Identify the Brain Regions Projecting to Subthalamic Nucleus Using an AAV Retrograde Tracer

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Background

Deep Brain Stimulation (DBS) is used in healthcare as a supplement to medication in treating and managing movement disorders such as Parkinsons' Disease (PD). DBS in PD targets the Subthalamic Nucleus (STN) located in the Basal Ganglia. This study uses an AAV retrograde tracer to identify the approximate brain regions projecting toward the STN. Its effect brings the activation of antidromic brain regions.

Methods

Subject: Three female Sprague Dawley rats (weight 250-275g).

- **Retrograde GFP expression**

AAVretro-hSyn-GFP(0.6ul,1.5ul/min) was injected unilaterally into STN (AP -3.6mm, ML 2.6mm, depth 6.8mm). Four weeks after the virus injection, the brain was extracted after being fixed by 4% paraformaldehyde.

- **Cryostat Sectioning**

A machine used to thinly section frozen tissue. Tissue samples are sectioned to approximately 40 um in thickness. Samples are placed into 12-well plates, containing 1x PBS (Phosphate Buffered Saline), in series for plating.

- **Tissue plating**

After transferring each well's worth of tissue samples to a larger well plate of 1x PBS. Sections are transferred onto glass slides starting from the anterior to posterior of the brain. They are then covered with a DAPI mounting medium to preserve fluorescence and sealed with clear nail polish.

- **Microscope imaging**

Sealed tissue samples are placed under a Nikon microscope that uses a program, NIS Elements, to automate imaging. Tissue samples are fluorescently imaged and stitched together using a programmed function in the NIS Elements software. Sections are imaged at 20x magnification.

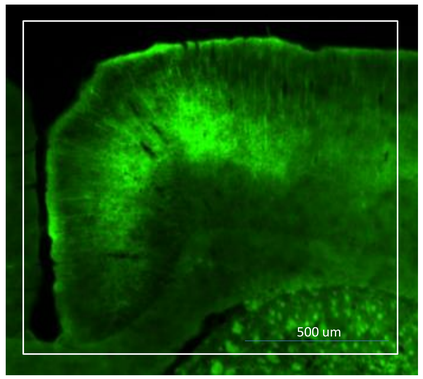
- **Identifying**

Each image is placed under an Atlas to identify the specific corresponding section where GFP is displayed and then labeled.

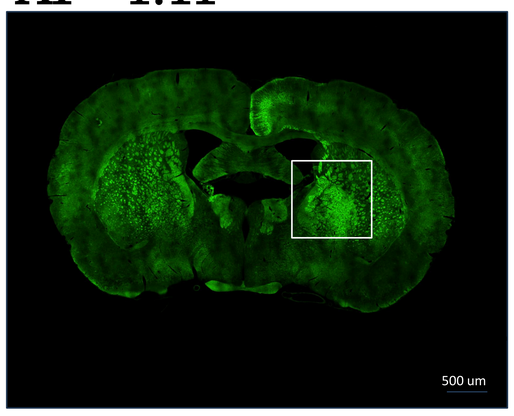
Results: Levels of GFP per brain section

AP 1.39

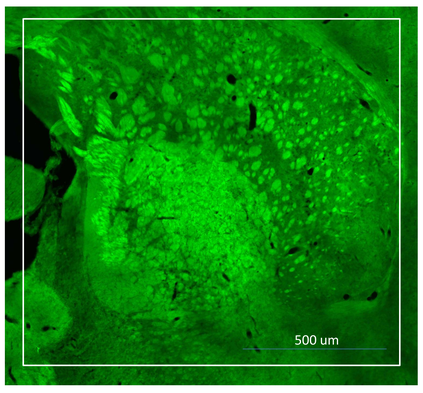
AP 1.39: **M1**



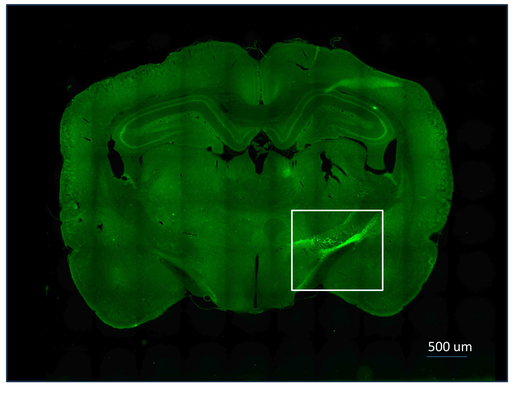
AP –1.11



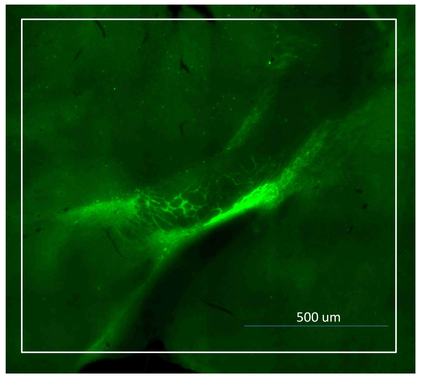
AP –1.11: **CPu**



AP –2.86



AP –2.86: **STN**



20x Dapi, Nikon, AAV Fluorescent

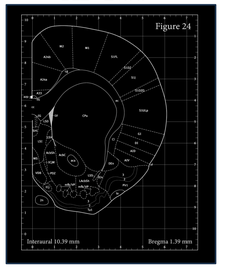
Results: Significant region tracing

AP 1.39:
Effected Area(Left Hemisphere): M2 (secondary motor cortex), M1 (Primary motor cortex)

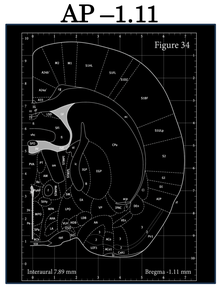
AP –1.11:
Effected area(Left hemisphere): IGP(internal segment of globus pallidus), EGP(External segment of globus pallidus), ic(internal capsule), CPu(caudate putamen[striatum]), A24b(cingulate cortex, area 24b), A33(cingulate cortex, area 33),A24a(cingulate cortex, area 24a)

AP –2.86:
Effected Area(Left Hemisphere): RI(rostral interstitial nucleus of medial longitudinal fasciculus), MV(medial vestibular), VPM(ventral posteromedial thalamic nucleus),
VPL(ventral posterolateral thalamic nucleus), ml(medial lemniscus)

AP 1.39



AP –1.11



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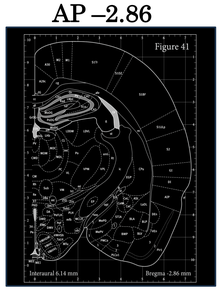

Summary

We found STN receives projections from M2, M1, IGP, EGP, RI, VPM, VPL, CPu, ic, ml, A24a, A24b. Our results suggest these brain regions could be retrogradely activated during STN DBS and potentially mediate the therapeutic effects.

References

Paxinos, G. and C. Watson (2006). The rat brain in stereotaxic coordinates: hard cover edition, Elsevier.

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