

Science Education Collection

Murine *In Utero* Electroporation

URL: <https://www.jove.com/science-education/5208>

Abstract

In utero electroporation is an important technique for studying the molecular mechanisms that guide the proliferation, differentiation, migration, and maturation of cells during neural development. Electroporation enables the rapid and targeted delivery of material into cells by utilizing electrical pulses to create transient pores in cell membranes. Although electroporation has traditionally been used in *in vitro* studies, scientific advancements have now broadened its utilization to intact organs, such as those found in mouse embryos developing *in utero*.

This video will introduce the key principles behind *in utero* electroporation in addition to reviewing the basic surgical techniques required to access developing embryos within a pregnant rodent. Details of the injection and electroporation steps are provided along with important considerations for directing gene delivery to specific brain regions. Finally, neurobiological applications of *in utero* electroporation are presented, such as investigating how specific genes contribute to neural development and how connections form between developing neurons.

Transcript

In utero electroporation is a key technique for studying the molecular mechanisms that guide neurodevelopment. By injecting DNA to alter gene expression in specific regions of the developing rodent brain, researchers can study the proliferation, differentiation, migration, and maturation of neural cells in the context of their natural environment.

This video will introduce the key principles behind *in utero* electroporation, a basic overview on how to perform the technique, and its applications in neurobiology research.

Before we delve into how to perform this procedure, let's first go over some principles of electroporation. This procedure utilizes electrical pulses that create transient pores in cell membranes, allowing DNA to enter the cell. Since the negatively charged DNA will move towards the positive electrode, different cell populations can be targeted depending on the positioning of the electric field.

Though electroporation has traditionally been used in *in vitro* studies, scientific advancements have broadened its utilization to intact organs, including those found in mouse embryos developing *in utero*.

In utero electroporation, unlike cell culture or *ex vivo* techniques, has the advantage that transfected cells will continue to be exposed to all the physiological cues that guide normal development. This is particularly important in the brain, where structures such as axons require guidance cues from other cell types in order to develop properly.

Brain development in particular involves a programmed series of proliferation and migration events, meaning that different tissue layers can be targeted based on the timing of electroporation.

Lastly, the availability of different types of electrodes allows researchers to access both regions near the surface of the brain as well as deeper areas. Paddle electrodes are used when targeting superficial parts of the brain, such as the cortex and hippocampus, while smaller needle electrodes are used when targeting deep structures like the thalamus and hypothalamus.

Now that we have discussed some basic principles behind the technique, let's get our preparations out of the way so we can delve into the surgical procedure and *in utero* electroporation.

First, sterilize all instruments and wipe down the surgical area with 70% ethanol. Next, make an injection solution by dissolving plasmid DNA in sterile PBS containing 0.1% Fast Green. The Fast Green is added to allow for visualization of injection solution in the embryo. Lastly, using a needle puller, an ultra thin needle must be created to reduce the loss of amniotic fluid and subsequent fetal mortality.

Now that we have everything prepared, let's go over the basic steps for electroporating brain tissue in developing rodents. Start by anesthetizing a pregnant dam using 5% isoflurane then transferring it to a respirator tube delivering 2.25% isoflurane for the entirety of the procedure. Next, for postoperative pain relief, deliver an analgesic like buprenorphine subcutaneously. Then shave the abdomen and sterilize the incision site.

To surgically expose the uterus, make a vertical incision along the midline in the skin and, using scissors, cut through the peritoneum. To prevent the embryos from drying out, cover the opening with sterile saline soaked gauze. Next, gently pull the embryonic chain out of the abdominal cavity being sure to keep the embryos wet by covering with sterile pre-warmed saline.

Using a dissection microscope, identify embryos properly positioned for easy access to the brain. Using your fingers or forceps stabilize the head of the embryo and inject the DNA solution through the uterine wall and into the brain. Place an electrode on each side of the head, with the positive electrode in the direction to which you want the DNA electroporated. Once all appropriately positioned embryos are electroporated, carefully place the uterine horn back into the abdominal cavity.

Before closing the incision, add 2 - 3 ml of warm saline to the cavity. Sew the peritoneum together with absorbable sutures, then close the skin using staples. Lastly, transfer the mouse to a cage with a heating pad and monitor.

Now that we have gone over how to perform *in utero* electroporation, let's examine some downstream applications of this technique.

In utero electroporation is a useful tool for investigating how specific genes contribute to neural development. Electroporated constructs can guide overexpression of wildtype or mutant proteins or block protein expression completely. Neurological phenotypes can then be assessed at either the microscopic or organismal level. For example, these experimenters determined that transient overexpression of the schizophrenia-associated gene, DISC-1 in the developing mouse brain, resulted in hypersensitivity to amphetamine later in life.

In utero electroporation can also be useful for visualizing specific cell populations and the connections they make by delivering sequences encoding fluorescent proteins into neural tissue. For example, these experimenters electroporated the green fluorescent protein gene into E13.5 retinas to study retinal ganglion cells, or RGCs, which transmit visual information from the eye to the brain. Examination of retinas at E18.5 shows how this technique can be used to trace the path of the RGC axon through the optic nerve, optic chiasm, and optic tract.

Finally, many important events in neural development occur after birth, inspiring scientists to develop a modified technique called *in vivo* electroporation. This procedure follows the same basic steps as *in utero* electroporation, but it is usually performed within the first few days following birth. Postnatal genetic manipulation is particularly useful for studying later-born cell types, such as those found within the olfactory bulb shown here.

You've just watched JoVE's video on *in utero* electroporation. This video contained key principles and a basic overview on how to perform *in utero* electroporation, as well as a few downstream applications that allow researchers to investigate the molecular mechanisms that guide neurodevelopment.

Thanks for watching!