Journal of Neuroscience Methods 201 (2011) 177–179

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Short communication

Visualization of virus-infected brain regions using a GFP-illuminating flashlight enables accurate and rapid dissection for biochemical analysis

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ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 13 July 2011
Accepted 18 July 2011

Keywords:
Lentivirus
EGFP
Bluestar flashlight
Tissue dissection
Viral vector

ABSTRACT

Engineered viral vectors tagged with a fluorescent protein such as enhanced green fluorescent protein (EGFP) have been widely used to study neuronal function after intracranial injection into specific brain regions. A rapid dissection of the virus-expressing region is required for certain biochemical analyses. To improve the accuracy of the rapid dissection, we developed a method that employs a *Bluestar* flashlight in combination with barrier filter glasses to visualize the expression of EGFP lentivirus that has been microinjected into the nucleus accumbens. Processing of dissected tissue for EGFP immunoblotting further validated the approach.

1. Introduction

Genetic approaches have been widely used in recent years for probing the function of neural circuits (Luo et al., 2008). A common approach is to deliver viral vectors that express a protein of interest or interfering RNAs that disrupt protein expression into a specific brain region via intracranial injection. In many instances, the viral vector is engineered to also express a fluorescent protein such as enhanced green fluorescent protein (EGFP) to enable visualization of its expression with the aid of fluorescence microscopy.

A problem arises when the virus-infected brain region must be selectively and rapidly dissected for biochemical analysis. The use of microscopy is often not practical in these experimental scenarios, since the tissue has to be processed rapidly to prevent biochemical changes. Therefore, tissue is typically dissected based on anatomical landmarks for the targeted injection site rather than actually visualizing the infected area based on expression of fluorescent protein. If the injection is not completely accurate, these landmarks may be misleading. Therefore, there remains a need for a practical method to easily visualize, during the dissection process, tissue that is fluorescent and therefore has been infected.

Here we describe a simple and inexpensive method in which a GFP-illuminating *BlueStar* flashlight and barrier filter glasses were used to visualize infected areas three weeks after injection of EGFP-expressing lentiviral vector into the rat nucleus accumbens (NAc). Analysis of the dissected tissue by immunoblotting further validated EGFP expression in the region identified with the flashlight. This approach is easy to use and will facilitate the study of neurochemical changes resulting from intracranial injection of viral vectors.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (250–275 g on arrival; Harlan) were housed in groups of three under a 12 h/12 h light/dark cycle with food and water available *ad libitum*. They were allowed a 7-day period of acclimation to the colony before intracranial injection. All procedures were performed during the light phase and were approved by the Institutional Animal Care and Use Committee of Rosalind Franklin University of Medicine and Science.

2.2. Intracranial injection of lentivirus

Rats were anesthetized by injecting (i.p.) a cocktail composed of ketamine (80 mg/kg; Bioniche Teo Ltd., Galway, Ireland) and xylazine (10 mg/kg; Akorn Inc., Decatur, IL, USA). After being mounted onto a stereotaxic frame, rats received bilateral microinjections of EGFP-expressing lentivirus (titer: 7.1×10^7 pg/ml; 2 μl/side, 0.2 μl/min). The lentivirus was prepared at the Ernest Gallo Clinic and Research Center (University of California, San Francisco, Emeryville, CA, USA) based on pLL3.7 (Rubinson et al., 2003); pLL3.7 was obtained from Addgene Inc. (Cambridge, MA; Addgene plasmid 11795). Lentivirus was injected into the core subregion of

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doi:10.1016/j.jneumeth.2011.07.016

the NAc [AP, +1.2 mm; ML, 2.6 mm (6° angle); DV, 7.0 mm; relative to Bregma; Paxinos and Watson, 1998] using a Hamilton syringe (84851; Hamilton, Revo, NV, USA). After injection, the syringe was left in place for 5 min.

2.3. Visualization of EGFP and validation by immunoblotting

Three weeks after intracranial injection of EGFP-expressing lentiviral vector into the NAc, rats were decapitated and brains were placed into an ice-cold metal brain matrix (ASI Instruments, Warren, MI, USA). A 2 mm coronal section containing the NAc was obtained and placed on a cold glass slide. After turning off the room lights, EGFP was visualized using a *BlueStar* flashlight by an experimenter wearing a pair of VG2 barrier filter glasses (flashlight and glasses were developed by Nightsea, Bedford, MA, USA). The *BlueStar* flashlight has a blue LED to excite green fluorescence. The VG2 glasses block reflected light and transmit fluorescence. A *DFP-1 Dual Fluorescent Protein Flashlight* is also available (Nightsea) that includes both blue and green LEDs to visualize both green and red fluorescence. After detecting EGFP fluorescence, a tissue puncher was used to dissect the fluorescent region in the core of the NAc. A punch of the same size was obtained from a non-fluorescent control region in the dorsal striatum. Tissue punches were quickly sonicated in lysis buffer (Ferrario et al., 2010). After brief centrifugation to remove debris, supernatants were frozen at −80 °C until used for immunoblotting, which was performed as described previously (Ferrario et al., 2010). Immunoblots were probed with antibodies to GFP (sc-9996; 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase, CB1001; 1:10,000, Calbiochem, San Diego, CA, USA). GAPDH was used as a loading control.

3. Results and discussion

Our goal was to develop a method to selectively dissect infected brain tissue, following intracranial injection of viral vectors, based on EGFP fluorescence. As a test case, we injected an EGFP-expressing lentiviral vector into the core subregion of the NAc. Three weeks later, rats were killed, brains were removed rapidly, and a 2 mm coronal section containing the NAc was obtained using a brain matrix. By shining a GFP-illuminating *Bluestar* flashlight on the coronal section, we were able to observe the EGFP expression pattern, enabling rapid and selective dissection of EGFP-expressing tissue. A total of 5 rats were analyzed, all of which exhibited EGFP expression that could be visualized in this manner. The photograph in Fig. 1A depicts two typical patterns of EGFP expression observed in these rats. In the right hemisphere, the entire track is within the plane of the section, and is therefore visualized. Extensive labeling along the track reflects the relatively high volume of lentiviral vector injected. In the left hemisphere, the track is apparently at an angle to the plane of the section, so only the injection target at the tip of the track is fluorescent (NAc core).

To further validate our method of detecting EGFP expression, we used a tissue punch (internal diameter of ~1.75 mm) to sample fluorescent and non-fluorescent regions as indicated in Fig. 1A, and processed the tissue for immunoblotting. As shown in Fig. 1B, the fluorescent region (punch 1) contained EGFP protein, which was absent in the non-fluorescent region (punch 2).

Although all data in this study were obtained after injection of a lentivirus expressing only EGFP, we have successfully used the same approach to visualize the area of NAc infected by intracranial injection of a lentiviral vector that expresses both siRNA to a target protein and EGFP (Wang et al., in press). We have also adapted this approach to solve the challenging problem of accurately dissecting brain tissue injected with a "non-fluorescent" viral vector, i.e., one that expresses a protein or RNA of interest but no fluorescent tag. This can be accomplished by mixing the "non-fluorescent" vector with an EGFP-expressing viral vector prior to intracranial injection, and then using the GFP-illuminating flashlight to localize the injection area.

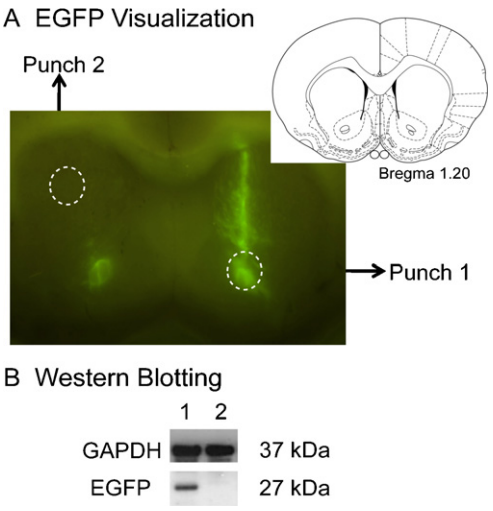

Fig. 1. Visualization of the area of tissue infected by an EGFP-expressing lentiviral vector using a GFP-illuminating *BlueStar* flashlight. (A) Unmodified photograph of a NAc coronal section taken with a Nikon camera equipped with a filter (62Y12; Tiffen, Hauppauge, NY) while illuminating the section with a *BlueStar* flashlight. The camera view duplicates the view of an experimenter equipped with filter glasses. On the right side, the entire track is within the plane of the section. On the left side, the track was apparently at an angle to the section, so that only the tip (injection site) is visualized. A schematic showing the corresponding coronal section is also shown (Paxinos and Watson, 1998). (B) Further validation of EGFP expression in a tissue punch from the fluorescent region (punch 1) but not a non-fluorescent region (punch 2) by immunoblotting.

Our dissection method is useful for situations in which brain tissue must be rapidly dissected for biochemical analysis. For example, freshly dissected tissue, in which cell membranes are intact, must be used to accomplish selective labeling of cell surface receptors with membrane-impermeant protein crosslinking and biotinylating reagents (e.g., Boudreau and Wolf, 2005). Freshly dissected tissue is also required for analysis of enzymatic activity or labile biochemical changes.

In conclusion, dissecting tissue based on visualization of EGFP fluorescence increases the accuracy and speed of the dissection, thereby providing results that more accurately reflect the biochemical consequences of infection with the viral vector.

Financial disclosures

Marina E. Wolf has no biomedical financial interests but has a patent on A Possible Therapy For Cue-Induced Cocaine Craving Leading to Relapse in Abstinent Cocaine Abusers Based on Blockade of GluR2-lacking AMPA Receptors in the Nucleus Accumbens. Xuan Li reports no biomedical financial interests or potential conflicts of interest.

Acknowledgements

This work was supported by DA015835 and DA029099 to M.E.W. We thank Dr. Amy Lasek for preparing the lentiviral vector and advising us on its use. We also thank Dr. Joseph B. Justice, Jr., for performing the photography and Dr. Jessica A. Loweth for helpful comments on the manuscript.

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