

## Protocol

# Single-Cell Electroporation of Neurons

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Single-cell electroporation allows the transfection of a small number of neurons in an organotypic culture with a single plasmid or a defined mixture of plasmids. Desired protein expression levels can vary depending on the experimental goals (e.g., high expression levels are needed for optogenetic experiments); however, when too much protein is expressed, cellular toxicity and cell death may arise. To a large degree, protein expression can be controlled by adjusting the concentration of plasmid DNA in the electroporation pipette. Here, we present a protocol for transfecting individual neurons in hippocampal slice cultures by electroporation. Essentially, a patch-clamp setup is required that includes an upright microscope with infrared differential interference contrast or Dodt contrast with a camera and a specialized amplifier that is able to deliver large-voltage pulses to the electroporation pipette.

## MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

## Reagents

Alexa Fluor 594 (Invitrogen A33082, or other dye compatible with available filter sets)

Hippocampal slice cultures on inserts (at least 5 d in vitro [DIV], optimally 2 wk)

*For preparation of hippocampal slice cultures, see Protocol: [Preparation of Slice Cultures from Rodent Hippocampus](#) (Gee et al. 2016).*

K-gluconate-based intracellular solution <R>

Plasmid DNA in TE buffer (pH 7.4)

*Prepare using any standard commercial purification kit.*

Saturated FeCl<sub>3</sub> solution

Slice culture medium <R>

Slice culture transduction solution (sterile, prewarmed to 37°C) <R>

## Equipment

Centrifuge (table-top, refrigerated, e.g., Eppendorf 5415 R)

Culture plates (six-well; e.g., Corning 3516 or Sarsted 83.1839)

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### Electroporation setup

20×–40× water immersion objective (20× with a variable magnifier tube before the camera)

Axoprotor 800A with pipette holder (Molecular Devices)

Epifluorescence illumination and filters (optional: see Step 11)

Headphones or speakers

Microscope chamber consisting of glass microscope slide (70 × 100 × 1 mm) onto which a Teflon ring (inner diameter ~35 mm, ~2 mm high) is affixed with silicone aquarium sealant

Motorized micromanipulator (e.g., from Luigs & Neumann or Sutter)

Silver wire (~0.25 mm diameter)

Upright microscope with motorized stage, CCD/CMOS (charge-coupled device/complementary metal oxide semiconductor) or video camera and IR-DIC (infrared-differential interference contrast) or Dodt contrast

Forceps (coarse; e.g., Fine Science Tools 11002-16)

Hot bead sterilizer (e.g., Fine Science Tools 18000-45)

Incubator (37°C/5% CO<sub>2</sub> with rapid humidity recovery, copper chamber recommended; e.g., Hera-cell 150i/160i, Thermo Scientific)

Micropipette puller (e.g., PC-10, Narishige)

Thin-walled borosilicate glass capillaries (WPI TW150F-3)

Tissue culture dishes (60-mm, sterile; Sarstedt 83.1801)

Tissue culture hood

Ultrafree centrifugal filter units (UFC30GV0S, Millipore)

## METHOD

### Preparation of DNA

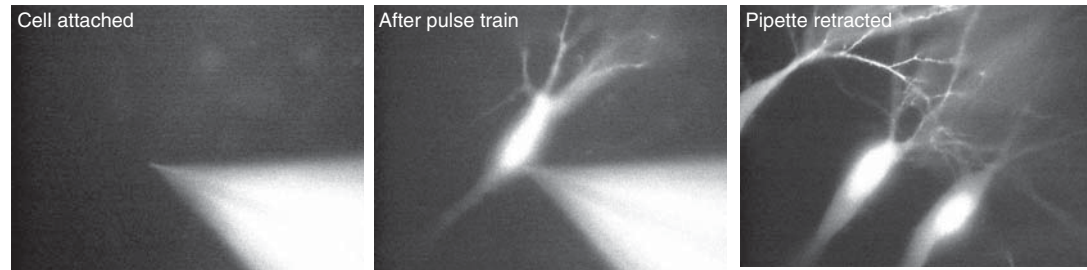
1. Dilute Alexa Fluor 594 to a final concentration of 20 μM in K-gluconate-based intracellular solution and filter sterilize the solution through a Millipore Ultrafree centrifugal filter unit by centrifugation at 16,100g for several seconds in a table-top centrifuge at 4°C.
2. Remove the filter insert and add plasmid DNA to the desired final concentration. Centrifuge the solution again for 5 min at 16,100g, 4°C to pellet debris.

*One hundred microliters of solution will be enough for more than 50 slices and can be stored between uses at –20°C. It is important that the DNA-containing solution is not passed through the Millipore Ultrafree centrifugal filter unit. The optimal final DNA concentration must be determined empirically and is usually 1–100 ng/μL.*

### Single-Cell Electroporation

*The hippocampal slice cultures used in this protocol must have been prepared under stringent sterile conditions and all steps in this procedure have to be performed under sterile conditions. For precautions to avoid contamination, see Protocol: **Preparation of Slice Cultures from Rodent Hippocampus** (Gee et al. 2016). It is recommended that the electroporation setup be close to the tissue culture hood. Furthermore, construction of a cabinet around the electroporation microscope with a fan and HEPA (high-efficiency particulate arrestance) filter to blow clean air down over the setup will reduce the incidence of contamination to almost never (for further description of this setup, see Protocol: **Viral Vector-Based Transduction of Slice Cultures** [Wiegert et al. 2016]).*

3. Coat the tips of silver wires for the electroporation and grounding electrodes with Cl by bathing them in FeCl<sub>3</sub> solution for a few minutes (when scratched) or overnight (when new).
4. Place a fresh six-well plate containing 1 mL of slice culture medium in each well in the incubator for pre-equilibration.
5. Fabricate electroporation pipettes using a micropipette puller to pull thin-walled borosilicate capillaries to a resistance of 10–15 MΩ when filled with the intracellular solution. For each slice to



**FIGURE 1.** Single-cell electroporation. Fluorescence images taken during electroporation procedure. (Left) Pipette filled with DNA solution and Alexa Fluor 594 touching target cell. (Middle) Immediately after applying pulse train, target cell is filled with DNA solution and Alexa Fluor 594. (Right) Successfully electroporated neurons after retraction of the pipette.

be electroporated, back-fill one electroporation pipette with 1.5  $\mu\text{L}$  of DNA/Alexa Fluor 594 solution (from Step 2).

*Several pipettes can be filled at once and kept upright (tip down) for 1–2 h.*

6. Working in a tissue culture hood, pipette 1 mL of transduction solution prewarmed to 37°C into the microscope chamber. Use sterile forceps to transfer one slice culture insert into the chamber and add another 2 mL of transduction solution on top of the slice cultures. Place forceps into the hot bead sterilizer for  $\sim 10$  sec to resterilize between handling of inserts.
7. Cover the microscope chamber with a sterile 60-mm dish and transfer to the microscope.
8. Approach the selected cells with the electroporation pipette while applying positive pressure to the pipette by mouth. Monitor the tip resistance, which should be 10–15  $\text{M}\Omega$ , by the audio output of the Axoporation 800A amplifier. Make sure the tip is not clogged by monitoring tip resistance and expulsion of Alexa Fluor 594 fluorescence if needed.
9. Move the tip of the electroporation electrode close to a cell of interest while reducing pressure (easily controlled by mouth by blowing less hard). Monitor the tip resistance acoustically.
10. Approach the cell without sealing the electrode with membrane from other cells in the tissue.
11. Lightly touch the plasma membrane, which will cause a rise in pitch and tip resistance.
12. Release the pressure (do not apply suction) and wait for resistance to increase to 25–40  $\text{M}\Omega$ ; however, avoid the formation of a  $\text{G}\Omega$  seal.
13. Apply pulse train (e.g., voltage:  $-12$  V, pulse width: 500  $\mu\text{sec}$ , frequency: 50 Hz, train duration 500 msec; the optimal settings may differ depending on cell type).
14. Slowly retract the pipette and begin applying very light pressure when 2–5  $\mu\text{m}$  away from the soma. Increase pressure at larger distances.  
*Do not apply too much pressure while retracting the pipette, otherwise the cell may rupture. Electroporation success can be instantly evaluated by assessing Alexa Fluor 594 fluorescence in electroporated cells (Fig. 1).*
15. Repeat Steps 8–14 to electroporate more cells.
16. Change the pipette before electroporating the second culture on insert and when the pipette becomes clogged.
17. Cover the chamber with a 60-mm dish and transfer to the tissue culture hood. Aspirate all solution and return the insert to the slice culture medium in the six-well plate (from Step 4).

*The optimal time between electroporation and starting an experiment has to be determined empirically for each plasmid.*

## RELATED INFORMATION

This protocol is based upon the electroporation of single neurons in organotypic cultures described by Rathenberg et al. (2003).

## RECIPES

### *K-Gluconate-Based Intracellular Solution*

Reagent	Final concentration	Amount (for 50 mL)
Aldrich K-gluconate (Sigma-Aldrich G4500)	135 mM	1.581 g
Aldrich EGTA (Sigma-Aldrich E0396)	0.2 mM	0.004 g
Aldrich HEPES (Sigma-Aldrich H4034)	10 mM	0.119 g
MgCl <sub>2</sub> (1 M; Fluka 63020)	4 mM	0.200 mL
Aldrich Na <sub>2</sub> -ATP (Sigma-Aldrich A3377)	4 mM	0.121 g
Aldrich Na-GTP (Sigma-Aldrich G8877)	0.4 mM	0.010 g
Aldrich Na <sub>2</sub> -phosphocreatine (Sigma-Aldrich P7936)	10 mM	0.128 g
Aldrich Ascorbate (L-ascorbic acid; Sigma-Aldrich A5960)	3 mM	0.026 g

Adjust pH to 7.2 with KOH, and check osmolality (it should be 290–300 mOsm/kg). After mixing, filter-sterilize (0.2- $\mu$ m pore size) and divide into 100–500- $\mu$ L aliquots. Store at  $-20^{\circ}\text{C}$ .

### *Slice Culture Medium*

Reagent	Final concentration	Amount (for 500 mL)
MEM (Sigma-Aldrich M7278)		394 mL
Heat-inactivated horse serum <sup>a</sup>	20%	100 mL
L-glutamine (200 mM; Gibco 25030-024)	1 mM	2.5 mL
Insulin (1 mg/mL; Sigma-Aldrich I6634)	0.01 mg/mL	0.5 mL
NaCl (5 M; Sigma-Aldrich S5150)		1.45 mL
MgSO <sub>4</sub> (1 M; Fluka 63126)	2 mM	1 mL
CaCl <sub>2</sub> (1 M; Fluka 21114)	1.44 mM	0.72 mL
Ascorbic acid (25%; Fluka 11140)	0.00125%	2.4 $\mu$ L
D-glucose (Fluka 49152)	13 mM	1.16 g

<sup>a</sup>The serum is often a critical factor in slice quality and it is often necessary to test several batches (lots); three products that have been successfully used are Sigma-Aldrich H1138, Gibco 26050070, and Gibco 16050122. Gibco 16050122 must be heat-inactivated for 30 min at  $55^{\circ}\text{C}$ .

After mixing, filter-sterilize (0.2- $\mu$ m pore size), and store at  $4^{\circ}\text{C}$  in 50-mL aliquots. (The solution should be orange-red [i.e., pH  $\sim$ 7.3] and osmolality should be  $\sim$ 320 mOsm/kg.)

### *Slice Culture Transduction Solution*

Reagent	Final concentration	Amount (for 500 mL)
NaCl (Sigma-Aldrich S5150)	145 mM	4.23 g
HEPES (Sigma-Aldrich H4034)	10 mM	1.19 g
D-glucose (Fluka 49152)	25 mM	2.25 g
KCl (1 M; Fluka 60129)	2.5 mM	1.25 mL
MgCl <sub>2</sub> (1 M; Fluka 63020)	1 mM	0.5 mL
CaCl <sub>2</sub> (1 M; Fluka 21114)	2 mM	1 mL

Adjust pH to 7.4 with NaOH. After mixing all ingredients, check the osmolality—it should be 310–320 mOsm/kg. Filter-sterilize (0.2- $\mu$ m pore size) and store at  $4^{\circ}\text{C}$ .

## REFERENCES

- Gee CE, Ohmert I, Wiegert JS, Oertner TG. 2016. Preparation of slice cultures from rodent hippocampus. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot094888.
- Rathenberg J, Nevian T, Witzemann V. 2003. High-efficiency transfection of individual neurons using modified electrophysiology techniques. *J Neurosci Methods* 126: 91–98.
- Wiegert JS, Gee CE, Oertner TG. 2016. Viral vector-based transduction of slice cultures. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot094896.



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