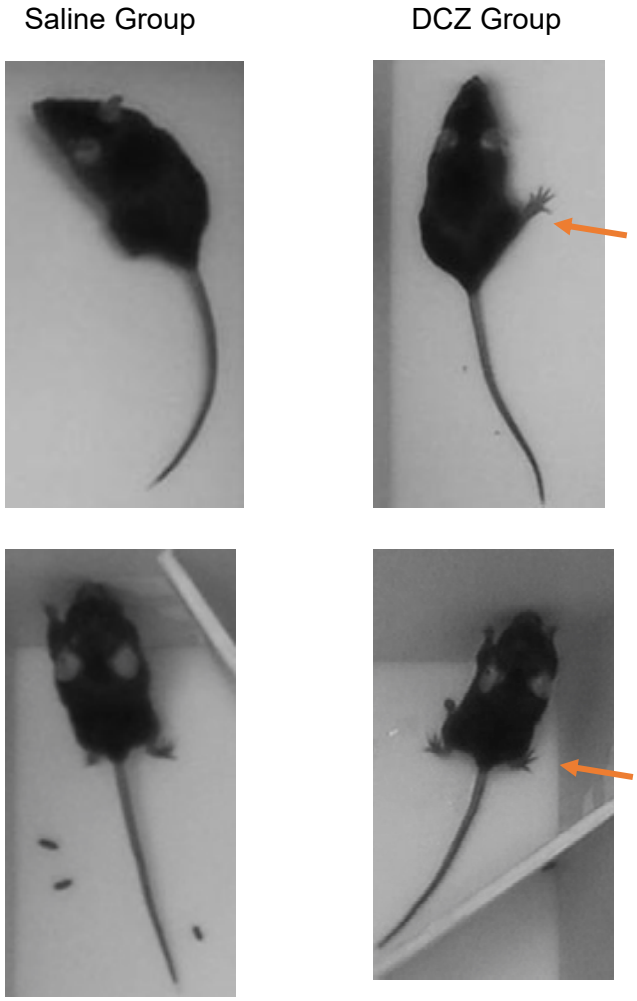
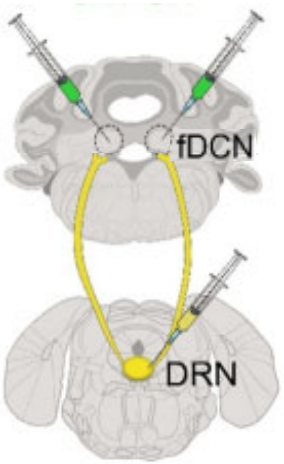




DRN-FN Chemogenetic experiments:

Pilot test: Bilateral DRN and FN excitation + Bilateral FN inhibition studies

DRN excitation



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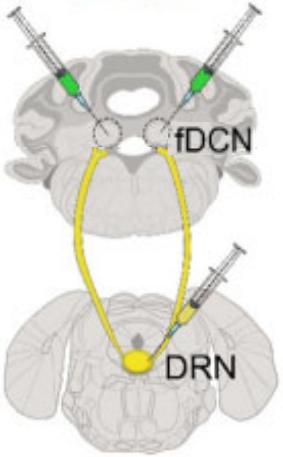
FN excitation



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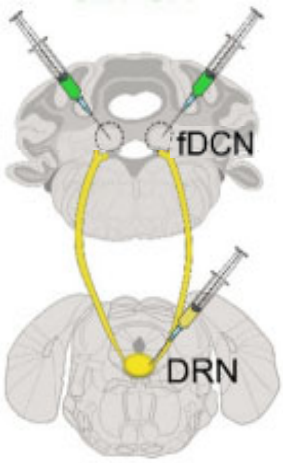

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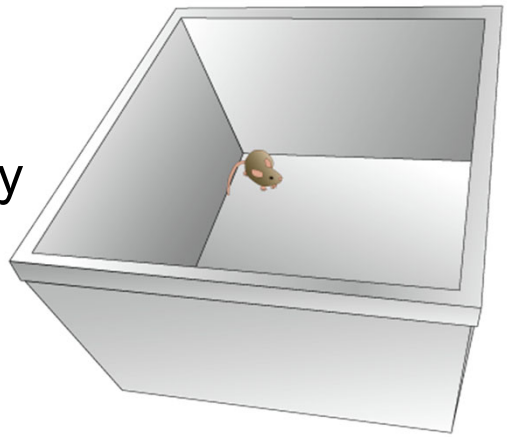

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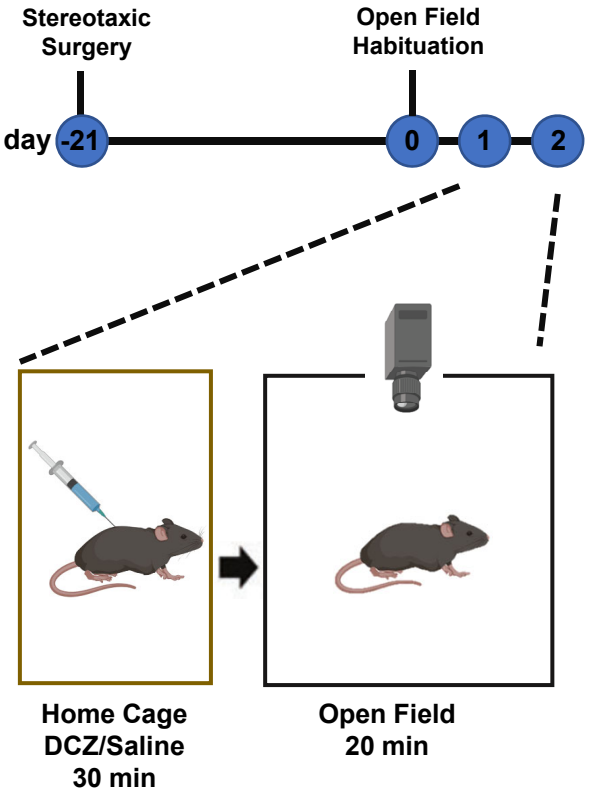
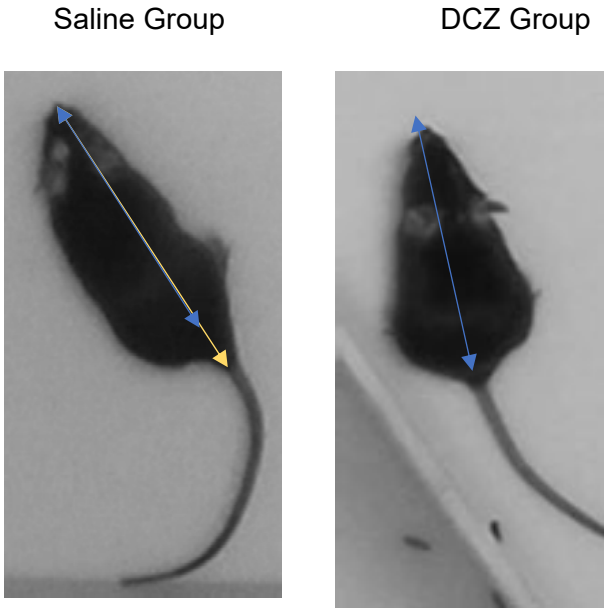
DRN-FN Chemogenetic experiments:

Pilot test: Bilateral FN inhibition





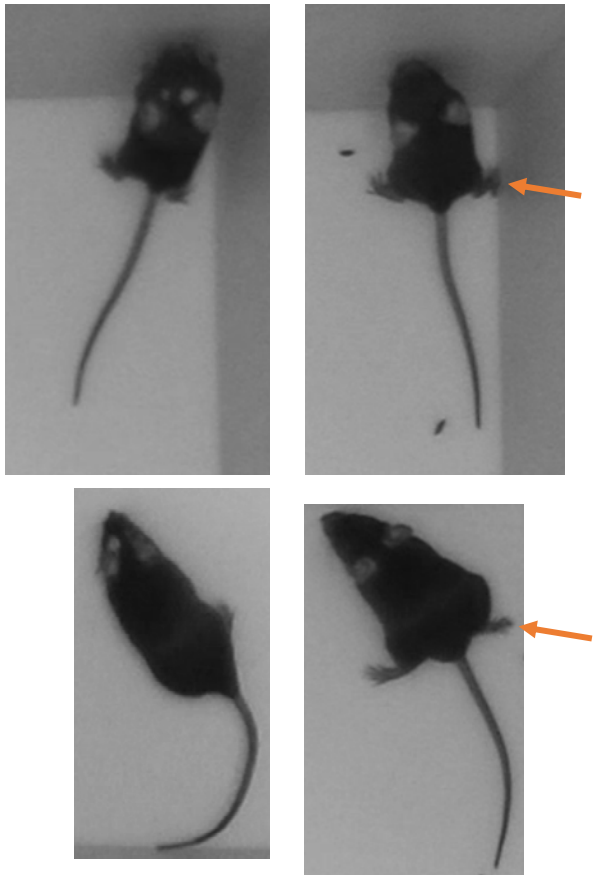
DRN-FN Chemogenetic experiments:

Bilateral DRN and FN excitation + Bilateral FN inhibition studies

DRN excitation

Ctrl Group

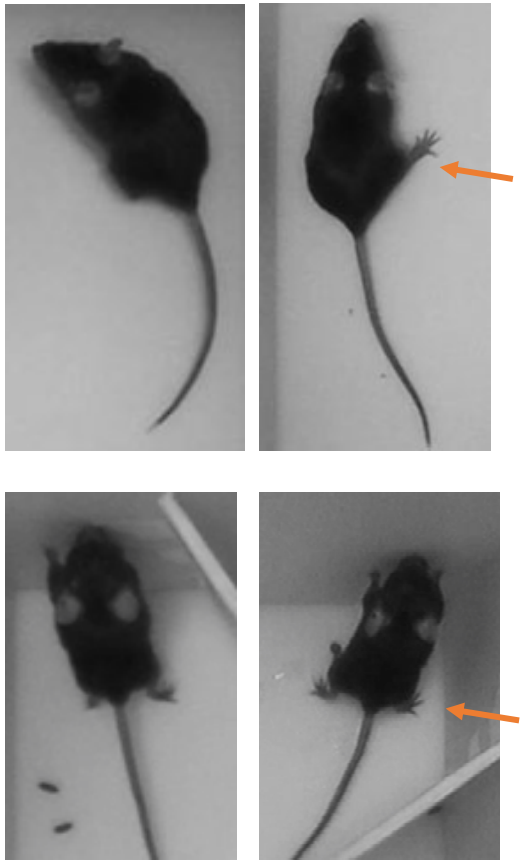
Exp Group



FN excitation

Saline Group

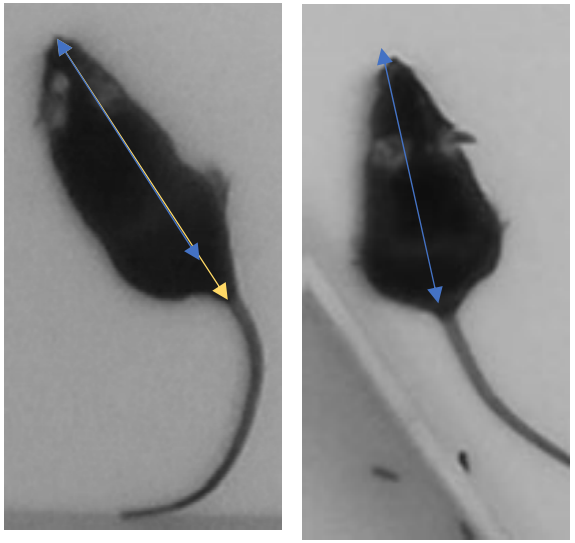
Exp Group



FN inhibition

Ctrl Group

Exp Group



Outline

1. Conducted Experiments
    1. Circuit tracing and cell population

        Result: Majority of dorsal DRN neurons projecting to the fastigial nucleus are serotonergic.
    2. Chemogenetic study
    3. Fiber photometry study
    4. Electromyography study
2. Future Experiments

Questions:

1. Could serotonin that regulates motor control from the DRN?
2. What information do these serotonergic neurons encode?

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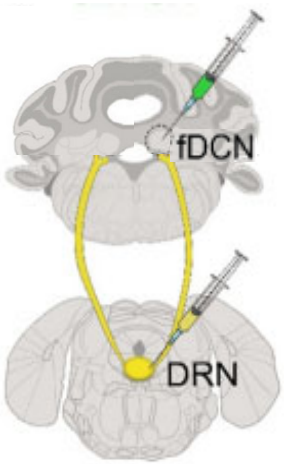
2. DRN-FN Chemogenetic experiments:

Unilateral DRN and FN excitation + Bilateral FN inhibition studies

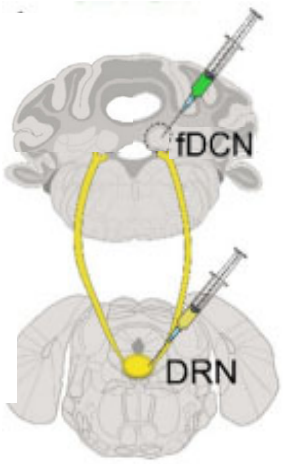
Unilateral DRN



AAVrg-hSyn-Cre

AAV5-hSyn-DIO-hM3D(Gq)

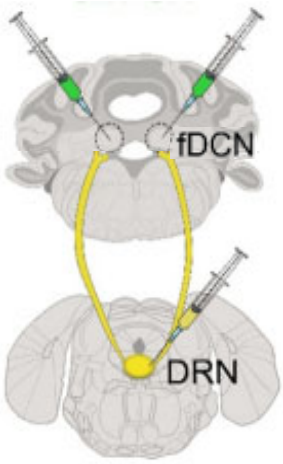
Unilateral FN



AAV5-hSyn-DIO-hM3D(Gq)

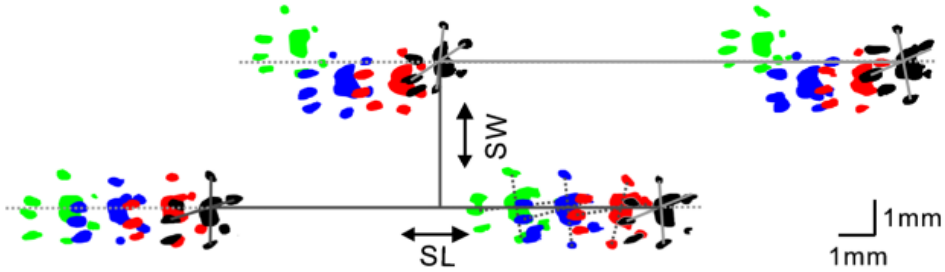
AAV1-hSyn-Cre

Bilateral FN



AAV5-CaMKII-DIO-hM4D(Gi)

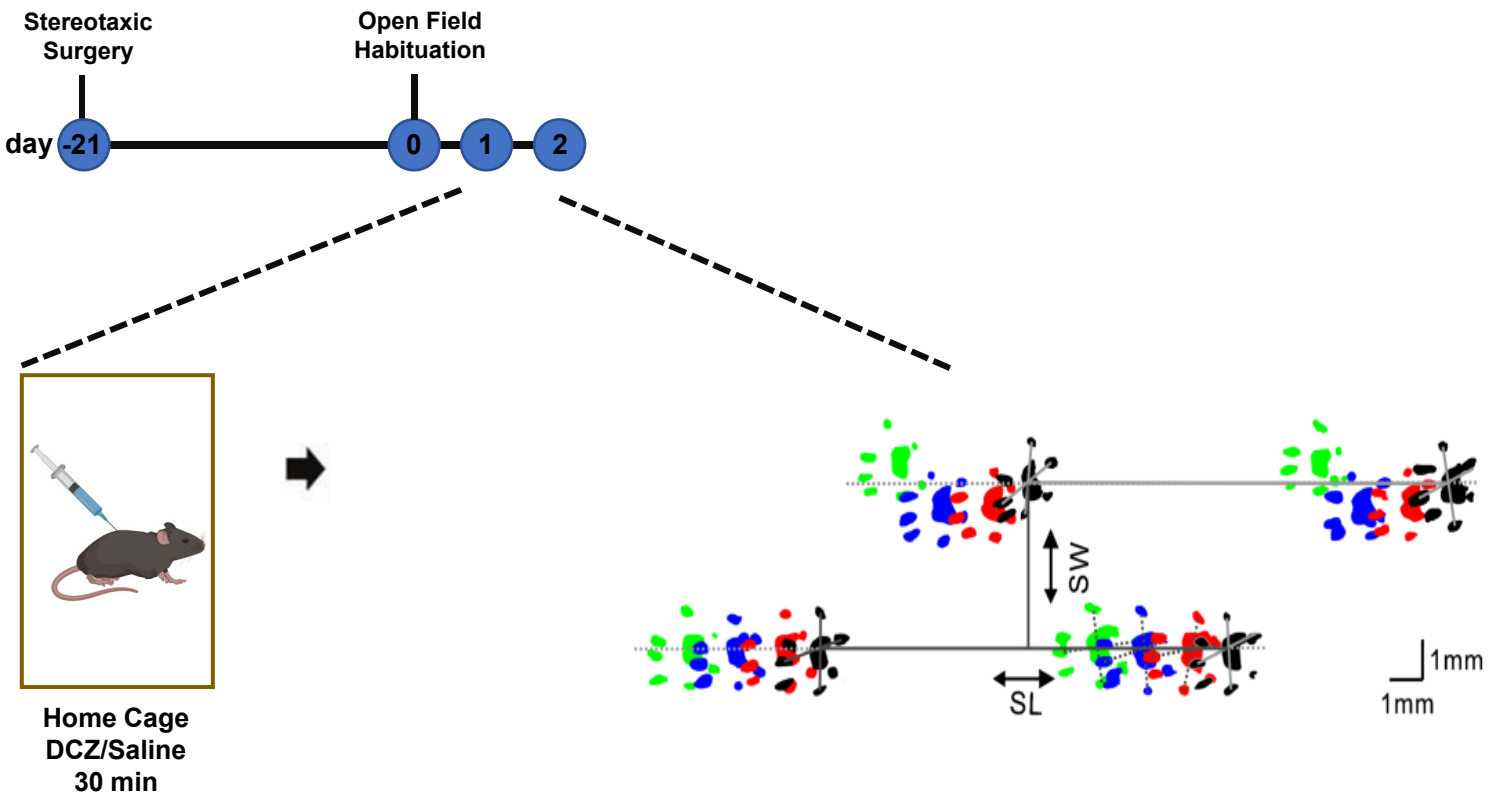
AAV1-hSyn-Cre



DCZ: 0.2mg/kg in 0.9% saline; pre-experiment 30min IP injection

2. DRN-FN Chemogenetic experiments:

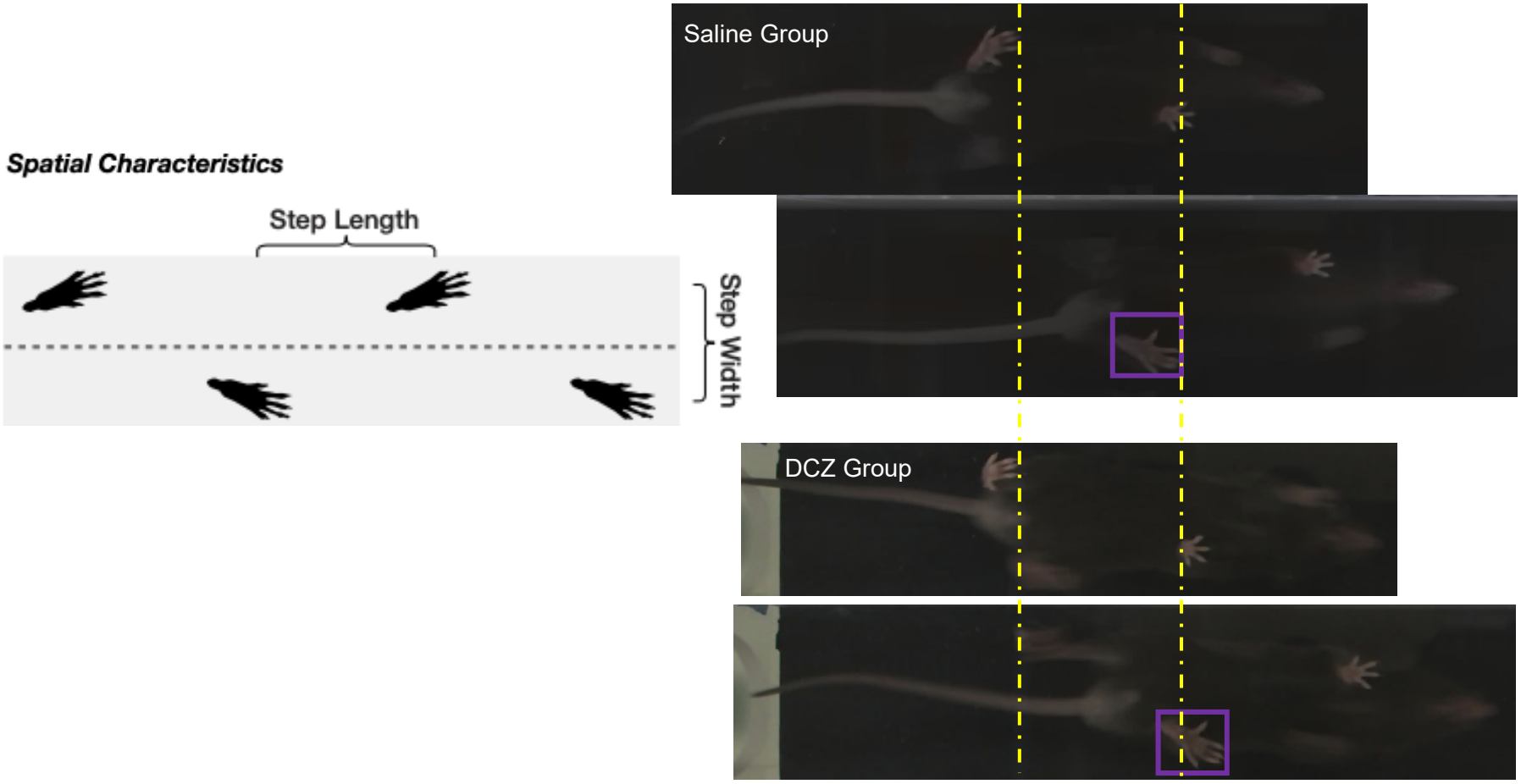
Gait analysis were conducted during chemogenetic manipulation



DCZ: 0.2mg/kg in 0.9% saline;
pre-experiment IP injection

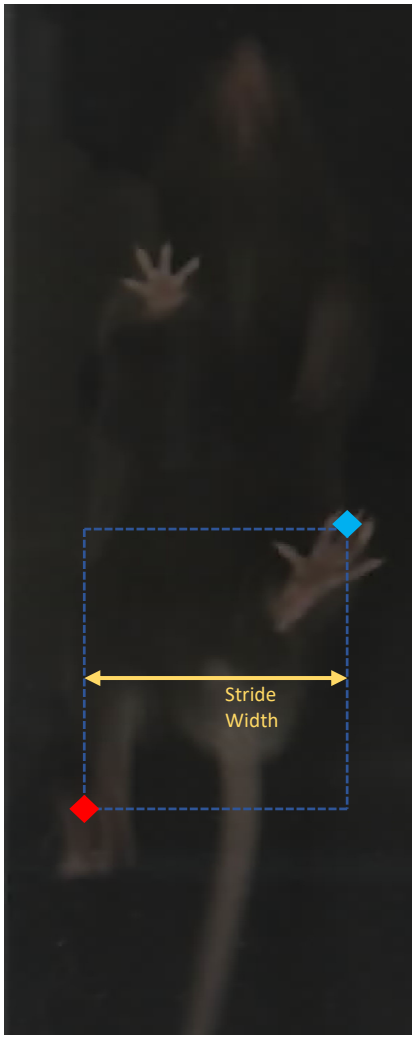
2. DRN-FN Chemogenetic experiments:

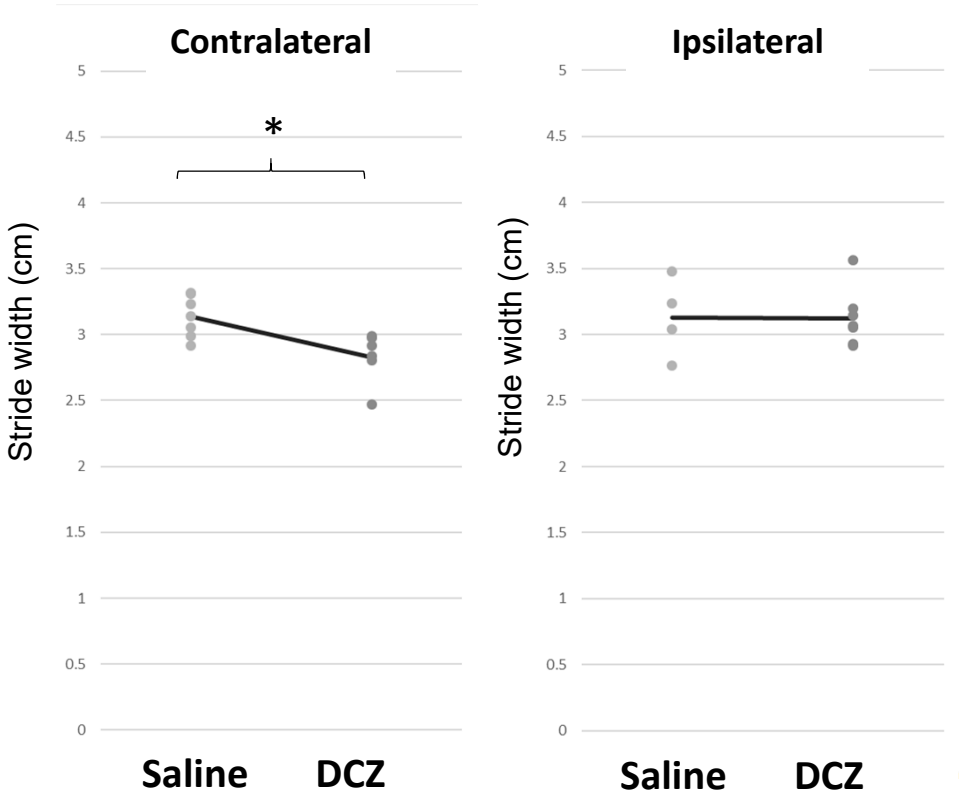
Catwalk: Unilateral DRN (Right) excitation



2. DRN-FN Chemogenetic experiments:

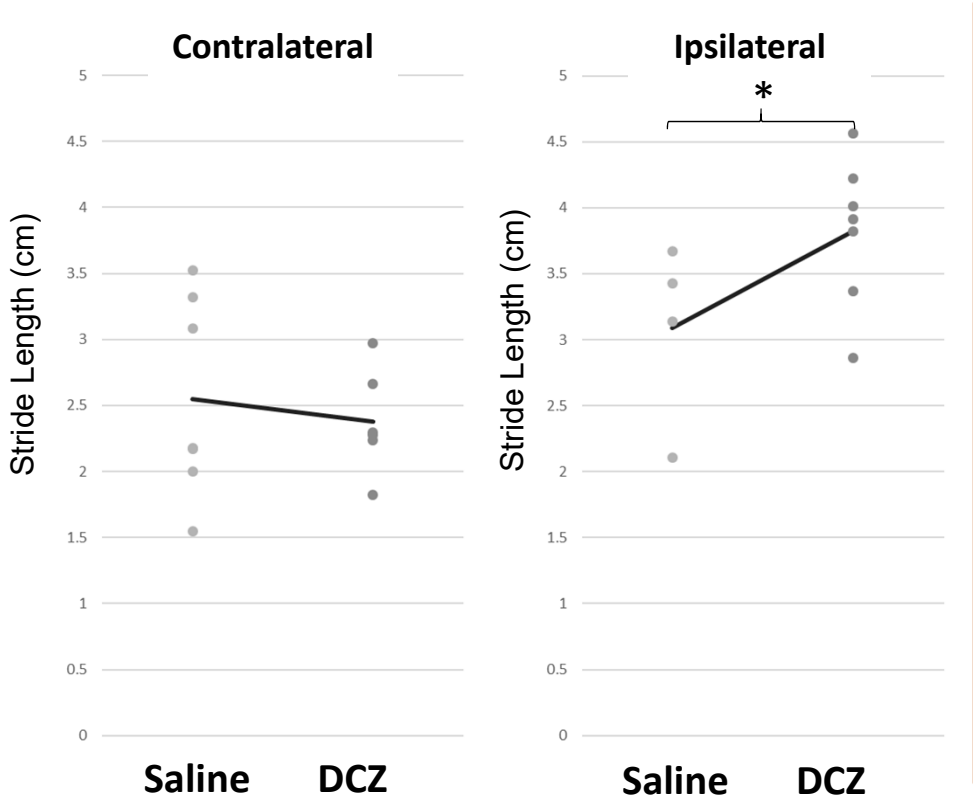
Unilateral DRN (Right) excitation induced narrower stride width on contralateral side





2. DRN-FN Chemogenetic experiments:

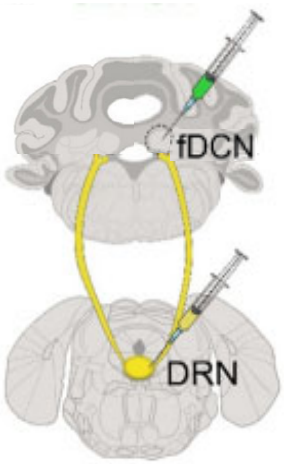
Unilateral DRN (Right) excitation induced longer stride length on ipsilateral side



2. DRN-FN Chemogenetic experiments:

Unilateral DRN and FN excitation + Bilateral FN inhibition studies

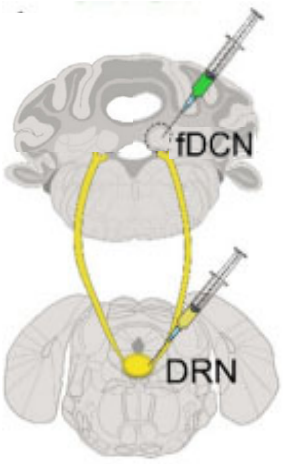
Unilateral DRN



AAVrg-hSyn-Cre

AAV5-hSyn-DIO-hM3D(Gq)

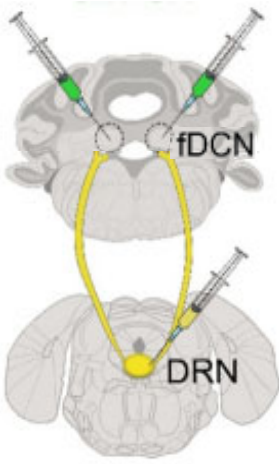
Unilateral FN



AAV5-hSyn-DIO-hM3D(Gq)

AAV1-hSyn-Cre

Bilateral FN

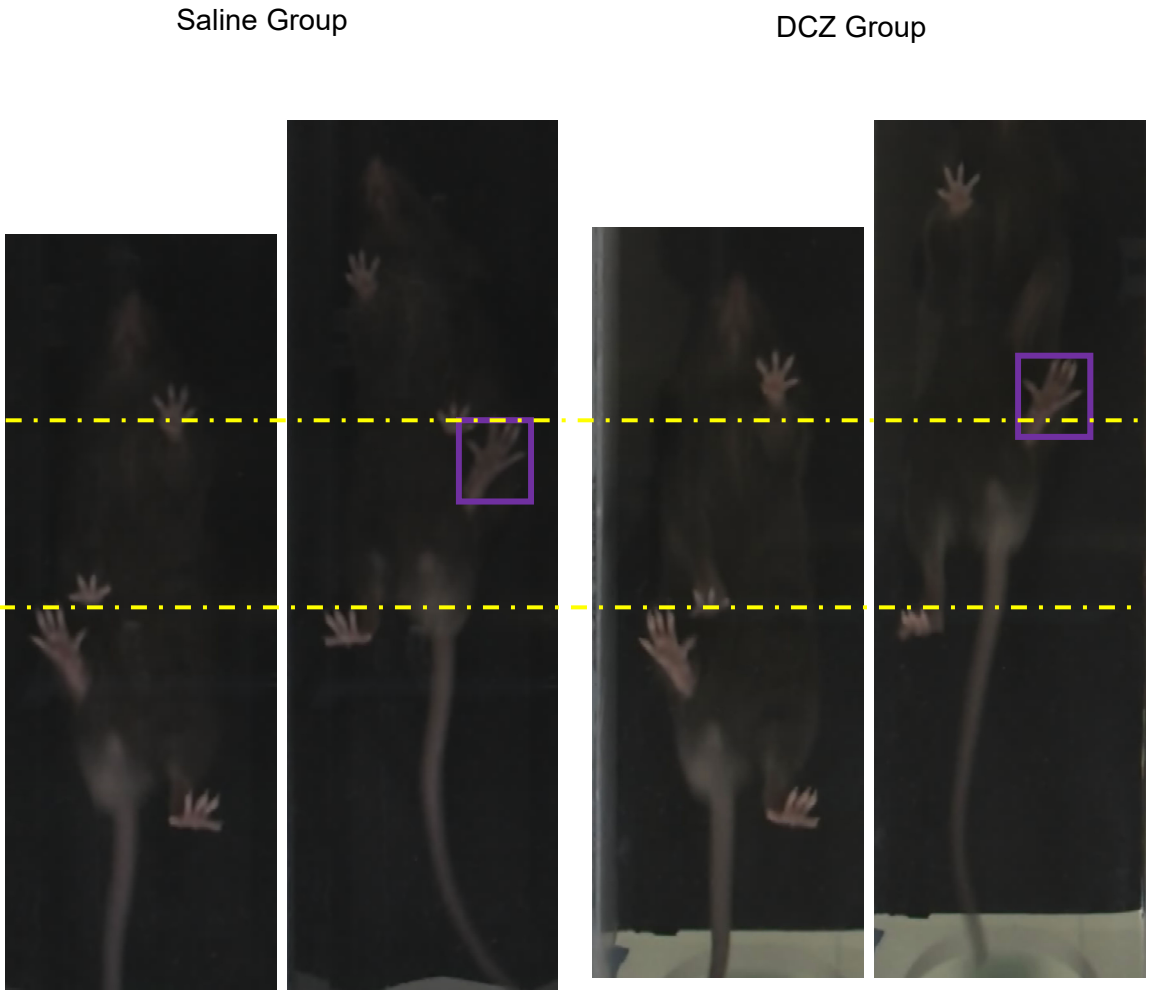


AAV5-CaMKII-DIO-hM4D(Gi)

AAV1-hSyn-Cre

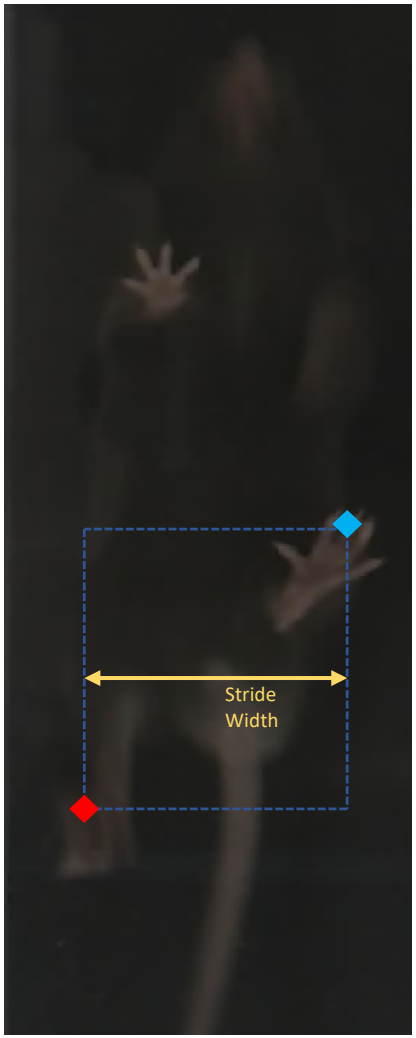
2. DRN-FN Chemogenetic experiments:

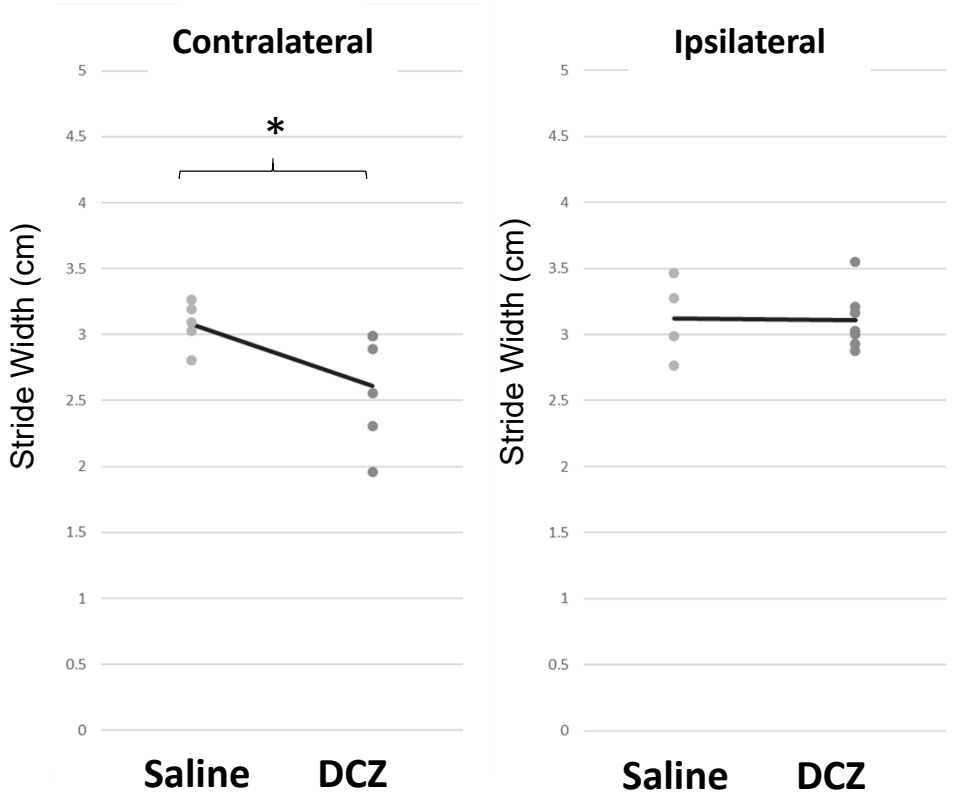
Unilateral FN excitation induced stride differences in the same pattern as DRN excitation



2. DRN-FN Chemogenetic experiments:

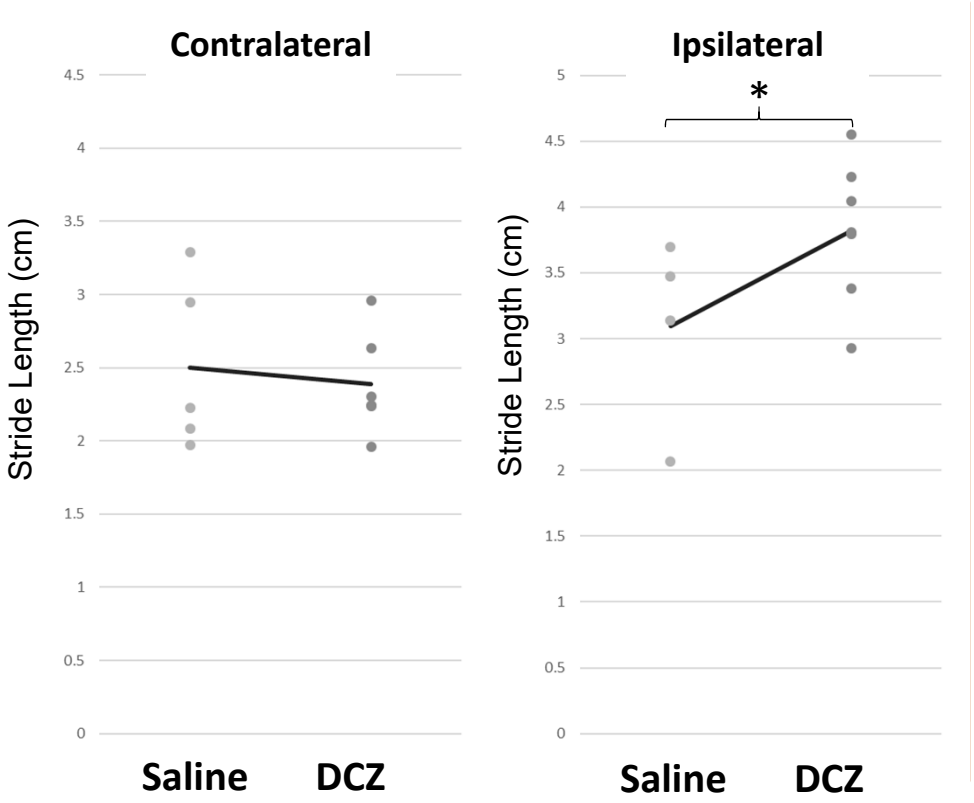
Unilateral FN (Right) excitation induced narrower stride width on contralateral side





2. DRN-FN Chemogenetic experiments:

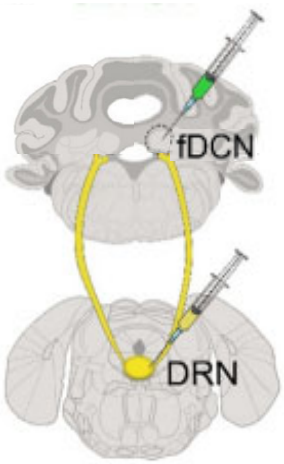
Unilateral FN (Right) excitation induced longer stride length on ipsilateral side



2. DRN-FN Chemogenetic experiments:

Unilateral DRN and FN excitation + Bilateral FN inhibition studies

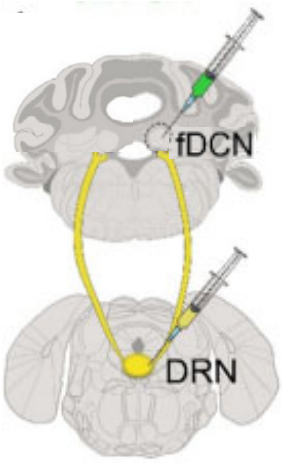
Unilateral DRN



AAVrg-hSyn-Cre

AAV5-hSyn-DIO-hM3D(Gq)

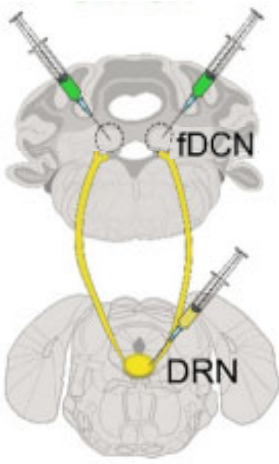
Unilateral FN



AAV5-hSyn-DIO-hM3D(Gq)

AAV1-hSyn-Cre

Bilateral FN

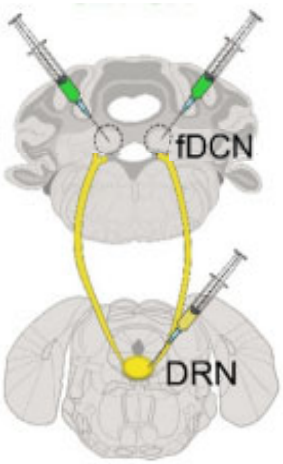


AAV5-CaMKII-DIO-hM4D(Gi)

AAV1-hSyn-Cre

2. DRN-FN Chemogenetic experiments:

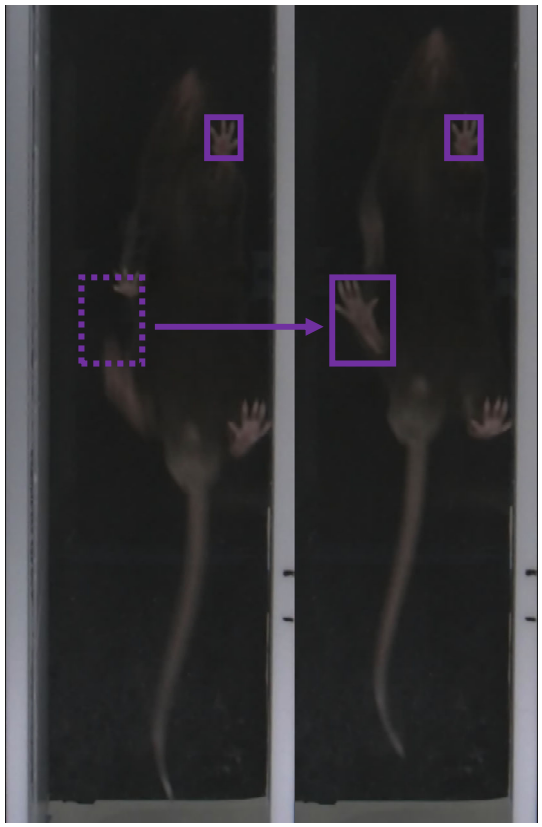
Bilateral inhibition of FN glutamatergic neurons results lagging of hindlimb but not different in gait pattern



AAV5-CaMKII-DIO-hM4D(Gi)

AAV1-hSyn-Cre

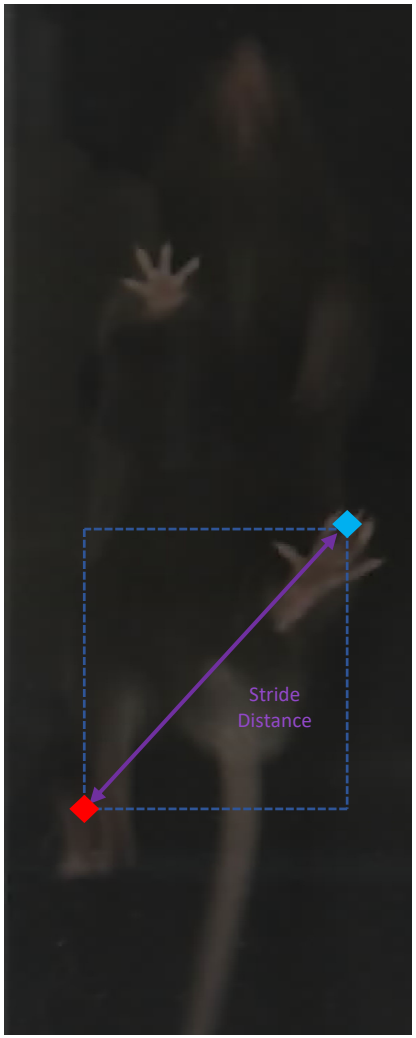
Saline Group

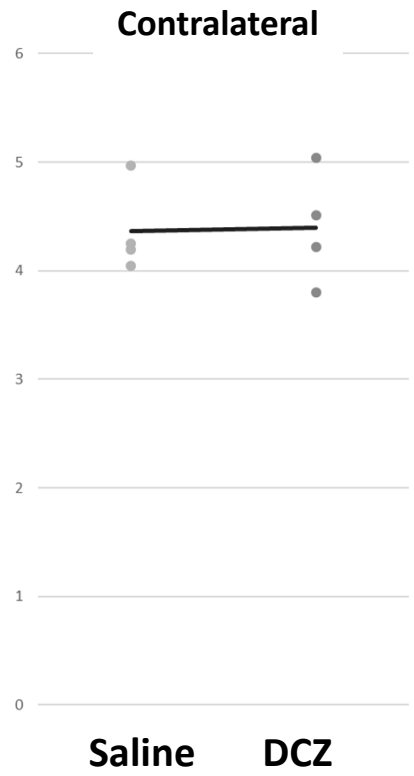


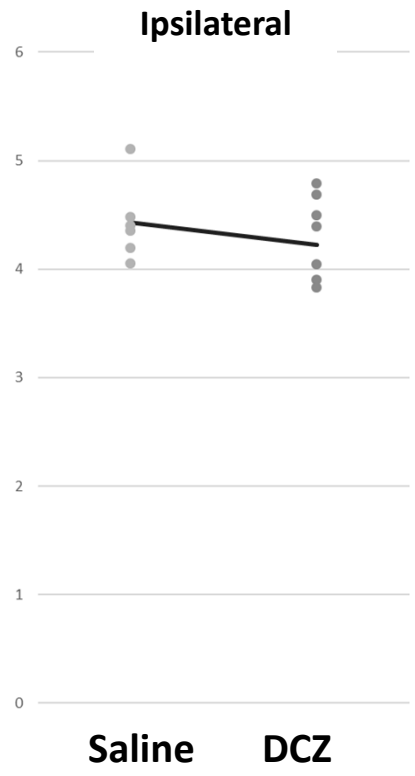
DCZ Group

2. DRN-FN Chemogenetic experiments:

Bilateral inhibition of FN glutamatergic neurons did not induce different in stride distance

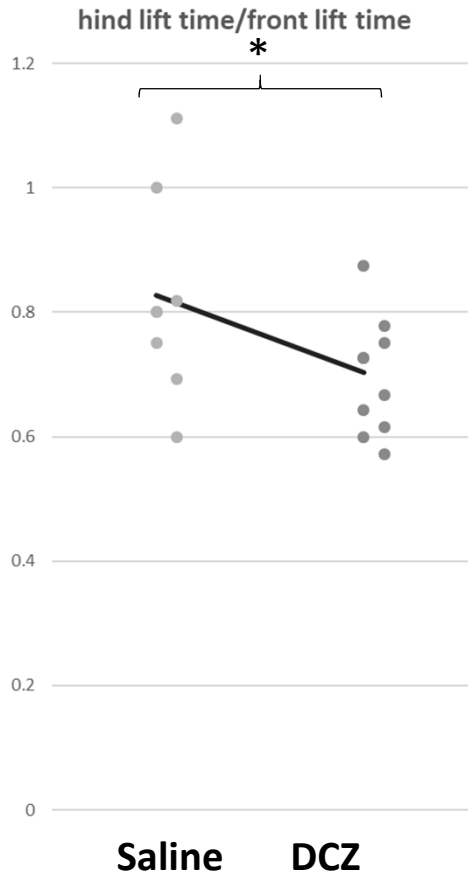






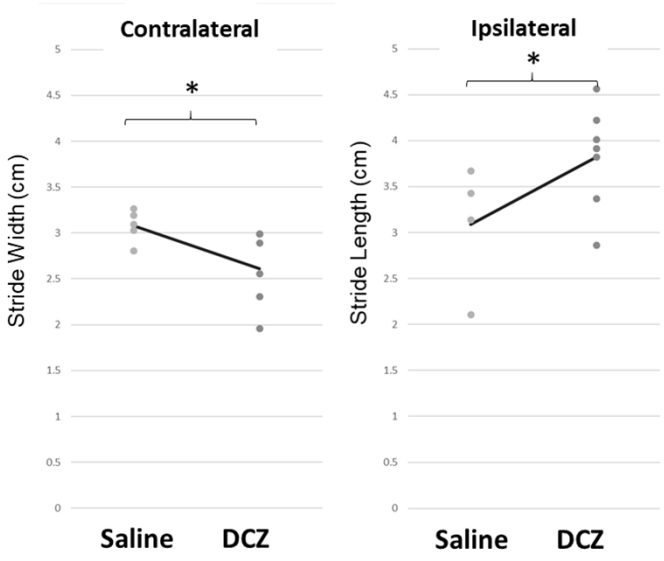
2. DRN-FN Chemogenetic experiments:

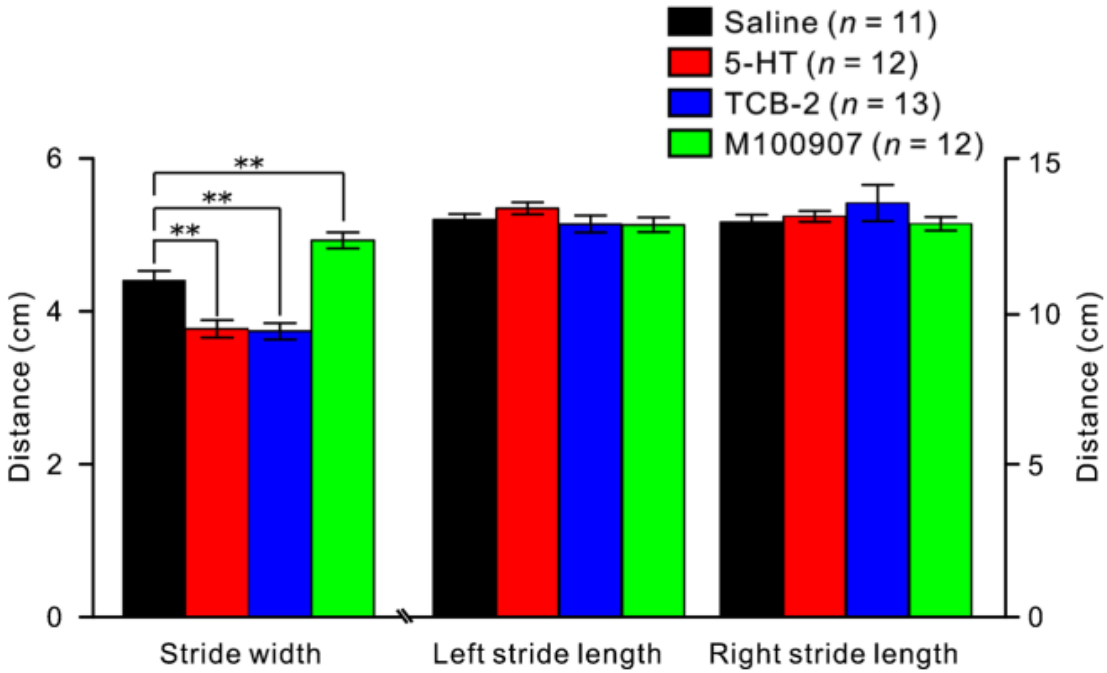
However, FN glutamatergic neuron inhibition decreased time that hindlimbs are lifted during strides



2. DRN-FN Chemogenetic experiments:

DRN (Right) excitation showed similar patterns to decrease stride width as previously reported but not stride length





Outline

1. Conducted Experiments
    1. Circuit tracing and cell population

       Result: Majority of dorsal DRN neurons projecting to the raphe nucleus are serotonergic.
    2. Chemogenetic study

       Result: DRN neurons projecting to the fastigial nucleus changes gait pattern.
    3. Fiber photometry study
2. Future Experiments

Questions:

1. Could serotonin that regulates motor control from the DRN? Likely!
2. What information do these serotonergic neurons encode?

Outline

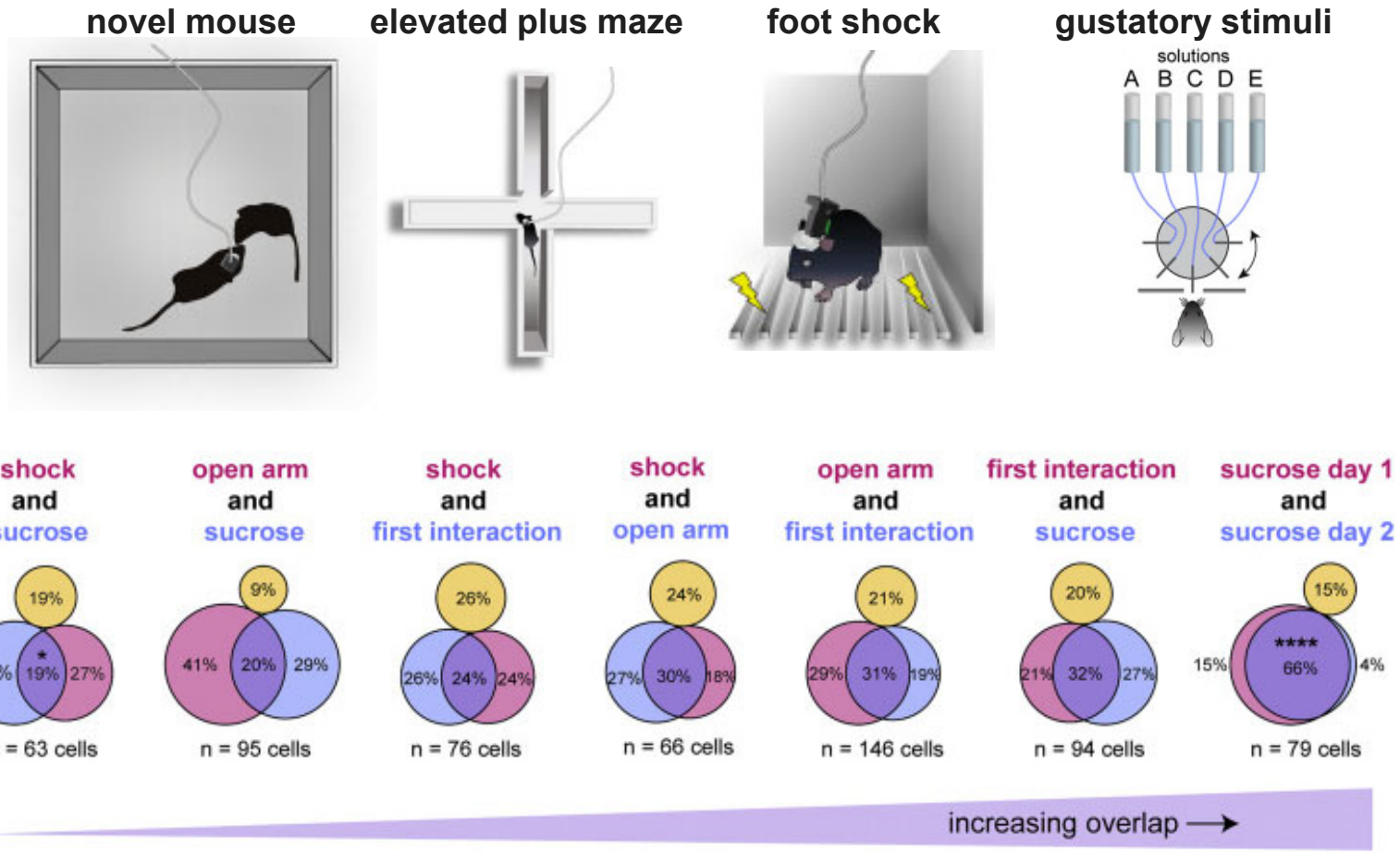
1. Conducted Experiments
    1. Circuit tracing and cell population
    2. Chemogenetic study
    3. Fiber photometry study
2. Future Experiments

Questions:

1. Could serotonin that regulates motor control from the DRN? Likely!
2. What information do these serotonergic neurons encode?

3. Fiber photometry experiments:

Serotonergic neurons show heterogenous activities to different stimuli



Paquelet, Grace E et al., 2022

3. Fiber photometry experiments:

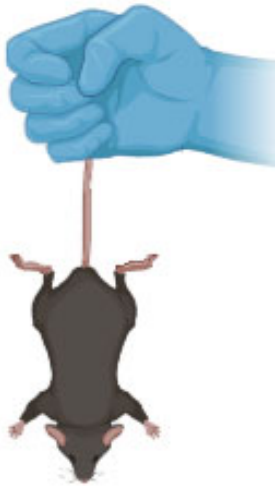
Five different stimuli given during fiberphotometry recording

Juvenile interaction

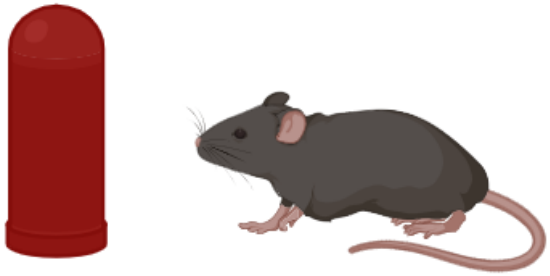
Chow food interaction

Novel mouse interaction

Tail picking

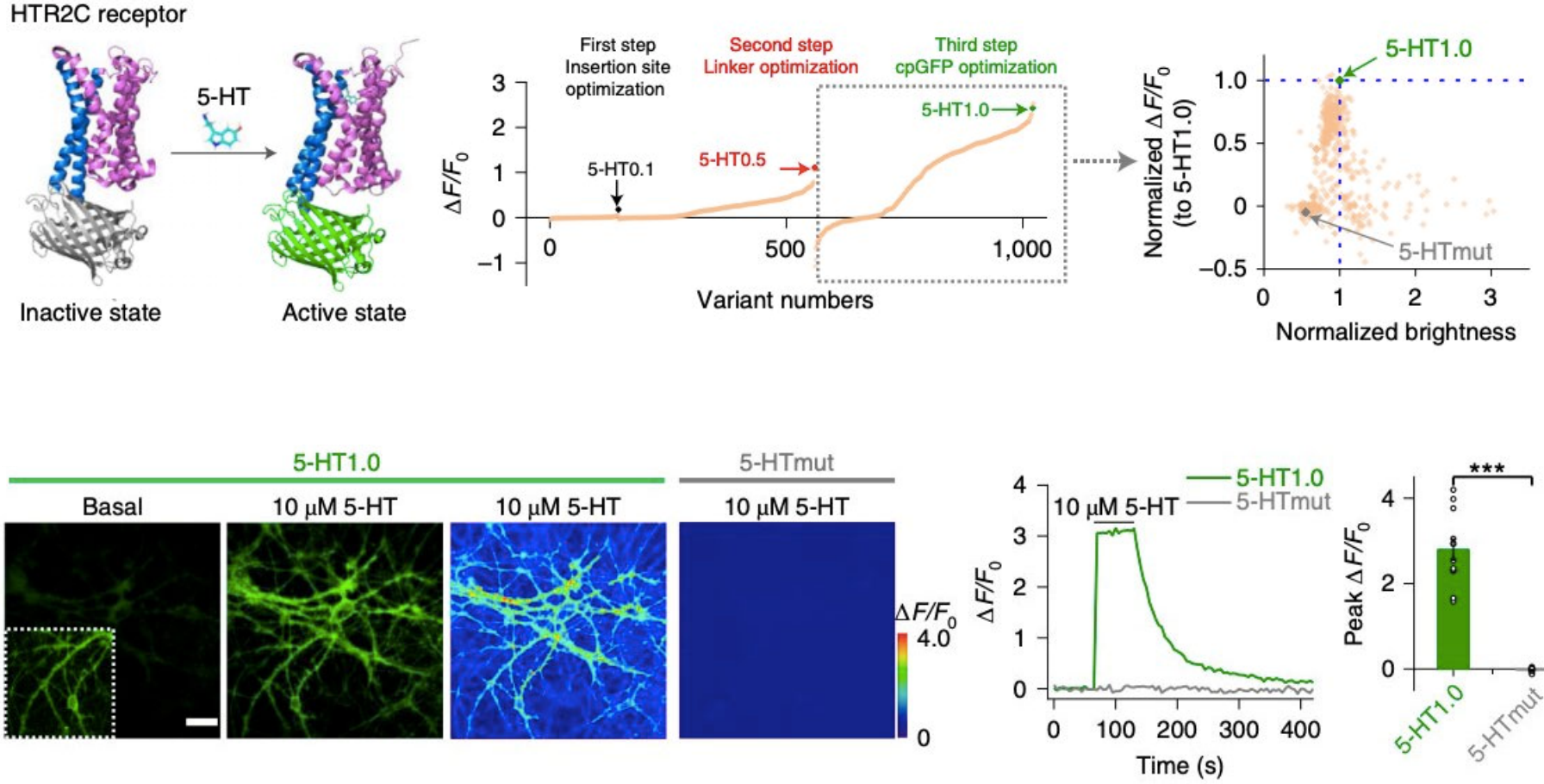


Novel object interaction



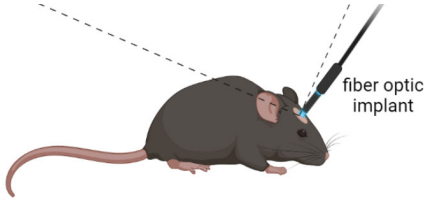
3. Fiber photometry experiments:

Use of GRAB 5-HT1.0 for alternative recording method due to low axon terminal expression of DRN axon terminal on FN



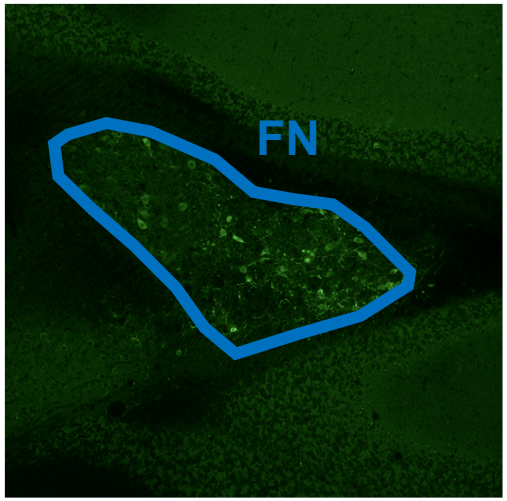
3. Fiber photometry experiments:

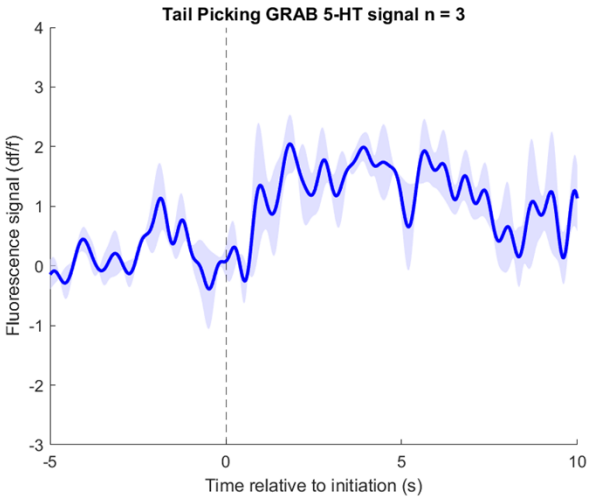
Use of GRAB 5-HT1.0 expressed on FN

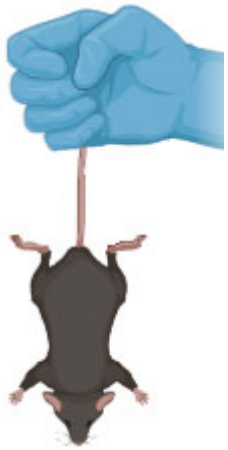


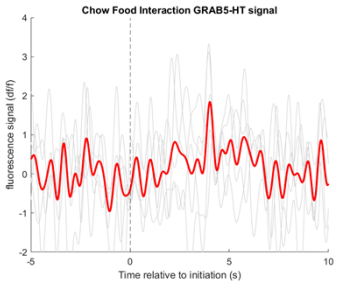
GRAB soma recording
from FN
(B6J wt)

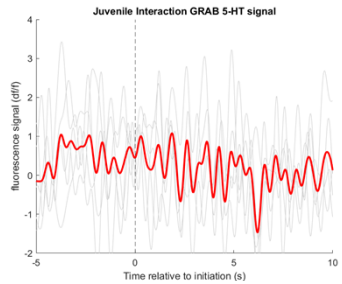
AAV9-hSyn-GRAB_5-HT1.0

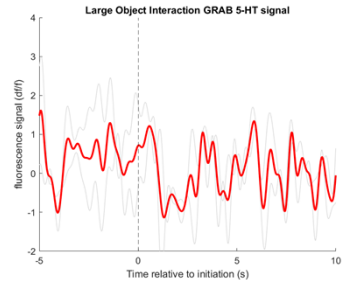


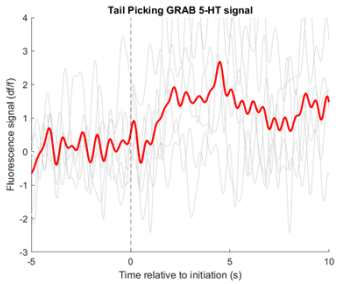






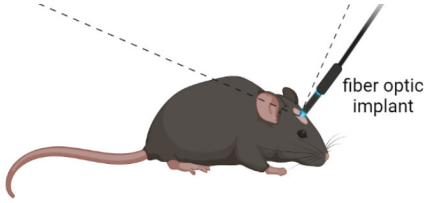






3. Fiber photometry experiments:

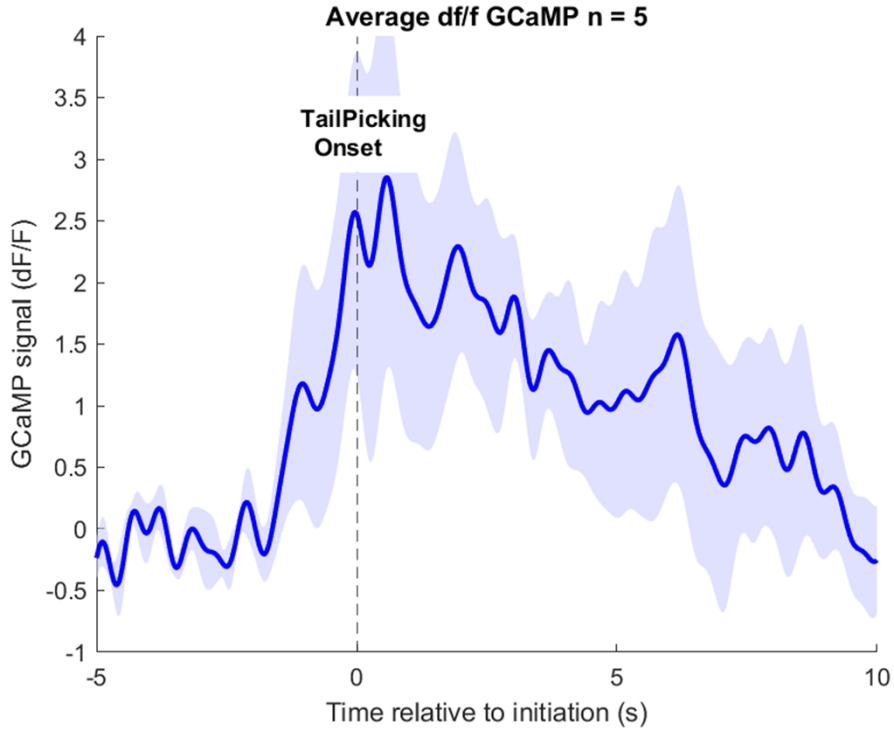
Soma recording from DRN by retrogradely expressing AAV9-DIO-GCaMPs6 from Fastigial Nucleus

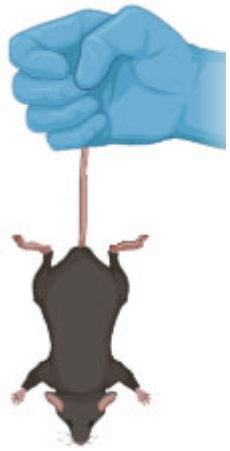


DRN neurons projecting to
fastigial nucleus
(B6J wt)

AAV9-CAG-FLEX-GCaMP6m

AAVrg-ef1a-cre-mCherry-IRES

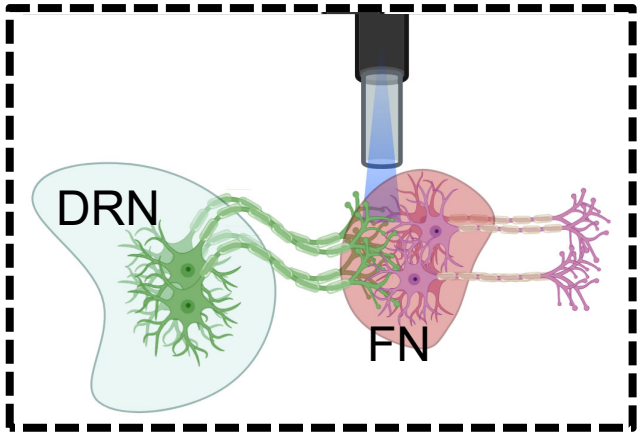


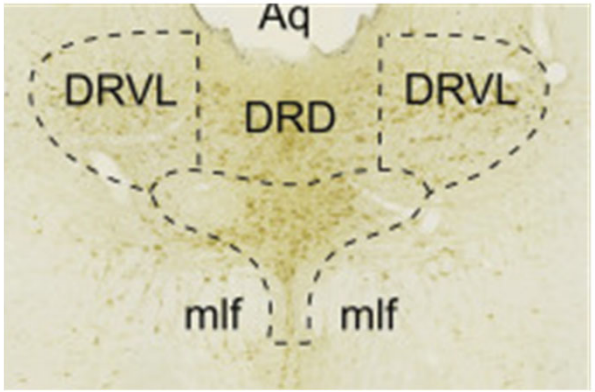


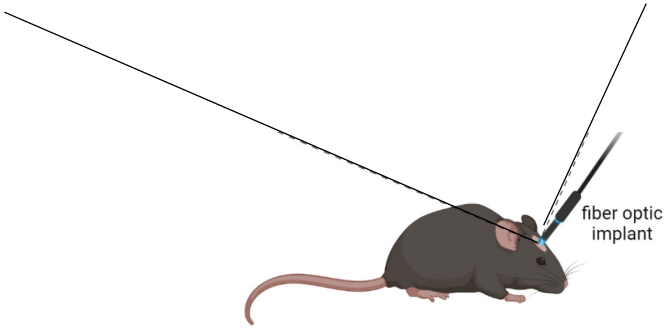
3. Fiber photometry experiments:

Low axon terminal expression of AAV9-GCaMPs6 virus from dorsal raphe nucleus

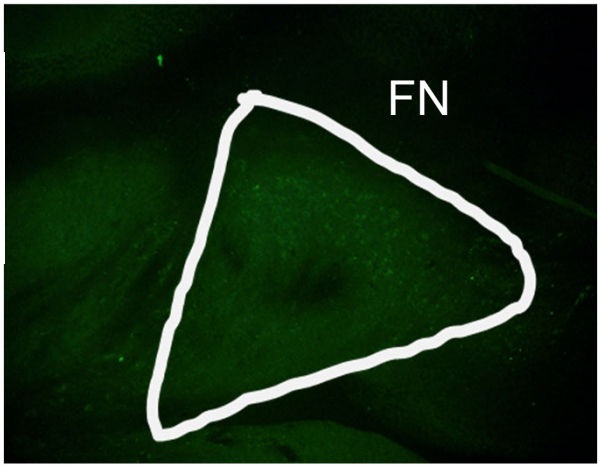
AAV9-hSyn-DIO-GCaMP6s



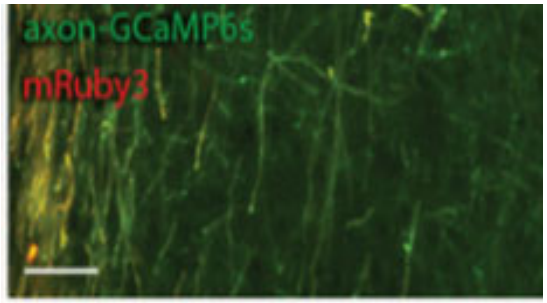




Axon terminal recording from DRN
(ePet-cre)



To be continued



3. Fiber photometry experiments:

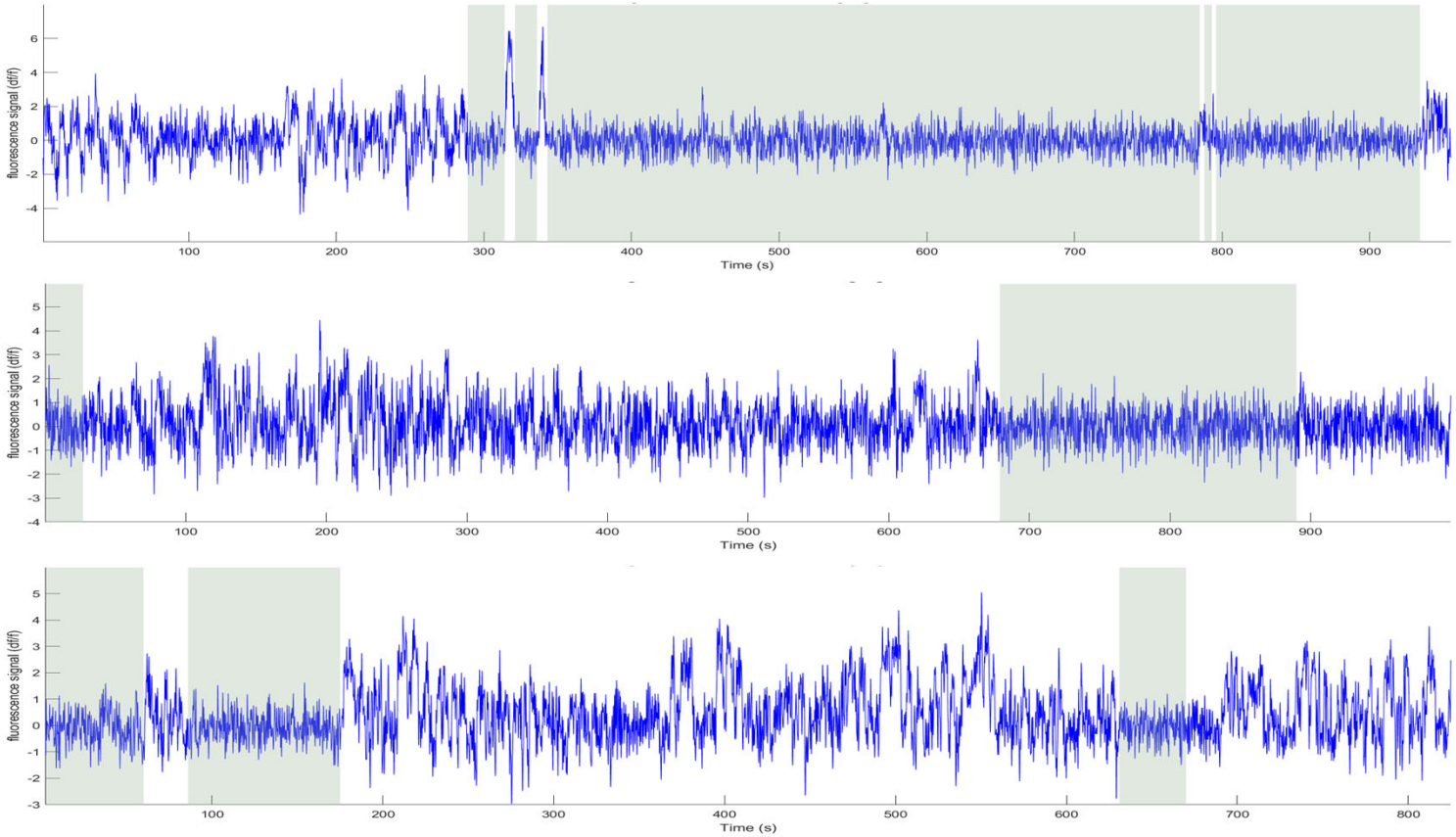
Serotonin activity was recording home cage to reduce human interference and measure more “natural” behaviors

3. Fiber photometry experiments:

Serotonin levels decreased in the fastigial nucleus during sleep periods

Homecage

Sleeping periods in gray



Outline

1. Conducted Experiments
    1. Circuit tracing and cell population
    2. Chemogenetic study
    3. Fiber photometry study

        Result: Serotonin released in fastigial nucleus at tail picking session might come from DRN neurons.
2. Future Experiments

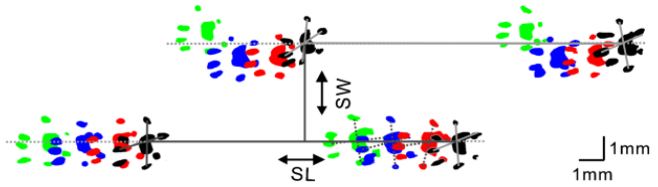
Questions:

1. Could serotonin that regulates motor control from the DRN? Likely!
2. What information do these serotonergic neurons encode? Ongoing ☺

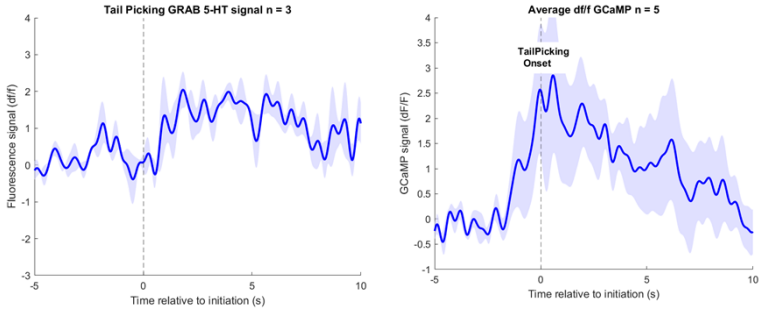
Summary:

DRN neurons release serotonin to the fastigial nucleus for motor control during awake period and tail picking session

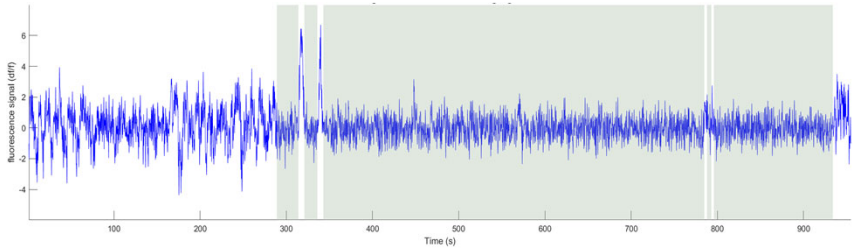
1. DRN activation regulates gait patterns in mice.



2. Serotonin release from the DRN increases in the fastigial nucleus at tail picking.

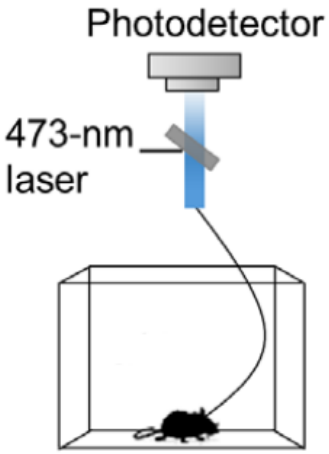


3. Serotonergic activities are silenced during sleep and increased when awake.

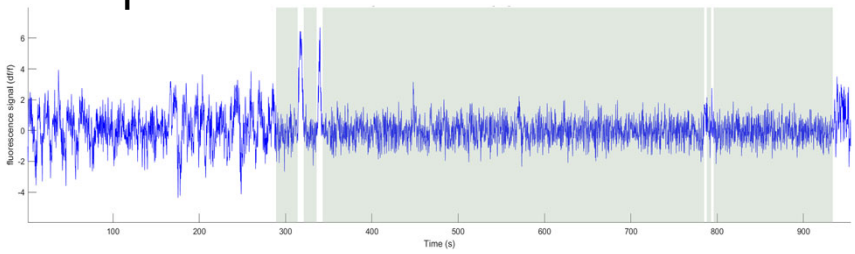


Future plan:

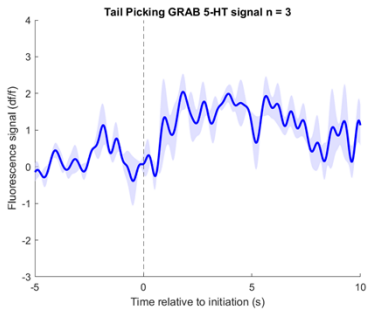
Further recordings of serotonin activities at the fastigial nucleus are necessary to decipher the information encoded via serotonin for motor control

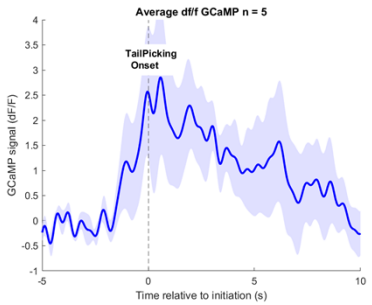


Sleep < awake



awake < tail picking





Alert?

Arousal?

Threat?

? ? ? ? ? ?

Acknowledgement