Video Article

Preparation of Neuronal Cultures from Midgastrula Stage Drosophila Embryos

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URL: http://www.jove.com/video/226
DOI: doi:10.3791/226

Keywords: Neuroscience, Issue 5, neuronal culture, insects, Drosophila, embryo, primary neurons, defined medium

Date Published: 7/4/2007

Citation: Sicaeros, B., O'Dowd, D.K. Preparation of Neuronal Cultures from Midgastrula Stage Drosophila Embryos. *J. Vis. Exp.* (5), e226, doi:10.3791/226 (2007).

Abstract

This video illustrates the procedure for making primary neuronal cultures from midgastrula stage Drosophila embryos. The methods for collecting embryos and their dechorionation using bleach are demonstrated. Using a glass pipet attached to a mouth suction tube, we illustrate the removal of all cells from single embryos. The method for dispersing cells from each embyro into a small (5 l) drop of medium on an uncoated glass coverslip is demonstrated. A view through the microscope at 1 hour after plating illustrates the preferred cell density. Most of the cells that survive when grown in defined medium are neuroblasts that divide one or more times in culture before extending neuritic processes by 12-24 hours. A view through the microscope illustrates the level of neurite outgrowth and branching expected in a healthy culture at 2 days in vitro. The cultures are grown in a simple bicarbonate based defined medium, in a 5% CO_2 incubator at 22-24°C. Neuritic processes continue to elaborate over the first week in culture and when they make contact with neurites from neighboring cells they often form functional synaptic connections. Neurons in these cultures express voltage-gated sodium, calcium, and potassium channels and are electrically excitable. This culture system is useful for studying molecular genetic and environmental factors that regulate neuronal differentiation, excitability, and synapse formation/function.

Video Link

The video component of this article can be found at http://www.jove.com/video/226/

Protocol

I. Drosophila Embryo Collection

1. Prepare egg collection plates
    1. Boil 200 ml dH_2O with 8 g agar
    2. Cool to 50°C
    3. Add 2 ml EtOH and 2 ml Acetic acid
    4. Pour into 35 mm plastic petri dishes, tops
    5. Cool and store in air tight container at 4°C
    6. Makes approximately 80 plates

2. Prepare Yeast paste
    1. Dissolve Fleishmans active baking yeast in water to form paste
    2. Microwave for 20-30 seconds to kill yeast (watch out for boiling over)
    3. Store in 10 cc syringe (without needle) at 4°C for 2 weeks

3. Flies: The adults used for egg collection are generally most productive between 3 and 14 days after emerging, providing that they have had access to fresh food. Many mutant stocks have decreased fertility that can manifest itself in a lowered rate of egg laying and a change in the age at which they are most productive. Generally, several hundred adults make for a good egg-laying population.
4. Procedure: Spread a thin layer of yeast paste on an egg collection plate. This provides a favorable substrate for the laying of eggs. Transfer adult flies to a clean egg collection bottle and tape the collection plate to the mouth of the bottle. Don't leave the flies in these bottles longer than 3-4 hours as the humidity rises and the flies get stuck in paste and on sides of bottle.

II. Embryo Dechorionation with Bleach

1. Set up a Buchner funnel connected to a filter flask attached to an aspirator. Put a piece of Whatman #1 paper in the funnel. With suction running, wet the paper with sterile distilled water.
2. Rinse embryos from collection plates onto the filter paper with a stream of water from a wash bottle.
3. Rinse them in several volumes of sterile water, turn off aspirator, and then add a funnel volume of 50% bleach. Let eggs sit 5-10 minutes. The chorions will be dissolved in 1-2 minutes, but the longer you leave the embryos in the bleach the more of the contaminating yeast will be destroyed. Pick out any adults or larvae that have rinsed off dish.

4. Rinse the embryos into a sterile petri dish (NOT tissue culture) with sterile distilled water. Many of the embryos will stick to the bottom of the dish if it has not been pre-wetted. Rinse several times with sterile water in this dish.
5. Examine the embryos with transmitted light to pick out mid-gastrula stage embryos.

III. Preparation of single embryo cultures

1. Make DDM1 (see recipes in section IV)
2. Pour water off dish of embryos just prior to culturing. Cover embryos with sterile media.
3. Set out 3, 35 mm petri dishes. Put in 4 autoclaved round coverslips in each dish. On each coverslip, put 5 µl of medium. Use Bellco coverslips-low lead glass coverlips.

   Bellco Biological Glassware

   800-257-7043

   Cat #1943-00012

4. Pull some fairly fine pipets. The pipets we use are pulled on a Narishige PP-83 puller. The exact dimensions of the pipet are not crucial and we usually pull them finer than we want and them break off the tip in the same field of view as the embryo to gauge the size. You don't want to suck up all of the embryos in one go, and your don't want the tip so small that it takes 5 minutes to suck up the cells. Aim for somewhere in between. Examine the embryos with transmitted light and use a mouth suction tube attached to the pipet to remove the contents of the embryo. Disperse the cells onto the prepared coverslip by gently expelling the cells as close to the coverslip as possible. You may want to examine the coverslips at higher power and further disperse the cells in the same manner.
5. Let the cells sit for no longer than 10 minutes. Flood the dish with media and put in incubator, 4-5% CO_2 environment, if using defined medium. Temperature between 22-24°C. We routinely use 23°C and 95% humidity. Humidity is important, as you want to avoid evaporation. You can use a standard mammalian incubator placed in a coldroom with temperature control set to 23°C.

IV. DDM1 Defined medium for growing the cultures

1. Make DMEM: To 100 mls of Ham's F12/DME Media (DMEM) (Irvine Scientific #9052).
   1. Add 0.476 g Hepes (20mM) (Sigma #H-3375). Mix.
   2. Add 1.25 ml L-Glutamine 200 mM (Irvine Scientific #9317). Mix.
   3. Filter in hood with 0.2 µm acetate syringe filter (need 2 filters).
   4. The pH is 6.64 and Osm 288.
   5. Make aliquots of 10ml in 15ml centrifuge tubes.
   6. Store at 4°C for not more than 2 weeks.

   Note: Always use autoclaved filtered water and make in containers that are reserved for Media and Supplements only. Never put a pH electrode or stir bar used for other purposes in Media.To make DDM1: Add supplements listed below to DMEM just prior to culturing.

2. To 10 mls of DMEM add:
   - 100 µl Transferrin
   - 100 µl Putrescine
   - 100 µl Selenium
   - 100 µl Progesterone
   - 50 µl Insulin

Discussion

In Drosophila cultures prepared from midgastrula stage embryos the neurons arise from neuroblast precursors, many of which divide prior to differentiation in vitro. This system thus provides a unique opportunity for exploration of genetic and environmental factors important in very early phases of neuronal development (Rohrbaugh et al., 2003). We have shown that neurons grown in a simple defined medium differentiate into electrically excitable neurons that also form functional synaptic connections (O Dowd, 1995; Lee and O Dowd, 1999). Using analysis of mutants and/or pharmacological manipulations, in combination with standard whole cell recording techniques this model system can be used to explore the role genes and environmental factors involved in development of electrical excitability and synaptic transmission (Hodges et al., 2002; Lee and O Dowd, 2000; Lee et al., 2003).

Acknowledgements

This work was supported by NIH grant NS27501 to DKOD. Additional support for this work from an HHMI Professor Grant to DKOD.

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