Take a good, close-up photograph of the craniotomy after making it and bleeding has stopped. It should show the craniotomy itself along with the small burr holes along each side of it (used as reference marks), and LAMBDA. Print out this image full size on a single sheet of paper. During every single recording, make an X mark on the craniotomy image with the approximate location of the probe. Draw a line to the X mark and write down the relevant information for that recording: (1) date (2) DiI, if used (3) Lesion, if made, and (4) current (5) duration (6) which TT channel, and anything else useful, like what type of neuron was recorded from (AHV or EYE), and which structure we are estimated to be in (NPH, Gi, SGN, DTN). This photograph and notes makes it much easier later to estimate which probe tracks / lesions/ DiI tracks correspond to which recording days.

Start the probe off at LAMBDA, as best as you can determine the exact position of lambda. Aim to have the center point of the probe on the center point of lambda. Record these stereotax values (A/P, M/L, D/V). Record the stereotax values again before descending into the brain.

Estimate your zero D/V point and record the stereotaxic value → where you think the skull surface would lie (if it were not removed). This can become less accurate over time if there is any infection of the skull, or if the brain swells, or if the tissue changes over time. It’s still more helpful than nothing. Write down the D/V depth of the recording once you’ve reached the final recording spot. If a second recording is made during the same probe penetration, write down that recording depth, and whether it is above or below the first. File names with multiple recordings on the same day should end with ‘-1’ and ‘-2’, etc.

Consider driving the probe down after recording to see the tracks better. Keep note of recording depth and deepest probe depth.

Use EYFP filter immediately on the Olympus scope to visualize fluorescence in SGN/NPH from the ChR construct. Puncatate green labelling of cell nuclei from the retro-AAV doesn’t necessarily indicate expression of ChR.

Use the FAR-red dye at the DTN injection site. Make a composite on the Keyence scope using the green and far-red channels to visualize the injection site. Using FAR-red should allow a little more room to use red DiI paint during SGN recordings that don’t interfere too much with the nearby FAR-red fluorescence.

Alternate green and red DiI dyes on alternate recording days, moving from posterior in the craniotomy to more anterior.

Use a fair amount of current for the electrolytic lesion. As much as 10uA for 20 sec. I have used different intensities and durations and there is no right answer. Whether a lesion shows up in the tissue (i.e. is successful) seems to have an element of randomness to it. Using DiI with electrolytic lesions can help to locate the recording site when the lesion is absent. In general though, the DiI technique is not as precise.

For very important recordings, consider making a second lesion further down below the initial recording site. If the second lesion shows up and the 1st does not, you can backtrack with the known D/V difference.

Make note of brain tracks (especially any with DiI) that I do NOT record from. They should have their own recording sheet. Definitely record those penetrations in the master craniotomy image.

I typically move from posterior to anterior over days. But since SGN and anterior NPH are most important, it may be worth starting there and jumping around a little bit. Careful notes can help reconstruct later the relative A/P positions of each recording day. Try not to make recordings that are very close together (in anterior/posterior space). It will be hard to differentiate them later. Few recordings may be better because having even some confusion between close recording sites and threw several sessions into some doubt.

During slicing, write down the thickness of the sections. This can go in the mouse’s surgery document where there is info about perfusion and histology. This could serve as a useful reference later to figure out a distance between two slices when one has obvious anatomy that can localize it.

For mounting the tissue do one round of every other brain slice for fluorescence. Do a second round of the remaining sections (every other) for thionin staining. If possible, take a sneak peak at the fluorescence mounted slides first and see if they look ok. If there are any glaring problems, you can choose to do a second round for fluorescence instead of thionin. Strong electrolytic lesion marks should show up in the DAPI channel (all channels, really).

Slices should start from the top left of the microscope slide and go downward, and then begin in the next column. The slide can usually fit 3 mouse brain slices per column, and 5-6 columns total. With 6 columns, its not possible to capture everything in one Keyence composite, if you are looking to do that.

Do microscope imaging the same day or the next day after mounting the tissue. No need to wait. Tissue will gain autofluorescence over time.

Microscope images taken with the Leica scope (thionin) and single or composite images with the Keyence scope should be named with the same naming convention:  Mouse name + date + slide number + row number + column number. This three-number system should allow you to know which brain slice the image came from. It helps when trying to compare adjacent thionin and fluorescence slides, or when referring a single fluorescence image to a large composite of the whole microscope slide.

If stitching together images on the Olympus scope for a composite, make sure to set the exposure time high enough so that the overall features of the brain section are also visible. Use Microsoft Ice on desktop to stitch the images together.

After all of the histology images have been gathered, transfer them to the office desktop. There should be one folder for thionin images and one folder for fluorescence images, often broken up into a Keyence folder and an Olympus folder.

The key step at the end of this process is to make a ppt document for each mouse that has select microscope images and annotation that indicates which recording session you think the image originates from. The images will be of lesions or DiI tracks or other salient features. Use the craniotomy cheat sheet to draw a correspondence between recording sites in the craniotomy (relative positions across days, plus estimated distances from LAMBDA). Assign a recording session to each lesion. Give a confidence rating from 1 to 3 (1 = most confident, 3 = least confident). Include images of the DTN injection site and anything else useful.

Parameters for Doing the Gliosis after a Recording

Red output = contacting channel to lesion Black output = connected to drive ground

|  |  |
| --- | --- |
| **parameter** | **value** |
| power: | on |
| audio: | on |
| mode: | unipolar |
| DC/test: | on |
| range: | 100uA |
| % range: | e.g. 15 (for 15 uA) |
| Polarity select: | red light on right/red for positive current |
| Output: | on |

Use a stopwatch to time the stimulation. Flip the DC test switch to MOM or ON during the stimulation time period.