Video Article

Homarus Americanus Stomatogastric Nervous System Dissection

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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 crustaceans[1,2]. While it is possible to study this system *in vivo*[3], 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 article[4]. 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.

6. Clean the *ion* and *son* from lateral to medial. Leave the labial nerve intact to anchor the nervous system to the stomach. The esophageal ganglion sits were the *ion*s intersect. You can clear below it by grabbing the inferior ventricular nerve and pulling up to cut underneath.

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 *agn*s 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 *agn*s, 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 *agn*s have been dissected, cut all the muscles and tissue connecting the STG to the stomach.

9. Isolate the *mvn*s. 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 *lvn*s. Here you are far from the *mvn*s 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 *lvn*s and posterior nerves.
    1. Starting where the *lvn*s 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 *psn*s posteriorly and laterally, and pinning them down.
    4. Remove the pyloric dilator muscle from the *pdn*s, and pin the *pdn*s.
    5. Remove the pyloric segment from the *pyn*s, and pin the *pyn*s.
    6. Pin down any remaining isolated nerves such as the labial nerves, the *mvn*s and *agn*s.

19. Desheath the STG.
    1. Ensure the STG is flat against the Sylgard and held taut by the lateral *agn*s, the anterior *stn* and the posterior *dvn*.
    2. Arrange the lighting to enable visualization of the axons within the *dvn*.
    3. Using a small pin or fine tungsten needle, make a small shallow hole in sheath surrounding the *dvn*, just posterior to the STG.
    4. Pull the needle across the *dvn*, 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:	
STNS	Stomatogastric nervous system
STG	Stomatogastric ganglion
CoG	Commissural ganglion
stn	stomatogastric nerve
mvn	medial ventricular nerve
ion	inferior oesophageal nerve
son	superior oesophageal nerve
agn	anterior gastric nerve
dvn	dorsal ventricular nerve
lvn	lateral ventricular nerve
psn	posterior stomach nerve
vpln	ventral posterior lateral nerve
pyn	pyloric nerve
pdn	pyloric dilator nerve

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 *agn*s (containing Gastric Mill (GM) axons), the *mvn*s (containing Lateral Gastric (LG) and Inferior Cardiac (IC) axons), the *pyn*s (containing Pyloric neuron (PY) axons), the *pdn*s (containing Pyloric Dilator (PD) axons), and the *lvn*s (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 neuron 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].

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