BOX 1. Optimal parameters and electroporation efficiency for *in utero* electroporation with triple-electrode probe.

MOUSE

Targeted zone	Electroporation age	Voltage	Tweezer-like electrode size	Number of litters	Survival	Number of pups	% EGFP$^+$ pups
	E14.5-E15.5	30V	10 mm	7	74.9 ± 6.1	60	82. 1 ± 4.9
Hippocampus	E15.5	30V	5 mm	1	84.6	13	45
	E15.5	30V	3 mm	4	80.9 ± 19.1	42	18.6 ± 5.9
Visual cortex	E15.5	30V	5 mm	7	72.8 ± 5.4	51	87.7 ± 4.7
Motor cortex	E15.5	30V	5 mm	5	58.2 ± 13.9	41	83.8 ± 9.2
Prefrontal cortex	E15.5	30V	5 mm	9	64.4 ± 9.0	57	86.9 ± 5.0

RAT

Targeted zone	Electroporation age	Voltage	Tweezer-like electrode size	Number of litters	Survival	Number of pups	% EGFP$^+$ pups
Hippocampus	E17.5	50V	10mm	15	79.5 ± 4.4	132	90.3 ± 1.9
Visual cortex	E17.5	50V	10mm	8	72.8 ± 9.8	51	93.8 ± 3.6
Motor cortex	E17.5	50V	10mm	8	67.6 ± 10.1	49	92.5 ± 4.0
Prefrontal cortex	E17.5	50V	10mm	4	78.8 ± 8.0	46	89.2 ± 4.7
Cerebellum	E14.5	35V	10mm	8	53.1 ± 12.3	41	57.7 ± 7.7

BOX 2. Third electrode crafting (Fig. 2c,d)

(A) Prepare:

(i) Cell scraper (Falcon; Cat. No. 353085)
(ii) Platinum plates (3 mm x 5 mm for mouse or 5 mm x 6 mm for rat)
(iii) Male-plug cable
(iv) Soldering
(v) Aluminum soldering flux
(vi) Epoxy glue
(vii) Labeling tape
(viii) Scissors

(B) Procedure

(i) Solder platinum plate with male plug cable compatible with the connector cable of the electroporator.
(ii) With the scissors, cut the both endings of the cell scraper's head to prepare optimal squared surface to glue the platinum plate.
(iii) Use epoxy glue to stick platinum plate to the cell scraper head.

(iv) Wait until the glue is dry and the plate is stably attached to the cell scraper's head.

(v) Use insulating tape to attach the wire to the scraper's handle.

BOX 3. After-surgery cleaning of surgical tools

(A) Prepare a cleaning solution:
- **(i)** 1% sodium dodecyl sulfate (SDS, Sigma, cat. no. L4390)
- **(ii)** 0,2 N NaOH in water.

(B) Leave the instruments in the solution under agitation for few hours or overnight.

(C) Rub the instruments with a brush.

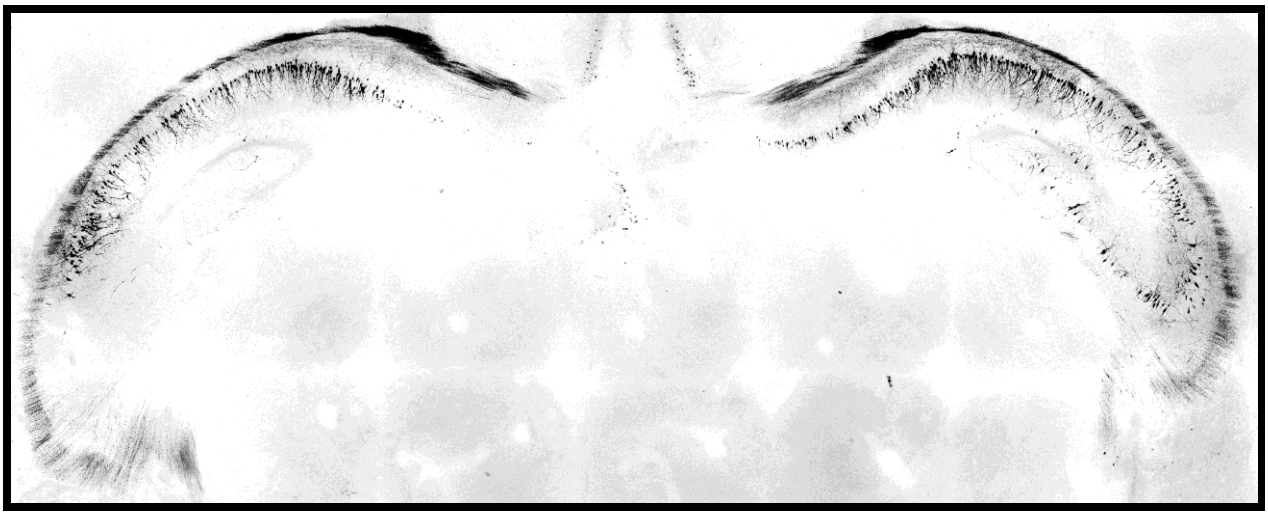
(D) Rinse very well with milliQ water.

(E) Rinse with ethanol 70 % and dry.

(F) Autoclave.

BOX 4. Bilateral transfection.

Hippocampus, and motor, visual and prefrontal cortices are structures symmetrically located in both hemispheres of the brain. The tripolar configuration of the electrodes during electroporation *in utero* distributes the electric field symmetrically and equally in both hemispheres, thus allowing for bilateral transfection of brain structures during a single electroporation episode[4,6]. For bipolar transfection, both ventricles must be filled with the DNA (by two consecutive injections in each lateral ventricle or by a single injection in one only ventricle until diffusion of DNA to the other ventricle) and the third electrode must be placed at an equal distance between tweezer-like electrodes.



Confocal image example of bilateral transfection of EGFP in rat hippocampus with the usage of the tripolar electrode configuration. Black cells are neurons expressing GFP.

BOX 5. Stitching mouse's and rat's abdominal cavities

Rodents will often try to remove their stitches. In order to prevent them from succeeding:

(A) First suture the muscles.

(i) Use absorbable vicryl-coated suture thread with gauge 5-0 for mouse and 4-0 for rat.

(ii) Shorten the remaining ends of the suture thread. Leaving them too long will irritate the rodent skin and will increase chances of the animal trying to remove the stitches.

(iii) Make three knots, every time in opposite direction, and pull them tight.

MOUSE: while making the first two knots, make two loops before tightening. For the last knot, make one loop only before tightening.

RAT: while making each knot, make one loop with the stitching thread on the mosquito forceps before tightening the knot.

(B) Suture the skin in the same way as for stitching. However, you can use surgical staples commercially available for both mice (Fine Science Tools; cat. no. 12032-07) and rats (Fine Science Tools; cat. no. 12032-09).

(C) Every time you stitch, make sure that under the abdominal wall there are no embryos. It is very easy to sting them during stitching.

BOX 6. After-surgery care

It is highly important to take care of dams after surgery. Sometimes, the success of the whole surgery depends on few after-surgery care details.

(A) After the surgery, shut down the isoflurane flow, and leave the oxygen flow alone for 1-2 minutes to increase the speed of the animal to awake.
(B) Place the animal in a clean cage with its belly down to favor breathing.
(C) Inside the cage, put wet food since the animal may have initial difficulties to reach the water source and food.
(D) After surgery, the animal is usually wet (blood and PBS) and under distress. Inside the cage put a piece of towel paper. The animal will dry faster and feel safer.
(E) Place the cage with the animal under a heating lamp until the animal fully recovers from anesthesia.
(F) Keep the animal separate from any source of stress (e.g., noise, smells).

(G) Control the general health condition of the dam few hours after surgery and the day after. Inject painkiller, if necessary.

(H) Pay attention to stitches conditions and possible bleedings.

(I) When possible, we recommend performing surgery on CD1 mice. After delivery, it is very common of C57BL/6 mouse females to cannibalize their pups. In the case that C57BL/6 are necessary for the experimental needs, it is highly important to have a foster mother. We recommend CD1 mice. Remove from natural litter as many pups as you want to foster. Nevertheless, add a maximum of six C57BL/6 pups for each foster mother. If possible, use the foster mother with a different fur color (e.g., CD1 mice foster for C57BL/6 experimental animals) that delivered not earlier than 2 days before the experimental mother.

BOX 7. Equivalent embryonic days for mice and rats

Animal	**Embryonic day**														
MOUSE	1	4	6	8	9.5	11	12	12.5	13	13.5	14	14.5	15	15.5	16
RAT	1	5	7.5	9	11	12.5	13.5	14	14.5	15	15.5	16	16.5	17	17.5

BOX 8. Increasing survival of embryos and transfection efficiency

(A) Being sterile during surgery is very important since contaminated embryos will not survive.

(B) Do not touch anything unsterile with the sterile gloves.

(C) Use sterile gauzes to hold the unsterile needle and electrodes. In case of contamination, change the gloves or use ethanol to clean them.

(D) Embryos are very delicate and need extreme care during handling. Never squeeze them with forceps, fingers or electrodes.

(E) Keep the total surgery time as short as possible (typically below 30 min)

(F) Never use sharp tools which may cause bleeding of the wound or the uterus. Bleeding always decreases embryos' survival.

(G) Always stop any kind of bleeding:

(i) Use the sterile gauze to buffer the blood flow.

(ii) Strongly compress the origin of bleeding (if it's in the muscles) with your fingers or with curved mosquito-forceps. You can also use the cauterizing pen.

(iii) Never remove the clot.

(H) Make sure there are no air bubbles in the needle with DNA. Injection of the air into the brain of the embryo will result in embryonic death.

(I) Use appropriate size of the syringe needle (32G – mouse; 30G – rat). Too big needle can damage brain and decrease survival of embryos.

If the efficiency of the transfection is low, you may increase the DNA concentration or slightly increase the voltage of the pulses during electroporation. Nevertheless, increases of voltage above 15% may result in increased embryonic death.