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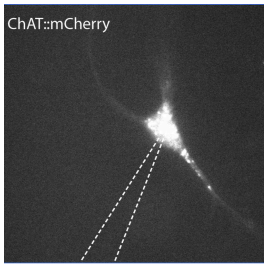
# Whole-cell Patch-Clamp Recordings from Striatal Cholinergic Interneurons in ex vivo Mouse Brain Slices V.1

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

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## Abstract

This protocol describes the steps to perform whole-cell patch-clamp recordings of striatal cholinergic interneurons (ChIs) previously labelled with the fluorophore mCherry from acute, *ex vivo* mouse brain slices.

## Image Attribution

Stedehouder, Roberts et al. (In Review)

## Guidelines

The high quality of whole-cell patch-clamp recordings critically depends on four, partially interrelated, variables:

**First**, all solutions have to be made fresh, in clear, rinsed glassware, with particular care on inclusion of all ingredients and carefully weighed amounts.

**Second**, slice quality is extremely important. The brain has to be removed from the skull quickly without nicking, damaging or putting any type of pressure on the brain and transferred to an ice-cold solution. Then, with minimal manipulation, 300 coronal slices should be cut with a sharp blade, and the slices, individually, are only manipulated to a minimal amount (touched, moved etc.).

**Third**, selection of healthy-looking cells is paramount, that do not look bloated, damaged, too deep, or are obscured by other cells.

**Four**, high giga-seal (>GOhm) should be attained before breaking in followed by proper access (low MOhm) of the cell.

## Materials

**Reagents:**

- AAV5-hSyn-DIO-mCherry **(ETH Zurich Viral Vector Facility)** or equivalent
- Salts
- Sodium pentobarbital

**Equipment:**

- **VT1200S Vibrating blade microtome** (Leica)
- **Multiclamp 700B amplifier** (Molecular Devices Corp.)
- **Digidata 1440A acquisition board** (Molecular Devices Corp.)
- P-1000 Horizontal Pipette Puller (Sutter Instruments)

**Mouse Lines:**

- **Heterozygous ChAT-Cre mice (B6;129S6-Chattm2(cre)Lowl** (The Jackson Laboratory) or equivalent

**Preparation of Preparation of NMDG-based cutting solution (~310 mOsm, pH 7.4) :**

- N-methyl-d-glucamine (NMDG), 93 mM
- HCl, 93 mM
- $NaHCO_3$, 30 mM
- D-glucose, 25 mM
- HEPES Buffer, 20 mM
- Na-ascorbate, 5 mM
- Thiourea, 2 mM
- $MgCl_2$, 7mM
- Na-pyruvate, 3 mM
- KCl, 5 mM
- 1.25 $NaH_2PO_4$, 1.25 mM
- $CaCl_2$ 0.5 mM

Adjust pH to 7.4 using HCl. Made fresh on the morning of the experiment

**Preparation of artificial cerebrospinal fluid (aCSF):**

- NaCl, 127 mM
- NaHCO3, 25 mM
- D-glucose, 25 mM
- KCl, 2.5 mM
- NaH2PO4, 1.25 mM

- MgSO4,  5 mM
- CaCl2, 1.6 mM

Made fresh on the morning of the experiment

**Prepare intracellular solution ( ~290 mOsm, 7.4 pH):**

- K-gluconate, 120 mM
- KCl, 10 mM
- HEPES, 10 mM
- K-phosphocreatine, 10 mM
- ATP-Mg, 4 mM
- GTP, 0.4 mM

Adjust pH to 7.4 using KOH. Stored in 500-1000 µl aliquots in a -20 freezer. Thaw a single aliquot for use on the day of experiment, filter through a 20 µM filter.

**Software:**

- Python
- Clampex 10.0 (Molecular Devices Corp.)
- Linlab

## Troubleshooting

## Safety warnings

Blades!

Acids!

Sharps!

## Ethics statement

Experiments involving animals must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Usa Committee (IACUC) or equivalent ethics committee(s). **Prior ethics approval should be obtained before performing these experiments.**

## Injection of mCherry virus to label cholinergic interneurons

1 Inject AAV5-hSyn-DIO-mCherry (~ 1.3 × $10^{13}$ genome copies/mL,) of equivalent AAV bilaterally or unilaterally into the Caudate Putamen (CPu) (ML ±1.75 mm from bregma, AP +0.8 mm from bregma, DV -2.4 mm from brain surface) or Nucleus Accumbens core (NAc) (ML ±1.2 mm from bregma, AP +1.3 mm from bregma, DV -3.75 mm from brain surface) of heterozygous adult ~8-16 weeks old *ChAT*-cre male or female mice.

## Preparation of *ex vivo* mouse brain slices

1h 15m

2 At 3-5 weeks post-injection, induce anaesthesia using i.p. sodium pentobarbital (200 mg/kg)

3 Decapitate mouse and quickly remove the brain in ice-cold, NMDG-based cutting solution (see **Materials**) oxygenated with 95% $O_2$/5% $CO_2$.

4 Slice striatum into 300 µm coronal slices using a vibrating blade microtome (Leica VT1200S)

5 Incubate slices in a heat bath at 34°C for 15 mins in NMDG-based cutting solution (see **Materials**). If fluorescence is present in the slices, keep slices in the dark as much as possible.

15m

6 Carefully transfer slices with minimal fluid transfer to artificial cerebrospinal fluid (aCSF; see **Materials**) oxygenated with 95% $O_2$/5% $CO_2$ and incubate at 34°C for another 15 mins

15m

7 Take the slices out of the heat bath and allow slices to recover at room temperature for at least 1 hour in aCSF oxygenated with (95% $O_2$/5% $CO_2$). Use slices within ~6 hours from cutting

1h

## Whole-Cell Patch-Clamp Recordings

8 Gently place a coronal slice in a rig recording chamber perfused with aCSF oxygenated with 95% $O_2$/5% $CO_2$ at 32-33 °C at a rate of ~3 ml/min. Weigh the slice down with a harp or metal clips

9 Use the DIC mode on the microscope at 40x to identify your region of interest based on gross landmarks (e.g. anterior commissure for NAc)

10 Turn off the brightfield illumination. Turn on your red wavelength LED or laser, switch to the TXRED (or equivalent wavelength) filter cube and briefly illuminate the slide by opening the shutter and look for mCherry+ somata. Keep the illumination brief (<1 s) to prevent phototoxicity

11 Center and focus over the soma of this cell, check if the cell looks healthy and patchable (between ~25 to 150 µm from surface; clearly visible and approachable)

12 Pull a glass pipette (1.5 mm OD x 0.86 mm IK x 100 mm L; Harvard Apparatus ,30-0057) using an appropriate puller (e.g. P1000 Horizontal Pipette Puller, Sutter Instruments)

13 Backfill the glass pipette with ~10-15 µl of freshly thawed intracellular solution (See **Materials**). Avoid bubbles near the tip of the pipette. If bubbles do occur, gently tap the pipette with the tip facing downwards

14 Load the pipette on the headstage and place the pipette near the slice in aCSF. The pipettes should have resistance ranging from 3–5 MΩ. Log the exact pipette resistance in your lab journal

15 Perform whole-cell recordings from mCherry-positive neuronal somata. Record access resistance, capacitance and 'resting' membrane potential of the cell. Fully compensate for bridge balance and capacitance. Remove recordings in which the series resistance exceeds ~25 MΩ.

16 Run stimulation protocols tailored for the specific experimental question. For example, run depolarizing current steps in current clamp of 750 ms from -100 pA to +200 pA.

Record data using Clampex 10.0 using a Multiclamp 700B amplifiers and Digidata 1440A acquisition board digitized at 20 kHz sampling rate.

17 Run additional protocols. Keep an eye out for changes in 'resting' membrane potential, holding potential or series resistance

## Data Analysis

18 Transfer data from recording computer to a server or drive with mirrored back-up. Perform data analysis offline using custom written Python software

19 Determine basic physiological characteristics from voltage responses to square-wave current pulses of 750 ms in duration ranging from -200 pA to +300 pA in 25 pA steps for ChIs.

20 Determine input-resistance by the slope of the linear regression through the voltage-current curve from -200 pA to 0 pA.

21 Determine Sag from the ratio voltage difference between the lowest voltage response and the steady-state response at the last 100 ms to a square-wave current pulses of 750 ms duration from 0 pA to –200 pA.

22 Determine single action potential (AP) characteristics from the first elicited action potential.

23 Calculate 'Resting' membrane potential was calculated as the average membrane voltage during the 500 ms interval before current injection. No correction is required for liquid junction potential.

24 Define AP threshold from the inflection point at the foot of the regenerative upstroke, where the first derivative exceeded 10 mV/ms.

25 Define AP amplitude as the voltage difference between the threshold and peak voltage.

26 Measure AP half-width at half of the peak amplitude.

27 Measure the after-hyperpolarizing potential (AHP) amplitude as the peak hyperpolarizing deflection from AP threshold following AP initiation.

28 Determine spike frequency adaptation from the first ISI and last ISI in response to a +100 pA square-wave current pulse of 750 ms duration.

## Protocol references

Stedehouder *et al.* (2019) Local axonal morphology guides the topography of interneuron myelination in mouse and human neocortex. **eLife**. Nov 19;8:e48615. doi: 10.7554/eLife.48615.