Protocol

Viral Vector-Based Transduction of Slice Cultures

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Transgenes can be introduced into the cells of organotypic slice cultures using different delivery methods, such as biolistic transfection, electroporation, and viral vector-based transduction. These methods produce different patterns of transgene expression. Local injection of recombinant adeno-associated virus (rAAV) produces a small cluster of transgene-expressing neurons around the injection site. Expression in individual cells varies with the distance from the injection site, indicating that many neurons take up several rAAV particles. The serotype and promoter also play a role in transgene expression. Here, we present a protocol for the transduction of previously prepared hippocampal slice cultures with rAVV.

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

Hippocampal slice cultures on inserts (at least 3 d in vitro [DIV])

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

Recombinant adeno-associated virus (rAAV) suspension (concentration: 10^{11}–10^{13} GC/mL)
Slice culture medium (sterile) <R>
Slice culture transduction solution (sterile) <R>

Equipment

Compressed/pressurized air (for Picospritzer)
Culture plates (six-well; e.g., Corning 3516 or Sarstedt 83.1839)
Forceps (coarse; e.g., Fine Science Tools 11002-16, sterile)
Forceps (fine, No. 5 Dumont; e.g., WPI 500342, sterile)
Incubator (37°C/5% CO_2 with rapid humidity recovery, copper chamber recommended; e.g., Heracell 150i/160i, Thermo Scientific)
Microelectrode holder (e.g., WPI MPH6S)
Micromanipulator (e.g., LN Junior 3 or 4 axis; Luigs & Neumann or PatchMan, Eppendorf)
Micropipette puller (e.g., Sutter P-1000)

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Microscope (either an upright microscope with camera, wide-field illumination, and 4× objective or a USB microscope [e.g., dnt DigiMicro Profi] and movable stage)
Picospritzer III with foot switch (Parker Hannafin)
Sharps container
Thin-walled borosilicate glass capillaries with filament (WPI TW150F-3)
Tissue culture hood

METHOD

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 injection setup be close to the tissue culture hood. Furthermore, construction of a cabinet around the injection microscope with a fan and HEPA (high-efficiency particulate air) filter to blow clean air down over the setup (Fig. 1C) will reduce the incidence of contamination to almost never. A dedicated viral vector injection setup can be constructed using an inexpensive USB microscope and the required ancillary parts. This setup is shown in Figure 1. Viral vector injections can be performed equally well on an upright microscope using wide-field illumination and a low-magnification objective (i.e., a standard patch-clamp setup).*

1. Place a fresh six-well plate containing 1 mL of slice culture medium in each well in the incubator to allow for temperature and pH equilibration.
2. Fabricate injection pipettes using a micropipette puller to pull thin-walled borosilicate capillaries to obtain a long and narrow tip with a shallow taper. Insert an empty injection pipette into the holder attached to the microscope and focus on the tip (Fig. 1A). Break off the tip with fine forceps under visual guidance to achieve a tip diameter of ~10 µm.

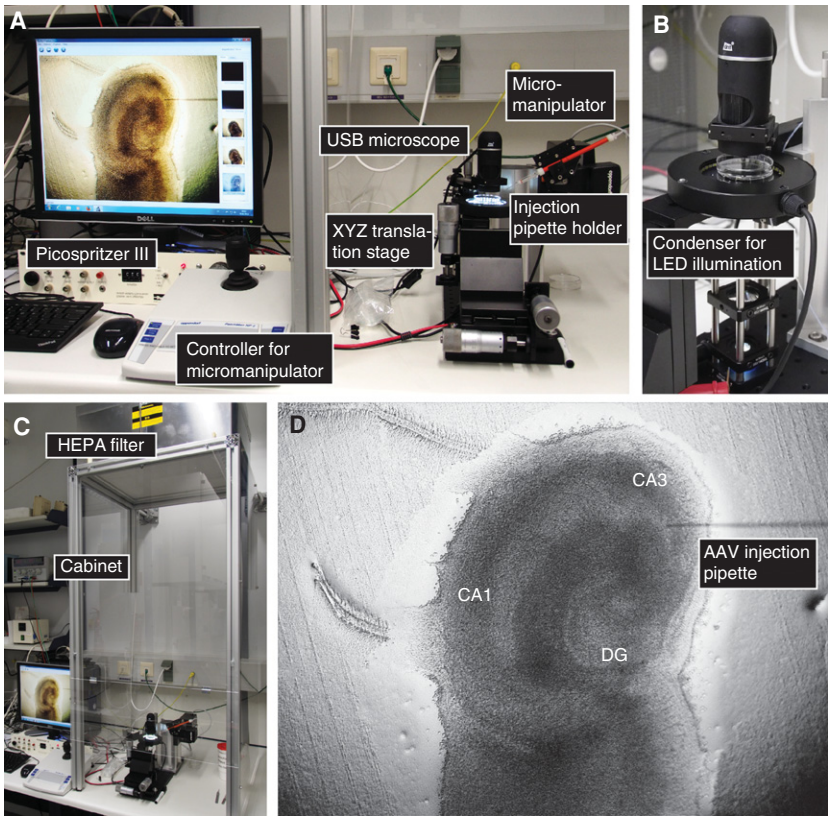

FIGURE 1. Injection of rAAV into CA3. (*A*) Photograph of USB microscope–based injection setup. (*B*) Detail of LED (light-emitting diode) illumination condenser. (*C*) View of setup showing surrounding cabinet. (*D*) Hippocampal slice culture with tip of injection pipette in area CA3.

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3. Remove the pipette from the holder and back-fill with 1.2 µL rAAV suspension (this typically lasts for 12 slice cultures). Hold the pipette tip down and wait until all liquid has migrated along the filament into the tip. Set the pressure at the Picospritzer to 1.8 bar (25 p.s.i.) and the pulse duration to 50 msec.
4. Insert the pipette into the holder, focus on the tip, and apply a pressure pulse to test whether the rAAV suspension is expelled from the tip. If the tip is open, a small droplet should emerge. This check is most easily and safely done with a membrane insert already in the chamber (see Step 5). If the tip of the pipette is positioned to just touch the membrane (away from the slice cultures to be injected), then the drop will form on the membrane. The pulse duration/pressure should be adjusted if larger/smaller injection volumes are desired.

   Perform all subsequent steps quickly to avoid degradation of the rAAV at room temperature and to minimize drying out of air-exposed slice cultures.

5. Working in a tissue culture hood, fill the microscope chamber with 800 µL transduction solution prewarmed to 37°C and, using coarse forceps, place an insert with slice cultures into the chamber.

   When using a USB camera setup, the lid of a sterile 35-mm tissue culture dish serves as the chamber. For injecting cultures on the stage of an upright microscope a large glass microscope slide (70 × 100 × 1 mm) onto which a Teflon ring (inner diameter 34 mm, 2 mm high) is fixed with silicone aquarium sealant should be used as the chamber. Before use, the chamber should be wiped with 70% ethanol and rinsed with sterilized transduction solution.

6. Transfer the chamber to the microscope and focus on the slice culture. Bring the pipette tip into view and move tip axially into the cell body layer of CA3 (Fig. 1A,D). Depending on the desired infection density, one to three pressure pulses should be given. If desired, this procedure can be repeated several times at neighboring sites until the whole target area is covered. Axially retract the pipette until it no longer touches the culture and move to the second slice culture on the insert to repeat the injection procedure.
7. Transfer the insert to the six-well plate newly prepared in Step 1. Repeat Steps 5 and 6 until all cultures are injected with viral constructs; the same injection pipette may be used for many slice cultures. When finished, remove the injection pipette immediately from the holder and dispose in a sharps container.

   The ideal time between rAAV infection and when cells may be stimulated with light depends on many factors including the promoter, rAAV serotype, and the construct itself. We recommend testing cultures at various intervals before beginning actual experiments.

RECIPES

Slice Culture Medium

Reagent	Final concentration	Amount (for 500 mL)
MEM (Sigma-Aldrich M7278)		394 mL
Heat-inactivated horse serum[a]	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
$MgSO_4$ (1 M; Fluka 63126)	2 mM	1 mL
$CaCl_2$ (1 M; Fluka 21114)	1.44 mM	0.72 mL
Ascorbic acid (25%; Fluka 11140)	0.00125%	2.4 µL
D-glucose (Fluka 49152)	13 mM	1.16 g

[a]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°C.

After mixing, filter-sterilize (0.2-µm pore size), and store at 4°C in 50-mL aliquots. (The solution should be orange-red [i.e., pH ~7.3] and osmolality should be ~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
$MgCl_2$ (1 M; Fluka 63020)	1 mM	0.5 mL
$CaCl_2$ (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-µm pore size) and store at 4°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.

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