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# GRAB Acetylcholine (ACh) sensor imaging in *ex vivo* mouse striatal slices

Forked from GRAB sensor imaging in mouse striatal slices

In 1 collection

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

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## Abstract

This protocol describes how to image GRAB Acetylcholine (ACh) sensor expression in *ex vivo* mouse striatal slices, and measure changes in fluorescence signals resulted from electrically-evoked ACh release.

## Materials

**Equipment:**

- Iris 9 Scientific CMOS (sCMOS) camera **Teledyne Photometrics**
- Vibratome VT1200S **Leica Biosystems**
- Microscope BX50WI Olympus
- Microscope C-mount Camera Adapter U-CMAD-2
- Gilson MINIPULS 3 Peristaltic Pump (SKU: GFAM00051)
- Multichannel Systems stimulus generator (STG4004)
- **Cairn Research OptoLED Light Source**
- **Digitimer DS3 Isolated Current Stimulator**
- **FHC stimulating electrode (SKU: 30200)**

**Virus:**

- AAV5-hsyn-ACh3.0(ACh4.3) virus (WZ Biosciences, Cat #YL001003-AV5) - Titre: ~$8\times10^{12}$ GC/ml in saline

**Reagents:**

- Sodium Chloride (Sigma-Aldrich, Catalog #31434, CAS #7647-14-5)
- Sodium Bicarbonate (Sigma-Aldrich, Catalog #31437-M, CAS #144-55-8)
- HEPES (Sigma-Aldrich, Catalog #H3375, CAS #7365-45-9)
- HEPES Sodium Salt (Sigma-Aldrich, Catalog #H7006, CAS #75277-39-3)
- Potassium Chloride (Sigma-Aldrich, Catalog #P9541, CAS #7447-40-7)
- Calcium Chloride Solution (Sigma-Aldrich, Catalog #21114, CAS #10043-52-4)

**Software:**

- ImageJ (1.5)
- ImageJ plugin: **Micro-Manager Reader 1.3.38, May 3, 2009**
- **MATLAB** (R2020b)

## Troubleshooting

## Before start

Stereotaxic intracranial injections were performed to deliver AAV5-hsyn-ACh3.0(ACh4.3) virus in the mouse striatum following the **Protocol: Intracranial injections of viral vectors in mouse striatum**.

## Slice Preparation

1 Sacrifice mice by cervical dislocation and exsanguination, and collect the brain.

2 Cut 300 µm thick coronal slices using a vibratome in ice-cold HEPES-based buffer saturated with 95% $O_2$/ 5% $CO_2$, containing (in mM): 120 NaCl, 20 $NaHCO_3$, 6.7 HEPES acid, 5 KCl, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, 1.2 $KH_2PO_4$, 10 glucose.

3 Keep slices in a holding chamber for at least 1 hour at room temperature (20-22°C) in HEPES-based buffer before transferral to the recording chamber.

4 While slices are incubating, prepare the recording solution (artificial cerebrospinal fluid, aCSF). Bicarbonate-buffer based aCSF contains (in mM): 125 NaCl, 26 $NaHCO_3$, 3.8 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.2 $KH_2PO_4$, 10 glucose.

5 Clean the recording chamber and perfusion system with dH2O and then run aCSF through (perfusion rate ~ 2 ml/min).

Note

Ensure that the recording chamber temperature is stable at 32°C (fluctuation range 31-33°C is acceptable).

6 Following 1 hour incubation, bisect slices and transfer to the recording chamber. Leave slice to equilibrate in the bath for for 30 minutes prior recording.

## Check GRAB expression

7 Place brain slice under a x10/0.3 NA water-immersion objective (Olympus) using an Olympus BX51WI microscope equipped with a 470 nm OptoLED light system (Cairn Research), Iris 9 Scientific CMOS camera (Teledyne Photometrics), and 525/50 nm emission filter (Cairn Research).

8 Turn on blue LED light (470 nm, ~ 10-11 mW; OptoLed, Cairn Research) to check whether GRAB sensor is expressed in the desired region.

Note

Iluminate the slice through the microscope turret and objective.

## Image Processing and Data Extraction

9 Acquire images under a x10/0.3 NA water-immersion objective (Olympus) using the ImageJ plugin MicroManager.

10 After choosing a region of good sensor expression, position the stimulating electrode on tissue.

10.1 Electrically stimulate acetylcholine (ACh) release using a surface bipolar concentric Pt/Ir electrode (FHC Inc., outer/inner diameter 125/25 µm), applying pulses at 0.6 mA and for 200 µs.

Note

Electrical stimulations, LED light, and image acquisition were syncronised using TTL-driven stimuli via Multi Channel Stimulus II (Multi Channel Systems).

10.2 If needed, change the camera acquisition rate.
**10.2.1.** The camera default acquisition rate (30 frames per second, fps) can be modified to obtain higher sampling frequency.
**10.2.2.** To achieve the highest sampling rate (100 fps), lower the camera exposure to 10 ms, and define a rectangular recording window smaller than the field of view.
**10.2.3.** The recording window should include the tip of the stimulating electrode and be large enough to allow sampling from multiple regions.

11 Once the acquisition parameters have been finalised, proceed with acquisition.

## Image Processing and Data Extraction

11.1 Acquire images at 10 Hz (100 ms exposure duration) during continuous blue LED light for a 10 second recording window.

11.2 Electrically evoke acetylcholine (ACh) release every 2.5 minutes to ensure full recovery of terminals between pulses.

## Image Processing and Data Extraction

12 Open the image saved for each stimulation in ImageJ to extract fluorescence information.

13 Acquire one image for each stimulation condition applying continuous blue light for the time needed for the signal to reach baseline.

Note

The baseline depends on the off rates of different GRAB sensors.

14 Draw a square (100 µm x 100 µm) region of interest (ROI) and position 50 µm away from the tip of the stimulating electrode.

## Image Processing and Data Extraction

15 Draw an ROI of the same size and position on the tip of the stimulating electrode to use as background.

## Image Processing and Data Extraction

16 Select Images > stacks > plot z-axis profile to extract fluorescence and frame values from the ROIs. Save the data in Excel.

17 It is possible to select multiple ROIs at different distances from the electrode to record signals further away from the stimulation source.

## Data Analysis

18 Analyse frame and fluorescence values with a **custom written MATLAB script**.

19 Convert the frames into time (s), knowing the acquisition sampling rate.

20 Subtract the background from the raw fluorescence values. Background was chosen as a ROI with no fluorophore expression (i.e. cortex) to account for non-specific

changes in ambient light.

21 The baseline ($F_0$) fluorescence was calculated as the average fluorescence intensity over a 1 second period before the onset of stimulation.

22 Calculate $\Delta F/F_0$ as $(F-F_0)/F_0$.