Whole-brain quantitative 3D imaging of robot-assisted intracerebral AAV delivery: A Light sheet fluorescence microscopy study in mice

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Background & Aim

Precision-targeted drug delivery is critical in the treatment of CNS diseases. Stereotaxic injection is a primary method for delivering agents into specific brain regions. Ensuring the accuracy of these injections and effectively visualizing their impact is essential for study outcomes. This study seeks to:

(1) Evaluate the precision of robot-guided stereotaxic AAV injections using a multimodal mouse brain atlas[1].

(2) Apply whole-brain 3D light sheet imaging to visualize, map and quantify AAV-infected cells and axonal projections.

Methods

C57BL/6JRj male mice (6-8 weeks of age, n=4) were stereotaxically injected with AAV9-CAG-eGFP (500nL; 10^{13} gene copies/mL) using a semi-automated, robot-assisted system (Neurostar). 4 brain areas were targeted (1 for each animal): Hippocampus (HIP): anterior-posterior (AP): -3.3mm, dorso-ventral (DV): 3.3mm, medio-lateral (ML): 2.6mm; Entorhinal cortex (ENT): AP: -3.8mm, DV: 4.9mm, ML: 3.7mm; Intracerebroventricular (ICV): AP: -0.25; ML: +1.0; DV: -2.5; Substantia nigra pars compacta/ventral tegmental area (SNc/VTA): AP: -3.1mm, DV: 4.2mm, ML: 1.4mm. Two weeks after injection, the animals were transcardially perfused with 4% PFA, brains were collected, stained against GFP, optically cleared (iDISCO) and imaged using light sheet microscopy. Segmentation was performed using DeepTraCE and DeepCOUNT[2], and analyzed using a custom Python pipeline.

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1 Study design and workflow

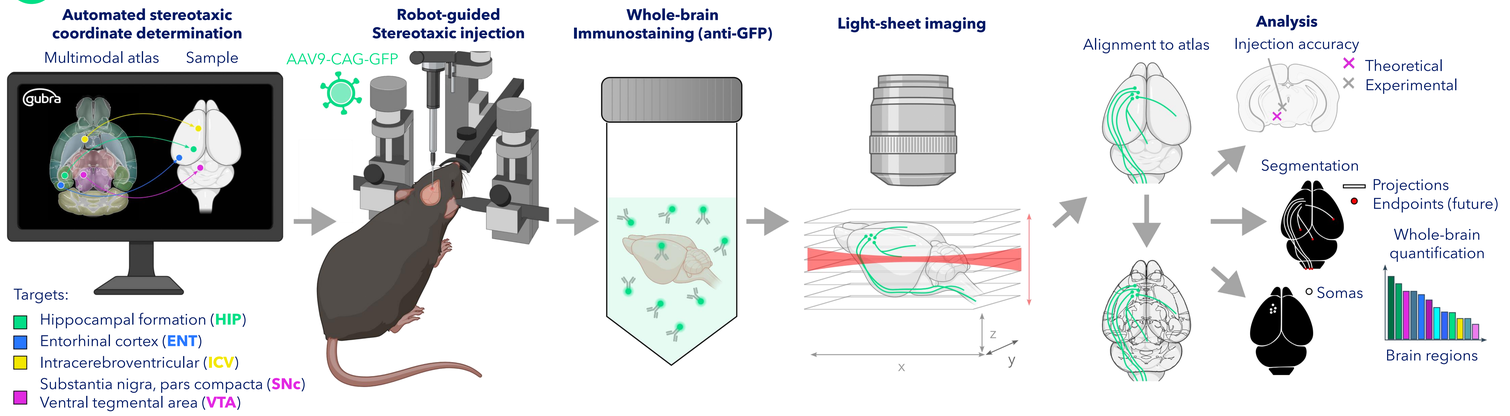

Figure 1. Experimental design and LSFM pipeline.
First, the precise stereotaxic coordinates of the specific targets in the brain are automatically determined using the Gubra multimodal atlas. Following this, a robot-guided stereotaxic injection (Neurostar) is used to introduce a virus (AAV9-CAG-GFP) to the designated regions. Post-injection, the brain is harvested, immunostained with an anti-GFP antibody, and cleared (iDISCO+) to visualize the infected cells as well as their projections. The stained and cleared brains are then scanned using light-sheet microscopy. The captured images are subsequently aligned with an atlas for future quantification. The final phase is the analysis, where the accuracy of the injection is assessed. Additionally, segmentation techniques using state-of-the-art deep neural networks distinguish projections, their endpoints, and somas. The entire brain data is quantified, with results differentiated by specific brain regions.

3 Accuracy of stereotaxic injections and comprehensive whole-brain labeling of infected cells and processes

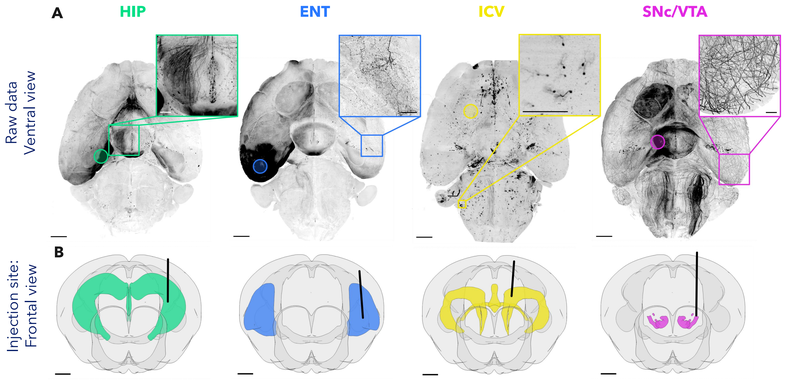

Figure 2. Accuracy of stereotaxic injections and comprehensive whole-brain labeling of infected cells and processes. (A) Representative ventral-view images of the entire brain, showing the staining of infected cells and their projections from various experiments. These include injections into the hippocampus (HIP), entorhinal cortex (ENT), intracerebroventricular (ICV), and the substantia nigra pars compacta (SNc)/ventral tegmental area (VTA). Circled areas indicate the approximate injection sites. The scalebar is 1mm. **(B)** Mapping of the injection site for each experiment. The mouse brain (grey) is represented in the frontal view using brainrender[3]. The target region is pseudo-colored. The injection path is depicted as a black rod. Scalebar, 1mm.

4 Segmentation of infected cells and their axonal projections following the SNc/VTA Injection

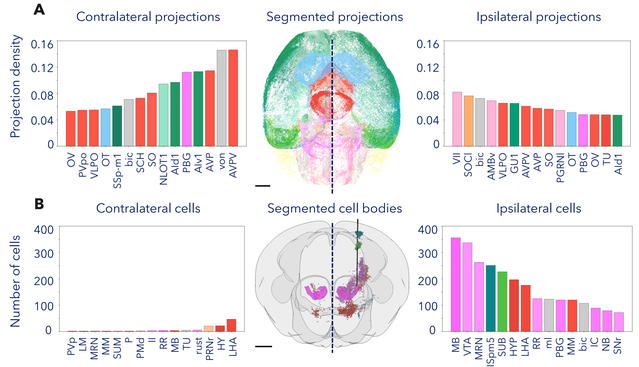

Figure 3. Segmentation of infected cells and their axonal projections in the SNc/VTA injection. (A) Whole-brain segmentation and quantification of axonal projections from the SNc/VTA injection using DeepTraCE[2]. Widespread projections were observed across the brain, both ipsilaterally and contralaterally to the injection site. The scalebar is 1mm. **(B)** Whole-brain segmentation and quantification of infected cell bodies from the SNc/VTA injection using DeepCOUNT[2]. While infected cells were detected primarily in the vicinity of the injection site, some were also present along the injection path and even on the contralateral side of the injection site. The target region is pseudo-colored. The injection path is depicted as a black rod. Scalebar, 1mm.

Conclusion

- Using a multimodal mouse brain atlas and robot-guided stereotaxic AAV injections enables precise and reproducible targeting of SNc/VTA, HIP, ENT, and ICV.
- Light-sheet imaging and atlas alignment allows for the evaluation of stereotaxic injection accuracy in 3D.
- Comprehensive whole-brain 3D mapping and detailed segmentation of AAV-infected cells and axonal projections facilitates objective assessment of infection dispersion and an in-depth exploration of the projectome of targeted neurons.

References

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2. Gongwer, M. W. et *al. Brain-Wide Projections and Differential Encoding of Prefrontal Neuronal Classes Underlying Learned and Innate Threat Avoidance. J. Neurosci.* ***43***, *5810-5830 (2023).*
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