Video Article

Protocol for Culturing Sympathetic Neurons from Rat Superior Cervical Ganglia (SCG)

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Abstract

The superior cervical ganglia (SCG) in rats are small, glossy, almond-shaped structures that contain sympathetic neurons. These neurons provide sympathetic innervations for the head and neck regions and they constitute a well-characterized and relatively homogeneous population (4). Sympathetic neurons are dependent on nerve growth factor (NGF) for survival, differentiation and axonal growth and the wide-spread availability of NGF facilitates their culture and experimental manipulation (2, 3, 6). For these reasons, cultured sympathetic neurons have been used in a wide variety of studies including neuronal development and differentiation, mechanisms of programmed and pathological cell death, and signal transduction (1, 2, 5, and 6). Dissecting out the SCG from newborn rats and culturing sympathetic neurons is not very complicated and can be mastered fairly quickly. In this article, we will describe in detail how to dissect out the SCG from newborn rat pups and to use them to establish cultures of sympathetic neurons. The article will also describe the preparatory steps and the various reagents and equipment that are needed to achieve this.

Video Link

The video component of this article can be found at https://www.jove.com/video/988/

Protocol

1. Preparation for the dissection:

1. Select the appropriate culture dish (10 cm or 6-well or 24-well plates, etc.) depending on the nature of your experiments, and coat them with rat-tail collagen 24 hours before dissection. The methods for preparing rat-tail collagen and using it to coat culture dishes have been described elsewhere (1).
2. Prepare a 0.25% Trypsin solution in phosphate buffered saline (PBS) (no EDTA) and filter sterilize. Prepare 1 ml aliquots and store at -20ºC.
3. Prepare a solution containing 2.5 mM each of Uridine and 5-FDU in distilled/deionized H_2O. Filter-sterilize and prepare 50 µl aliquots. Store at -20ºC.
4. Prepare Complete Medium: RPMI 1640 culture medium with 10% heat-inactivated horse serum (heat inactivate by incubating in a 56ºC waterbath for 30 minutes) and 5% fetal bovine serum.
5. Prepare Final Medium: RPMI 1640 culture medium with 1% heat-inactivated horse serum + NGF (100ηg/ml final concentration) + penicillin/streptomycin (1/250) + 1/250 uridine/5-FDU solution (10 µM final concentration). Note: this medium should be made up fresh before use every time. For an experiment in a 24-well plate format, one will need to prepare 12 ml of this medium. It is recommended that you prepare a little extra. NGF can be purchased from a variety of commercial sources (see Table at the end of this protocol).
6. Equipment: One will need a dissecting microscope and light source. A dual gooseneck fiber optic illuminator with focusing tips is preferred. In addition, one will need to clean and sterilize (by autoclaving or soaking in 70% ethanol for at least 20 minutes) the following dissection tools: two pairs of micro-dissecting stainless steel forceps, and a pair of dissecting scissors (also stainless steel and both from Roboz). Two sterile syringe needles (26 gague x 1 ½ inches or similar) will also be needed. It is necessary to set up a dissection board, which is a 3”x3” piece of styrofoam wrapped in aluminum foil and covered with a Kimwipe. Spray the board with 70% ethanol to decontaminate. Finally, prepare a fire-polished glass pasture pipette. In order to fire-polish, take the glass pipette and hold the tip in the flame for a few seconds, while rotating. Upon inspection, the tip will look smoother and the opening will be narrower. This is necessary for the step where the cells will be dissociated. Perform fire-polishing in a cell culture hood so that the pipette remains sterile.

2. Dissection of Superior Cervical Ganglia:

1. Collect neonatal rat pups that are of age P0 or P1.
2. Rapidly wipe the pup for dissection with 70% ethanol to reduce chances of contamination.
3. Rapidly decapitate pup with a sharp pair of scissors (this step will not be shown in the video). Hold the head using sterile forceps against sterile gauze to briefly drain excess blood.

4. Place the head on the dissection pad with the ventral side facing up and the caudal end oriented toward you. The severed trachea should be readily visible. Use the sterile syringe needles to secure the severed head to the dissecting pad in the following manner: push one pin though the roof of the mouth into the pad and push the second pin though the severed trachea into the pad. Place the pad under the dissecting microscope and begin dissection.
5. Using both forceps (one in each hand) clear away skin and fat around the trachea, taking care not to go too deep. Doing this will expose a pair of muscles running almost parallel to each other on either side of the trachea. Note: during dissection, you will need to irrigate with cold sterile PBS or cold, serum free RPMI 1640 medium delivered with a sterile, Pasteur pipette to wash away excess blood and to keep the area clean and moist. This may be done only once or more than once depending on how fast one works. For an experience dissector, it takes only about two to three minutes to dissect out a pair of ganglia.
6. Use the forceps to cut and remove the muscle on one side first.
7. Once the muscle is removed, the carotid artery will become visible. Often it is light pinkish and contains blood. It may appear lighter in color than other blood vessels that you may see in the vicinity and for this reason the carotid artery may not be noticeable right away. This artery runs alongside the trachea and bifurcates into what looks like a "Y"-shaped ribbon. This bifurcation is an important landmark and once it is located, it will be relatively easy to find the SCG.
8. Sever the artery at the most caudal end and lift it up slightly with the forceps (the other end is still attached). Once you do this, you will see an almond-shaped, glossy looking small mass of tissue that is loosely attached to the artery just at the point of bifurcation and that has thread-like pre- and/or post-ganglionic connectives. This is the superior cervical ganglion. Use a second pair of forceps in the other hand to gently detach it and place in a small culture dish (35 mm) containing cold, serum free RPMI 1640 medium.
9. Next, extract the ganglion on the other side of the trachea following the same protocol.

3. Cleaning the Ganglia:

1. Once all the ganglia have been dissected out, they should be freed as much as possible of extraneous tissue.
2. Under the dissection microscope, you may see that the ganglia have attached pieces of carotid artery, fat or other tissue. Work with forceps in each hand to tease away these unwanted materials. Place cleaned ganglia into a sterile 15ml conical, polypropylene tube, containing RPMI 1640 medium.
3. The pre- and/or post-ganglionic connectives should also be trimmed away. Each ganglion has about 10,000 neurons. Once the neurons are plated, they will regrow their neurites.

4. Establishing Sympathetic Neuron culture:

1. Centrifuge the ganglia at 200xg for 2 minutes and discard the supernatant.
2. Resuspend the ganglia in 0.5 to 1 ml of 0.25% trypsin solution. Place in a 37˚C waterbath or incubator for 30 minutes. This will help dissociate the cells in the ganglia.
3. After 30 minutes, add 10 ml of Complete Medium to dilute out and neutralize the trypsin and centrifuge at 200xg for 2 minutes.
4. Discard supernatant and resuspend in 2 ml of Final Medium.
5. Further dissociate the cells by triturating the ganglia up and down with the fire-polished glass pasture pipette. Triturate the ganglia 20 times and place the tube on ice for a few minutes.
6. Meanwhile, fire-polish the pipette more to make the opening narrower (approximately 2/3 to 1/2 mm in diameter). Wait a few minutes until it cools and triturate the ganglia 20 times more. As you continue, the medium will become progressively cloudier indicating that the cells are being dissociated. Repeat this step once or twice more depending on how well the cells dissociate. After some time, you will notice that all the ganglia have dissociated into neurons and no intact ganglia are visible in the tube. There may be some tissue material that will not dissociate. At this point, let the tube sit in ice for a few minutes so that any undissociated debris can settle to the bottom. Now carefully collect almost all of the medium from the top and place in another tube. To ensure that you do not collect the undissociated debris, leave about 50 µl at the bottom of the tube. Add more of the Final medium to the tube containing dissociated cells: this volume depends on the type of culture plate one is using to culture the neurons. For example: if a 24-well dish is to be employed, then plate 0.5 ganglia per well (~10,000 neurons per well) in 0.5 ml of Final medium per well. A typical rat litter consists of 12 pups, which would give 24 ganglia. This is enough to seed one 24-well plate and the total volume of Final medium is 12 ml. Note: the ganglia also contain a small population of glia and other cell types that will be present in the culture. Using Uridine/5-FDU in the Final medium will prevent their proliferation and promote their death.
7. Feed cells every 48 hours by replacing 2/3 of the medium with fresh RPMI +1% horse serum + NGF and uridine/5-FDU. Note: At first, the culture contains sympathetic neurons that look round with no neurites. Short neurites become visible 24 hours after dissection and progressively become denser and more branched over time.

Discussion

We use Sprague Dawley rats for our cultures.

Take time and care when cleaning the ganglia. Remove all debris, blood vessels and fat. This step is important in ensuring a culture that is more or less homogeneous and free of extraneous cell types. The addition of uridine/5-FDU will largely eliminate any remaining non-neuronal (mitotic) cells contaminating the culture or at least suppress their proliferation.

In addition, during the dissociation step, be patient and take the time to separate the neurons so that they are not found in large clumps once they are seeded onto the plates. Having large clumps of cells will not yield good experimental results. For example, large clumps will make it difficult for the neurons to be transfected or infected. Immunostaining may also be hindered and any experiments that require counting the neurons will be difficult to carry out, as well.

It is not necessary to supplement the medium with pen/strep every time the cultures are fed. Addition of pen/strep the very first time the cells are plated is sufficient. Do add fresh uridine/5-FDU to the medium every time the medium is exchanged.

We have used sympathetic neuronal culture to study apoptosis in response to NGF-withdrawal. For example, in Biswas et al. 2007 (2), sympathetic neurons were transfected with shRNA against several molecules that play important roles in promoting apoptosis. Deckwerth et al. 1996 (5) have used sympathetic neurons from wildtype mice and mice lacking the pro-apoptotic gene, Bax. They have looked at how these neurons behave when they are deprived of NGF. Figure 6 from Biswas et al. 2007 (2) and Figure 3 from Deckwerth et al. 1996 (5) show what transfected and non-transfected sympathetic neuronal cultures look like, respectively.

Disclosures

The authors have nothing to disclose.

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