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Miniscope calcium signal recording of cortical activity in freely moving mice

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol was used to record motor cortical activity in mice that had received AAV-GCaMP6f injections and Prism GRIN Lens Implantation (dx.doi.org/10.17504/protocols.io.4r3l21rrqg1y/v1) and were trained on the Mouse reach-to-grab task training protocol (dx.doi.org/10.17504/protocols.io.ewov11bb2vr2/v1).



Habitation

5d

- 1 Following post-op recovery, bring the mouse to the behavior room for a 1-hour habituation.
- 2 Gently handle the mouse by the scruff of the neck.
- 3 Slightly loosen the side screw on the baseplate and remove the baseplate cover with blunt forceps.
- 4 Attach the nVue 2.0 miniscope to the baseplate and slightly tighten the side screw.

Note

You must hear a click to make sure the miniscope is securely attached to the baseplate.

- 5 Place the mouse back in its home cage and allow it to walk freely to habituate to the weight of the miniscope for about 30 min, 2 days in a row.
- 6 At the end of the habituation session, take the mouse out of the home cage.
- 7 Slightly loosen the side screw on the baseplate, remove the miniscope, and replace it with the baseplate cover.
- 8 Return the mouse to its home cage.
- 9 Repeat the same process on the next 3 days, but place the mouse in the reaching box for habituation.

Recording

20m

- 10 The day before the recording session, remove all food from the mouse's home cage at the end of the light phase, but keep water.

- 11 On the day of the recording session, bring the mouse to the behavior room and allow it to habituate again to the room for about 30 min.
- 12 Then, gently handle the mouse by the scruff of the neck.
- 13 Slightly loosen the side screw on the baseplate and remove the baseplate cover with blunt forceps.
- 14 Attach the miniscope to the baseplate and slightly tighten the side screw.

Note

you must hear a click to make sure the miniscope is securely attached to the baseplate.

- 15 Place the mouse back in its home cage.
- 16 Start Inscopix data acquisition software, and turn on the green channel LED to check parameters for the best signal quality.

Note

Green channel parameters: for GCaMP6f imaging with the prism GRIN lens in M1, we use a frame rate of 20 Hz, LED power between 0.2 and 0.4 mW/mm², exposure time 50 ms, and sensor gain of 2 to 4. Digital focus is manually chosen such as to have as many cells as possible in focus, typically between 0 and 1000 Inscopix units (i.e., ~ 0-300 μm).

- 17 Once your parameters are set, turn off the LED and start the recording on the green channel.
- 18 Gently place the mouse in the reaching box and start the behavior video recording.
- 19 Record mouse reaching behavior with concomitant calcium imaging for 20 minutes, then stop both recordings and leave the mouse in the testing box.
- 20 On Inscopix data acquisition software, switch to the red channel.



- 21 Record multiple videos at different digital focal planes, about 5 seconds each.

Note

Red channel parameters: for tdtomato imaging with the prism GRIN lens in M1, we use a frame rate of 10Hz, LED power between 0.2 and 0.4 mW/mm², exposure time 50-100 ms, and sensor gain of 2 to 4. Digital focus is manually chosen such as to have as many cells as possible in focus, typically between 0 and 1000 Inscopix units (i.e., ~ 0-300 μm), with steps of 25 or 50 Inscopix units. The number of videos depends on the quality of the signal.

- 22 Remove the mouse from the test box.
- 23 Slightly loosen the side screw on the baseplate, remove the miniscope, and replace it with the baseplate cover.
- 24 Return the mouse to its home cage and provide food ad libitum.