

Video Article

An *In-vitro* Preparation of Isolated Enteric Neurons and Glia from the Myenteric Plexus of the Adult Mouse

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Abstract

The enteric nervous system is a vast network of neurons and glia running the length of the gastrointestinal tract that functionally controls gastrointestinal motility. A procedure for the isolation and culture of a mixed population of neurons and glia from the myenteric plexus is described. The primary cultures can be maintained for over 7 days, with connections developing among the neurons and glia. The longitudinal muscle strip with the attached myenteric plexus is stripped from the underlying circular muscle of the mouse ileum or colon and subjected to enzymatic digestion. In sterile conditions, the isolated neuronal and glia population are preserved within the pellet following centrifugation and plated on coverslips. Within 24–48 hr, neurite outgrowth occurs and neurons can be identified by pan-neuronal markers. After two days in culture, isolated neurons fire action potentials as observed by patch clamp studies. Furthermore, enteric glia can also be identified by GFAP staining. A network of neurons and glia in close apposition forms within 5–7 days. Enteric neurons can be individually and directly studied using methods such as immunohistochemistry, electrophysiology, calcium imaging, and single-cell PCR. Furthermore, this procedure can be performed in genetically modified animals. This methodology is simple to perform and inexpensive. Overall, this protocol exposes the components of the enteric nervous system in an easily manipulated manner so that we may better discover the functionality of the ENS in normal and disease states.

Video Link

The video component of this article can be found at <https://www.jove.com/video/50688/>

Introduction

The enteric nervous system (ENS) is vast network of nerves and glia that runs the entire length of the gastrointestinal (GI) tract. The ENS functionally controls all aspects of digestion, including peristalsis, fluid absorption/secretion, sensation of stimuli, *etc* (for review see ¹). It contains over 500 million neurons, more than found in the spinal cord, and contains every neurotransmitter class found in the brain. Furthermore, the ENS is unique in that it can function reflexively without input from the central nervous system ². Understanding of the ENS is crucial, not only to understand its normal physiological role, but to understand its involvement in a variety of neuropathies which can be congenital (Hirschsprung's disease), acquired (Chagas), secondary to disease states (diabetic gastroparesis), drug-induced (Opioid bowel syndrome), or due to injury (postoperative ileus) ¹. In addition, enteric neurons can be a reservoir for viral infection (varicella zoster) ³. Because of its similarities to the brain and the high levels of serotonin in the gut, medications aimed at treating central nervous system defects often have unwanted side effects on the ENS ². It is also noteworthy that many neuropathies such as Alzheimer's disease and Parkinson's disease show similar cellular changes in the enteric neurons long before their appearance in central neurons, making the ENS an accessible model to study the pathogenesis of these diseases ⁴. Therefore, a thorough understanding of the ENS is a necessity in understanding disease states and preventing/predicting pharmacological side effects.

The neurons of the ENS have been traditionally studied in the guinea pig using wholemount preparations ^{5–7} or cultured neurons ⁸. Despite the ease at which neurons can be studied in this large animal, this model has many limitations including lack of genetically modified strains, lack of reagents specific to this species, and the high cost associated with ordering and housing these subjects. The development of a murine enteric nervous system model has the unique advantage of various knock out systems, a vast array of other established methodologies that can be used in conjunction with the cell culture technique, and the ability to provide a validation for the guinea pig model.

The ENS is comprised of three plexi that run the length of the gastrointestinal tract: the outer myenteric plexus (between the longitudinal and circular muscle) which is mainly responsible for the peristaltic actions of the gut, as well as the submucosal and mucosal plexi, (found under and within the mucosa, respectively) which largely controls fluid absorption/secretion and the detection of stimuli ¹. This method begins with the isolation of the longitudinal muscle/ myenteric plexus (LMMP) preparation by peeling off the outer muscle layer of the GI tract. This dramatically

cuts down on contamination issues that arise when the mucosal layer is involved in the isolation. As a result, this process is ideal for the study of neuronal control of motility rather than secretory actions of the ENS.

The method presented here results in a mixed culture of enteric neurons and glia. At least two different types of neurons are present based on previous electrophysiological and immunocytochemical observations⁹. The presence of glia is highly advantageous, as they are not only an important cell type to study in their own right, but they contribute to the survival of the enteric neurons¹⁰ and maintain native receptor expression on the neuronal cell surface¹¹. Furthermore, deficiencies of enteric glia may lead to abnormal gastrointestinal motility disease states, coined 'neuro-gliopathies'¹². Therefore, the ENS culture presented here results in several cell types that are ripe for investigation.

The advantages to this methodology are ease of isolation, inexpensive tool requirements, and a short time to master the technique by experienced lab personnel. Limitations of the methodology include low overall cell yield from high tissue volumes and the exclusion of ENS neurons from mucosal and submucosal plexi. This procedure will be highly advantageous to scholars specializing in electrophysiology, immunohistochemistry, single-cell PCR, and other methodologies.

Protocol

All animal care and experimental procedures were in accordance with and approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

1. Preparation of Sterile Poly-D-Lysine- and Laminin-Coated Glass Coverslips in 24-Well Plates

1. All procedures for step 1 are performed in sterile conditions; under a hood, and with sterile reagents. Glass coverslips and double deionized water (ddH₂O) should be sterilized in advance. Preparation of plates can be done up to two weeks before neuron isolation.
2. Under the hood, use sterile forceps to place autoclaved glass coverslips in 12 wells of a 24-well plate.
3. Prepare poly-D-lysine stock ahead of time. Add 50 ml of sterile ddH₂O to 5 mg poly-D-lysine. Vortex and store in 3 ml aliquots at -20 °C. Thaw aliquots before use.
4. For a final concentration of 1 ml poly-D-lysine stock per 25 cm²: pipette 80 µl of poly-D-lysine stock on top of each glass coverslip (2 cm²). Let solution settle for 10 min.
5. Remove poly-D-lysine using vacuum and rinse coverslips 3x with sterile ddH₂O.
6. Allow plates to dry for at least 2 hr under the hood.
7. Plates may be stored at 4 °C or -20 °C before proceeding to laminin coating.
8. Prepare laminin stock ahead of time. Thaw laminin on ice and keep on ice during use. Dilute to a concentration of 50 µg/ml; pay attention to lot concentration; each lot will be different. Depending on lot concentration, approximately 20 ml ddH₂O will be added to each vial of laminin. Aliquot (5 ml) and store at -80 °C. Thaw aliquots on ice before use.
9. Coat coverslips with 5 µg/cm² laminin; pipette 200 µl of laminin stock on each coverslip.
10. Incubate laminin solution on coverslips for 1 hr.
11. Aspirate remaining laminin solution and rinse coverslips once with ddH₂O. Avoid scraping surface of coverslips.
12. Store plates at 4 °C for up to two weeks.

2. Advance Preparation of Neuron Isolation Solutions

1. Prepare Krebs solution (in mM: 118 NaCl, 4.6 KCl, 1.3 NaH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 glucose, and 2.5 CaCl₂). Place 13.79 g NaCl (FW 58.44), 0.686 g KCl (FW 74.55), 0.312 g NaH₂PO₄ (FW 120), .289 g MgSO₄ (FW 120.4), 4.20 g NaHCO₃ (FW 84.01), 3.96 g glucose (FW 180.2), and 0.555 g CaCl₂ (FW 111) in 2 L ddH₂O. Chill to 4 °C.
2. Prepare rinse media (F12 media with 10% fetal bovine serum (FBS) and antibiotic/antimycotic): To a 500 ml bottle of F12 media, add 50 ml FBS and 5 ml of Antibiotic/Antimycotic 100x Liquid (Gibco).
3. Prepare enteric neuron media (Neurobasal A media with B-27, 2 mM L-glutamine, 1% FBS, 10 ng/ml, Glial Derived Neurotrophic Factor (GDNF), and Antibiotic/Antimycotic 100x Liquid). To make GDNF stock, add 1 ml ddH₂O to 10 µg GDNF and freeze back in 50 µl aliquots, store at -80 °C. For a 50 ml vial of complete neuron media, combine 47.5 ml Neurobasal A media, 1 ml B-27 (50x), 500 µl FBS, 500 µl 200 mM L-glutamine (Gibco) 50 µl GDNF stock, and 500 µl Antibiotic/Antimycotic 100x Liquid. Media is made in small 50 ml batches to ensure freshness of GDNF and to avoid contamination of large amounts of this expensive cell media mixture.

3. Harvest Longitudinal Muscle/Myenteric Plexus (LMMP) Preparation from Mice

1. Appropriate national and institutional ethics should be in place before performing animal experiments. Adult Swiss Webster mice weighing over 25 g (typically over 8 weeks of age) are housed in groups of 6 prior to experiments. Tissues from two mice are used for the isolation of ileal enteric neurons and glia and three mice are used for isolation of colonic cells.
2. Prepare surgical area. Gather small surgical scissors, angled forceps, cotton swabs, three 200 ml glass beakers, and a glass/plastic rod (paintbrush). Ensure all supplies are thoroughly washed and rinsed before use to minimize contamination. This step can be performed the day before the experiment.
3. Place Krebs solution on ice and bubble with carbogen (95% oxygen, 5% CO₂) for at least 30 min to stabilize pH.
4. Turn on various equipment and warm chemicals to stabilize at desired temperatures; heat water bath(s) to 37 °C, cool centrifuge to 4 °C, place Hank's balanced salt solution (HBSS) and 0.5% trypsin in water bath (37 °C). Complete neuron media and rinse media should remain refrigerated.
5. Place 150 ml ice cold bubbled Krebs in three 200 ml glass beakers labeled 'dirty', 'clean', and 'LMMP'. Bubble beaker labeled 'LMMP' with carbogen.

6. Following approval from ethics committee, euthanize mouse by cervical dislocation or CO₂ asphyxiation.
7. Place mouse in dorsal recumbence on surgical surface, clean skin with 70% EtOH, and lift abdominal skin using forceps. Using scissors, open abdominal cavity and reveal internal digestive organs.
8. Remove the length of the gastrointestinal tract by lifting a section of ileum and revealing its mesentery. Snip through mesentery with scissors to gently remove and unravel ileum and colon, being careful not to pull mesentery from the ileum/colon.
9. After the full length of intestine is unraveled, remove the ileum by cutting through the intestine distal to the stomach and proximal to the cecum. **Note:** this procedure can also be performed with colonic tissue by removing the colon from distal to the cecum to proximal to the anus.
10. Tissues can be further divided between proximal and distal colon, and jejunum, and ileum. The rest of this procedure will refer to isolation of the ileum; the procedure is the same for the isolation of colon LMMP. Place the entire ileum in a glass container with ice cold Krebs marked 'dirty'.
11. Create a tool to clean the ileum; blunt a large 20 G needle using a filing stone and attach it to a large syringe (10 ml) containing ice cold Krebs.
12. Clean fecal matter from the ileum by sectioning the ileum into three or more large pieces. Remove an ileal section from the beaker and place the blunted needle into the end of an ileal piece.
13. Gently run Krebs through the gut section until all fecal matter is removed into a separate waste container. Place the cleaned section into the container of Krebs marked 'clean'. Repeat until entire ileum is cleaned.
14. To remove the LMMP, cut the ileum into small segments, approximately 2 - 4 cm. Place a segment of ileum on a plastic or glass rod; ileum should fit snugly but not be loose or taut (~2 mm). Begin removal of the LMMP by gently removing bits of mesentery still attached to the gastrointestinal (GI) tract using a forceps. Prevent the GI tract from rotating around rod by gently pinning the tube to the rod using the thumb.
15. Separate the LMMP from the underlying circular muscle; first gently rub the edge of the forceps along the entire line where the mesentery was attached, from top to bottom of the segment, gently creating a gap in the longitudinal muscle. Then gently tease away the longitudinal muscle using a cotton swab wetted with Krebs.
16. Begin at the top of the gap in the longitudinal muscle and tease away using the lightest horizontal stroke while applying very light pressure until the longitudinal muscle just begins to separate from the circular muscle; do this down the entire strip along the mesentery attachment point.
17. Then gently begin to work around the GI tube; moving from top to bottom and back as the longitudinal muscle is slowly separated from the circular muscle all the way around the tube. When complete, the LMMP will naturally come off the remainder of the GI tube.
18. Place the resulting thin strip of longitudinal muscle in the beaker marked 'LMMP'. Repeat for all the segments.
19. After all the strips of LMMP have been gathered from one mouse, repeat procedure for any other mice being used. Thoroughly rinse the 'dirty' and 'clean' beakers before re-use.
20. Rinse the LMMP 3x to remove biological contamination. Fill three 2 ml Eppendorf tubes with ice cold Krebs. Place the LMMP strips in the first tube and spin for 30 sec at 356 x g in a centrifuge cooled to 4 °C. Carefully remove the supernatant with a pipette and move the LMMP strips to next clean Krebs filled tube. Repeat this procedure for each remaining tube.

4. Digest LMMP

1. Prepare digestion solution by placing 13 mg collagenase type 2 and 3 mg BSA in 10 ml carbogen-bubbled Krebs solution.
2. Place segments of rinsed LMMP into digestion solution and use scissors to snip LMMP into tiny pieces.
3. Digest LMMP for 60 min at 37 °C in water bath with shaker while being gently bubbled with carbogen.
4. After digestion is complete, gather cells by centrifugation for 8 min at 356 x g in centrifuge cooled to 4 °C.
5. **From this point forward all procedures should be done under sterile conditions in a cell culture hood!** All reagents and containers should be sterilized before use.
6. During centrifugation, prepare 0.05% trypsin solution by placing 1 ml warmed 0.25% trypsin and 4 ml warmed HBSS into a sterile 50 ml cell culture tube.
7. After centrifugation, remove and discard supernatant. Do not use a vacuum; this will likely suck up the cell pellet due to low centrifugation speeds. Carefully remove the cell pellet and place into clean tube with 5 ml of 0.05% trypsin solution.
8. Digest cells in 0.05% trypsin solution in 37 °C water bath with shaking for 7 min. Do not exceed 7 min of total trypsin treatment or neurons will perish.
9. Neutralize trypsin with 10 ml of cold rinse media after 7 min of trypsin treatment.
10. Centrifuge cells for 8 min at 356 x g. Remove and discard media.
11. During centrifugation, balance a section of sterilized Nitex mesh on top of a sterile 15 ml cell culture tube.
12. After centrifugation, remove and discard supernatant. Gently resuspend cell mixture by triturating in 3 ml complete neuron media. Avoid generating air bubbles during this step and all future steps in which cell mixture is triturated. All triturations should be done very gently. Filter cell solution through Nitex mesh into clean 15 ml cell culture tube.
13. Optional: Cap cell culture tube and place in refrigerated Hula mixer (or any mixer that provides rotation) with gentle rolling and tilting for 30 min. This step is optional but recommended.
14. Optional: After Hula mixer, add 2 ml cold rinse media to wash cells.
15. Gather cells by centrifugation (8 min, 356 x g). Remove and discard supernatant.
16. Resuspend cells in 1,200 µl complete neuron media. Triturate cells gently using 1 ml plastic pipette tip. Do not generate air bubbles. Pipette slowly and gently until most chunks are broken up and cells are suspended into liquid.
17. Add 750 µl of complete media to each of the 12 wells containing a pre-coated glass coverslip and add 100 µl of the triturated cell solution.
18. Incubate neurons in cell culture incubator at 37 °C with 5% CO₂.
19. Change half of the cell media every 2 days.
20. Neurons are ready for electrophysiological recordings after 1 - 2 days in culture.

Representative Results

Immediately following isolation of LMMP-derived cells, neurons and other cell types will not be readily evident. Living, round cells of indistinct phenotype can be seen as well as tissue detritus from incompletely digested tissue fragments and connective tissue. This flotsam is of no concern and will be largely removed with the first media change in two days. Do not attempt to clean the slides before this as the healthy, viable cells will be removed as well.

After one day in culture, neurons will begin to show neurite outgrowth. Specific identification of neurons may still be indistinct at this time. Provided the cells are adherent enough to transfer to an experimental chamber, they will be ready for electrophysiological study after one day of culture. However, the cells are more adherent after two days in culture and ideal for function studies (electrophysiology, calcium imaging) from approximately days 2 - 5.

Morphological features of neuron become distinct after approximately a week in culture (**Figure 1**). Ideal immunocytochemical features can be identified after about ten days, when neurons display long projections and grow in an almost 'ganglionic' like fashion interspersed with glia (**Figure 2**). This staining suggests it may be possible to study the synaptic interactions of these cells using this methodology.

After one day in culture, neurons are recognized by their sine que non or 'defining feature', the action potential (**Figure 3**). Electrophysiologically, they can be functionally classified into at least two neuronal types: neurons that contain an after-hyperpolarization and increased current/density relationships of sodium and potassium and neurons that do not have an after-hyperpolarization and significantly less sodium and potassium conductance (**Figure 4**). AHP positive and negative neurons appear to correlate with AH and S neurons seen in previous guinea work, respectively. AHP positive neurons (AH neurons) have multiple long projections originating from around the cell body, while the AHP negative neurons (S neurons) have one long projection which branches many times. This, in conjunction with the immunocytochemical coding in **Figure 1**, suggests that neuronal population is very heterogeneous.

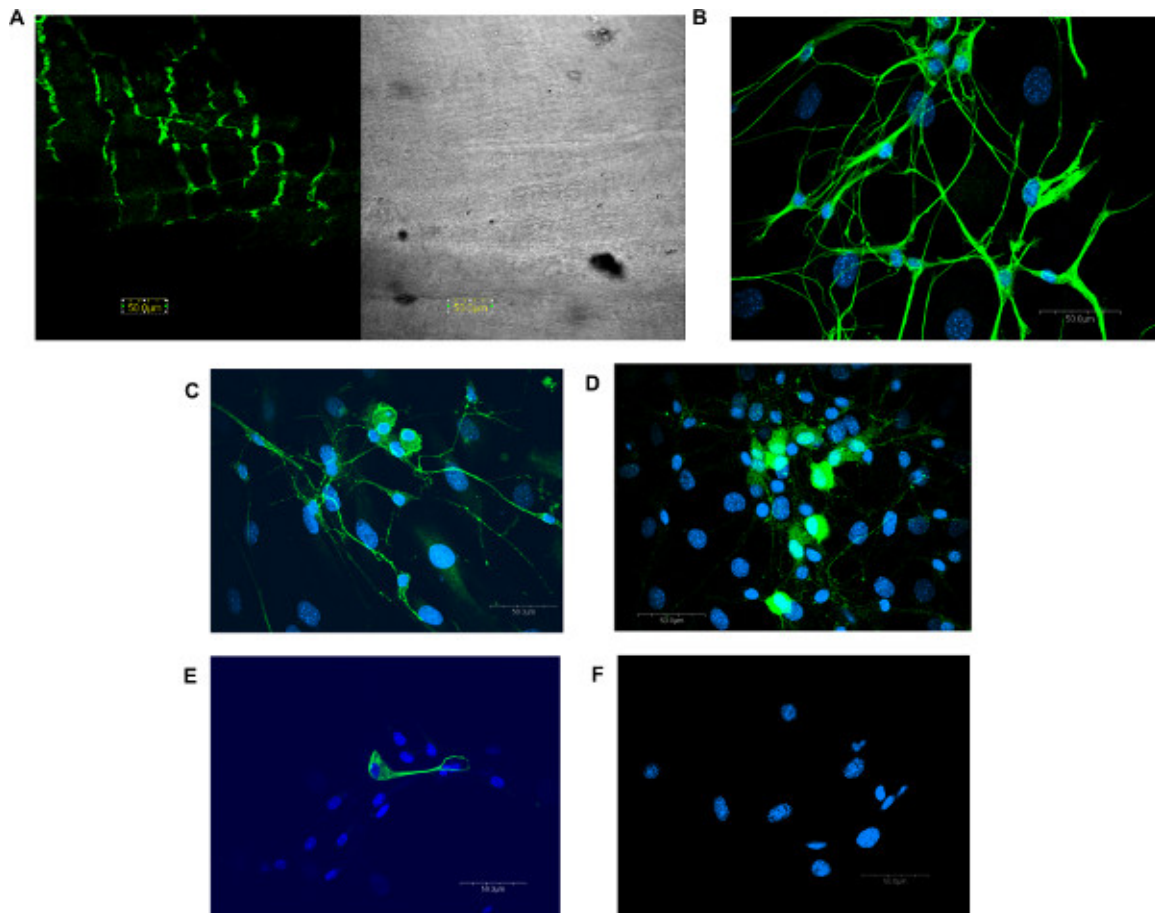


Figure 1. Immunohistochemical characterization of enteric neurons and glia isolated from the mouse longitudinal muscle. Confocal microscopy revealed neuronal-specific β -III-tubulin (Abcam, rabbit, 1:1,000) staining in whole mount ileal longitudinal muscle (**A**) preparation from the mouse. Cells isolated from longitudinal muscle/myenteric plexus (LMMP) preparations contain neurons (**B**; β -III-tubulin, Abcam, rabbit, 1:1,000) that stain positively for calbindin (**C**) (Chemicon, rabbit, 1:1,000) and calretinin (**D**) (Swant, rabbit, 1:2,000). Glia cells (**E**) were visualized with the glia-specific marker GFAP (Chemicon, mouse, 1:500). Antibodies were visualized via appropriate goat secondary antibody Alexa 488 (green, Molecular Probes, 1:1,000). Nuclei were visualized using Hoechst 33342 (blue, **C-G**, 1 μ g/ml). No staining was seen when primary antibody was omitted (**F**). Modified and reprinted from Smith, T.H., *et al.* Morphine Decreases Enteric Neuron Excitability via Inhibition of Sodium Channels. *PLoS One*, doi:10.1371/journal.pone.0045251.g001 (2012). [Click here to view larger figure.](#)

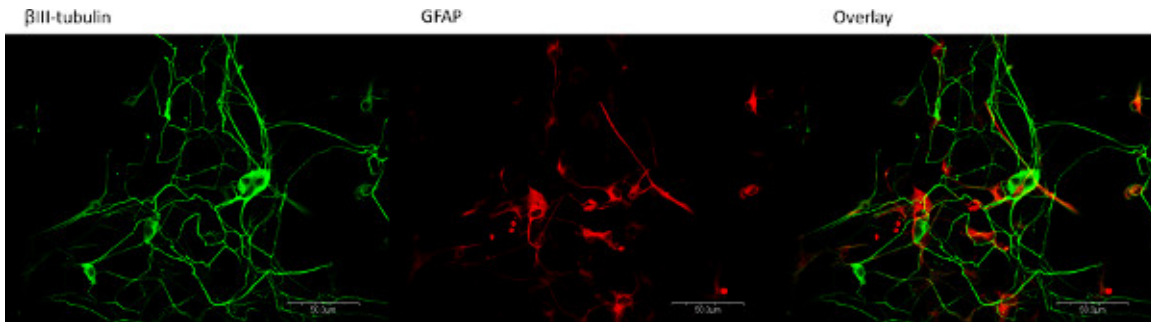


Figure 2. Neurons and glia isolated from the mouse longitudinal muscle grow in close proximity to one another. Confocal microscopy images indicate the neurons (green, β -III-tubulin, Abcam, rabbit, 1:1,000) and glia (red, GFAP, Chemicon, mouse, 1:500) readily grow adjacent to one another and appear to interact *in vitro*. [Click here to view larger figure.](#)

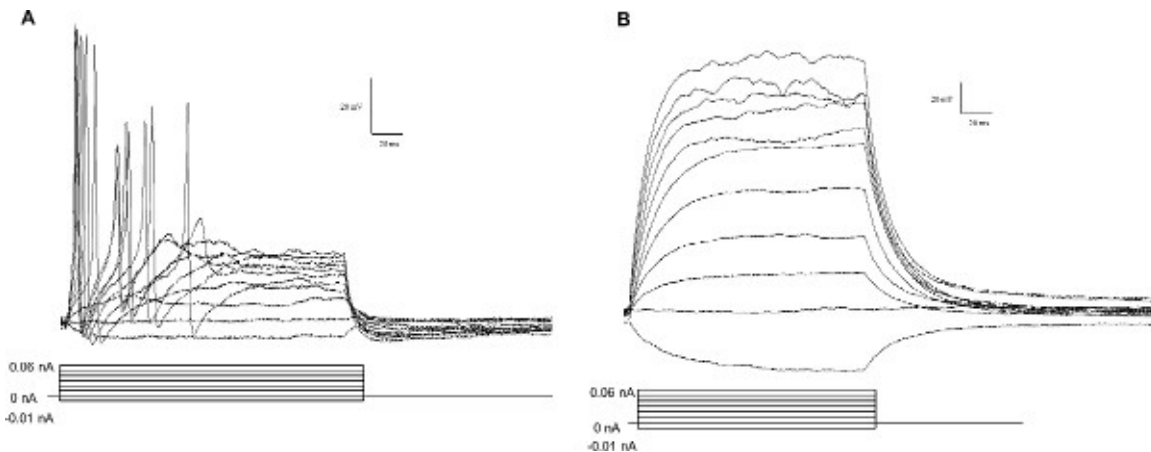


Figure 3. Electrophysiology of cultured enteric neurons and glia. In current clamp mode, all neurons (A) displayed action potentials upon current injection. Glia (B) do not have action potentials but do display large electrotonic potentials in response to current injection. Protocols in A & B start with a current injection of -0.01 nA and increase to 0.09 nA in eleven 0.01 nA steps. [Click here to view larger figure.](#)

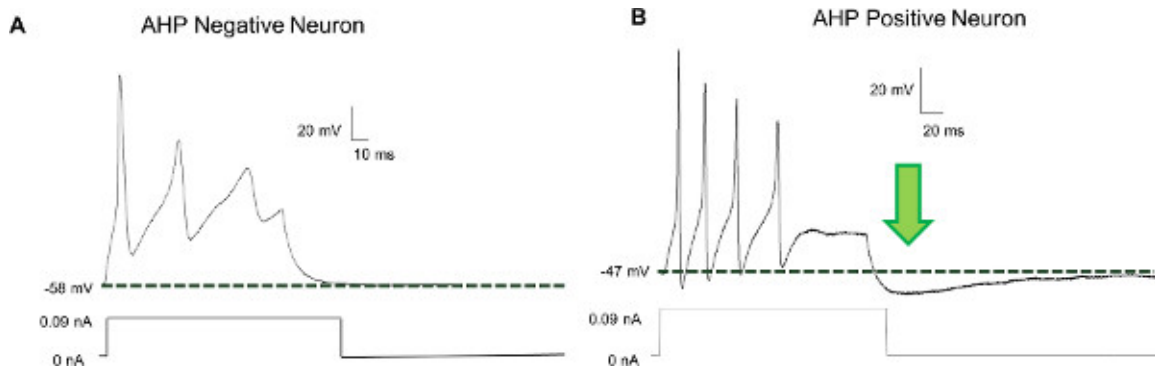


Figure 4. Neurons cultured from the mouse ileum are an electrophysiologically heterogeneous population. In current clamp mode, a current injection of 0.09 nA into neurons results in action potentials. S neurons (A), immediately return to the resting membrane potential following stimulation. AH-type neurons (B) display an afterhyperpolarization (AHP) following stimulation in which the resting membrane potential falls below baseline before slowly returning to the initial value. Modified and reprinted from Smith, T.H., *et al.* Morphine Decreases Enteric Neuron Excitability via Inhibition of Sodium Channels. *PLoS One.*, doi:10.1371/journal.pone.0045251.g001 (2012).

Discussion

Animals Used

This protocol has been optimized for Swiss Webster mice. However, this method is easily adaptable to other small-sized mammals such as rats and to other strains of mice. We have successfully performed preliminary isolations with C57 mice and μ -opioid receptor knock-outs. However, it is also possible that other strains of mice may be problematic due to morphological variations in the GI tract. Furthermore, there are known differences between mouse strains (C57Bl/6 vs. Balb/c) in the neuronal circuitry of the LMMP preparation¹³, which can affect the resulting mix of neurons in the final isolation. Additionally, age should be taken into consideration when using this method, as age-related neuronal losses occur in the myenteric plexus that are specific to cholinergic neurons and their corresponding glia, and can be seen as early as 12 months of age¹⁴.

Finally, when adapting this protocol to other species of animals, care should be taken to ensure that the myenteric plexus comes away with the longitudinal muscle rather than remaining attached to the circular muscle.

Tissue Used

Neurons and glia can be cultured from both the ileum and colon using this protocol. Ileal isolations are easier to perform due to a greater amount of available tissue and the ease at which the longitudinal muscle separates from the thick circular muscle. A minimum of two animals are needed to occupy 12 wells of the 24-well plate for applications such as electrophysiology or immunocytochemistry. The isolation of colonic material is more problematic. The thick colonic longitudinal muscle is more difficult to separate from the circular muscle. Furthermore, the underlying circular muscle is thinner in the colon, making it more susceptible to tears. Also, the colon is significantly shorter than the ileum so three animals, at minimum, are required to occupy 12 wells of a 24-well plate. Both the ileum and the colon can be harvested and plated separately from the same animal, but additional animals or the use of less cell plating area will be required for the colon isolation.

Duration of Cell Culture

Electrophysiological recordings are optimally performed in isolated enteric neurons/glia after 2 to 6 days in culture; at this time cells are rounded and pliant, ideal conditions for making a whole-cell seal. After a week in culture cells become flatter and more visually differentiated as the cells adhere to the plate. Immunocytochemical experiments are best done when cells are more firmly attached to the plate, around day 10, to prevent cell loss during numerous wash steps. Mouse enteric cells have been maintained in culture for up to three weeks. Patterns of receptor and neurotransmitter expression over time have yet to be studied in these cultures. However, previous studies on neuronal survival and biochemical/morphological differentiation in traditional guinea pig enteric neuron isolations have found that neurons survived up to 15 days and maintain a high degree of histochemical and morphological properties up to three weeks⁸, which suggests that neurons derived from other animals sources may do the same.

Cell Yield

The overall yield of cells from this technique is 12 wells of a 24-well plate from two mice when isolating from the ileum. This is a relatively low number of neurons compared to tissue volume, as the myenteric plexus consists of less than 1% of the total gut wall. One drawback to this method is that to increase the number of cells obtained, one must increase the number of animals used. When animal numbers are increased, it is suggested that several lab members collaborate in the first step of the isolation of the LMMP. This reduces the amount of time the cells are in a disrupted state before final plating and this increases cell survival.

Cell Density

As written, this protocol uses two animals to yield an ileal isolation of low-density cell confluence (~10 - 40% measured after one day in culture). Low-density cell plating is ideal in this methodology to reduce the risk of contamination in the final culture. It is also useful when single cell electrophysiology is performed. Cell number can be increased by using more animals or reducing the final plating area, but risk of contamination increases. To off-set this problem when higher cell densities are required, either increased washing steps or the increased use of antibiotics is suggested.

Cell Heterogeneity

Neurons acquired using these protocols are comprised of at least two functionally distinct populations, as indicated by electrophysiological characterization (**Figure 3**). However, there are many known types of neurons with specific neurochemical coding patterns found in the guinea pig¹⁵ and the same is probably true of the mouse. This cell heterogeneity is both an advantage and disadvantage to this methodology. Cell heterogeneity is beneficial when performing single-cell functional studies, observing cell-cell interactions or in immunocytochemistry, among others. Cell heterogeneity may be particularly advantageous when studying neuron/glia interactions in culture (**Figure 3**). However, cell heterogeneity is a hindrance to methodologies such as immunoblotting, where changes in protein expression, *etc.*, cannot be contributed to any one cell type or neuron type. Examination of glia will be less problematic as only two subtypes are shown to exist in the LMMP preparation: intraganglionic and intramuscular enteric glia¹⁶. Other cell types may also be present in this culture, such as interstitial cells of Cajal or fibroblasts.

Plating Cells

In this methodology, cells are plated in twelve wells. Occasionally, one or two of these wells will develop contamination in the first day after isolation. If this occurs, the slides from the contaminated wells are promptly discarded and the well is rinsed with 70% ethanol to kill the remaining contamination. This protocol can be modified to plate all of the isolated cells in fewer wells or in a single dish, and as a result, the risk of contamination will increase. Again, when the risks of contamination increase, extra wash steps or increased antibiotic use is suggested. The antibiotic/antimycotic liquid (Gibco) used in the protocol consists of Amphotericin B, Streptomycin, and Penicillin. Other antibiotic combinations or increased concentrations can be used to optimize the reduction of contamination while preparing the rinse and complete neuron media.

Attaching Cells

Cells are plated on coverslips coated with laminin and poly-D-lysine. Laminin is important in the survival of enteric neurons and glia, and encourages neurite outgrowth and neuronal development¹⁷, and as such, is considered essential to this isolation. However, alternative attachment factors to poly-D-lysine, such as ornithine or Matrigel, may be tried as desired.

In conclusion, this isolation provides a mixed culture of enteric neurons and glia suitable for a wide range of study techniques. This methodology is easy to master, inexpensive, and time efficient. It is our hope that this cell model will contribute to the understanding of various pathologies associated with the ENS.

Disclosures

The authors declare that no major competing interests exist.

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