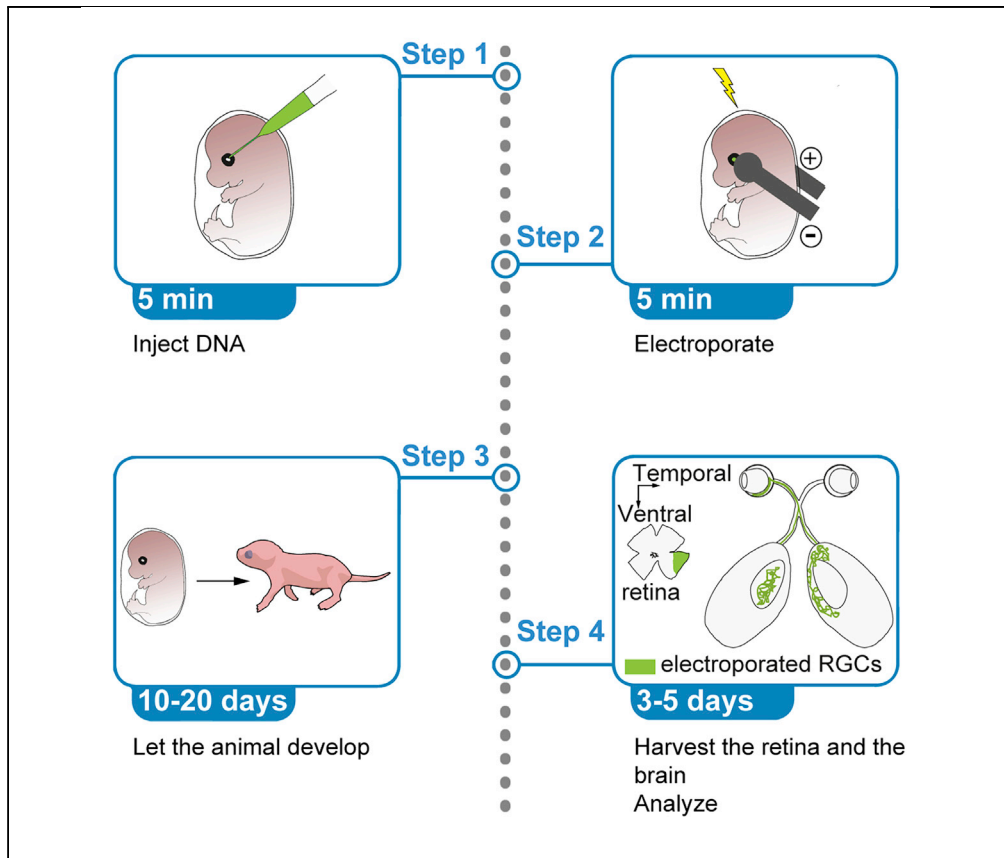


Protocol

Targeted *in utero* electroporation of the ventro-temporal mouse retina



Alice Louail, Ahlem Assali, Xavier Nicol

alicelouail@gmail.com
(A.L.)
xavier.nicol@inserm.fr
(X.N.)

Highlights

Electroporate the ventro-temporal embryonic retina in mice

Manipulate developing ipsilateral retinal ganglion cells with genetically encoded tools

Trace axons of ipsilateral and neighboring contralateral retinal ganglion cells

Techniques enabling DNA delivery into mouse retinal cells using *in utero* electroporation are available. However, these techniques target the central retina and do not enable the electroporation of the ventro-temporal retina where ipsilateral retinal ganglion cells are located. Here, we describe a protocol to specifically electroporate the ventro-temporal retina, a critical approach to manipulate ipsilaterally projecting retinal ganglion cells and contralaterally projecting neurons located in the same region of the retina. The procedure is adaptable to target other retinal quadrants.

Louail et al., STAR Protocols 2, 100516

June 18, 2021 © 2021 The

Author(s).

<https://doi.org/10.1016/j.xpro.2021.100516>

j.xpro.2021.100516



Protocol

Targeted *in utero* electroporation of the ventro-temporal mouse retina

Alice Louail,^{1,3,5,6,*} Ahlem Assali,^{2,4,5} and Xavier Nicol^{1,7,*}

¹Sorbonne Université, Inserm, CNRS, Institut de la Vision, 17 rue Moreau, 75012 Paris, France

²Sorbonne Université, Inserm, Institut du Fer à Moulin, 17 rue du Fer à Moulin, 75005 Paris, France

³Present address: Achucarro Basque Center for Neuroscience, Science Park of the UPV/EHU, Sede Building, 48940, Leioa, Spain

⁴Present address: Department of Neuroscience, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425, USA

⁵These authors contributed equally

⁶Technical contact

⁷Lead contact

*Correspondence: alicelouail@gmail.com (A.L.), xavier.nicol@inserm.fr (X.N.)
<https://doi.org/10.1016/j.xpro.2021.100516>

SUMMARY

Techniques enabling DNA delivery into mouse retinal cells using *in utero* electroporation are available. However, these techniques target the central retina and do not enable the electroporation of the ventro-temporal retina where ipsilateral retinal ganglion cells are located. Here, we describe a protocol to specifically electroporate the ventro-temporal retina, a critical approach to manipulate ipsilaterally projecting retinal ganglion cells and contralaterally projecting neurons located in the same region of the retina. The procedure is adaptable to target other retinal quadrants.

For complete details on the use and execution of this protocol, please refer to Louail et al. (2020).

BEFORE YOU BEGIN

This protocol describes the procedure and material to specifically electroporate embryonic retinal cells located in the ventro-temporal (VT) retina. This technique was established to investigate the development of the ipsilateral and contralateral projections from the VT retina in their brain targets. The original procedure is described in Louail et al., 2020, and was adapted from a previously described approach for *in utero* retinal electroporation (Garcia-Frigola et al., 2007; Petros et al., 2009). Of note, the described protocol does not specifically target ipsilaterally projecting retinal ganglion cells (RGCs), but also leads to electroporation of contralaterally projecting RGCs of the VT retina. In addition, only a subset of ipsilaterally projecting RGCs will express the construct of interest.

△ **CRITICAL:** The success of the procedure relies on a precise targeting of the VT retina and on an efficient survival rate of the electroporated pups, both during their embryonic and early post-natal life. It is therefore recommended to first practice this technique on wild-type animals using plasmids expressing a fluorescent protein to assess electroporation efficiency, the successful target of the area of interest, as well as the survival rate of the electroporated embryos, before performing the experiments on a transgenic mouse line using the plasmids of interest. This requires following strict surgical asepsis. Minimal manipulation of the animals after the surgery and during the week after the birth of the pups is also



critical to improve the survival rate. Manipulations should be limited to analgesia and post-procedure monitoring.

Preparation of mice, reagents, and capillaries

⌚ Timing: [up to 1 week]

1. Use embryonic day 14.5 (E14.5) timed-pregnant dams with a pigmented genetic background to avoid the alterations of retinal projections carried by albino mice. Allow timed-pregnant mice to adapt to the animal facility five to seven days prior to the surgery. To improve the pup survival rate, use Swiss timed-pregnant mice as foster dams. To be ready to lactate the electroporated pups, the foster dams should be placed in the same cage as the mice carrying the embryos to electroporate during the period of adaptation to the animal facility. The foster dam should give birth one day before the experimental dam. One foster dam can take care of up to two litters of pigmented pups. Allow timed-pregnant mice to adapt to the animal facility five to seven days prior to the surgery. Euthanize all the pups of the foster dams at birth, except two to trigger lactation and maternal behavior.
2. Prepare the DNA solution. Although it is preferable to prepare a fresh solution for each experiment, DNA solutions can be used for up to a month if stored at -20°C .
 - a. Use a DNA plasmid coding for the gene of interest under the control of the CAG promoter, or any specific promoter for a retinal subtype. RGCs can be targeted by γ -synuclein (Snca) promoter (Chaffiol et al., 2017; Wang et al., 2020). Specific promoters such as U6 or H1 can be used to express shRNAs. To visualize the electroporated cells, use plasmids that include the expression of fluorescent proteins (GFP, tdTomato...) or peptide tags (HA, Flag, Myc...).
 - b. Prepare a plasmid solution in PBS1X containing $2\text{ }\mu\text{g}\cdot\mu\text{L}^{-1}$ of plasmid and $0.014\text{ }\mu\text{g}\cdot\mu\text{L}^{-1}$ of Fast Green as a reporter of a successful injection. If more than one plasmid is electroporated, limit the total amount of DNA to $2\text{ }\mu\text{g}\cdot\mu\text{L}^{-1}$. Lower DNA concentrations can be used to get sparse electroporated cells.
3. Elongate the glass capillaries.
 - a. Use 1.2 mm outer diameter glass capillaries (Harvard Apparatus, 30-0043).
 - b. Calibrate the resistance temperature and pulling force of the micropipette puller to obtain thin, sharp but still rigid capillary tips (diameter $\sim 50\text{ }\mu\text{m}$, Figure 1). In our hands, and with our capillary puller, the resistance temperature was set at 61.2°C , with 3 weight blocks. These parameters need to be adapted for each capillary/puller combination.
 - c. Pull a set of several glass capillaries and store them with the tip pointing up (use modeling paste or lab tape).

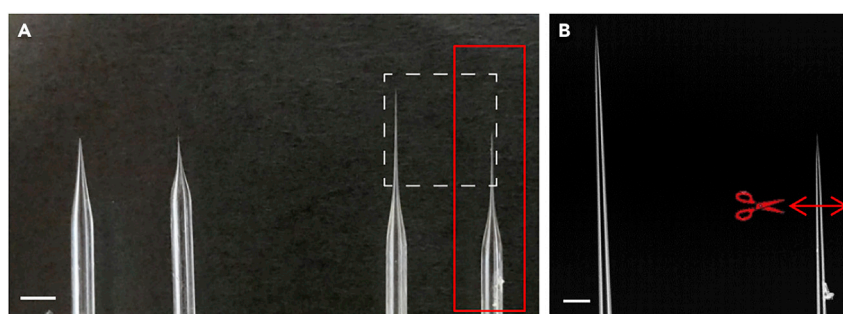


Figure 1. Choosing the optimal elongated glass capillary

(A) Examples of elongated glass capillaries with different tips length. Dotted square corresponds to (B). Red rectangle indicates the correct capillary tip to use for electroporation.

(B) High magnification of the capillary tips. Arrows represent where to cut the capillary. At this point, the capillary diameter is around $50\text{ }\mu\text{m}$. Scale bars: A, 2 mm ; B, $200\text{ }\mu\text{m}$.

Bench preparation

⌚ Timing: [10 min]

4. Clean the bench for the surgery using a disinfectant spray.
5. Lay a heating pad on the bench and set it at $\sim 37^{\circ}\text{C}$. Place a surgical drape on the heating pad. Turn on a small animal incubator which will be used for animal recovery after surgery and set it at 30°C . Alternatively, set a second heating pad at $\sim 37^{\circ}\text{C}$ for animal recovery.
6. Warm sterile 0.9% NaCl at 30°C ($\sim 25\text{--}50$ mL per mouse). It will be used to keep the embryos continuously wet during the surgery.
7. Prepare the following instruments:
 - a. Sterilize surgery tools (autoclave, hot bead sterilizer or 70% ethanol): 2 standard forceps with straight tip head, Dumont forceps, scissors, and needle holder.
 - b. Prepare: hair trimmer, clean gauze compresses, ocular protecting gel (lubrithal), povidone iodine (vetedine) and 70% ethanol solutions, non-absorbable braided silk sutures (wire diameter 4-0, needle C-3).
8. Turn on the electroporator:
 - a. Use the following settings: 5 pulses of 45 V during 50 ms every 950 ms. To get sparse electroporated cells, the number of pulses or voltage can be decreased.
 - b. Plug the electrodes and submerge them in a Falcon tube containing PBS1X. Check for the formation of bubbles at the surface of the submerged electrodes when the current is delivered (Troubleshooting 1).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Ketamine	Axience	Cat# Ketamidol 100 mg/mL
Xylazine	Centravet	Cat# Rompun 2%
Buprenorphine	Axience	Cat# Buprécure multidose 0.3 mg/mL
Oxybuprocaine	CSP	Cat# Chlorhydrate d'oxybuprocaine 1.6 mg/ 0,4 mL
Povidone iodine	Centravet	Cat# Vetedine Solution (Vetoquinol)
Lubrithal	Centravet	Cat# LUB001
Fast Green	Sigma	Cat# F-7252
PBS	Thermo Fisher Scientific	Cat# 14200-067
Experimental models: organisms/strains		
Mouse: C57BL/6NRj	Janvier Labs	RRID:MGI:6236253
Mouse: SWISS	Janvier Labs	RRID:MGI:2168141
Recombinant DNA		
Plasmid: CAG::GFP	This manuscript	N/A
Other		
Capillaries	Harvard Apparatus	Cat# 30-0043
Aspirator tube for calibrated microcapillaries	Sigma	Cat# A5177-5EA
Electroporation paddles	Sonidel	Cat# CUY650P2
Electroporator	Nepa Gene	Cat# CUY21EDIT
Micropipette puller	Narishige	Cat# PC-10
Ultrasound transmission gel	AquaSonic	Cat# 01-08
Standard forceps	FST	Cat# 11006-12
Dumont forceps	FST	Cat# 11251-30
Scissors	FST	Cat# 14381-43
Hair trimmer	Wella Professionals	Cat# Contura
Clean gauze compresses	LCH Medical Products	Cat# Pure SN30
Nonabsorbable braided silk sutures	Ethicon	Cat# F736

STEP-BY-STEP METHOD DETAILS

Animal anesthesia and analgesia

⌚ Timing: [15–20 min]

1. Prepare a solution of Xylazine/Ketamine ($2 \text{ mg} \cdot \text{mL}^{-1}$ and $10 \text{ mg} \cdot \text{mL}^{-1}$, respectively) diluted in sterile 0.9% NaCl. Warm the solution on the heating pad.
2. Prepare a solution of buprenorphine ($0.3 \text{ mg} \cdot \text{mL}^{-1}$) diluted in sterile 0.9% NaCl. Warm the solution on the heating pad. Alternatively, ketoprofen ($0.5 \text{ mg} \cdot \text{mL}^{-1}$) can be used for analgesia.
3. Anesthetize the timed-pregnant mouse via an intra-peritoneal injection of Xylazine/Ketamine solution ($10 \text{ mg} \cdot \text{kg}^{-1}$ and $100 \text{ mg} \cdot \text{kg}^{-1}$, respectively). The anesthesia provides about 30 min to complete the procedure. Alternatively, gas anesthesia using isofluorane can be used. This type of anesthesia enables a faster recovery of the mouse. However, keep in mind that the constraints of using gas anesthesia with a mask on the mouse may complicate the manipulation of the embryos.
4. Inject subcutaneously the anesthetized mouse with the analgesic solution ($0.0125 \text{ mg} \cdot \text{kg}^{-1}$ buprenorphine).

⚠ **CRITICAL:** Insert the needle in the middle of the abdomen, between the lower nipples, at a depth of about 6 mm. The intraperitoneal injection should be done with the needle at 45° to the skin to avoid damaging the embryos.

Preparation of the surgery

⌚ Timing: [5 min]

5. Cut as short as possible (0.5 mm maximum) the tip of a capillary using the Dumont forceps oriented in order to obtain a beveled tip (Figure 1B). Fill the capillary with the DNA solution, using a mouth aspirator tube. A microinjector can also be used for this step.

⚠ **CRITICAL:** Cut the capillary tip as thin as possible to keep it sharp and able to easily penetrate the amniotic sacs. However, too thin capillaries clog easily. Too thick capillaries create a hole in the amniotic sac that will lead to a leak of the amniotic solution and lead to the death of the embryo. This step needs to be calibrated and adjusted based on the settings used to pull the micropipettes (Figure 1). Before injecting the DNA in the embryo's retina, make sure that the capillary is not clogged by ejecting some of the DNA solution out of the micropipette.

6. Prepare the mouse for the surgery.
 - a. Using the hair trimmer, shave the lower part of the mouse abdomen. Shave an area of about 2 cm long and 1 cm large, and try not to rip the nipples.
 - b. Place the mouse on the surgical drape, with the abdomen towards the experimenter. Apply ocular protecting gel.
 - c. Disinfect the abdomen with povidone iodine first and then 70% ethanol. Place a gauze pad as surgical drape on top of the shaved abdomen. By using a Pasteur pipette, help the gauze pad adhering to the shaved abdomen by wetting it with warmed 0.9% NaCl (30°C).

Surgical procedure

⌚ Timing: [20 min]

7. Exposing the embryos.

- a. Perform a midline laparotomy (Figure 2A) by doing a longitudinal incision of about 1.5 cm long through the *linea alba*. First cut the skin and then cut the muscle. Do not cut past the lower nipples. Cut as straight as possible to easily affix both parts of the muscle and skin together during the suturing and help the scarring. After this step, the uterine horns are exposed.
- b. Very gently, pull one uterine horn out of the abdomen cavity, by grabbing the uterine horn in between embryos. Importantly, rough manipulation and pulling of the last embryo (most distal from the ovary) can trigger abortion after the surgery. Be extra careful not to pinch any of the embryos, they are often in very close contact with the muscle (Troubleshooting 2).

△ CRITICAL: Keep all the embryos wet during the entire surgery using warm and sterile 0.9% NaCl delivered with a Pasteur pipette.

8. DNA injection in the eye.
 - a. Using the 2 pairs of standard forceps, gently grab the first embryo and immobilize it with one pair of forceps (ring forceps can also be used here). Gently position the embryo so the eye gets in close apposition with the amniotic membrane and uterine wall.
 - b. Using the capillary filled with DNA, pierce the uterine wall, the amniotic sac and the eye in one time (Troubleshooting 3). Fill the eye with 1 μ L of the DNA solution. Avoid injecting with the capillary perpendicular to the eye, instead inject with the capillary almost parallel to the uterine wall, with only a small angle, and pierce the eye through the side of the eye (Figure 2B). Resistance will usually be felt when piercing the eye, while no resistance will be felt while piercing the embryo outside of the eye. The eye turns green in case of successful injection (Troubleshooting 4). If the capillary is in the amniotic sac but not in the eye, fast green will be observed in the amniotic sac.
9. Electroporate the retina.
 - a. Immerse the electrodes in highly conductive, multi-purpose ultrasound transmission gel (AquaSonic 01-08).
 - b. Place the head of the embryo between the electrodes.
 - i. Always approach the paddles from the back of the embryo.
 - ii. Place the negative electrode on the ventro-temporal part of the injected eye; and the positive electrode on the other side of the head. Adjust the angle between the electrode and

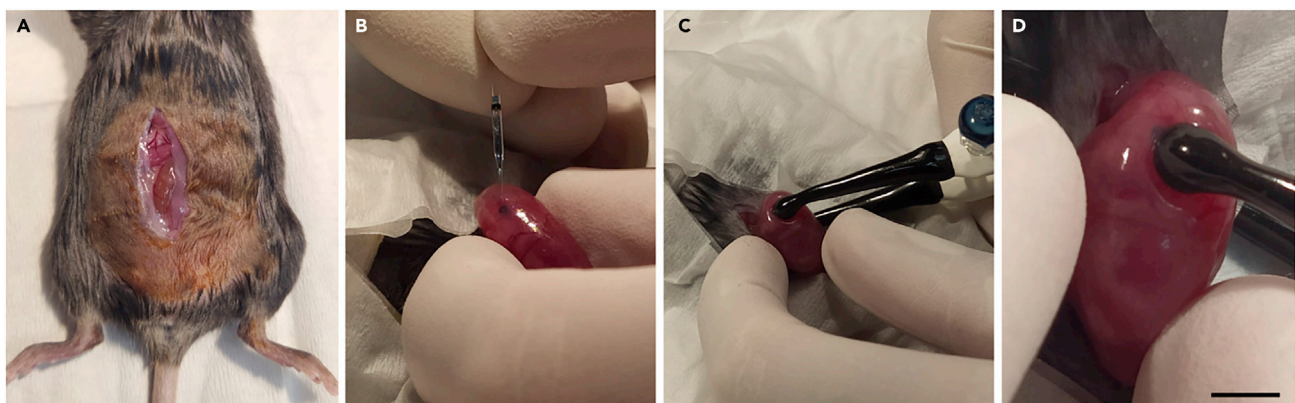


Figure 2. Illustrations of the key steps of the surgical procedure

(A) Skin and muscle incision.

(B) To inject the plasmid in the eye, the capillary should be almost parallel to the uterine wall, with a small angle, in order to facilitate the capillary's piercing the uterine wall, the amniotic membrane and the side of the eye. The closer the eye is to the uterine wall, the easier it is to pierce the eye with the capillary.

(C) The negative electrode is placed on the eye to be electroporated, covering the ventro-temporal (VT) region, and the positive electrode is placed on the other side of the head. The surface of both electrodes should entirely touch the uterine wall.

(D) The negative electrode is placed on the VT retina, covering almost half of the eye. Scale bar: D, 2 mm.

the head axis so that the ventro-temporal retina is between the cathode and the anode (Figures 2C and 2D).

- c. Apply five electric pulses of 45 V during 50 ms every 950 ms using the paddle of the preprogrammed electroporator.

⚠ **CRITICAL:** It is crucial to stay still (embryo and electrodes) during the electroporation. Any shift would result in a wider electroporated area, or a missed VT targeting (Troubleshooting 5).

10. Repeat steps 8 and 9 for all of the embryos. Repeat these steps for the second uterine horn. To assess the survival rate, take note of the total number of embryos electroporated. Since it can be hard to recognize a non-electroporated embryo from an electroporated embryo, start with the first embryo (the closest to the ovary) and then electroporate them in order, from the closest to the most distant from the ovary.

⚠ **CRITICAL:** Wet profusely all the embryos with warm and sterile 0.9% NaCl after the electroporation of each embryo.

⚠ **CRITICAL:** It is critical to avoid any damage to the placenta during the manipulation of the embryos, or to inject through a blood vessel. During the electroporation, the experimenter should gently hold the embryos with the electrodes with minimal force.

11. Closing the abdominal cavity.
 - a. Gently place the uterine horns back in the abdominal cavity.
 - b. Fill the abdomen with warm and sterile NaCl 0.9%.
 - c. Close the muscle with continuous sutures.
 - d. Close the skin with lock-stitch sutures.
 - e. If the mouse endures pain during the sutures, apply oxybuprocaine as local anesthetics.
 - f. Apply povidone iodine to the sutures.

⚠ **CRITICAL:** The mouse should not have its abdomen open for more than 30 min to ensure a high survival rate of the embryos, and for anesthesia constraints.

Post-surgical care

⌚ **Timing:** [3 days]

12. Leave the mouse in an incubator at 30°C until it wakes up before placing it back to its cage. The cage should not be cleaned at least two days before and five days after the surgery, so that the mouse recovers in a familiar environment. Leave the embryos to develop normally.
13. Minimize mouse disturbances and check for signs of pain during the first 48 h after the surgery (hunched position, reduced activity, squinted eyes). If signs of pain are detected, inject the analgesic solution (0.0125 mg·kg⁻¹ buprenorphine). If the analgesia does not improve the mouse welfare, proceed to euthanasia.
14. Carefully check for the suture integrity until the skin is properly closed. Re-suture if needed (although repeated anesthesia can be detrimental for the embryos' survival).

Expected outcomes

This protocol for ventro-temporal retinal *in utero* electroporation enables the expression of a gene of interest in both ipsilaterally projecting RGCs and their neighbors projecting contralaterally. A survival rate of 50% or more is expected from a trained experimenter. One of the key points is the survival of the pups after birth since cannibalism is not uncommon. This survival rate is obtained with C57BL/6NRj pups and Swiss foster dams. Among the surviving pups, about 60% are expected to

exhibit electroporated RGCs specifically in the ventro-temporal retina when using the CAG promoter. An extensive training might be necessary to reach this success rate.

Using pan-cellular promoters (e.g., CAG) will not exclusively target RGCs, but all retinal cell types. The successful targeting and efficiency of the electroporation can be assessed by dissecting and imaging the retina and retinal brain targets (Figure 3). A successful electroporation labels cells in the VT retina (Figure 3B). A longer incision in the ventral retina can be made during the dissection to orient the retina. Optic chiasm dissection and imaging validate the targeting of ipsi- and/or contralaterally projecting RGCs. The projections of the electroporated RGCs can be visualized at the optic chiasm level (Figure 3C) and in their brain targets, in both the ipsilateral and contralateral hemispheres (Figures 3D–3G).

This protocol can be combined with post-natal injections of cholera toxin β subunit conjugated to different fluorophores in each eye to visualize the entire visual projections of both eyes. This technique enables assessing the position of axonal projections of the electroporated RGCs in the visual targets (Figures 3D–3G). The procedure can be adapted to target other retinal quadrants, by adjusting the position of the electrodes.

LIMITATIONS

Since ipsilaterally projecting RGCs are located in the peripheral end of the VT retina, a more medial targeting of the retina would lead to electroporation of cells projecting to the contralateral hemisphere of the brain. One limitation of this technique is that only a random subset of retinal neurons of the VT retina is electroporated. This technique is therefore not appropriate to answer questions that require the manipulation of most ipsilaterally projecting RGCs. Finally, each electroporation is unique for number of cells electroporated, number of plasmids integrated, and precise retinal area targeted. Therefore, this technique is not optimal for experiments requiring a highly reproducible number of electroporated cells.

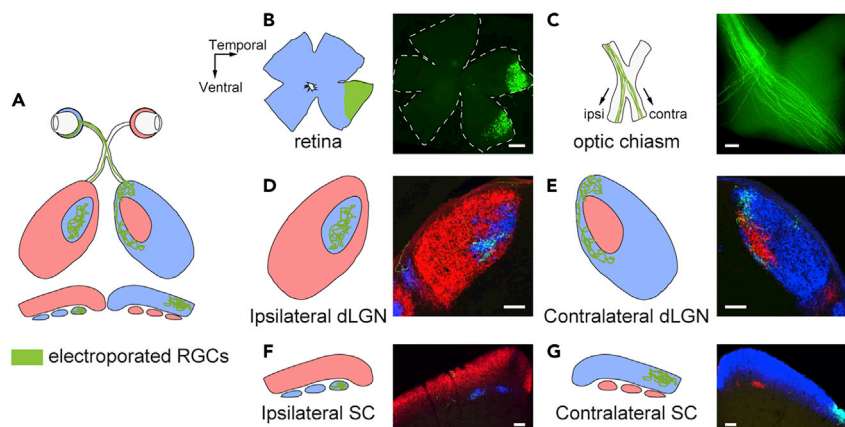


Figure 3. Ventro-temporal retinal *in utero* electroporation with a GFP plasmid

(A) Drawing depicting the localization of the projections of electroporated RGCs in the mouse visual system, following this protocol. In this example, post-natal injections of cholera toxin β subunit conjugated to different fluorophores were performed in each eye to visualize the axons of electroporated RGCs (in green) in the visual targets and compare their position with the projections of all RGCs of the electroporated eye (blue) and with the projections of the opposite retina (red).

(B) Electroporated RGCs are found in the ventro-temporal retina, projecting their axons to the optic disc.

(C–G) (C) At the level of the optic chiasm, some electroporated visual axons avoid the midline (ipsilaterally projecting); while others cross the midline (contralaterally projecting axons). This protocol for ventro-temporal retinal electroporation targets axons projecting to the ipsilateral visual nuclei: (D) the dorso-lateral geniculate nucleus of the thalamus (dLGN) and (F) the superior colliculus (SC). A subset of axons projecting to the contralateral visual nuclei are also electroporated: (E) dLGN and (G) SC. Scale bars: 100 μ m.

TROUBLESHOOTING

Problem 1

Bubbles are not observed on the surface of the electrodes immersed in PBS when the current is delivered (Before you begin step 8).

Potential solution

Absence of bubbles might reflect defective electroporation paddles or altered wiring with the electroporator.

Solution 1: Verify that the current is passing through the paddles.

Solution 2: Verify that the paddles are properly plugged to the electroporator.

Solution 3: Verify that the paddles are functioning, by plugging another pair of paddles.

Problem 2

One of the amniotic sacs gets damaged during the procedure (*i.e.*, with amniotic liquid leaving the sac), or is difficult to pierce (step 7).

Potential solution

The integrity of the amniotic sac is a key factor of the surgery. The embryo in a damaged amniotic sac will very probably not survive. To avoid this:

Solution 1: Very gently use the forceps to grab the membranes in between the embryos, avoiding grasping the embryo or the amniotic sac.

Solution 2: Be extra careful when manipulating the embryos. The more gentle the experimenter, the higher the survival rate.

Solution 3: Keep the embryos wet with 0.9% NaCl at all times.

Solution 4: If the amniotic sac does not pierce easily with the capillary, the tip is not sharp enough. Prepare a new capillary.

Solution 5: Do not pierce the amniotic sac more than one time. Leave an embryo not electroporated rather than try to re-inject an already injected embryo.

Problem 3

The embryo is not well positioned to properly visualize the eye and to favor its apposition to the amniotic sac/uterine wall (step 8).

Potential solution

Gently, turn the embryo inside of the amniotic sac using the standard forceps to reach a position that facilitates the targeting of the eye. Always manipulate the back of the embryos, avoiding grasping them from the ventral side. One turning direction is usually easier than the other.

Problem 4

The brain ventricles turn green after DNA injection or electroporation (step 8).

Potential solution

If the brain ventricles turn green, the injection was too deep, the capillary overshot the eye.

Solution 1: Cells outside the retina are likely to be electroporated. Avoid analyzing these animals.

Solution 2: Make a new sharper capillary to inject the other embryos. The force to apply in order to pierce the sac will be lower and thus will reduce the risk of injecting beyond the eye.

Problem 5

The electrodes move after the electroporation has started (step 9).

Potential solution

Quickly remove them from the embryo, while noting how many electric pulses have occurred. Wait until the end of the five pulses, place the electrodes correctly, and start again the electroporation process with only the number of pulses that have not been applied to the embryo the first time.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xavier Nicol (xavier.nicol@inserm.fr).

Materials availability

This study did not generate new unique material.

Data and code availability

For dataset and analysis generated using this protocol, please refer to [Louail et al., 2020](#).

ACKNOWLEDGMENTS

We are grateful to members of the Nicol laboratory for thoughtful discussions and to the members of the animal and imaging facilities of Institut de la Vision. This work was supported by grants from Agence Nationale de la Recherche (ANR-15-CE16-0007-01), UNADEV (17UU1166-00), and Sorbonne Université (FCS-SU IDEX SUPER SU-15-R-PERSU-17) to X.N. This work was performed in the framework of the LABEX LIFESENSES (ANR-10-LABX-65) and IHU FOReSIGHT (ANR-18-IAHU-0001) supported by French state funds managed by the Agence Nationale de la Recherche within the Investissements d'Avenir program. A.L. was supported by fellowships from the ED3C doctoral program (Sorbonne Université) and Fondation de France. A.A. was supported by fellowships from the ED3C doctoral program (Sorbonne Université) and Retina France.

AUTHOR CONTRIBUTIONS

A.L. wrote the initial draft. A.A. and X.N. edited the manuscript. A.A. adapted the retinal *in utero* electroporation approach to specifically target the VT retina. A.L. further developed, validated, and stabilized the technique. A.L. and A.A. generated the illustrative images.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Chaffiol, A., Caplette, R., Jaillard, C., Brazhnikova, E., Desrosiers, M., Dubus, E., Duhamel, L., Macé, E., Marre, O., Benoit, P., et al. (2017). A new promoter allows optogenetic vision restoration with enhanced sensitivity in macaque retina. *Mol. Ther.* 25, 2546–2560.
- Garcia-Frigola, C., Carreres, M.I., Vegar, C., and Herrera, E. (2007). Gene delivery into mouse retinal ganglion cells by *in utero* electroporation. *BMC Dev. Biol.* 7, 103.
- Louail, A., Sierksma, M.C., Chaffiol, A., Baudet, S., Assali, A., Couvet, S., Nedjam, M., Roche, F., Zagar, Y., Duebel, J., et al. (2020). cAMP-dependent co-stabilization of axonal arbors from adjacent developing neurons. *Cell Rep.* 33, 108220.
- Petros, T.J., Rebsam, A., and Mason, C.A. (2009). *In utero* and *ex vivo* electroporation for gene expression in mouse retinal ganglion cells. *J. Vis. Exp.* 31, 1333.
- Wang, Q., Zhuang, P., Huang, H., Li, L., Liu, L., Webber, H.C., Dalal, R., Siew, L., Fligor, C.M., Chang, K.-C., et al. (2020). Mouse γ -synuclein promoter-mediated gene expression and editing in mammalian retinal ganglion cells. *J. Neurosci.* 40, 3896–3914.