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Acute striatal or midbrain fiber photometry in head-fixed mice

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We use this protocol and it's working

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Funders Acknowledgements:
Maite Azcorra

Disclaimer

Appropriate ethics approval must be obtained before undertaking these experiments.

Abstract

This protocol describes how to:
- Implant head plates on mice for head-fixation during behavior
- Train mice for head-fixed running on a cylindrical treadmill, and to receive rewards and air puffs while head-fixed
- Record changes in neural activity during behavior using acute fiber photometry.
- Perform histology to identify the recording location.
While this protocol is focused on recordings from striatum with GCaMP, it can be easily modified to record from other brain regions and with other fluorescent reporters.

Troubleshooting

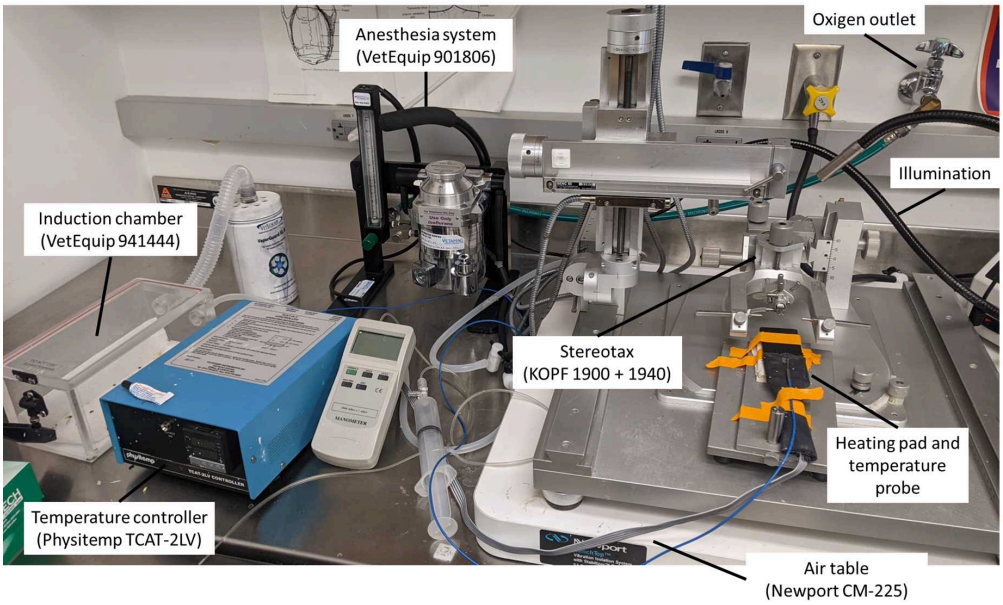
Outline & comments

1 This protocol is designed to record changes in neural activity by measuring changes in GCaMP fluorescence in dopaminergic axons in striatum, though it can also be used for dLight/GrabDA and other green fluorescent reporters. Recording of red reporters requires adapting the photometry setup (different excitation LEDs and filters). Recording from other brain areas only requires editing the recording coordinates.

The first step of this protocol already assumes GCaMP is expressing in striatum. This can be achieved through:
- transgenic mice expressing GCaMP (for example Calb1-Cre/DAT-tTA/Ai93 mice). Implant the head plate as explained below at 2-3 months of age.
- AAV injections (for example DAT-Cre mouse + AAV FSF-GCaMP). In this case implant the head plate at the same time as the AAV injection at 2-3 months. Follow the protocol "Midbrain viral injections for striatal fiber photometry in mice". For recording axons of midbrain dopamine neurons in striatum, allow 4 weeks from viral injection for ramping up of reporter expressing to the axons before performing fiber photometry.

Head plate implant

2 **Surgery preparation**



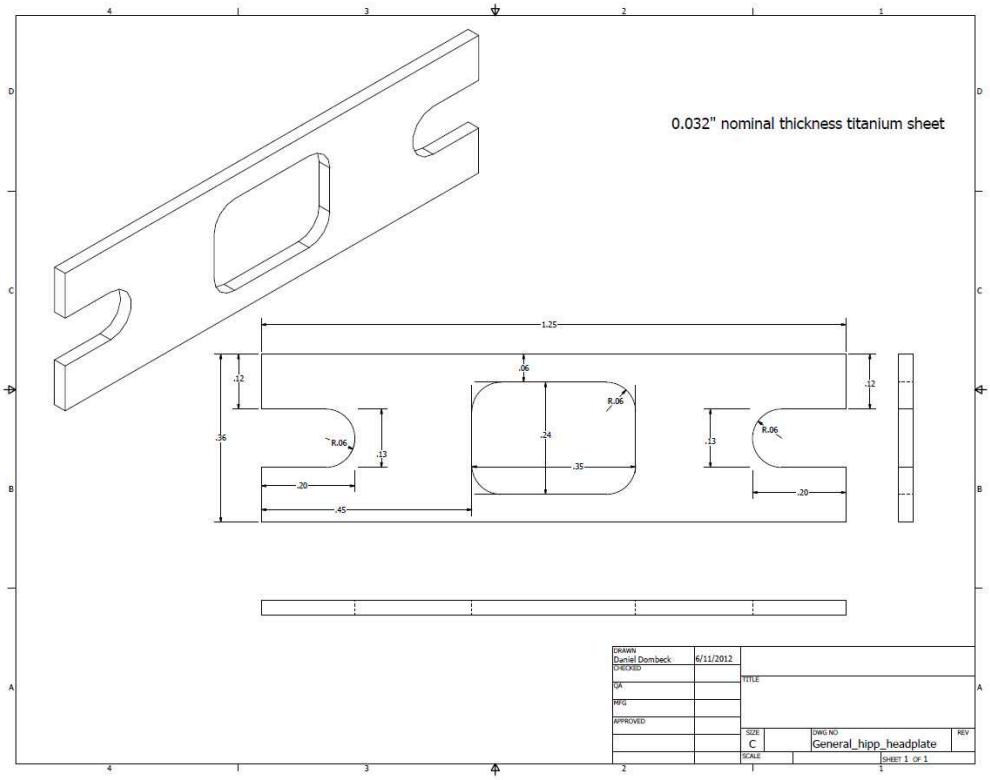
Surgery setup for head plate implant (same for craniotomies)

2.1 Turn on heat sterilizer ahead of time. When hot, sterilize tools (same for craniotomies):
- Flat spatula

- Coarse forceps
- Large scissors
- Fine scissors
- Fine forceps

2.2 Make sure that you have the following equipment/tools at hand (same for craniotomies):
- Isoflurane
- Betadine vial
- Ethanol vial
- Cotton swabs
- Artificial tears (Puralube Ophthalmic ointment)
- PBS vial
- Disposable transfer pipette
- Buprenex SR (slow release) 1mg/ml vial
- Insulin syringes (30 gauge)
- Metabond (Parkell): mixing dish (in freezer), radiopaque L-powder, "B" quick base, "C" universal TBB catalyst, scoop

2.3 Clean a medium sized head plate for implantation and ring using a razor.



Dimensions for mouse medium head plate

3 **Anesthetize mouse.** Redirect the anesthesia system (VetEquip 901806) to the induction chamber (VetEquip 941444) and turn on oxygen flow at 0.8 liters per minute. Turn on

isoflurane to 3% for induction, and place mouse inside. Wait until mouse's breathing has slowed down.

4 **Place mouse on the stereotax.** Redirect flow to nose cone on the stereotax (KOPF 1900 + 1940, mounted on a Newport CM-225 air table), take mouse out of the induction chamber and place on nose cone: hold mouse from neck skin, and use flat spatula to open its mouth, pushing down on the tongue and lower teeth. Slide nose cone plate into open mouth until top teeth fit into small hole on plate. Make sure mouse is secure by gently pulling on tail, without mouth slipping off. Slide nose cone over nose and secure, but not too tight yet as it would difficult ear bar placement. Reduce isoflurane (around 1.5/2%, but adjust to keep the mouse anesthetized and breathing consistently).

5 Cover eyes completely with **artificial tears** (Puralube Ophthalmic ointment) to prevent the eyes from drying. Use this also to keep whiskers away from surface of head, to prevent cutting them during shaving.

6 Place **temperature probe** in mouse's rear and hold down to table with tape. Turn on the temperature controller (Physitemp TCAT-2LV), which should be set at 35.7^{o}C. Inject 0.02 ml of Buprenex SR (slow release) 1mg/ml using insulin syringe subcutaneously between mouse's shoulders.

7 Make sure mouse is anesthetized by checking the foot pinching reflex. **Adjust ear bars into ears**, making sure they are centered. Careful not to put them too low (into ear canal, as it will hurt the mouse) or too high (head might slip from bars when pressure is applied). Normally when ear bars are properly placed the 10-mark on the stereotax is near the 6-mark on the bars (on our stereotax). Use coarser forceps to hold ears away for easier visualization. Tighten the nose cone to help fixate the head.

8 **Shave mouse's head**, from a bit before the eyes until between the ears, as wide an area as possible. First cut hair off with large scissors. Then smear shaving cream (Nair) thoroughly in area, wait 1 min and remove all of it with cotton swabs.

9 **Sterilize** mouse's skin first with Betadine and then with EtOh. Use cotton swabs, and always swipe skin in one direction.

10 **Cut the skin off the top of the skull** by pulling up with the coarse forceps and cutting around with small scissors. Do not make a single incision, but instead cut out a whole section of skin, from between the eyes until lambda is exposed. Make the hole as big as possible, to allow the head plate to be securely attached later, but without getting too close to the eyes or ears.

11 Dip two cotton swabs in PBS and **rub off the connective tissue** layer above skull, removing out of the way to expose both bregma and lambda. Let skull dry off for a bit, as this will allow better identification of landmarks. Note: if the mouse is not deeply anesthetized, this process can trigger a reflex, moving their paws every time the connective tissue is touched.

12 **Level the mouse skull:**

12.1 Use skull leveler tool on the stereotax (KOPF 1905) to level skull surface (bregma and lambda should be in the same plane). Also check right-left level, although it is usually good when the ear-bars are properly in place.

12.2 Once leveled, re-check by using the fine drill stereotax tool (KOPF 1911): move it until it is centered on bregma, and touching the skull. Zero the coordinates at this point. Move the drill towards lambda and check the leveling with the coordinates (should be under +-0.1mm off).
Use this chance to write down bregma-lambda distance for the mouse, as this can help adjust coordinates during any future troubleshooting.

13 **Dry up the skull .** You can use air from a pneumatic drill or just wait for it to dry own its own. This is very important, as the Metabond will not dry properly on a wet skull and thus will not atatch the head plate properly.

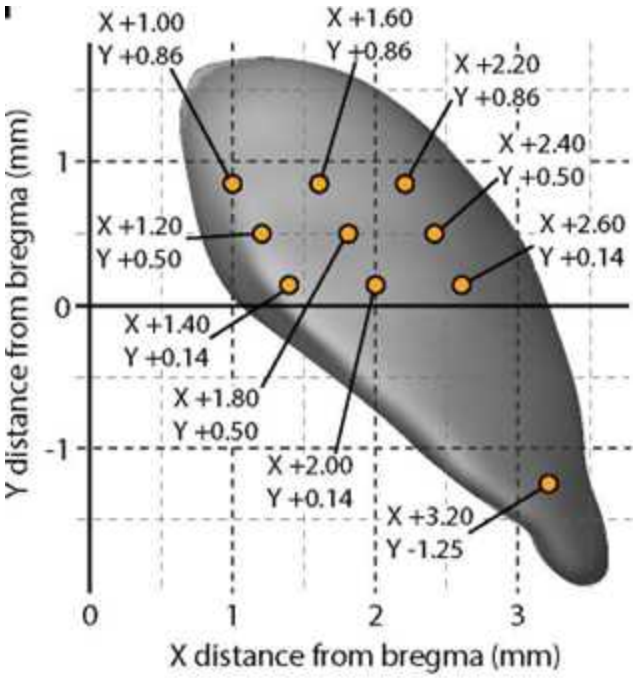
14 **Place the head plate** on the skull**.** It must be straight but mainly leveled to bregma-lambda (use the ear-bars for reference), so that when the mouse is head restrained the fibers will go down perpendicularly. Move the stereotax drill to the coordinates of the future craniotomy for fiber access (above your target region). Use the position of the drill to make sure that the head plate does not cover this location.

15 **Fix the head plate with Metabond.** Mix white Metabond (Parkell) in the cold mixing dish: 1 scoop of powder (Parkell Radiopaque L-powder), 4 drops of liquid base (Parkell “B” quick base) and 1 drop of catalyst (Parkell “C” universal TBB catalysis). Quickly mix it with the back of a cotton swab and apply it to the skull, covering all the exposed bone and joining it to the head plate so that no gaps are left, and noTry to apply a thinner layer around the area when the future craniotomy will be for drilling ease. Clean the mixing dish before it dries.

16 When the white Metabond is dry, **add a thin layer of black Metabond**. Mix black Metabond - same recipe as white Metabond above but add half a scoop of activated charcoal powder. Cover the white Metabond with a very thin layer of black Metabond. This serves to reduce light scattering off the head plate.

17 When the Metabond is dry, **mark the locations of future craniotomies** with the drill. To facilitate their future identification, use a sharpie to draw over the markings, and draw a cross centered on it to locate its center once the new craniotomy is made. Also draw a large number to identify the animal easily.
Common recording/marking locations:
- **CPu anterior: X: 1.4Y:1.8**
- **CPu medial: X: 1.8Y:0.5**

- **CPu tail:** **X: 3.2Y: -1.25**
- **SNc:** **X: 1.1Y: -3.25**



Common recording locations and coordinates in striatum

18 **Remove the mouse from the stereotax.** Remove the ear bars and the temperature probe (remember to turn of the thermometer too), and push back the nose cone. To remove the mouse from the nose cone, use the flat spatula again, introducing it in between the plate and the mouse's top jaw. Push up slightly until the top teeth come out of the hole, and pull the mouse off. Turn off the isoflurane and oxygen flow.

19 **Recovery from anesthesia.** Return the mouse to its cage, leaving it lying on its belly. Be careful to hold the mouse's tail throughout the whole process: although usually it takes a few minutes for them to wake up, they can sometimes jump out of your hand. Keep watch over the mouse until it starts to move. If for any reason it is taking more than a few minutes, place a hot mat under the cage to keep the mouse warm until it does wake up, and to provide extra care.

Training and behavior

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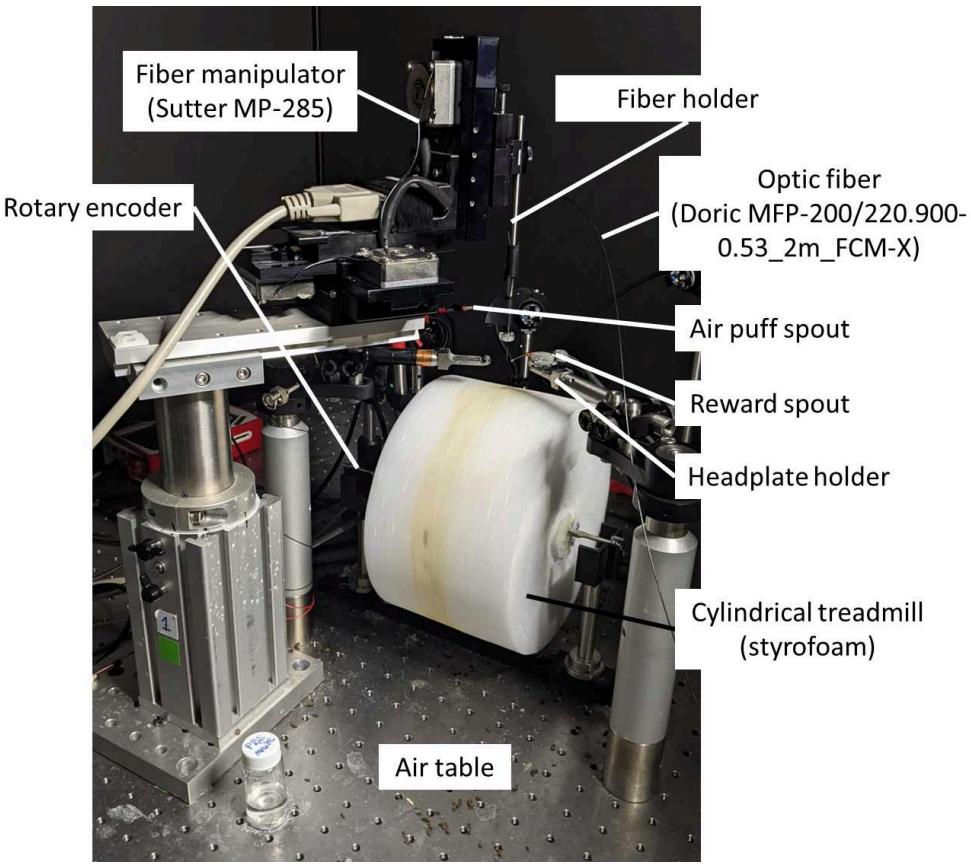


Behavior and fiber photometry setup.

Rotational velocity of the treadmill during locomotion is sampled at 1,000 Hz by a rotary encoder (E2-5000, US Digital) attached to the axel of the treadmill and a custom LabView program.

21 **Habituate mice to run on the treadmill.** Starting 1-2 weeks after injection or at least 24 hours after head plate implant, head-fix mice with their limbs resting on a 1D cylindrical Styrofoam treadmill ~20 cm in diameter by 13 cm wide in the dark with a white noise machine for noise cancelling – see image above. Habituate the mice on the treadmill for 3-10 days in 45-60 min sessions per day until they run freely and spontaneously transition between resting and running.

22 **Other training and stimuli.** After mice run freely, they can be trained to receive unexpected, unconditioned rewards or conditioned rewards. They can also receive unexpected aversive stimuli or neutral light or sound stimuli, but these do not require training and can be delivered for the first time during a recording session.

Mice can be trained for these stimuli after running habituation and before recording. If you want to record during only running (prior to any association of the treadmill with rewards has formed) and then after rewards in the same mice, you can habituate the mice to run, then record, then train them for rewards. Beware however that the

craniotomy made for the first recording will slowly deteriorate, so trainings that take more than a couple days should be done before the first recording.

All stimuli were delivered electronically using a custom LabView program, alternating between stimuli at random, and delivered at pseudo-random time intervals (10-30s between any two stimuli).

22.1 **Aversive stimuli - air puffs to the face.** Air puffs were delivered to the head-fixed mice through a small spout pointed at their left whiskers, which was connected to a ~20 psi compressed air source and triggered electronically through the opening of a solenoid valve for 0.2s. Triggering of this solenoid was accompanied by a 'click' noise, but this can be silenced by placing the solenoid inside a noise cancelling container.

22.2 **Neutral stimuli - light flash.** A blue LED was placed ~30 cm in front of the head-fixed mouse and electronically triggered for 0.2s.

22.3 **Unexpected water rewards.** Mice were water restricted for at least 3 days (1 ml water per day, making sure their weight always stays above 75% of their pre-water-restricted weight but below 85%). Then mice received unexpected water rewards while head fixed through a waterspout placed directly in front of their mouth but not touching it, though close enough for their tongue to reach comfortably. The spout was connected to a syringe filled with water and placed ~1 meter above the spout for gravity to push water through the tubbing, and was gated electronically through a solenoid valve, which was accompanied by a short 'click' noise. The water spout was connected to a sensor to detect when the mouse licked.

Mice were first trained to associate the spout with water by delivering a drop of water and pushing the spout closer to the mouse's mouth until they touched the water. This was done a couple times until the mice started to lick spontaneously.

Then mice were trained to associate the click noise with the water reward by delivering small rewards (4 µl) at random (10-30s intervals). Mice were considered trained when they licked consistently after the reward delivery times with a very short delay (reaction time). Usually a single 45 min session is enough for mice to learn.

This solenoid's click noise can be silenced by placing the solenoid inside a noise cancelling container. However, this prevents the mice from immediately realizing a reward is available, and will result in a delay in reward consumption that must be accounted for during analysis.

Large rewards - small 4 µl rewards were used for training, but during recording sessions 1/3 rewards were larger (16 µl). This was used for testing whether neuronal responses scaled for larger unexpected rewards.

22.4 **Conditioned rewards (or air puffs).** The light flash was used as the unconditioned stimulus (US) that predicted the reward (CS). The light was turned on of 4 seconds then the reward was delivered as the light turned off. Alternatively for fixed-interval schedule conditioning, the light can be turned on briefly (0.2s) and the reward delivered 3 seconds later. Mice are considered trained when they reproducibly perform pre-licking: anticipatory licking that ramps up between the cue/US and the reward. In these cases, the reward solenoid needs to be silenced, as otherwise the mice will just wait to hear the click to lick.
Alternatively the cue can also be used to predict an aversive air puff. No silencing of the solenoid is needed in this case.

Craniotomy

23 **One day** before fiber photometry experiment make a craniotomy to expose the brain. This requires the same setup and tools as the head plate implant procedure - go to step #2 - Follow steps 2, 2.1, & 2.2 .

24 Anesthetize the mouse, place it on the stereotax, cover its eyes with artificial tears, set the temperature probe and place the ear bars in position, as for the head plate implant - go to step #3 - follow steps 3, 4, 5, 6, & 7 .

25 Use the hand drill (Midwest Tradition 282-9205) to indent a large circle (about 2mm in diameter) around each marking on the Metabond covering the head plate - these markings were previously made during the injection and/or head plate surgery to mark the striatal recording locations. If more than one location in each hemisphere will be recorded from, make separate craniotomies – this will make it easier to target the correct locations.

26 Once the craniotomy has been outlined, use the hand drill to deepen the outline, slowly and consistently throughout. If the Metabond is too thick and it difficult the process, you can drill off the Metabond inside the craniotomy outline. Keep drilling the outline until the skull is thinned down. This might be indicated by the central bone moving slightly every time the drill touches the skull, or by a slight cracking of the skull. Do not thin the central bone as it will be much harder to remove it. Then use the fine scalpel to poke around the outline, both making sure that the bone is thin enough all around and weakening the area so that it is easier to remove the center. If an area is too thick, drill again. Then use the thin scalpel as a lever to pull up the central bone from one side, separating it from the brain. Once it sticks out, use the fine forceps to pull it off.

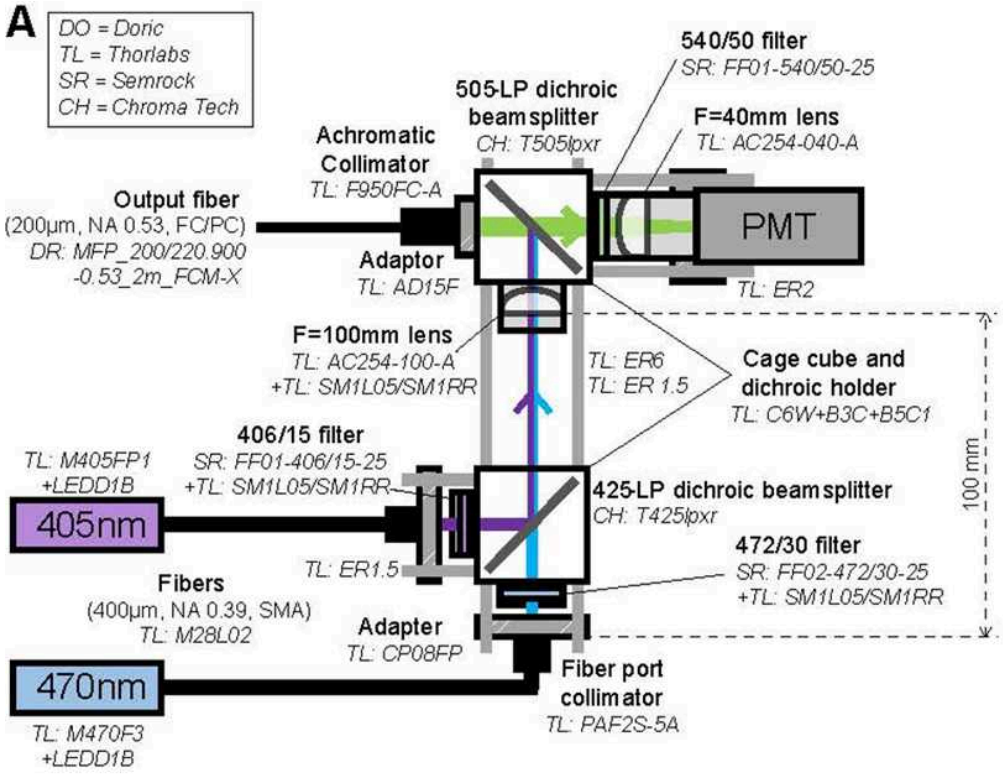
27 This large craniotomy often starts bleeding; there is a large blood vessel over the striatum. To stop the bleeding, first add PBS with the transfer pipette and remove it with a kimwipe (rolled into a fine tip).

28 **Cover it with KWIK-SIL** (WPI) to prevent the brain from being exposed to air until the time of recording. For this, first dry the craniotomy with kimwipes then with the air from the hand drill. Push out a drop of each component from the KWIK-SIL (no need to use the special tips) and mix them with the back of a cotton-swab. Apply to the craniotomy, letting it slowly cover it so that no large bubbles form. Let it dry before drilling other craniotomies (or the air from the hand drill will mess up the KWIK-SIL).

Fiber photometry I - setup and preparation

29 A custom-made photometry setup was used for recording (shown below). Blue excitation (470 nm LED, Thor Labs M70F3) and purple excitation light (for the isosbestic control) (405 nm LED, Thor Labs M405FP1) were coupled into the optic fiber such that a power of 0.75 mW emanated from the fiber tip. 470 and 405 nm excitation was alternated at 100 Hz using a waveform generator (Rigol DG812) plus an inverter powered by 5V triggered by a Labview program, each filtered with a corresponding filter (Semrock FF01-406/15-25 and Semrock FF02-472/30-25) and combined with a dichroic mirror (Chroma Tech Corp T425lpxr). Green fluorescence was separated from the excitation light by a dichroic mirror (Chroma Tech Corp T505lpxr) and further filtered (Semrock FF01-540/50-25) before collection using a GaAsP PMT (H10770PA-40, Hamamatsu; signal amplified using Stanford Research Systems SR570 preamplifier). A Picoscope data acquisition system was used to record and synchronize fluorescence and treadmill velocity at a sampling rate of 4 kHz. The output of the waveform generator was also input into Picoscope, as well as the trigger signals used for reward, air puff, and light stimuli delivery.

30



Custom fiber photometry setup including part numbers.

31 Despite the use of low auto-fluorescence fibers, the setup does build up auto-fluorescence over time, which can saturate the PMTs after a few days of no use. To prevent this, the LEDs were turned on for 15-45 min before each recording day to **bleach the setup**, with the time depending on how recently the setup was used (45 min at least if it wasn't used for over 3 days).

32 **Clean the fiber** tips with a wet kimwipe before every recording.

33 **Check the fiber** to make sure it has a clean edge (on occasions it can accidently break) - turn the blue LED on and point the fiber towards the black wall of the chamber or a white paper, and look at the shape that the light makes: it should be round without light scattering sideways. If this is not the case, re-cleave the fibers. For this, first remove the outer protective layer with a wire-stripper, to expose about 4cm of fiber plus its jacket. Then remove the inner protective jacket to expose about 1 cm of clear optic fiber, using a razor at a ~30 degree angle to shave off the material. Make sure the edge of the removed protective jacket is smooth, as anything sticking out will prevent the fiber from fitting through the holder. Cleave the fiber by chopping off a small amount (~1 mm) with the plastic fiber scribe (Thorlabs S90R). Check the cleaved end and repeat if the light output is not round.

34 **Measure the power** coming out of each individual fiber for each LED (470 vs 405 nm) individually. Turn on the power meter and uncover the sensor. Make sure the meter's wavelength is set at 470nm or 405nm appropriately. Hold the fiber perpendicular to the sensor and bring it as close to its center as possible but trying to not touch it. The power should be around 0.75mW (± 0.01mW) for both fibers and for each wavelength, so adjust the LED power control accordingly.

35 Place optic fiber(s) on holder, threading them through the appropriate holes. Make sure the inner protective layer comes out of the holder, as this will make sure the fiber is straight. Tape the fibers to the holder using electrical tape, making sure to tape the inner protective jacket to keep the fiber fixed (the outer layer can slide over the fiber). Also be careful to avoid sharp bends on the fiber when taping it.

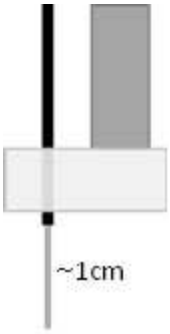
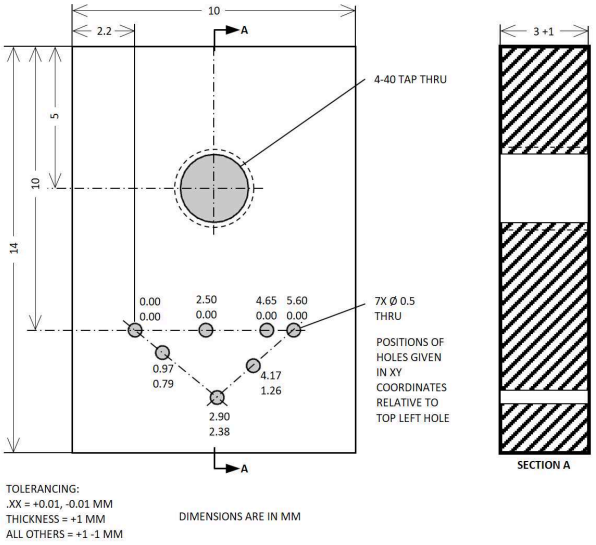


Diagram of fiber in holder.



Fabrication diagram for fiber holder. Fiber holes might have to be slightly enlarged manually by progressively larger micro-drill bits.

Fiber photometry II - recording

36 Turn the white noise machine on, place the mouse head fixed on the treadmill and remove the Kwik-sil to expose the brain. Position the reward spout and air puff spout if needed.

37 Rotate the fiber holder arm so that fibers are directly above the craniotomy (or as close as possible), fix it, and use the micromanipulator in coarse mode to center the fibers above the craniotomy, then move them down until the fibers barely touch the surface of the brain. Use a flashlight to illuminate the fiber while moving it down, as the light will shine off the flat tip of the fiber until it touches the brain surface, making it easy to identify. Then zero the manipulator location.

38 Cover the craniotomy with PBS to prevent it from drying, turn the light off and close the curtain.

39 Turn on the recording equipment:
- Make sure the micromanipulator is on, on moving mode and zeroed at brain surface
- Make sure the custom micromanipulator controller program is open on VisualStudio
- Load correct recording settings on PicoScope
- Make sure the rotary encoder LabView program is running
- Turn on the waveform generator and set it to the correct program (a 100Hz square wave)
- Turn on both amplifiers

- Turn on the PMT power source
- Turn on the PMTs (ONLY when all room lights are off, or they will be damaged)

40 Use the micromanipulator program to move the fiber down to the desired location at a speed of 5 microns per second. Faster movements will cause grater tissue damage.

41 When the fiber is in the correct location, wait 2 min to allow the tissue to settle, then turn on the LEDs by turning on the Waveform generator output and the Labview program powering the inverter (that way the 470 and 405 nm LEDs will be turned on alternatively at 100 Hz). Click "record" on Picoscope and look at the fluorescence recorded live on the program.

- The florescence from the 405 nm excitation should be flat - otherwise it shows there are movement artifacts. In that case, move the fiber 100 microns down and check again.
- The fluorescence from the 470 nm excitation should show transients - otherwise it means there is no GCaMP expression in the current recording location.

42 For **recording from striatum**, start recording at 1.6 mm from brain surface (the dorsal-most region), then go down to 1.9 mm, and from then down in 0.5 mm steps: 2.4, 2.9, 3.4, and 3.9 mm. Deeper locations than 3.9 mm get into the Nucleus Accumbens. These are reference locations for the striatum, but they will need to be adjusted based on the recording goals - for example, if recording from axons of Calb1+ dopamine neurons, no axons (and thus transients) will be present at more dorsal depths. We recommend checking for transients at 1.6 mm or a bit above from where GCaMP is expected to be found, and either record at that location or move further down until transients are found. Keep moving down until no transients are observed.

For **recording from SNc**, move the fiber down to 3.4 mm and check for transients. If no transients are seen, move down in 0.2 mm steps until

Never move the fiber up before recording, as the hole left behind by the fiber can unpredictably affect the recording volume/area, cause movement artifacts, and result in recordings from damaged tissue.

43 If stimuli (e.g. rewards) will be delivered during this recording, turn on the Labview program once in the correct location, to avoid satiating or habituating the mice during the fiber movement phases.

44 Start recording on Picoscope, and record during 15-20 min. More than that can lead to bleaching of the fluorescent transients.

45 Once the recording is done, save it and move the fiber down to the next recording location, and repeat until the last recording location is done.

46 **Remove the fiber from the brain SLOWLY**, at a speed of 5 microns per second.

47 Once the fiber is outside the brain, use the manipulator to move it up fast and rotate the hold arm to get the fiber away from the mouse.

48 Dry the craniotomy well with a kim-wipe and cover it with KWIK-SIL (see go to step #28), to protect the brain from damage between recordings.

49 Remove the mouse from the rig and return it to its home cage.

50 Clean the fiber tip with a wet kim-wipe, as often there can be blood or tissue stuck to it.

51

Note

You can record 3-4 times from the same mouse before too much tissue damage occurs. These recording sessions must not be too far apart in time, as the craniotomy will swell and difficult the identification of the brain surface and thus result in inaccurate depth measurements.
A standard experiment consists of:
- Mon: craniotomy
- Tue: recording day 1 - running only
- Wed: train mouse for unexpected rewards
- Thu: recording day 2 - running, rewards, and air puffs
- Fri: recording day 3 - same as 2.
- Mon: optional, recording day 4

Histology and identification of recording location

52 **Perfuse** the mouse transcardially with PBS (Fisher) then 4% paraformaldehyde (EMS).

Note

ALWAY PREFUSE THE MOUSE ON THE SAME DAY AS THE LAST RECORDING. Otherwise, the fiber track with heal and fade and it will be hard to identify the recording location.

52.1 Dissect the brain immediately after perfusion and store in paraformaldehyde 4% overnight at 4 °C .

52.2 Transfer the brain to a 40% sucrose solution in PBS and store it for at least 2 days at 4 °C (it must sink to the bottom of the solution)

53 **Cut coronal slices** 50 µm thick on a freezing microtome. Right before sectioning cut the cerebellum off the brain with a razon at an angle perpendicular to bregma-lambda, and flash freeze the brain in dry ice. Mount the frozen, cut brain on the microtome with cortex facing away from the blade, and cut coronal slices 50 microns thick. Some blood should be visible on the cortex which will mark the craniotomy locations, indicting the regions to slice to get the fiber tracks. Place the slices in PBS.

54 Mount the slices directly, as fiber tracks will be clearly visible without staining. Alternatively, stain the tissue following any standard immunostaining protocol - for example, stain for GFP to amplify the GCaMP fluorescence. It is enough to mount 1 in every 4 slices, as the fiber tracks will be visible.

55 Take images of the brain slices using any fluorescence microscope using a 10X objective.

56 Select the slice with the clearest fiber track. You will be able to identify it by a straight hole in the tissue most often surrounded by a thin layer of scar tissue which will be auto-fluorescent green.

57 Identify the closest matching slice from a reference Atlas like the Paxinos Brain Atlas, and scale the slice until it matches the reference - paying particular attention to matching the brain region of interest (for example the striatum).

58 Mark the fiber track from the brain image to the reference and use the scale in the Atlas to measure depth from the surface of the brain along the fiber track to locate each recording's location.

Note

For multiple recording days, often only the last day's fiber track will be visible. If you have used the same craniotomy for all recordings, the last fiber track should be a good approximation for identifying recording locations for previous days, but keep in mind it will be less accurate.

Note

Brain tissue can deform during sectioning and mounting, and damage to the brain surface across days can make it harder to identify the surface of the brain, so use the reference slice. to address this.