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Stereotactic Viral Injection into Subthalamic Nucleus in Mice

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Elizabeth Phelan[1], Maite Azcorra[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

E Elizabeth Phelan
Northwestern University

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We are still developing and optimizing this protocol

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Abstract

This is a protocol describing the surgical procedure of injecting AAV viruses into the subthalamic nucleus of a mouse. It contains information regarding the stereotactic setup, surgery preparation, anesthesia, craniotomy, injection, and recovery. This protocol has been adapted from Maite Azcorra's protocol for midbrain viral injections.

Troubleshooting

Preparation

1 Turn on heat sterilizer ahead of time. Sterilize the following tools:
- Large scissors
- Large forceps
- Fine forceps
- Rod or flat spatula

2 Have the following tools and substances at hand:
- Isoflurane
- Glass micropipettes
- Plastic tubing
- Hand syringe
- Insulin syringes
- Cotton swabs
- PBS
- Artificial tears (Puralube Ophthalmic ointment)
- Buprenex SR (slow release) 1mg/ml vial
- Rimadyl
- Virus aliquot (keep on ice)

3 Insure anesthesia system (RWD-R540) has sufficient isoflurane for the duration of the procedure.

4 Turn on heating pad.

Surgery

5 Anesthetize mouse. Redirect the anesthesia system to the induction chamber and turn on oxygen flow at 0.8 liters per minute. Turn on isoflurane to 3% for induction, and place mouse inside. When mouse's breathing has reached approximately one second in between breaths, move to the stereotactic apparatus (RWD 68526). Use a rod or flat spatula to open its mouth and lift its teeth to the proper position.

6 Apply lidocaine cream to the inside of mouse's ears and insert ear bars, being careful not to insert to deep or too low. The mouse's head should be centered firmly and shouldn't wiggle if the body is moved slightly. Adjust nose cone snugly. Turn isoflurane down to 1.5%.

7 Apply artificial tears to cover mouse's eyes.

8 Administer analgesia. Inject 0.02mL of Buprenex SR subcutaneously.

9 Shave fur away from mouse's head with an electric razor. Remove as much fur as possible.

10 Apply betadine to skin surface to disinfect.

11 Make incision into skin by pulling skin surface up slightly with forceps and making one clean snip with large scissors. The resulting incision should be close to the midline.

12 Clear tissues to expose skull using a cotton swab dipped in PBS.

13 Once skull is cleanly exposed, level the mouse's head so that bregma and lambda are in the same plane. Also check right-left level, although it is usually good when the ear-bars are properly in place. Zero the apparatus on the mouse's bregma.

14 Using a hand-held dental drill, make a mark on the mouse's skull with the coordinates. Verify correct coordinates with the stereotax, then drill all the way through the skull, until a small hole appears. Once the brain surface can be seen, do not drill further.

The coordinates for the STN are below:

	Location	X	Y	Z (depth 1)	Z (depth 2)
	STN	1.50 mm	-2.155 mm	-5.00mm	-5.10 mm

15 Attach the glass micropipette to the stereotactic arm and fill with the desired amount of virus. Attach plastic tubing connected to the syringe.

16 Make sure the craniotomy is completely dry so that the dura is clearly visible, and lower the pipette until it touches the surface of the brain centered to the craniotomy. Zero the Z coordinate at this point to measure depth from dura.

17 Pierce the dura with the pipette and then continue to lower the pipette slowly, approximately 500µm per minute, until you arrive at the injection depth.

18 Once you reach the first injection depth, wait 5 minutes for the tissue to settle.

19 When you are ready to inject the virus, slowly press the syringe while looking through the microscope at the meniscus within the pipette. Inject 0.15uL in 5 minutes. Wait 1 minute for the virus to diffuse. Proceed to the second injection depth and inject 0.15uL in 5 minutes.

20 Once all injections have been completed, wait 10 minutes for the virus to completely diffuse before bringing the pipette up, to prevent it from sucking up the virus away from the intended location.

21 Move the pipette up until it exits the brain at an approximate speed of 500 µm per minute, then remove from the pipette holder. I do not recommend using the same pipette for multiple injections, either in the same animal or otherwise.

Recovery

22 Turn off isoflurane and oxygen. Remove mouse from stereotax (withdraw ear bars, remove teeth from hole by lifting mouse head).

23 Return mouse to cage lying face-down. It should wake up after a few minutes. Place the cage half-on a heating pad so that the mouse can choose to lie on the warm or cool side of the cage.

24 Monitor mice for the length of time specified in IACUC protocols.