Viral-mediated gene delivery for *in vivo* circuit manipulation in neonatal mice

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[Abstract]

Understanding the cellular and circuit basis of early activity-dependent brain development is key to addressing basic and translational neuroscience questions. To achieve this goal, we need to manipulate the activity of specific neuronal subtypes while assaying activity *in vivo* – but this is a daunting task in newborn mice. The major bottleneck has been the technical difficulty of delivering, expressing, and utilizing activity manipulation tools in the fragile neonatal mouse. Adeno-associated viruses (AAV) are a common gene transfer system in adults but generally take several weeks to achieve high expression; thus, they have not been widely used for *in vivo* studies of very young animals. We have refined an AAV-mediated approach for optogenetic and chemogenetic tool delivery at early developmental stages. By injecting AAV into the mouse brain at postnatal day 0 (P0), our approach allows manipulation of neuronal subtypes in the cortex, thalamus, and hippocampus in un-anesthetized neonatal mice as early as P3. This protocol describes the general guideline of viral-mediated gene delivery for *in vivo* circuit manipulation in neonatal mice.

Keywords: Optogenetics, Chemogenetics, Adeno-associated virus, Virus injection, Neonatal mouse, *in vivo* neuronal manipulation

[Background]

The developing brain spontaneously generates oscillatory network activity, which is fundamental to functional circuit formation. However, the cellular and circuit mechanisms underlying early activity generation have been poorly understood mainly due to a substantial lack of detailed studies in very young animals. In particular, the technical barrier in applying neuronal and circuit manipulation techniques, such as optogenetics and chemogenetics, to the study of the intact developing brain has hindered the field from advancing. Viruses like AAVs and lentiviruses are commonly used to express

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proteins in adults but generally need an extended time to achieve high expression level, thus have not been widely used in neonates. To address this issue, we have optimized, validated, and established a viral-mediated gene delivery method using AAV. We have had good success with AAV injected into the cortex, thalamus, or hippocampus of newly born pups in order to manipulate activity of a specific population of neurons as early as postnatal day 3, and have been able to directly observe the effects on network activity *in vivo*. We used this approach to provide *in vivo* evidence that GABAergic neurons shift their action from excitatory to inhibitory in hippocampus between postnatal day 3 and 7, while GABA remains inhibitory in cortex throughout the early postnatal period. This approach also helped to reveal that a developmentally-transient corticothalamic feedback excitation amplifies early thalamic activity and facilitates information transfer from retina to cortex. Although a supplemental histological assessment and *in vitro* validation assays are needed for each tool to be used in an individual research project, this general viral-mediated approach for *in vivo* circuit manipulation will facilitate questions about neural circuit function during early development.

Materials and Reagents

1. AAV8-hSyn-dF-HA-KORD-IRES-mCitrine (Addgene, 65417-AAV8) (Vardy et al., 2015)
2. AAV8-hSyn-DIO-hM3D(Gq)-IRES-mCitrine (Addgene, 50454-AAV8) (Armbruster et al., 2007)
3. AAV1-hSyn1-SIO-stGtACR2-FusionRed (Addgene, 105677-AAV1) (Mahn et al., 2018)
4. AAV1-Syn-Chronos-GFP (Addgene, 59170-AAV1) (Klapoetke et al., 2014)
5. AAV8-Syn-FLEX-ChrimsonR-tdTomato (Addgene plasmid#62723, produced by the UNC Vector core, courtesy of Dr. Klapoetke and Dr. Boyden) (Klapoetke et al., 2014)
6. AAV8-CAG-FLEX-Jaws-KGC-GFP-ER2 (Addgene plasmid# 84445, produced by the UNC Vector core, courtesy of Dr. Chuong and Dr. Boyden) (Chuong et al., 2014).
7. Mineral oil (Sigma, M-3516). Similar reagents are applicable.
8. Crushed ice

Equipment

1. Nanoject II (Drummond, 3-000-204): A newer or similar nano-injector is applicable.
2. Glass capillary (Drummond, 3-000-203-G/X).
3. Micropipette puller (Sutter Instrument, P-87): A newer or similar micropipette puller is applicable.
4. Stereotaxic frame (Narishige). A similar stereotaxic frame is applicable.
5. Heating plate (WPI, ATC-2000). A similar heating-plate is applicable.

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Procedure

A. Preparation of injection

1. Pull a glass capillary using a micropipette puller based on the manufacturer's manual. Any type of common micropipette puller can be used. We prefer to pull a glass pipette with a tip diameter of around 20-50 μm. The taper of glass capillaries can be optimized. For example, a long thin taper is desirable for targeting deep brain structures such as thalamus.
2. Set up Nanoject II or a similar nanoliter injector based on the manufacturer's manual. We prefer to use a nanoliter injector over a syringe injector or pressure injector because of the small injection volume and neonatal brain size. Follow the manufacturer's recommendation for aliquoting, storing, and preparing viruses. Follow the Institutional Biosafety guideline for handling viruses.

B. Brain microinjection

1. Take healthy P0-P1 mouse pups from a dam. We prefer to use newly-born pups delivered within the last 24 hours. Follow your approved Institutional Animal Care and Use Committee (IACUC) guidelines for survival surgery regarding analgesia, anesthesia, surgical preparation, and post-operative monitoring.
2. For cold anesthesia, we place an animal on an ice-cold petri dish on crushed ice for 2-5 minutes, so that it is not in contact with the ice. Time varies depending on the setup and needs to be optimized. Other setups or devices, such as electric cold-plates, may also be used.
3. Put an anesthetized animal on the stereotaxic frame. Because of the softness of the skull structures at this age, we do not use ear-bars. Instead, we put a carved Styrofoam block under the mouse to stabilize the head of P0-1 mouse. Any other setups or materials may be used to stabilize the mouse head.
4. Move the tip of a glass capillary over the injection area. Lower the glass capillary directly through the scalp. The scalp is thin and usually penetrable with a glass capillary at P0. For injection coordinates, we test several different coordinates to optimize targeting of the brain area of interest. In most cases, bregma and lambda are visible and recognizable through the scalp at P0 and can be used as a landmark. The brain atlas for neonatal mice is informative to optimize the injection coordinates (Paxinos et al., 2020; Sunkin et al., 2013).
5. Inject viral solution into the brain area of interest. We usually use viral solution without dilution. Optimal injection volume varies from 10 nl to 1μl depending on the goal of experiments. Optimal injection rate also varies from 10nl/min to 100 nl/min. Make sure the pipette tip is not clogged during the injection procedure.
6. Wait 1-5 min after injection.
7. Pull a glass pipette slowly from the brain.
8. Place the animal to recover in a small container or cage with cotton or bedding on a gentle heating plate and observe until recovered. Make sure to avoid over-heating animals.

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9. Bring animals back to the dam after full recovery from cold anesthesia. Make sure pups are warm, and that animals do not carry any extrinsic materials or smell to avoid disturbing or agitating a dam. Follow your institutional guidelines for post-operative monitoring.

Notes

Optimization and validation of each step is needed at the beginning of the research project according to the goal of project.

We list several tips for optimization below.

Quality of virus: Direct injection of adeno-associated viruses with a titer higher than 10^{12} GC (genome copies) /mL usually gives favorable results in our hands, but variability exists between distributors and batches. Also, it is important to make sure that the deep freezer for virus storage has experienced no issues, including temperature fluctuation and power outage, which can lead to the loss of viral potency.

Serotypes of AAVs: AAV1, AAV8, and AAV9 yield comparably similar expression in cortex, thalamus, and hippocampus of neonatal mice in our hands.

The promoter used for AAV constructs: We prefer to use the synapsin promoter for neuronal expression. Stronger and more ubiquitous promoters, such as CAG promoter, are more likely to cause toxicity issues after long-term expression (>2 weeks in our hands) due to the high expression level, thus require extra caution for use.

Amount of injection: The spread of viral infection from the injection site varies among the brain regions and AAVs in our hands. In addition, a type of injector and methodology affect the extent of spread. Injection volume needs to be optimized depending on these factors as well as the desirable infection area for each project.

Fluorescence signal: Fluorescence signal becomes visible 2-3 days after the virus injection; however, the signal intensity varies among the fluorescent molecules and constructs. It is important to note that the fluorescence signal intensity is not an ideal indicator of the strength of manipulation. In many cases, even very weak fluorescence signals provide profound yet specific manipulation effects both *in vitro* and *in vivo* in our hands.

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This protocol was derived mainly from our recent work (Murata and Colonnese, 2020) and partially from our previous work (Murata and Colonnese, 2016).

Competing interests

The authors declare no competing interests.

Ethics

All experiments were conducted with approval from The George Washington University School of Medicine and Health Sciences Institutional Animal Care and Use Committee, in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

References

Armbruster, B.N., Li, X., Pausch, M.H., Herlitze, S., and Roth, B.L. (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc. Natl. Acad. Sci. *104*, 5163–5168.

Chuong, A.S., Miri, M.L., Busskamp, V., Matthews, G.A.C., Acker, L.C., Sørensen, A.T., Young, A., Klapoetke, N.C., Henninger, M.A., Kodandaramaiah, S.B., et al. (2014). Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nat. Neurosci. *17*, 1123–1129.

Klapoetke, N.C., Murata, Y., Kim, S.S., Pulver, S.R., Birdsey-Benson, A., Cho, Y.K., Morimoto, T.K., Chuong, A.S., Carpenter, E.J., Tian, Z., et al. (2014). Independent optical excitation of distinct neural populations. Nat. Methods *11*, 338–346.

Mahn, M., Gibor, L., Patil, P., Malina, K.C.-K., Oring, S., Printz, Y., Levy, R., Lampl, I., and Yizhar, O. (2018). High-efficiency optogenetic silencing with soma-targeted anion-conducting channelrhodopsins. Nat. Commun. *9*, 4125.

Murata, Y., and Colonnese, M.T. (2016). An excitatory cortical feedback loop gates retinal wave transmission in rodent thalamus. ELife *5*, e18816.

Murata, Y., and Colonnese, M.T. (2020). GABAergic interneurons excite neonatal hippocampus in vivo. Sci. Adv. *6*, eaba1430.

Paxinos, G., Halliday, G., Watson, C., and Kassem, M.S. (2020). Atlas of the Developing Mouse Brain (Academic Press).

Sunkin, S.M., Ng, L., Lau, C., Dolbeare, T., Gilbert, T.L., Thompson, C.L., Hawrylycz, M., and Dang, C. (2013). Allen Brain Atlas: an integrated spatio-temporal portal for exploring the central nervous system. Nucleic Acids Res. *41*, D996–D1008.

Vardy, E., Robinson, J.E., Li, C., Olsen, R.H.J., DiBerto, J.F., Giguere, P.M., Sassano, F.M., Huang, X.-P., Zhu, H., Urban, D.J., et al. (2015). A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior. Neuron *86*, 936–946.