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The Use of Herpes Simplex Virus in Ex Vivo Slice Culture

Allyson K. Friedman and

Department of Pharmacology and Systems Therapeutics, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, New York, NY 10029

Ming-Hu Han

Department of Pharmacology and Systems Therapeutics, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, New York, NY 10029, (212)-659-1729

Allyson K. Friedman: Allyson.Friedman@mssm.edu; Ming-Hu Han: Ming-Hu.Han@mssm.edu

Abstract

Herpes simplex virus (HSV) can be used for a wide range of genetic manipulation in *ex vivo* slices of central nervous system tissue from both young and adult rodents. The fast expression of the HSV viral mediated gene transfer, which can be engineered to produce cell-type specificity, can be utilized in slice cultures for a variety of purposes over a 1-4 day period with spatial and temporal specificity. This protocol exploits the rapid expression of HSV viral vectors by utilizing slice culture for electrophysiological recordings, avoiding the need to do intra-cranial viral injections.

Brain slice cultures maintain many aspects of *in vivo* biology, including functional local synaptic circuitry with preserved brain architecture, while allowing good experimental access and precise control of the extracellular environment, making them ideal platforms for quick access to evaluate expression effects of HSV viral mediated gene transfer on the molecular and cellular properties of specific neurons. This protocol provides an easy way to study neuronal function following expression of these HSV viruses.

Keywords

Slice culture; HSV; gene transfer; neuronal property

Introduction

Viral mediated gene transfer utilizing the fast expression of HSV viral vectors in *ex vivo* slice culture is a useful tool for quickly manipulating and examining the molecular and physiological properties of neuronal circuits. Brain slice cultures retains the cytoarchitecture and provide high neuronal connectivity in which to examine gene manipulation on neuronal function compared to cultured dissociated neurons (Stoppini et al., 1991). Importantly, this *ex vivo* system permits direct treatment with HSV viral mediated gene transfer in any brain region without recourse to the whole animal. This protocol has been successfully used in a variety of studies to examine changes in neuronal properties following expression of a

variety short term HSV expressing viral vectors (Cao et al., 2010; Choi et al., 2011; Han et al., 2006; Huang et al., 2008; Iniguez et al., 2010; Krishnan et al., 2007; Mazei-Robison et al., 2011).

In this protocol we will discuss preparation of culturing acute brain slice and for use with HSV infection in slice. The use of viral vectors in neurobiological research has become common place due to their increasing efficiency and ability to selectively deliver viral vectors to particular brain regions and conditional cell-type selective gene expression. HSV viral vectors can express larger gene sequences up to 100kb as well as up to two different transcription cassettes making it an ideal tool for exploring expression of larger channel sequences (Neve and Lim, 2001). Further, the quick stable and non-toxic expression up to 4-5 days makes it an ideal tool to use in slice cultures. Therefore, *ex vivo* expression of HSV viral manipulation in slice culture provides a unique and beneficial experimental system to explore neuronal functions.

Basic Protocol 1

Herpes Simplex Virus in *Ex Vivo* Slice Culture—This protocol details how to utilize viral mediated gene transfer in a slice culture system. This procedure allows for molecular and electrophysiological characterization following targeted HSV gene expression, without being encumbered by intra-cranial injection, whole animal behavioral effects or difficulties with accuracy of brain region targeting.

Materials

Gloves, latex

Gibco MEM medium

30 mM Hepes

20 mM D-glucose

5% B27

5.0 mM L-glutamine

25 unit/mL streptomycin/penicillin

Artificial cerebral spinal fluid (ACSF) (see recipe below)

Sucrose artificial cerebral spinal fluid (sACSF) (see recipe below)

0.22 μ m filter (Corning, cat # 430758)

70% ethanol solution

Six-well plate (Corning, CellBIND® 6 Well Clear Multiple Well Plates, Flat Bottom, with Lid, Sterile) (Product #3335)

Millicell culture plate insert (0.4 μ m) (cat # PICM03050)

Isoflurane

Airstone

Surgical scissors
Spatula
Iris scissors
Paint brushes, size 1 and 2 (Dick Blick Wonder White, 2026)
Razor
Fresh tissue
Instant glue
Ice
Transfer pipette
Sterile stereological pipette (1 ml)
Herpes simplex virus (HSV) viral vector
Hood, tissue culture, laminar-flow
Carbogen tank (95% Oxygen and 5% carbon dioxide)
Microslicer (DTK-1000, Ted Pella)
Water bath
Microscope, dissection (Leica, WILD M3C)
Incubator (5% CO₂, 100% humidity, 34°C)

Protocol steps

Solution preparation: Contamination can occur at any step in when culturing acute brain slices. Care should be taken to maintain the sterility of six-well plates, pipettes and all tools that are used for solution preparation, brain tissue removal and HSV infection.

1. Prepare culture medium according to recipe using sterile technique.
2. Prepare sucrose artificial cerebral spinal fluid (ACSF) and ACSF according to recipe using sterile technique.
3. Filter 1× ACSF and sucrose ACSF with 0.22 µm filter (Corning, cat # 430758). Stock filtered solutions will last for a couple of weeks.
 - a. Make the solutions in standard lab conditions.
 - b. Filter solutions in laminar-flow hood and avoid contamination.
4. Using the airstone and carbogen tank, oxygenate the ACSF and the sucrose ACSF.
 - a. Chill sucrose ACSF
 - b. Place a beaker of oxygenated ACSF containing an immersed membrane into a 37° water bath.

5. Sterilize all dissection tools by placing all tools in a bath of 70% ethanol for at least 20 minutes; including air stones, slicing dish, razor, brain slice platform (Styrofoam), and small pipette.
6. Place culture medium in a water bath at 34°.
7. In a laminar flow hood or biosafety cabinet, fill each well of a six-well plate with a millicell culture plate insert (0.4 μ m) (cat # PICM03050) with 1 mL of warmed (34°C) culture medium solution.
8. Place six-well plate with millicell inserts immediately in incubator at 34 degrees.

Collection of brain tissue

9. Place surgical scissors, scalpel, razor, iris scissors, paint brush, spatula, slice chamber and suction pipette in 70% ethanol solution to sterilize.
10. Anesthetize rodent with isoflurane.
11. Swiftly decapitate the rodent with sterilized surgical scissors.
12. Utilizing sterilized iris scissors make a midline incision to remove the skin from the top of the skull.
13. Expose the brain by cutting the skull up the midline to the eye socket without damaging the underlying brain tissue.
14. Carefully remove the brain with a spatula by flipping the olfactory bulb up and out of skull.
15. Place the brain directly into a sterilized, chilled, oxygenated and filtered ACSF in which sucrose has replaced sodium chloride for 1 minute (sucrose ACSF).
 - a. Cold and oxygenated solutions function to firm the tissue prior to slicing.
16. Using a razor cut off the excess brain tissue and roughly isolate the tissue region of interest in a 5-10 cm block

Preparation of brain slice

17. Place a streak of instant glue on the sterilized slice cutting chamber.
18. Using sterilized spatula and paint brush to transfer the brain section, transfer the tissue to the slice cutting chamber.
19. Rapidly cover the tissue with the oxygenated and chilled sucrose ASCF.
20. Cut acute brain slices (250 μ m) using a microslicer (DTK-1000, Ted Pella) in sucrose-ACSF, that is saturated by 95% O₂ and 5% CO₂.
21. Slice a small section of desired brain region, and trim the section as 2-4 mm wide strips (make sure it is large enough to be held down by anchor if recording).
 - a. It is essential for the health of the slice in culture to reduce excess tissue allowing for culture medium and oxygen to reach target tissue.

22. Transfer brain slice into the oxygenated ACSF placed in a water bath (37° C) for one hour with a transfer pipette.

- a. Be careful not to expose tissue to the air.

23. Transfer tissue strips to culture inserts on a six-well plate and remove excess ACSF solution around the tissue strips with a transfer pipette (2-3 tissue strips per culture insert).

24. Place six-well plate into incubator at 34 °C.

HSV viral infection of brain slice

25. Place HSV virus on ice to defrost.

26. Remove six-well plate from incubator and place in laminar flow hood.

27. Under a dissection microscope in the hood pipette 0.4- 0.6 µl on each strip of tissue using a 0.1-10 µl pipette tip directly over the desired region.

- a. Ensure that during the HSV virus application the virus washes over the surface of the tissue.

28. Repeat this procedure at 5 minute intervals (in dissecting hood), placing the slice back in the incubator between pipetting virus.

- a. Depending on titer of the virus repeat 3-6 times. The higher the titer the fewer times it is necessary to apply the virus.

- b. Be sure to utilize a proper HSV control virus.

29. Twenty four hours later check for contamination (visible small dots). If proper sterile technique was used there should be no contamination.

- a. If visible contamination is present do not use the slice culture for neuronal characterization.

Electrophysiology on HSV infected brain slice

30. Cut membrane out of 6 well insert dish, place in petri dish with oxygenated ACSF (small volume). Do not let slice flip over.

- a. Only the surface of the slice that the virus was pipetted on will be infected.

31. Transfer to recording chamber that has a constant flow of oxygenated ACSF solution with the membrane (be sure to remove suction when anchoring the strip of tissue). Place anchor line in center of slice.

32. Wait 30 minutes to an hour for slice to acclimatize to the perfusing oxygenated ACSF and temperature differential.

33. Infection is seen 24 hours post dropping the virus on and cultured slices remain healthy up to 3-4 days post infection.

Reagents and Solutions

Sucrose artificial cerebral spinal fluid (sACSF)

254 mM Sucrose

3 mM KCl,

1.25 mM NaH₂PO₄,

10 mM D-glucose,

24 mM NaHCO₃,

2 mM CaCl₂

2 mM MgCl₂

(Oxygenated with 95% O₂ and 5% CO₂, pH 7.35, 295–305 mOsm) Make fresh daily

Artificial cerebral spinal fluid (ACSF)

128 mM NaCl

3 mM KCl,

1.25 mM NaH₂PO₄,

10 mM D-glucose,

24 mM NaHCO₃,

2 mM CaCl₂

2 mM MgCl₂

(oxygenated with 95% O₂ and 5% CO₂, pH 7.35, 295–305 mOsm) Make fresh daily

Culture Medium

Gibco MEM medium

30 mM Hepes

20 mM D-glucose

5% B27

5.0 mM L-glutamine

25 unit/mL streptomycin/penicillin.

Store 1 ml aliquots at -20 degrees

Commentary

Background Information

Viral vector gene manipulation is becoming a common tool in biomedical research. Significant advances in neurobiological research have been made with the expanded use of these HSV viral vectors to produce short-term gene expression in central nervous system

tissue. HSV viral vector expression is utilized for electrophysiological recordings, track tracing studies and introducing channelrhodopsin for optogenetic manipulations assisting in significant advances in our understanding of the neuronal function in a variety of disease models, including addiction and depression (Chaudhury et al., 2012; Friedman et al., 2014; Lobo et al., 2010). HSV viral vectors are commonly and successfully used for both *in vitro* or *in vivo* studies in rodent models (Christoffel et al., 2011; Coque et al., 2011; Dietz et al., 2012; Han et al., 2009; Koo et al., 2012; Krishnan et al., 2008; Kurita et al., 2012; Maze et al., 2014; Wallace et al., 2009). The use of these viral vectors tools in *ex vivo* acute brain slice culture is a growing technique with multitude of advantages and has allowed for further progress to be made in neuroscience research (Cao et al., 2010; Choi et al., 2011; Han et al., 2006; Huang et al., 2008; Iniguez et al., 2010; Krishnan et al., 2007; Mazei-Robison et al., 2011).

Critical Parameters

The preparation of this culture medium has been optimized for acute brain slice culture of mice and rats, and is slightly different compared to traditionally used culture medium recipes. Health of the neuronal function is dependent upon this culture medium, which is specifically important to maintain the native baseline firing activity of some neurons such as locus coeruleus neurons (Cao et al., 2010; Han et al., 2006). We observed that the baseline firing of locus coeruleus neurons in slice cultures obtained from young adult rats was sensitive to the culture medium and disappeared in traditional culture medium. Maintaining the incubator at 34° is also a critical component to maintain the health of the neurons. Adjusting these parameters will alter neuronal health and adjustments may be necessary depending on the brain region being investigated.

Troubleshooting

Acute brain slice health—Optimize the pH and osmolarity of the ACSF solutions to maintain health of the slice. Maintain brain tissue chilled and oxygenated throughout slicing process. Avoid contact with air or excessive mechanical stress to the tissue.

Contamination—Care should be taken to use sterile technique and to keep the sash as low as possible to minimize contamination throughout the brain slice preparation and when infecting slices with the HSV.

Viral expression—Increasing the number of applications of the HSV viral vector will increase the amount of infection. Pipette virus based on the level of titer of individual virus and the amount of expression desired.

Incubator—The slice culture must be maintained at 100% humidity in the incubator during infection to avoid cell death.

Proper control—Be sure to have proper control viruses for the desired experimental comparisons. Further, it is helpful to have recordings from non-infected neurons in the same slice as an additional control, which will provide confirmative information about the health of infected neurons.

Anticipated Results

The ideal result is infection of the desired population of neurons within 24 hours. Depending on the gene being inserted it may take up to 48-72 hours before the molecule is integrated into either the signaling pathway or membrane to effect overall neuronal function. Utilizing HSV vectors tagged with an eYFP can allow for quick confirmation.

Time Considerations

A tremendous advantage of this procedure is that infection can be observed as early as 3-4 hours post infection with HSV and can be observed up to 4-5 days. Solution preparation, acute slice preparation and HSV infection can take 4 hours. The following electrophysiological recordings can be performed for at least 5 hours with maintenance of healthy slice.

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