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An improved method of transducing retinal ganglion cells using AAV via transpupillary injection in adult mouse eyes

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Purpose: Intravitreal injection of adeno-associated virus (AAV) vectors is a good approach for transducing retinal ganglion cells (RGCs) in mice. It allows for high transduction efficiency and is relatively specific to RGCs. To deliver vectors, most studies use a transscleral approach that can have potentially negative effects, causing damage to the lens or retina. We optimized the intravitreal injection method using a transpupillary approach to minimize ocular damage and efficiently transfect RGCs.
Methods: C57BL/6J mice were anesthetized, and their irises were dilated. The eyeball was held with forceps while a small, full-thickness incision was made halfway between the center and periphery of the cornea. Using a bent 35-gauge blunt needle, the tip was navigated through the incision across the anterior chamber to reach the distal aspect of the pupil. The needle was inserted through the pupil, swept around the lens, and entered the vitreous, delivering expression vectors containing cytomegalovirus (CMV) promoter-driving green fluorescent protein (AAV-CMV-GFP) into the vitreous chamber. Fourteen days after injection, live fluorescent fundus images were taken, followed by immunostaining for GFP.
Results: With the improved injection technique, the lens remained clear and undamaged. Fundus imaging and GFP staining showed that over 90% of the mouse retinas sustained no visible damage. Retinas injected via the transpupillary approach also exhibited GFP transduction throughout the ganglion cell layer.
Conclusions: Transpupillary intravitreal injection reduces the potential risk compared to the transscleral approach, offering a promising and efficient method for delivering reporter genes to RGCs and ensuring high levels of gene expression without damage to the lens or retina.

The eye and retina provide a unique model system for studying the effects of genetic manipulation in neurons. The output cells of the retina are the retinal ganglion cells (RGCs), which are neurons that sit on the inner surface of the retina adjacent to the vitreous chamber of the eye [1,2]. RGCs project their axons down the optic nerve to transmit visual information from the retina to the brain [1,3]. Thus, distinct compartments allow a unique opportunity to deliver treatment to the RGCs via the vitreous and to monitor the effects of the treatment on axons within the optic nerve and terminal fields of RGCs in the brain. The normal function of RGCs is critical for maintaining vision, and injury to the RGCs or diseases such as glaucoma or optic neuropathies [4] can lead to vision loss. Intervening in the disease process of neuronal degeneration using genetic therapies can lead to RGC survival, potentially preserving or restoring vision. One approach to treating RGCs is to transduce these cells via injections of recombinant adeno-associated viral (AAV) vectors into the vitreous.

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AAV has become the favored vehicle for transducing RGCs with recombinant genes or antisense vectors [5]. As a delivery system for small viral vectors, AAVs are preferred in rodent studies due to their nonreplicating nature, low immunogenicity, and high titer. These viral particles can be injected into the eye with minimal complications [6,7]. This approach is used to overexpress genes within RGCs [8], knock down specific genes using shRNA [9], or mark transduced cells with reporter genes [5]. Intravitreal injection is a preferred method due to its high transduction success and specific selectivity for RGCs in various animal models [9–11], including rodents [4,12]. Most studies use a transscleral approach to intravitreal injection. In this commonly accepted procedure, a needle with a beveled tip is inserted through the sclera 1 mm below the limbus into the vitreous cavity [13,14]. While this procedure offers an easy approach and is effective for AAV delivery, some obvious inherent risks are associated with it, including potential injury to the lens or the retina. Damaging the lens during the execution of these studies can cause changes in gene expression [15], affect RGC development, or alter the RGC's response to insults and injury [16]. Injury in the retina may result in changes in gene expression or bleeding, which can significantly impact the experimental results.

Timmers et al. [17] described a transcorneal injection route into the eye of the rat. We have used this approach to deliver plasmids into the subretinal space aided by electroporation [18–20]. In this report, we demonstrate that an improved transpupillary approach for intravitreal injection minimizes damage to the eye and efficiently transfects RGCs. This method offers an alternative technique for transfecting RGCs in the mouse model, especially for those requiring the maintenance of an intact retina. We present photomicrographs and a schematic diagram of this procedure so that others can readily learn and repeat our approach.

METHODS

Mice: All procedures involving animals were approved by the Animal Care and Use Committee of Emory University and were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6J mice (The Jackson Laboratory, Strain #000664, n=7) were purchased from the Jackson Laboratory. All animals were housed in the standard 12h:12h light–dark cycle and were given standard mouse chow and water *ad libitum*.

AAV vector production: We used an AAV2 vector to express green fluorescent protein (GFP) under the cytomegalovirus promoter (AAV-CMV-GFP). The virus titer was 1.2×10^{12} genome copies per milliliter. All plasmids were made at Emory Integrated Genomic Core and were packed into AAV at Emory Viral Vector Core.

Transpupillary intravitreal injection:

Preparing the needle— The top 5 mm of the NanoFil 35G blunt needle (NF35BL-2, World Precision Instruments, Inc., Sarasota, FL) was bent to 30° (Appendix 1), making it easier to go around the lens in the following steps. The needle was attached to a Hamilton syringe (NanoFil 10 µl, World Precision Instruments, Inc., Appendix 1), and the syringe was carefully filled with 70% ethanol and thoroughly flushed out, deeply cleaning the syringe. Following the 70% ethanol, the needle was rinsed with phosphate-buffered saline (PBS).

Preparing the mice —C57BL/6J mice were deeply anesthetized with intraperitoneal injections of 100 mg/kg ketamine (Cat. #VINV-KETA-0VED, Vedco Inc., Saint Joseph, MO) and 10 mg/kg xylazine (AnaSed; NCD 46066-750-02, Pivetal, Greeley, CO), followed by 0.5% proparacaine eye drops (Cat. #24208–730–06, Bausch and Lomb, Rochester, NY) as local anesthesia. Pupils were dilated with 1% tropicamide drops (Cat. #17478–102–12, Akorn, Inc., Lake Forest, IL). Each mouse was placed on a heating pad (39 °C) during the surgery.

The following steps were taken to perform the transpupillary intravitreal injection:

1) The globe of the eye was held with forceps (HSC 702–93, Karl Hammacher (Solingen, Germany; Appendix 1) while a small, full-thickness incision was made using a 28-gauge needle halfway between the center and periphery of the cornea. The needle's bevel was oriented downward to minimize the risk of lens damage (Figure 1A–B).

2) The needle was retracted, and aqueous humor was allowed to flow out. A sterile cotton-tipped applicator was used to absorb the liquid.

3) A bent 35-gauge blunt needle (prepared previously) was attached to a Hamilton syringe and then preloaded with 2 µl of the injection solution. The needle was inserted through the incision (Figure 1C), avoiding puncturing the other side of the eye or damaging the lens. The tip of the needle was moved through the anterior chamber to the distal aspect of the pupil (Figure 1D). The needle passed through the pupil beneath the iris, sweeping around the lens (Figure 1E) and then into the vitreous chamber (Figure 1F). A schematic diagram of the procedure is provided in Figure 2.

4) At this point, 2 µl of cytomegalovirus promoter driving green fluorescent protein (AAV-CMV-GFP) was delivered into the vitreous chamber. The needle was then slowly removed.

5) Antibiotic ointment was applied to the eye. Following injection, the mouse was left on a heating pad until it fully recovered from anesthesia and then returned to a clean cage when it was fully awake. A video of the procedure can be found here (Appendix 2).

Advice for successful injection: It is very important to ensure that the needle is placed in the correct anatomic location before performing the injection. The needle must be carefully swept close to the lens. After the tip of the needle passes through the suspensory ligament of the lens, it is pushed approximately 1 mm further to place the needle tip in the vitreous chamber. At this point, the needle tip is clearly visible across the lens under a microscope. When the needle has passed beneath the lens and reached the middle of the pupil, as seen through the microscope (Figure 1F), it is in the correct position for the injection.

Spectral domain optical coherence tomography (SD-OCT): Fourteen days after injection, fluorescent fundus imaging was conducted on the live mice. The mice were anesthetized as previously described. Once anesthetized, proparacaine and tropicamide eye drops were administered to provide topical anesthesia and to dilate the pupils. A Micron IV SD-OCT system with a fundus camera (Phoenix Research Labs,

Pleasanton, CA, USA) was used to obtain fundus photographs for both eyes. Each mouse was placed on the OCT platform, and its head was adjusted to properly align with the camera lens. Topical Optixcare Eye Lube (Aventix Animal Health, Burlington, ON, Canada) was applied to the cornea before imaging. The focus and brightness were adjusted until the fundus became visible on the computer screen. Bright-field images were taken once the fundus was clear, with the optic nerve centered. To see the fluorescence, the filter and exciter knobs were set for GFP.

Immunohistochemistry: After live imaging, the mice were perfused through the heart with saline, followed by 4%

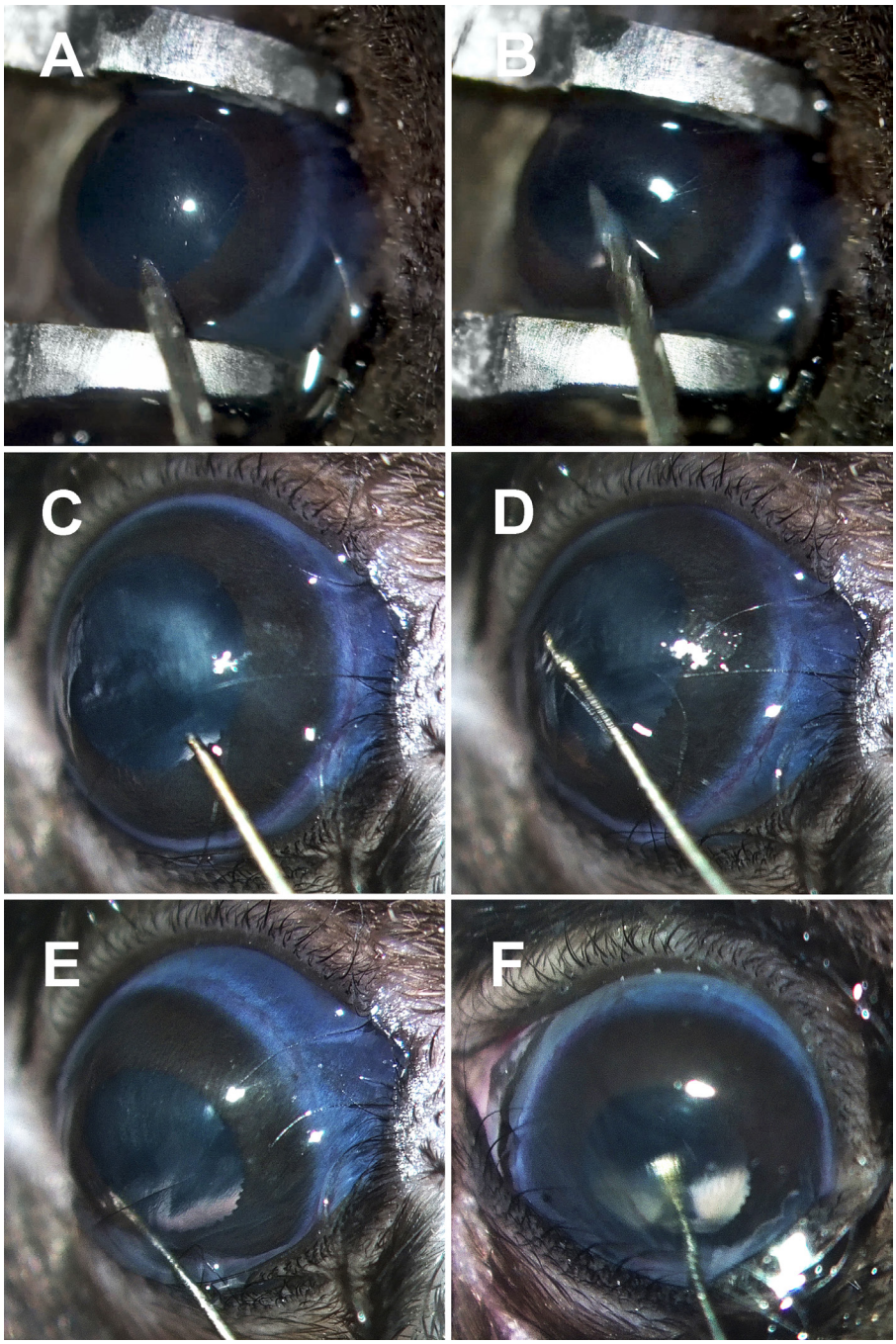

Figure 1. Transpupillary intravitreal injection technique. **A**–**B**: Make an incision in the cornea using a 28-gauge needle with the bevel oriented downward. **C**–**D**: Insert a bent 35-gauge blunt needle attached to a syringe preloaded with injection solution through the incision to enter the pupil. **E**–**F**: Sweep the needle around the lens beneath the iris to reach the vitreous chamber and then inject the solution.

Table 1. List of antibodies used in this study.

Antibodies	Dilution	SOURCE	IDENTIFIER
Rabbit anti-POU6F2	1:500	MyBiosource	Cat. #MBS9402684
Rabbit anti-BRN3A	1:500	Synaptic Systems	Cat. #411003
Chicken anti-GFP	1:500	Millipore Sigma	Cat. #AB16901
Donkey anti-rabbit, Alexa Fluor 594 conjugated	1:1000	Jackson Immunoresearch	Cat. #711–585–152
Donkey anti-chicken, Alexa Fluor 488 conjugated	1:1000	Jackson Immunoresearch	Cat. #703–545–155

paraformaldehyde in phosphate buffer (pH 7.3). The eyes were removed, and the retinas were dissected out and shaped into a cloverleaf pattern with four quadrants. Flat mounts of the retina were prepared for staining using a protocol similar to that previously described [21]. All antibodies and dilutions used are listed in Table 1.

The retinas were blocked in 3% bovine serum albumin (BSA) and 3% donkey serum in 0.5% Triton X-100 in phosphate buffered saline (PBS) for 1 h. The retinas were stained with a combination of antibodies, which has been reported to serve as a pan-nuclear RGC marker [21,22]. These included rabbit anti-POU6F2 at 1:500 and rabbit anti-BRN3A at 1:500. The retinas were also stained with chicken anti-GFP at 1:500. After staining with the primary antibodies for two nights at 4 °C, the retinas were rinsed three times in PBS with 0.1% Tween-20 (PBST) at room temperature. The retinas were then incubated with the corresponding secondary antibodies. These included Alexa Fluor 488 (Goat-Anti-Chicken IgY [H^+L]) and Alexa Fluor 594 (Goat-Anti-Rabbit IgG [H^+L]) at 1:1000 at 4 °C overnight. After three rinses in PBST, the retinas were placed on glass slides, and coverslips were placed over the retinas using Fluoromount-G (Cat. #0100–01, Southern Biotech, Birmingham, AL). All retinal flat mounts were imaged using a Nikon Eclipse Ti2 microscope (Nikon, Inc., Melville, NY) with a Nikon A1R confocal imager (Nikon, Inc., Melville, NY).

RESULTS

In this study, the retinas injected via the transpupillary approach demonstrated effective vector distribution throughout the ganglion cell layer (Figure 3A), which is critical for accurately assessing the impact of gene delivery on retinal function. Five of the 14 retinas had more than 90% of the ganglion cell layer surface with transduced RGCs, indicating that the transduction had spread across most of

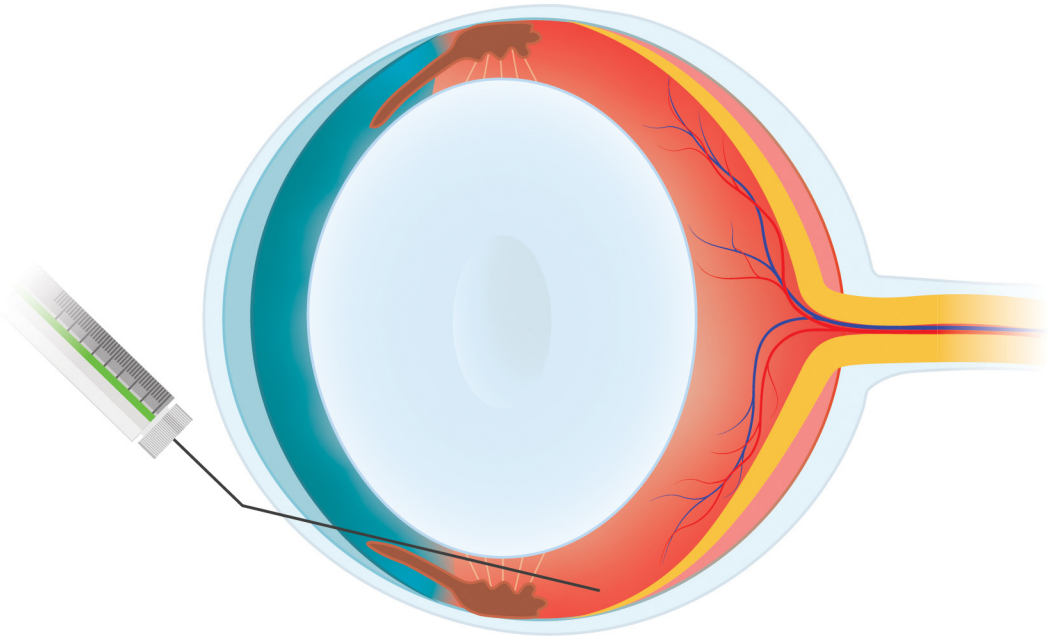

Figure 2. Illustration of the transpupillary method of intravitreal injection in the mouse eye, with a beveled needle inserted into the vitreous space through a punctured incision in the cornea. The length of the needle is not drawn to scale.

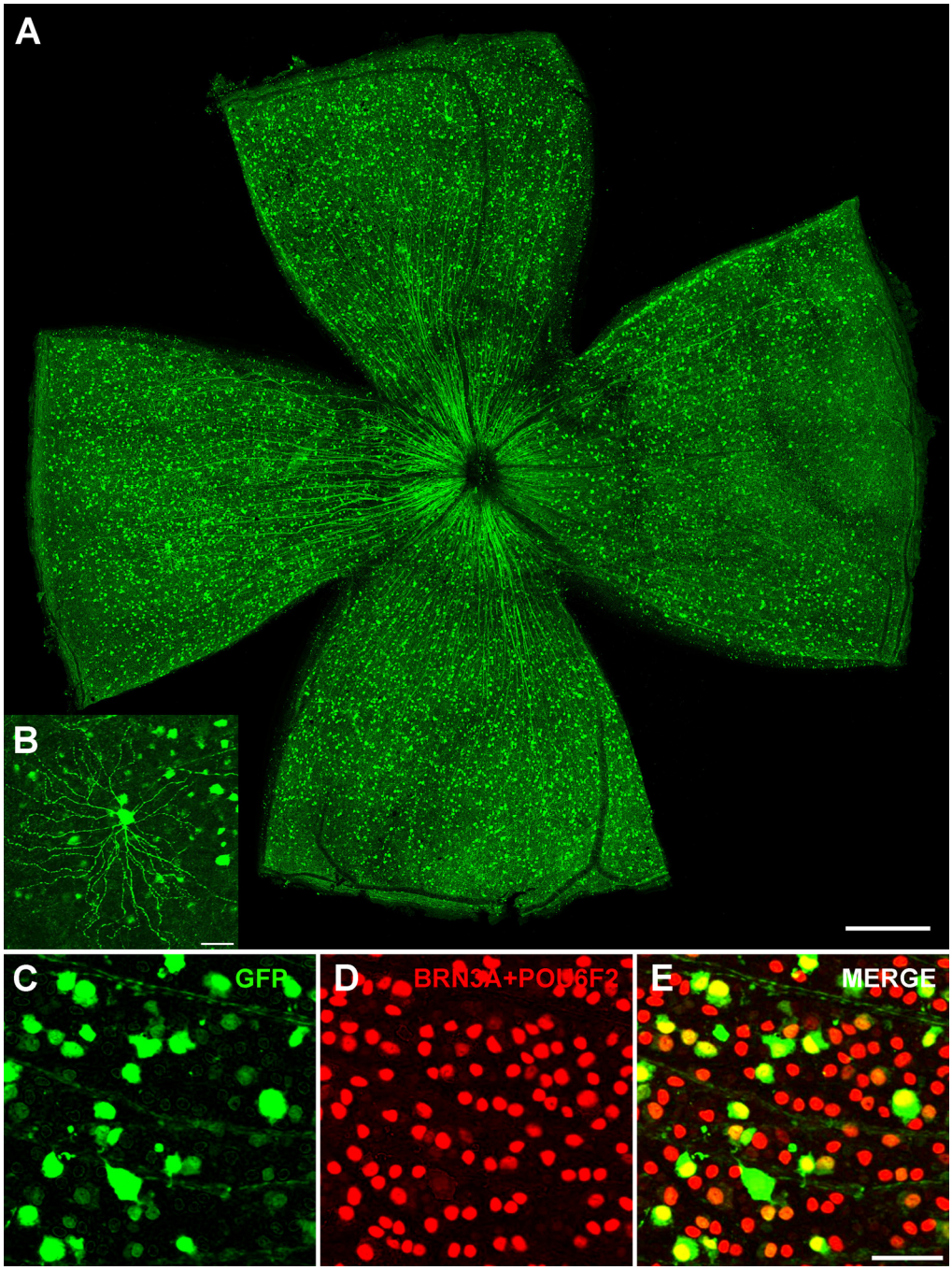

Figure 3. Immunostained retinal flat mount. **A**: Representative retinal flat mount showing strong GFP expression throughout the entire retina. **B**: Some GFP-positive cells exhibit the dendritic morphology typical of RGCs and have an axon extending from the cells to the optic disc, confirming their identity as RGCs. **C**–**E**: Immunostaining the retina with GFP, BRN3A, and POU6F2, indicating that virtually all the GFP-positive cells are RGCs. Scale bar in A = 500 µm. Scale bar in B–E = 50 µm.

the retinal surface. Only one retina had less than 50% of the retinal surface with transduced RGCs.

Transpupillary intravitreal injection effectively transduces RGCs: We took confocal images of retinal whole-mounts with a 20X magnification to image a 1024×1024 pixel area, with a resolution of 0.62 µm/pixel. The retinal whole-mount images showed extensive, strong GFP expression throughout the entire retina 14 days following injection (Figure 3A). Several cells had typical RGC dendrite morphology (Figure 3B). Many had axons extending to the optic disc (Figure 3A),

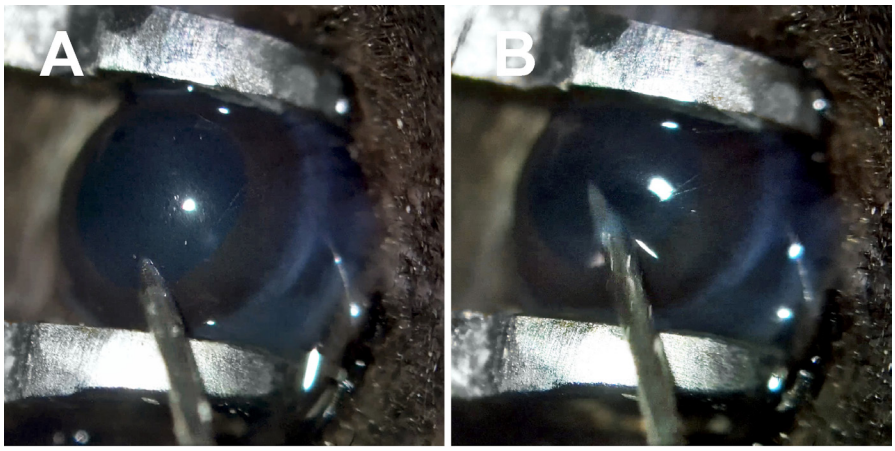

Figure 4. Representative fundus images of the same retina 14 days after injection of AAV-CMV-GFP. **A** is an image taken under bright light illumination. **B** represents the image with GFP signal only. Features in the retina are labeled as follows: BV = blood vessels, ONH = optic nerve head, and RGCs = retinal ganglion cells. The images have been enhanced using the sharpen tool on Adobe Photoshop. Scale bar = 200 µm.

confirming their identity as RGCs. When stained with a combination of BRN3A and POU6F2, which should represent virtually all RGCs [21], we found GFP-positive cells were mainly RGCs (Figure 3C–E).

Transpupillary approach reduces the risk of damaging the lens or retina: Unlike the conventional transscleral method, in which a sharp needle is used to penetrate the sclera to inject directly into the vitreous, our improved approach employed a sharp needle only to make an incision in the cornea. A blunt needle was used in the following steps, minimizing the risk of damaging the lens and avoiding puncturing the retina. After the transpupillary injection procedure, the lens remained clear and appeared undamaged. Fundus bright-field imaging (Figure 4A) and GFP fluorescence filter images (Figure 4B) were clear, which indirectly confirmed that the cornea and lens had no opacification. It is worth mentioning that, sometimes, the fluorescence fundus image did not correlate with the immunostaining image; that is, less fluorescence was observed in the fundus image compared to the immunostained flat mounts. Most (over 90%) of the retinas showed no visible damage to the lens or retina. None of the mice had retinal detachment or vitreous hemorrhage. Only one out of 14 retinas displayed minor damage, likely due to the blunt needle being inserted too deeply and hitting the retina. The cornea remained clear; no haze was observed around the site of the injection.

Potential pitfalls and trouble shooting: We have encountered a few problems when performing this procedure. Most of these issues are not severe and can be overcome with practice. Overall, the procedure is easy to learn. Table 2 outlines some of the challenges that we have encountered with this technique, along with suggestions to resolve these problems.

Table 2. Pitfalls and trouble shooting.

Problems	Probable cause	Suggestions
Localized cornea haze	Repeated cornea injection	When multiple injections are needed, try to use the same incision site each time.
Bleeding	Traumatizing iris blood vessel	Make sure the pupil is fully dilated. Sweep close to the lens to avoid injuring the iris.
Cloudy lens	Nicking lens capsule	Avoid touching the lens with needle tips.
Retina damage	Penetrating the retina	When the needle is in the vitreous, avoid pushing the needle too far toward the retina.
Air bubbles	Syringe not degassed	Flush thoroughly with ethanol and PBS before filling with vector solution. Degas the solution in the syringe.

DISCUSSION

Mouse models of human retinal diseases are commonly used to develop genetic therapies [23]. In this study, we used a recombinant AAV2-CMV-GFP virus in a mouse eye model via intravitreal injection. AAV2 has been widely researched and commonly employed in clinical trials due to its effectiveness [24–26]. AAV2-mediated gene delivery can transduce more than 95% of RGCs [27]. When the genes of interest were packaged into AAV2 backbone constructs, the transfection efficiency for these new vectors was the same as the AAV2-GFP, which was tested previously [28]. This study introduces a novel approach to intravitreal injection that uses a transpupillary method instead of the conventional transscleral method.

The conventional method involves scleral injection, which can be safe and efficient but has potential disadvantages. These include traumatic cataract and vitreous hemorrhage, similar to the complications observed in clinical intravitreal injections in humans [29]. Additionally, even with a small-needle sclerotomy, there is a risk of vector, medication, or vitreous escaping the eye or entering the subretinal space, leading to retinal detachment [30,31]. The resulting opening can be a potential entry point for bacteria [32]. Although the incidence of these complications is low, caution is warranted, particularly in experimental mouse models. Mice have large round lenses, making them more susceptible to injury during transscleral intravitreal injection. In our previous study, we used AAV to induce regeneration of RGC axons down the optic nerve [28], which requires multiple injections into the vitreous space in one mouse. Multiple injections can cause repeated damage to the retina, sclera, or lens, leading to changes in gene expression that complicate the ability to clearly define the effects of the experimental treatment. Using the transpupillary approach described in this study decreases the potential for confounding effects of ocular injury.

Frequent transscleral intravitreal injections have been associated with complications, including infection and vitreous hemorrhage [33]. In the mouse model, retinal hemorrhage after injection can occur. Some mice showed mild bleeding around the injection site at the sclera [34]. Our improved transpupillary approach eliminates the risk of posterior-segment bleeding since we do not make an incision through the sclera or retina. The only risk of hemorrhage occurs when the iris is accidentally touched. However, this can be easily controlled with practice. Overall, this procedure is easy to learn and master.

Conclusions: We describe a novel method for transducing RGCs using AAV via a transpupillary injection approach. The advantages of the transpupillary approach are that it reduces the impact of confounding variables on experimental data compared to the transscleral approach. This method represents a promising and efficient method of delivering reporter genes to RGCs, creating conditions for high levels of gene expression without damage to the lens or retina.

APPENDIX 1. SUPPLEMENTAL FIGURE 1.

To access the data, click or select the words "Appendix 1." Images of instruments used in this study. A: 35G blunt-ended 30 degree bent needle. B: NanoFil 10 µl Hamilton syringe with attached 35G blunt needle. C: Forceps with 3mm round ring tip used for holding the eyeball.

APPENDIX 2. VIDEO.

To access the data, click or select the words "Appendix 2." Demonstration of transpupillary approach of intravitreal injection in the mouse eye.

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REFERENCES

1. Yu DY, Cringle SJ, Balaratnasingam C, Morgan WH, Yu PK, Su EN. Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. Prog Retin Eye Res 2013; 36:217-46. [PMID: 23891817].

2. Munemasa Y, Kitaoka Y. Molecular mechanisms of retinal ganglion cell degeneration in glaucoma and future prospects for cell body and axonal protection. Front Cell Neurosci 2013; 6:60-[PMID: 23316132].

3. Nieuwenhuis B, Laperrousaz E, Tribble JR, Verhaagen J, Fawcett JW, Martin KR, Williams PA, Osborne A. Improving adeno-associated viral (AAV) vector-mediated transgene expression in retinal ganglion cells: comparison of five promoters. Gene Ther 2023; 30:503-19. [PMID: 36635457].

4. Nieuwenhuis B, Osborne A. Intravitreal Injection of AAV for the Transduction of Mouse Retinal Ganglion Cells. Methods Mol Biol 2023; 2708:155-74. [PMID: 37558970].

5. Nickells RW, Schmitt HM, Maes ME, Schlamp CL. AAV2-Mediated Transduction of the Mouse Retina After Optic Nerve Injury. Invest Ophthalmol Vis Sci 2017; 58:6091-104. [PMID: 29204649].

6. Costa Verdera H, Kuranda K, Mingozzi F. AAV Vector Immunogenicity in Humans: A Long Journey to Successful Gene Transfer. Mol Ther 2020; 28:723-46. [PMID: 31972133].

7. Whitehead M, Osborne A, Yu-Wai-Man P, Martin K. Humoral immune responses to AAV gene therapy in the ocular compartment. Biol Rev Camb Philos Soc 2021; 96:1616-44. [PMID: 33837614].

8. Chen Y, Yang X, Mao J. The Neuroprotective Effect of Activation of Sigma-1 Receptor on Neural Injury by Optic Nerve Crush. Invest Ophthalmol Vis Sci 2023; 64:9-[PMID: 37669061].

9. Madrakhimov SB, Yang JY, Ahn DH, Han JW, Ha TH, Park TK. Peripapillary Intravitreal Injection Improves AAV-Mediated Retinal Transduction. Mol Ther Methods Clin Dev 2020; 17:647-56. [PMID: 32300611].

10. Yin L, Greenberg K, Hunter JJ, Dalkara D, Kolstad KD, Masella BD, Wolfe R, Visel M, Stone D, Libby RT, Diloreto D Jr, Schaffer D, Flannery J, Williams DR, Merigan WH. Intravitreal injection of AAV2 transduces macaque inner retina. Invest Ophthalmol Vis Sci 2011; 52:2775-83. [PMID: 21310920].

11. Tshilenge KT, Ameline B, Weber M, Mendes-Madeira A, Nedellec S, Biget M, Provost N, Libeau L, Blouin V, Deschamps JY, Le Meur G, Colle MA, Moullier P, Pichard V, Rolling F. Vitrectomy Before Intravitreal Injection of AAV2/2 Vector Promotes Efficient Transduction of Retinal Ganglion Cells in Dogs and Nonhuman Primates. Hum Gene Ther Methods 2016; 27:122-34. [PMID: 27229628].

12. Dias MS, Araujo VG, Vasconcelos T, Li Q, Hauswirth WW, Linden R, Petrs-Silva H. Retina transduction by rAAV2 after intravitreal injection: comparison between mouse and rat. Gene Ther 2019; 26:479-90. [PMID: 31562387].

13. Cameron EG, Xia X, Galvao J, Ashouri M, Kapiloff MS, Goldberg JL. Optic Nerve Crush in Mice to Study Retinal Ganglion Cell Survival and Regeneration. Bio Protoc 2020; 10:e3559[PMID: 32368566].

14. Thomas CN, Bernardo-Colón A, Courtie E, Essex G, Rex TS, Blanch RJ, Ahmed Z. Effects of intravitreal injection of siRNA against caspase-2 on retinal and optic nerve degeneration in air blast induced ocular trauma. Sci Rep 2021; 11:16839-[PMID: 34413361].

15. Yin Y, Cui Q, Li Y, Irwin N, Fischer D, Harvey AR, Benowitz LI. Macrophage-derived factors stimulate optic nerve regeneration. J Neurosci 2003; 23:2284-93. [PMID: 12657687].

16. Lorber B, Berry M, Logan A. Lens injury stimulates adult mouse retinal ganglion cell axon regeneration via both macrophage- and lens-derived factors. Eur J Neurosci 2005; 21:2029-34. [PMID: 15869497].

17. Timmers AM, Zhang H, Squitieri A, Gonzalez-Pola C. Subretinal injections in rodent eyes: effects on electrophysiology and histology of rat retina. Mol Vis 2001; 7:131-7. [PMID: 11435999].

18. Johnson CJ, Berglin L, Chrenek MA, Redmond TM, Boatright JH, Nickerson JM. Technical brief: subretinal injection and electroporation into adult mouse eyes. Mol Vis 2008; 14:2211-26. [PMID: 19057658].

19. Nickerson JM, Goodman P, Chrenek MA, Bernal CJ, Berglin L, Redmond TM, Boatright JH. Subretinal delivery and electroporation in pigmented and nonpigmented adult mouse eyes. Methods Mol Biol 2012; 884:53-69. [PMID: 22688698].

20. Pang JJ, Boye SL, Kumar A, Dinculescu A, Deng W, Li J, Li Q, Rani A, Foster TC, Chang B, Hawes NL, Boatright JH, Hauswirth WW. AAV-mediated gene therapy for retinal degeneration in the rd10 mouse containing a recessive PDEbeta mutation. Invest Ophthalmol Vis Sci 2008; 49:4278-83. [PMID: 18586879].

21. Lin F, Lin ST, Wang J, Geisert EE. Optimizing retinal ganglion cell nuclear staining for automated cell counting. Exp Eye Res 2024; 242:109881[PMID: 38554800].

22. Lin F, Li Y, Wang J, Jardines S, King R, Chrenek MA, Wiggs JL, Boatright JH, Geisert EE. POU6F2, a risk factor for glaucoma, myopia and dyslexia, labels specific populations of retinal ganglion cells. Sci Rep 2024; 14:10096-[PMID: 38698014].

23. Sun YJ, Lin CH, Wu MR, Lee SH, Yang J, Kunchur CR, Mujica EM, Chiang B, Jung YS, Wang S, Mahajan VB. An intravitreal implant injection method for sustained drug delivery into mouse eyes. Cell Rep Methods 2021; 1:100125[PMID: 35128514].

24. Ghazi NG, Abboud EB, Nowilaty SR, Alkuraya H, Alhommadi A, Cai H, Hou R, Deng WT, Boye SL, Almaghamsi A, Al Saikhan F, Al-Dhibi H, Birch D, Chung C, Colak D, LaVail MM, Vollrath D, Erger K, Wang W, Conlon T, Zhang K, Hauswirth W, Alkuraya FS. Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum Genet 2016; 135:327-43. [PMID: 26825853].

25. MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, Clark KR, During MJ, Cremers FP, Black GC, Lotery AJ, Downes SM, Webster AR, Seabra MC. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet 2014; 383:1129-37. [PMID: 24439297].

26. Lam BL, Davis JL, Gregori NZ, MacLaren RE, Girach A, Verriotto JD, Rodriguez B, Rosa PR, Zhang X, Feuer

WJ. Choroideremia Gene Therapy Phase 2 Clinical Trial: 24-Month Results. Am J Ophthalmol 2019; 197:65-73. [PMID: 30240725].

27. Guo X, Starr C, Zhou J, Chen B. Protocol for evaluating the role of a gene in protecting mouse retinal ganglion cells. STAR Protoc 2021; 2:100932[PMID: 34806045].

28. Wang J, Li Y, King R, Struebing FL, Geisert EE. Optic nerve regeneration in the mouse is a complex trait modulated by genetic background. Mol Vis 2018; 24:174-86. [PMID: 29463955].

29. Patel D, Patel SN, Chaudhary V, Garg SJ. Complications of intravitreal injections: 2022. Curr Opin Ophthalmol 2022; 33:137-46. [PMID: 35266893].

30. Lin CH, Sun YJ, Lee SH, Mujica EM, Kunchur CR, Wu MR, Yang J, Jung YS, Chiang B, Wang S, Mahajan VB. A protocol to inject ocular drug implants into mouse eyes. STAR Protoc 2022; 3:101143[PMID: 35141566].

31. Mursalin MH, Livingston E, Coburn PS, Miller FC, Astley R, Callegan MC. Intravitreal Injection and Quantitation of Infection Parameters in a Mouse Model of Bacterial Endophthalmitis. J Vis Exp 2021; 168[PMID: 33616100].

32. Mehta MC, Finger PT. Angled transscleral intravitreal injection: a crossover study. Eur J Ophthalmol 2015; 25:173-6. [PMID: 25384968].

33. Bisht R, Jaiswal JK, Chen YS, Jin J, Rupenthal ID. Light-responsive in situ forming injectable implants for effective drug delivery to the posterior segment of the eye. Expert Opin Drug Deliv 2016; 13:953-62. [PMID: 26967153].

34. Da Costa R, Röger C, Segelken J, Barben M, Grimm C, Neidhardt J. A Novel Method Combining Vitreous Aspiration and Intravitreal AAV2/8 Injection Results in Retina-Wide Transduction in Adult Mice. Invest Ophthalmol Vis Sci 2016; 57:5326-34. [PMID: 27784063].

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