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# Imaging axonal calcium dynamics in *ex vivo* mouse brain slices

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**We use this protocol and it's working**

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## Abstract

This protocol describes how to image calcium dynamics in striatal dopaminergic axons in *ex vivo* mouse brain slices. We imaged calcium transients in response to single and trains (4 pulses, 100 Hz) of electrical stimulus pulses using genetically encoded calcium indicator GCaMP6f expressed in DAT-Cre:Ai95D mice.

## Materials

**Equipment:**

- Olympus BX51Wl microscope equipped with a OptoLED Lite system (CAIRN Research);
- Prime Scientific CMOS (sCMOS) Camera (Teledyne Photometrics);
- x40/0.8 NA water-objective (Olympus UK)
- ITC-18 A/D board (Instrutech)

**Software:**

- Micro-Manager v1.4
- Matlab vR2019b
- Fiji v1.5
- Igor Pro 6 (WaveMetrics)

## Before start

This protocol was performed in heterozygous DAT-Cre:Ai95D (4-7 weeks) mice. These mice were bred from homozygous DAT-Cre mice (B6.SJL-Slc6$^{a3tm1.1(cre)Bkmn}$/J, JAX stock number 006660) crossed with homozygous Ai95D mice (B6;129S-Gt(ROSA)26Sor$^{tm95.1(CAG-GCaMP6f)Hze}$/J, JAX stock number 028865).

Note

Ai95(RCL-GCaMP6f)-D (also called Ai95D; RRID:IMSR_JAX:028865) mice are a Cre-dependent, fluorescent, calcium-indicator tool strain. Ai95D has a floxed-STOP cassette preventing transcription of the GCaMP6 fast variant calcium indicator (GCaMP6f; a detector of single neuronal action potentials with fast response kinetics). After Cre exposure, bright EGFP fluorescence is observed following calcium binding (such as neuronal activation).

**This Ai95D allele is on a C57BL/6J genetic background.**

We prepare *ex vivo* mouse 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 16.6 Hz every 2.5 min using Micro-Manager v1.4. 16.6 Hz frame rate every 2.5 min using Micro-Manager 1.4.

3 Apply electrical stimulus pulses singly and in trains (4 pulses, 100 Hz) using custom-written procedures in Igor Pro 6 (WaveMetrics) and an ITC-18 A/D board (Instrutech).

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.

4 Record changes in fluorescence intensity using custom-written procedures in Igor Pro 6 (WaveMetrics) and an ITC-18 A/D board (Instrutech).

## Image Analysis

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

Extract fluorescence intensity from the region of interest 25 µm * 25 µm which was 50 µm away from the electrical stimulating electrode tip.

6 After background subtraction, bleach-correct the $Ca^{2+}$ transients by fitting an exponential curve function through both the baseline (2 s prior to stimulation) and the last 1 s in a 7.2 s recording window.

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