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Wide-field imaging of voltage sensors expressed in *ex vivo* mouse brain slices

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We use this protocol and it's working

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Disclaimer

This protocol uses the ASAP3 voltage sensor (AAV5-EF1α-DIO-ASAP3WPRE) kindly donated by the **Lin lab**.

Abstract

This protocol describes how to perform wide-field imaging of voltage sensors using high frame rates (660 Hz minimum every 2.5 minutes) in mouse midbrain using *ex vivo* brain slices.

Materials

Equipment:

- Olympus BX51WI microscope equipped with a OptoLED Lite system (CAIRN Research);
- iXon EMCCD Camera (ANDOR);
- x40/0.8 NA water-objective (Olympus UK)

Virus:

- AAV5-EF1α-DIO-ASAP3WPRE (ASAP3) from Stanford Gene Vector and Virus Core.

Software:

- Micro-Manager v1.4
- PClamp
- Matlab vR2019b
- Fiji v1.5

Troubleshooting

Before start

We injected the voltage sensor ASAP3 without a soma-targeting signal (AAV5-EF1α-DIO-ASAP3WPRE) following the steps described in **Protocol: Intracranial injections of viral vectors in mouse midbrain and striatum**. We injected the virus diluted to 2.4E+12 vg/ml in the midbrain (1 µL per site) of heterozygous DAT-IRES-Cre mice.

The coordinates used by us for targeting the midbrain were as follows:

Ventral tegmental area (VTA) (AP = -3.1 mm, ML = ± 0.5 mm, DV = -4.4 mm)
Substancia nigra pars compacta (SNc) (AP = -3.5 mm, ML = ± 1.2 mm , DV = -4.0 mm)

Animals were maintained for at least three weeks following surgery to allow virus expression in the midbrain. We then prepare *ex vivo* brain slices by performing **steps 1 to 11** from **Protocol: Fast-scan cyclic voltammetry to assess dopamine release in ex vivo mouse brain slices**.

Image Acquisition

1 Using a x40/0.8 NA water-objective (Olympus UK), position the stimulating electrode on the surface of the brain slice and centre it in the field of view.

2 Change the exposure time to reach a frame rate of around 600 Hz every 2.5 min using Micro-Manager v1.4.

3 Apply electrical stimulus pulses singly and in trains (4 pulses, 50 Hz) using PClamp.

Note

The order of single and train stimulations was alternated and equally distributed and data were collected in duplicate before and after a change in extracellular experimental condition.

Observations were time-locked to the deflection.

4 Record changes in fluorescence intensity using PClamp.

Image Analysis

5 **The following steps were performed in MATLAB vR2019b and Fiji v1.5.**

Extract fluorescence intensity from the region of interest (~5 µm * 5 µm).

6 Bleach-correct the ASAP3 transients by fitting an exponential curve function.

7 Expressed data as ΔF/F where F is the fitted curve.