Video Article

**Homarus Americanus Stomatogastric Nervous System Dissection**

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URL: https://www.jove.com/video/1171

DOI: doi:10.3791/1171

Keywords: Neuroscience, Issue 27, lobster, stomach, neural network, dissection, central pattern generator

Date Published: 5/28/2009


Abstract

With the goal of understanding how nervous systems produce activity and respond to the environment, neuroscientists turn to model systems that exhibit the activity of interest and are accessible and amenable to experimental methods. The stomatogastric nervous system (STNS) of the American lobster (Homarus americanus; also know was the Atlantic or Maine lobster) has been established as a model system for studying rhythm generating networks and neuromodulation of networks. The STNS consists of 3 anterior ganglia (2 commissural ganglia and an oesophageal ganglion), containing modulatory neurons that project centrally to the stomatogastric ganglion (STG). The STG contains approximately 30 neurons that comprise two central pattern generating networks, the pyloric and gastric networks that underlie feeding behaviors in crustaceans¹ ². While it is possible to study this system in vivo³, the STNS continues to produce its rhythmic activity when isolated in vitro. Physical isolation of the STNS in a dish allows for easy access to the somata in the ganglia for intracellular electrophysiological recordings and to the nerves of the STNS for extracellular recordings. Isolating the STNS is a two-part process. The first part, dissecting the stomach from the animal, is described in an accompanying video article⁴. In this video article, fine dissection techniques are used to isolate the STNS from the stomach. This procedure results in a nervous system preparation that is available for electrophysiological recordings.

Video Link

The video component of this article can be found at https://www.jove.com/video/1171/

Protocol

Prior to beginning this protocol the stomach of the lobster must be removed from the animal and prepared as described in the companion JoVE article (Bierman & Tobin, 2009). The majority of the steps outlined below are to be performed under a dissection microscope.

Part I- Isolating the Commissural Ganglia

1. Orient the dish so that the anterior end (by the lip) is away from you and the posterior end (by the pylorus) is towards you.
2. Ensure that the stomach is pinned so that it lies as flat as possible on the dish.
   1. Pin down the esophagus on each side of the lip.
   2. If necessary, rearrange the other pins placed during the gross dissection.
3. Remove the hypodermis.
   1. Grab the hypodermis near the gm2/gm3 muscles, detach it from these muscles and gently pull in the anterior direction.
   2. Separate the hypodermis from the underlying fat by snipping any connections. Use increased care around the central artery, which encloses a key part of the stomatogastric nervous system. Leave the hypodermis intact within about a 0.5 cm radius of the stomatogastric ganglion, which sits between the gm1b muscles.
   3. Cut off separated hypodermis.
4. Detach the brain from the underlying tissue.
   1. Identify the two large commissural nerves that project bilaterally from the anterior portion of the brain to the commissural ganglia in the anterior portion of the stomach.
   2. Leaving these nerves intact, gently lift the brain with forceps and cut to separate the brain from the rest of the tissue. Be careful as you do this, as the stomatogastric nerve is underneath the brain and must be kept intact.
5. Separate the commissural ganglia from surrounding the tissue.
   1. Follow one the commissural nerves to one of the commissural ganglia.
   2. Identify the ion and son – these nerves must remain intact. The commissural ganglion will have many nerves radiating out. Cut most of them (except the ion and son), but leave one extra nerve attached to hold the commissural ganglion in place while you finish the dissection.
   3. Repeat with the other commissural ganglion.
Part II- Isolating the Stomatogastric Ganglion (STG) and remaining nerves.

7. Separate the STG from surrounding the tissue.
   1. The STG sits inside an artery that runs between the gm1b muscles and down to the gm2a, b muscles. Look for the cut end of the artery near the gm2a, b muscles.
   2. Pull the cut end of the artery up and anteriorly, to expose the dvn. Continue pulling up until you reach the level of the remaining hypodermis. Once separated, the artery can be trimmed to the level where it touches the remaining hypodermis.
   3. Cut off the muscle flap that was situated in the rostrum of the lobster.
   4. Lift up the hypodermis above the artery, observing that the artery makes a tunnel inside the fat above the STG. Put one blade of your scissors inside the artery and cut the muscle above it. Check that you can see the dvn as you cut to be sure you are safely above the STG. Continue cutting anteriorly, following the artery. As you cut above the STG, you will be able to see the stn emanating anteriorly from the STG and diving down into the muscle. Continue cutting anteriorly to fully separate the muscle above the STG.
   5. Clear any tissue away from the stn.

8. Separate the agns from the surrounding tissue. (Even if you do not plan to record from the agn, you will need a portion of the intact nerve to pin down the ganglion for intracellular recordings.)
   1. To better see the agns, remove the remaining hypodermis and a thin layer of the underlying muscle.
   2. Just posterior to the STG, identify a dark tunnel in the fat and muscle on either side of the dvn.
   3. Stick one scissor blade inside the tunnel and cut the tissue above the nerve. Continue cutting laterally, following the nerve, to isolate it. (A couple millimeters is sufficient for pinning the ganglion, but expose at least 5 mm of the agn if you plan to record from it.)
   4. Detach the agn from the muscle it innervates by cutting the connections.
   5. Repeat on the other side to isolate the other agn.
   6. Once both agns have been dissected, cut all the muscles and tissue connecting the STG to the stomach.

9. Isolate the mvns. They are a bilateral pair of small nerves branching off the lvn and traversing laterally underneath the fat.
   1. Either use scissors or two forceps to cut through the fat on one side of the dvn, near where it branches into the two lvns. Here you are far from the mvns and won’t risk cutting them.
   2. Lift up the fat layer, and identify the small mvn underneath. These nerves run medial to laterally just posterior to the level of the gm1b muscles.
   3. Remove the layer of fat above the mvn, leaving the nerve intact for now so it won’t become tangled. You will cut its lateral end from off the stomach at the end of the dissection.
   4. Repeat the process on the other side to isolate the other mvn.

10. Isolate the lvns and posterior nerves.
    1. Starting where the lvns branch laterally off the dvn, follow an lvn by cutting the fat above it. Depending on the preparation, sometimes the entire fat layer above the nerve can be pulled off.
    2. Continue revealing the lvn until the point where the psn branches off.

11. Isolate the pyn.
    1. Locate the pyn, it is a nerve travelling posteriorly over the pylorus.
    2. Remove the thin membrane covering this region.
    3. Isolate the pyn by cutting a small section of the pylorus, attached to the pyn.
    4. Gently lift the pyloric section and cut away the tissue on either side of the pyn until you reach the branch that attaches to the pdn.

12. Isolate the pdn.
    1. Locate the pdn, it is a nerve travelling posteriorly to the pyloric dilator muscle.
    2. Cut a small section of the pyloric dilator muscle, attached to the pdn.
    3. Gently lift the pyloric dilator muscle section, cut away the tissue on either side of the pdn until you reach the branch that attaches to the pyn.

13. Isolate the ventral lvn, that connects the pyn and pdn to the lvn.
    1. At the triangular branch point where the pyn and pdn connect to the ventral lvn, isolate any small section of nerve that branches off the ventral lvn, such as the vpln. This will be used as a handle to pull on the ventral lvn.
    2. Lift on the handle and cut away the tissue on either side of the ventral lvn, working in the anterior direction until you reach the point where the psn branches off.

14. Repeat steps 10 – 13 to isolate the lvn and posterior nerves on the other side.

15. Detach the stomatogastric nervous system from the stomach.
    1. To fully detach the lvn from the stomach, pull on the psn and cut all tissue and nerves attached to the lvn and ventral lvn, except for the pdn and pyn. Repeat this process on the other side.
    2. Cut the lateral end of each mvn. Lift the lateral end and cut any extraneous attachments between the nerve and the underlying stomach tissue.
    3. At the anterior end, cut any extraneous attachments to the commissural ganglia, and cut the labial nerve on each side.
    4. Visually scan the stomatogastric nervous system system to identify and then cut any additional attachments to the stomach tissue.
    5. Grab onto the anterior end of both commissural nerves, above the commissural ganglia, and peel the stomatogastric nervous system off the stomach and move it to the side of the dish. As you do this, cut any remaining attachments to free the nervous system.
16. Prepare the small Sylgard dish.
   1. Rub some stomach tissue thoroughly on the surface of the Sylgard in the small dish. This will decrease the hydrophobicity of the
      Sylgard, so that saline will distribute evenly across the dish.
   2. Rinse and fill the Sylgard dish with chilled saline.

17. Transfer the nervous system to the prepared Sylgard dish by grabbing the anterior ends of both commissural nerves, lifting the nervous
    system out of the large dish, and laying it in the small dish.

18. Pin the STNS in the small dish using a combination of minuten pins and thinner pins cut from tungsten wire.
   1. Ensure that the preparation is dorsal side up by checking that the inferior ventricular nerve is pointing away from the Sylgard.
   2. Remove the brain and pin the commissural nerves.
   3. Gently pull the nervous system tight and flat by pulling each of the psns posteriorly and laterally, and pinning them down.
   4. Remove the pyloric dilator muscle from the pdns, and pin the pdns.
   5. Remove the pyloric segment from the pyns, and pin the pyns.
   6. Pin down any remaining isolated nerves such as the labial nerves, the mvens and agns.

19. Desheath the STG.
   1. Ensure the STG is flat against the Sylgard and held taut by the lateral agns, the anterior stns and the posterior dvns.
   2. Arrange the lighting to enable visualization of the axons within the dvns.
   3. Using a small pin or fine tungsten needle, make a small shallow hole in sheath surrounding the dvns, just posterior to the STG.
   4. Pull the needle across the dvns, dorsal to the axons, to create a horizontal tear in the nerve sheath.
   5. Grab the flap of nerve sheath posterior to the STG and pull anteriorly to remove the sheath from the dorsal side of the STG.

**Abbreviations:**

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>STNS</td>
<td>Stomatogastric nervous system</td>
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<tr>
<td>STG</td>
<td>Stomatogastric ganglion</td>
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<td>CoG</td>
<td>Commissural ganglion</td>
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<td>stn</td>
<td>stomatogastric nerve</td>
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<td>mvn</td>
<td>medial ventricular nerve</td>
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<td>ion</td>
<td>inferior oesophageal nerve</td>
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<tr>
<td>son</td>
<td>superior oesophageal nerve</td>
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<td>agn</td>
<td>anterior gastric nerve</td>
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<td>dvn</td>
<td>dorsal ventricular nerve</td>
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<td>lvn</td>
<td>lateral ventricular nerve</td>
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<td>psn</td>
<td>posterior stomach nerve</td>
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<td>vpln</td>
<td>ventral posterior lateral nerve</td>
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<td>pyn</td>
<td>pyloric nerve</td>
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<tr>
<td>pdn</td>
<td>pyloric dilator nerve</td>
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**Discussion**

The anatomy of lobster stomach and STNS has been well described previously\(^5,6\), and variations of this dissection procedure have been utilized
in numerous studies. We recommend becoming familiar with the anatomy of the stomach\(^5,7\) and STNS\(^5,8\) prior to beginning dissections. Such
familiarity will decrease the risk of accidental damage to nerves and tissue.

To maintain the health of the preparation, it is critical to maintain the preparation at a cool temperature. This is easily achieved by exchanging
the saline every 30 mins with 4°C saline kept in a refrigerator. When immersed in cold saline, the isolated STNS preparation is viable for many
hours. Recordings in related *Homarus gammarus* have demonstrated stable rhythmic activity from the STG throughout 5 days *in vitro*\(^9\); similar
robustness has been seen in *H. americanus* (pers. obs.).

Modulatory input to the STG from the commissural ganglia is required to preserve the ongoing motor patterns. Therefore, to maintain a normal
rhythmic activity these ganglia and the ion, son, and stn nerves must be kept intact. The dissection we describe preserves several motor nerves
(containing motor neuron axons): the agns (containing Gastric Mill (GM) axons), the mvens (containing Lateral Gastric (LG) and Inferior Cardiac
(IC) axons), the pyns (containing Pyloric neuron (PY) axons), the pdns (containing Pyloric Dilator (PD) axons), and the lvnns (containing Lateral
Pyloric (LP), Medial Gastric (MG), LPG axons as well as those from GM, PD and PY). By dissecting additional (or different) nerves, other motor
nerve axons may be recorded. Each individual type of motor neuron axon may be recorded individually by dissecting and recording from the
nerve innervating the muscle to which that type of neuron projects.

This preparation may be used for a variety of studies focused on nervous system function. The activity of the neurons may be recorded either
using intracellular, sharp electrode recordings from somata or axons, or by recording electrical impulses extracellularly from the nerves. Many ion
channels in the STG neurons have been partially cloned and mRNA can be measured from manually pulled somata\(^10,11\).
Acknowledgements

We would like to acknowledge our advisor, Dr. Eve Marder. This research was supported by National Institute of Health grants NS17813 to Eve Marder and NS059255 to A-E T.

References