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Confocal microscopy to assess TelC and iGluSnFR expression in fixed mouse brain slices

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We use this protocol and it's working

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Abstract

This protocol describes the tissue processing steps following (a) glutamate recordings using the genetically encoded glutamate sensor (iGluSnFR) and (b) behaviour experiments following tetanus toxin light chain (TelC) viral injection in the dorsal medial striatum.

Before start

This protocol was performed in **ChAT-IRES-Cre** mice (B6;129S6-Chattm2(cre)Lowl/J, JAX stock number 006410).

To block acetylcholine release from cholinergic interneurons, we injected **Cre-dependent Tetanus toxin light chain (TelC)** viral vector (RRID:Addgene_135391) in the dorsal medial striatum (aDMS) of ChAT-IRES-Cre mice.

To measure rapid changes in glutamate release, we expressed the **genetically encoded glutamate sensor iGluSnFR** selectively in anterior dorsal striatum (aDS) cholinergic interneurons of ChAT-IRES-Cre mice.

PFA Fixation by Intracardiac Perfusion & Brain Dissection

1 Perfuse intracardially with phosphate-buffered saline (PBS 1%, Fisher Scientific), followed by paraformaldehyde (PFA 4% in 1% PBS, Fisher Scientific).

2 Carefully extract brains and fix them overnight in 4% PFA dissolved in PBS.

3 Transfer brains to solution of 40% sucrose in 1% PBS (until the brains sank).

Cryosectioning & Mounting

4 Slice brains (50µm thickness) with a cryostat (Leica CM3050 S).

5 Mount coronal sections on super frost slides and cover slipped with Vectashield antifade mounting medium (Vector Laboratories, H-1900).

Image Acquisition using Confocal Microscopy

6 Acquire images on a Zeiss LSM 800 laser scanning confocal microscope.

Note

TelC and iGluSnFr fluorescence were not immuno-enhanced.