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Voltage sensor imaging in a population of dopaminergic axons in the mouse striatum

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We use this protocol and it's working

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Disclaimer

This protocol uses the ASAP5f voltage sensor (AAV9-EF1α-DIO-ASAP5-WPRE) kindly donated by the **Lin lab**.

Abstract

This protocol describes how to perform voltage sensor imaging from a population of striatal dopaminergic axons using high frame rates (600 Hz minimum) on coronal mouse brain slices. In particular, this protocol has been optimised for the use of ASAP5f voltage sensor (AAV9-EF1α-DIO-ASAP5-WPRE) from the **Lin lab** and changes in fluorescence are resulting from electrical stimulation.

Materials

SOLUTIONS:

Preparing Slicing Solution:

To dilute in dH_20 for final volume of 1 L:

- 194 mM sucrose
- 30 mM NaCl
- 4.5 mM KCl
- 1 mM $MgCl_2$
- 26 mM $NaHCO_3$
- 1.2 mM NaH_2PO_4
- 10 mM D-glucose

Preparing stock solution of artificial CerebroSpinal Fluid (aCSF) (10x):

To dilute in dH_20 for final volume of 1 L:

- 130 mM NaCl
- 2.5 mM KCl
- 26 mM $NaHCO_3$
- 1.25 mM $NaHCl_2$
- 2 mM $MgCl_2$,
- 1.25 mM NaH_2PO_4
- 10 mM glucose

Preparing working solution of aCSF (x1)

To dilute in dH_20 for final volume of 1 L:

- 100 mL of 10x aCSF
- 2.5 mM $CaCl_2$

REAGENTS:

- **Sodium Chloride (Sigma-Aldrich, Catalog #31434, CAS #7647-14-5)**
- **Potassium Chloride (Sigma-Aldrich, Catalog #P9541, CAS #7447-40-7)**
- **Magnesium Chloride Hexahydrate (Sigma-Aldrich, Catalog #M2670, CAS #7791-18-6)**
- **Sodium Bicarbonate (Sigma-Aldrich, Catalog #31437-M, CAS #144-55-8)**
- **Sodium Phosphate Monobasic (Sigma-Aldrich, Catalog #71496, CAS #7558-80-7)**
- **D-glucose (Sigma-Aldrich, Catalog #G8270, CAS #50-99-7)**
- **Sucrose (Sigma-Aldrich, Catalog #S0389, CAS #57-50-1)**
- **Calcium Chloride Solution (Sigma-Aldrich, Catalog #21114, CAS #10043-52-4)**

VIRUS:

- AAV9-EF1α-DIO-ASAP5-WPRE which corresponds to ASAP5f sensor; not currently commercially available, contact Lin lab for further details

EQUIPMENT:

Note

Equipment essential to our recordings are in **BOLD**.

- VT1200S Vibrating blade microtome (Leica)
- Water bath
- **Andor iXon Ultra 888 - high speed, high sensitivity camera**
- **Cairn OptoMask (product code: OPTMSK-L)**
- 10x objective
- 60x objective
- Blue LED light (λ = 470 nm, 10 mW)
- **Concentric bipolar Pt/Ir stimulating electrode** (FHC Inc., SKU #30200, outer/inner diameter 125/25 µm)
- **Gilson MINIPULS 3 Peristaltic Pump**
- **Digitimer DS3 Isolated Current Stimulator**
- Multichannel Systems stimulus generator (STG4004)

Software:

- **Andor Solis** software for data acquisition
- **MC_Stimulus II** (version 3.5.11) for stimulus generation

Before start

Intracranial injections:

Between 3 to 4 weeks prior to the experiments, we injected heterozygous DAT-Cre mice with the ASAP5f voltage sensor (AAV9-EF1α-DIO-ASAP5-WPRE). We injected the virus diluted to $1.92x10^{12}$ vg/mL bilaterally in the midbrain (1 µL per site).

To target mostly VTA (to record from NAcC) we used the following coordinates from Bregma: A/P: -3, M/L: +/- 1, D/V: -4.25, raising the needle to -4.2 by the end of the injection.
To target mostly SNc (to record from DLS), we used the following coordinates from Bregma: A/P: -3, M/L: +/- 1.25, D/V: -4.25, raising the needle to -4.2 by the end of the injection.

We found that using the same coordinates bilaterally to target VTA dopaminergic neurons and ultimately record from NAcC were giving the best results rather than targeting VTA in one hemisphere and SNc in the second one.

Brain slice preparation

1 Sacrifice mice using cervical dislocation, exsanguination and decapitation. Quickly open the skull and collect the brain in an ice-cold slicing solution saturated with 95% O_2/ 5% CO_2, containing (in mM): 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$-$6H_2O$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, 10 glucose.

2 Cut 300 µm thick coronal slices using a vibratome in ice-cold slicing solution saturated with 95% O_2/ 5% CO_2.

3 Immediately transfer each slice to a beaker in a water bath at 32°C, the beaker filled with aCSF saturated with 95% O_2/ 5% CO_2, containing (in mM): 130 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 glucose, 2 $MgCl_2$-$6H_2O$, 2.5* $CaCl_2$. Leave the beaker in the water bath at 32°C for 15 minutes. Then move the beaker to room temperature (20°C-22°C) for a minimum of 45 minutes before using the slices. This protocol also works well with aCSF containing 2 mM $CaCl_2$.

> **Note**
>
> The beaker containing the slices needs to be kept in the dark to avoid bleaching of the fluorescent sensor. We use foil to protect the beaker from light, or we use a beaker coated with a black opaque tape.

4 While the slices are equilibrating, turn on the rig perfusion system, the heater, and run through dH_2O. The temperature in the recording chamber should be 32°C ± 1°C and the perfusion rate should be ~ 2 mL/min.

5 Run aCSF through the perfusion system before putting your slices (or hemisected slices) in the recording chamber.

Choosing acquisition settings and placing stimulating electrode

6 Open the Andor Solis software and click on the Real Time icon to open the camera shutter and achieve visualisation of the slice.

Note

Ensure the cooler is on to maintain the camera temperature. You can find this option in the bottom left corner of Solis. We set our cooling to -70°C.

7 Use a 10x microscope objective to locate the region of the brain slice you are interested in. Keep the wide field light intensity to a minimum to avoid bleaching of your sample.

8 Briefly, turn on the blue LED light (λ = 470 nm, 10 mW) to confirm good viral expression.

9 Using the wide field light, position your stimulating electrode on the surface of the brain slice and centre it in the field of view.

10 Switch to a 60x objective, and locate the tip of the stimulating electrode.

11 Navigate to around ~ 150 µm (using the conversion between pixel size to actual distance that you can find in the specification documents of the camera) away from the stimulating electrode and place that region of interest in the bottom left corner of the sensor.

Note

Due to the nature of the camera sensor, selecting an ROI that is located in the bottom left corner (or the centre) of the sensor allows the fastest frame rate to be reached. Choosing between the bottom left corner, or the centre, will depend on several factors - such as, the size and shape of the ROI that you desire.

12 Click on the Acquisition Setup icon to open the acquisition settings. In the Camera Setup tab, choose Kinetic as the acquisition mode. Check the boxes for Frame Transfer and Crop Mode. Set the Electron Multiplier (EM) Gain Level to 200.

13 In the Crop Mode ROI tab, choose the size of your ROI (148 pixels high x 496 pixels long) and choose a pixel binning of 4x4.

Note

We used this size ROI because it allowed us to reach 600 fps while keeping a relatively big field of view.

14 In the Auto-Save tab, choose the folder you wish to save the data to, and any file naming options you desire.

15 Go back to the Camera Setup tab and change the exposure time to the minimum (0.0015870 seconds) to reach a frame rate of around 600 Hz (599.52 Hz). Close Acquisition Setup.

16 Click the Real Time icon and turn on the wide field light to position the blades of the OptoMask, ensuring that the edges of all four blades are just visible inside the ROI.

Note

If light falls outside of the ROI on the sensor, crop mode can not be used to obtain fastest frame rates without contamination of your data. Using an OptoMask allows light to be blocked whenever its blades are drawn. Once your ROI size and position are set for your experiments, the OptoMask allows for stoppers to be set, so that the blades can conveniently be drawn to the same position each time.

17 Briefly turn on the blue LED light to make sure that the surface of the brain slice is in focus.

Data acquisition

18 Go back to the Acquisition Setup menu and in the Triggering drop-down menu, change the trigger to External Start in order to have time-locked stimulations.

19 Calculate the right number of frames to be recorded (frame rate * seconds) and enter this value in the Kinetic Series Length box.

Note

For example, we record for 3.5 seconds at a frame rate of 600 Hz, so we use 2100 frames.

20 Navigate to the Auto Save tab and select the folder in which your want your files to be saved. Here, you can personalise the file naming structure.

21 When you are ready to begin, click on the Take Signal icon, and start your stimulation protocol in your preferred software/hardware (we use a Multi Channel Systems stimulus generator and the accompanying MC_Stimulus II software). We usually deliver the electrical

stimulation at 1.4 s into the recording, so that the initial “exponential” 'bleaching' decay has passed and the stimulation occurs when the 'bleaching' appears more linear.

Note

Do not forget to write down all recording parameters, the recording number, and the stimulation you used in your lab book for future reference!

22 Wait for at least 1 minute before the next stimulation.