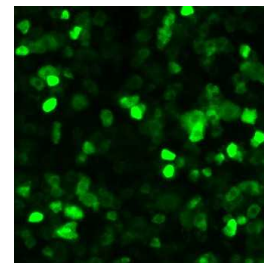


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In vivo calcium imaging of the L4 sensory ganglion in mice

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We use this protocol and it's working

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Abstract

This protocol describes the preparation and surgical exposure of the L4 dorsal root ganglion (DRG) in mice for in vivo calcium imaging with GCaMP6s.

Attachments



Figure 1. Diagram fr...

1MB



Figure 2. Diagram il...

79KB

Image Attribution

Figure 1 reused with permission from Yuan et al 2023

Guidelines

The surgery and clamping steps for this protocol are challenging. It is advised that you shadow someone who has experience performing this technique before attempting it on your own.

Materials

AAV9.CAG.GCaMP6s.WPRE.SV40 - RRID: Addgene_100844, Addgene

Drugs & Chemicals:

	Name	Dose
	Urethane	12.5% w/v
	Isoflurane	5% induction, 1-2% maintenance
	Saline	0.9%, sterile

Other equipment:

A
Name
Homeothermically controlled heat pad and rectal probe
Isofluorane anaesthetic rig
Electric Shaver
Tape
Cotton tips
Gauze pads
1ml syringes with 26g needles
Chlorhexidine
Bone wax



	A
	Gelfoam
	Kwik-Sil Silicone Elastomer (WPI)
	Dissection microscope
	Vaseline
	Sterile gloves

Recommended tool set:

	Name	Product code
	Rongeur (Friedman Pearson, curved)	FST 16221-14
	Forceps 2 teeth	FST 11027-12
	Serrated curved forceps	FST 11003-12
	Angled (45 degree) Dumont Forceps	FST 11253-25
	Sharply Curved Spring Scissors	FST 15017-10
	Black Tough cut Scissors	FST 14085-09
	Forceps 5.SA	Ideal-tek
	Vertebral clamps	Precision Systems and Instrumentation, VA, USA

Mice

- 1 Perform experiments in C57BL/6J mice (RRID:IMSR_JAX:000664).

Administration of GCaMP6s calcium indicator

- 2 Prepare the viral vector containing GCaMP6s calcium indicator: Use AAV9.CAG.GCaMP6s.WPRE.SV40 (available from Addgene, USA[1]; RRID:Addgene_100844) for injection.
- 3 Transport the virus to the animal facility on ice.
- 4 Select mouse pups at post-natal day P2–P5 and separate groups of 3–4 pups from their mother.
- 5 Fill a 10 μ L Hamilton syringe equipped with a 30G needle with 6 μ L of the AAV9 vector.
- 6 Administer 6 μ L of the AAV9 solution subcutaneously into the nape of the neck of each pup.

** We find that approximately 50% of sensory neurons from all sensory ganglia are labelled with GCaMP6s using this approach **
- 7 Place the injected pups back with their mother immediately after injection.

Preparation of mouse for surgery:

- 8 Select appropriate GCaMP6s injected mouse of at least 10 weeks of age.
- 9 Weigh mouse.
- 10 Perform intraperitoneal injection of urethane (~1g/kg dose using 12.5% w/v solution) to lightly anaesthetise mouse.



- 11 Place mouse into isoflurane induction chamber and set vaporiser to 5% until surgical plane of anaesthesia is achieved. Check depth of anaesthesia by checking pedal reflex in front hind paw (avoid squeezing hind paws if imaging lumbar DRGs).
- 12 In a separate preparation area place the anaesthetised mouse on a heated pad and place the nose of the mouse into an anaesthesia nose cone. Note that homeothermic control is not necessary for this brief shaving step; however, ensure that the pad is not too hot to the touch. Set the vaporiser to the appropriate level at which anaesthesia is maintained (by checking front paw pedal reflexes), but no more (usually around 2%).
- 13 Shave the back of the animal and remove loose fur.
- 14 Disinfect surgical area with chlorohexidine.
- 15 Move the mouse to a sterile surgical area with a dissection microscope and a homeothermally controlled heating mat with a rectal probe.
- 16 Place the mouse in a supine position on the heating pad and place the head in nose cone.
- 17 Using lubricating gel on the rectal probe (e.g. vaseline) and carefully insert the temperature probe into the rectal cavity.
- 18 Tape the temperature probe to the tail. It may be necessary to also tape the tail and/or probe to the bench or stage to avoid it moving the body of the mouse (depending on the flexibility of the cabling).
- 19 Check the depth of anaesthesia (front paw) to ensure surgical plane of anaesthesia is maintained.

Surgical dissection to expose the L4 DRG

- 20 Using black tough cut scissors, cut away skin on the back covering the lumbar part of the spinal cord.
- 21 Use thumb and index finger, covered by sterile gloves, to feel where the ileac crests are (hip bones). In C57BL6/J mice, the L5 vertebrae are approximately in line with the point where the hip bones line up. Use this as a reference point for the next steps.
- 22 Find the spinous processes of the dorsal spinal column and remove the soft tissue between the spinous processes and the transverse processes from L6-L3 DRG (Figure

1).

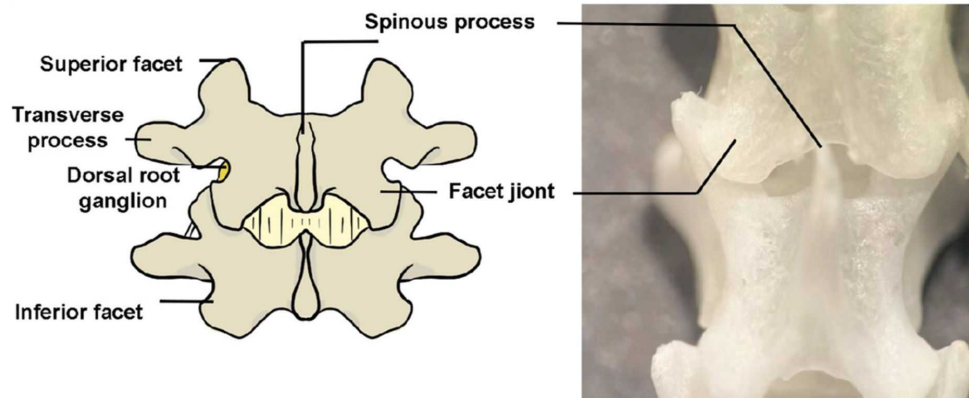


Figure 1: Diagram from Yuan et al 2023 [4] showing anatomy of bony structures surrounding the DRG

Note that the L6 vertebrae can be identified, because they are mobile when moved against L5, whilst S1 is fused to S2). You should be cutting close to the bone, removing the tendons connecting the surrounding muscle to the bone and clearing the dorsal part of the spinal column of all connective tissues, without damaging the bone underneath.

CAREFUL: do not cut below the level of the transverse processes, to avoid cutting or damaging the lumbar spinal nerves.

- 23 Carefully turn the mouse over until its position is between prone and lateral recumbent (lying slightly on its side).
- 24 Place vertebral clamps on the intact transverse processes (i.e. onto L5 and L3 vertebrae). *This step will take practice even for those experienced with rodent surgery. The clamps should be positioned so that the DRG is as close to horizontal as possible – see Figure 2.

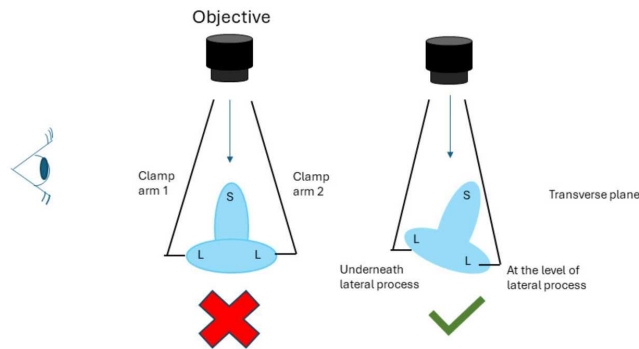


Figure 2: Diagram illustrating optimum positioning of clamps for imaging DRG on a horizontal plane

The dorsal processes should be angled away from you slightly.* *Ensure clamps are not overtightened or placed in the small gap between vertebrae, to avoid damaging the spinal cord or spinal nerves.*

- 25 Once the mouse has been clamped securely, carefully insert the rongeurs onto the facet joint between L4 and L5, with the tip of the rongeurs facing rostrally. Carefully clip away a small part of the L4 vertebrae using the rongeurs, in a caudal-rostral direction, thereby widening the gap between L4 and L5. This can cause bleeding, which can be stemmed using gelfoam.
- 26 Continue to carefully chip away the L4 vertebral bone until a hemilaminectomy of L4 is achieved. The laminectomy needs to be extended into the lateral direction, to expose the DRG which should lie just ventral of the transverse processes. Continue to use gel foam to stem any bleeding.

Note that there tends to be more bleeding from the caudal side so do not remove too much bone from this end.
- 27 Bleeding can be stemmed using gelfoam. Bone wax can be applied to stem bleeding from the bone.
- 28 Once the DRG is exposed, make sure all bleeding has stopped before coating with silicone elastomer. Apply a drop of saline onto the DRG to check for minor bleeding.
- 29 Apply a very thin coat of silicone elastomer to the DRG and wait ~5min for it to set and to ensure there is no bleeding. *Ensure that there are no bubbles in the silicone elastomer.

Should any bleeding occur while the silicone elastomer sets, remove the elastomer, stem the bleed and reapply.

30 Rehydrate mouse with 0.25mL saline subcutaneously. Repeat every hour to maintain hydration.

31 The optically cleared window over the DRG is complete, and the mouse can now be placed under the microscope for the visualisation of DRG.

Image acquisition

32 Image L4 sensory neurons using a 10x dry objective on a confocal microscope with the pinhole partially open (~154µm).

Note that a standard fluorescent microscope can be used but a confocal is preferred to minimise contamination from background fluorescent signal

33 A 488-nm Argon ion laser line can be used to excite GCaMP6s, and the signal is collected at 500–550 nm.

34 Perform time lapse recordings with an in-plane resolution of at least 512 ×512 pixels at ~ 3-4 frames per second.

Note that these acquisition parameters are sufficient to visualise GCaMP6s calcium transients produced by single action potentials[3].

Protocol references

[1] Chen, TW., Wardill, T., Sun, Y. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* 499, 295–300 (2013). <https://doi.org/10.1038/nature12354>

[2] Chisholm KI, Khovanov N, Lopes DM, La Russa F, McMahon SB. Large Scale In Vivo Recording of Sensory Neuron Activity with GCaMP6. *eneuro* 2018;5(1):ENEURO.0417-0417. <https://doi.org/10.1523/ENEURO.0417-17.2018>

[3] Ingram, Sonia; Chisholm, Kim I.; Wang, Feng; De Koninck, Yves; Denk, Franziska; Goodwin, George L. Assessing spontaneous sensory neuron activity using in vivo calcium imaging. *PAIN* 165(5):p 1131-1141, May 2024. | DOI: 10.1097/j.pain.00000000000003116

[4] Yuan X, Han S, Zhao F, Manyande A, Gao F, Wang J, Zhang W and Tian X (2023) Rapid injection of lumbar dorsal root ganglia under direct vision: Relevant anatomy, protocol, and behaviors. *Front. Neurol.* 14:1138933. doi: 10.3389/fneur.2023.1138933

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