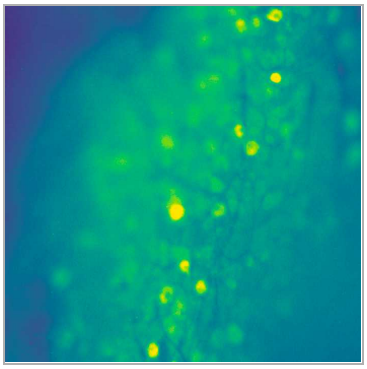

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We use this protocol and it's working

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Surgical window preparation to visualize the trigeminal ganglion in an anesthetized mouse

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RE-JOIN

Elizabeth A. Ronan

ABSTRACT

The trigeminal ganglion houses somatosensory neurons that innervate peripheral structures throughout the head and face. This protocol describes step-by-step instruction to perform a non-survival surgery for visualizing the ganglia. We apply this procedure to record calcium responses from the mouse trigeminal sensory neurons *in vivo.*

GUIDELINES

Animal subjects research was approved by the University of Michigan Institutional Animal Care and Use Committee. (IACUC protocol # PRO00010546).

Stereotaxic Surgical Apparatus Assembly

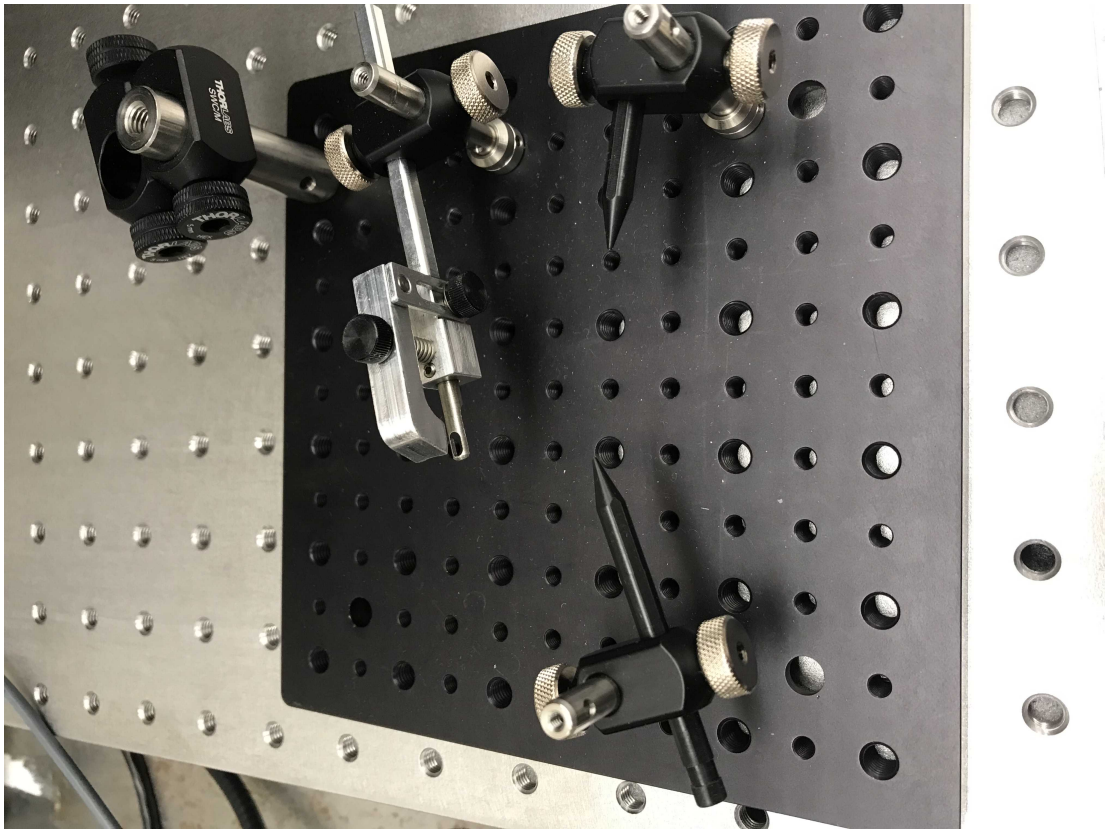
1 Any stereotax that stabilizes the skull and enables access to the mouse would be sufficient.

We assembled a custom stereotax from the following parts:

- Mini-Series Adapter with External M4 Threads and Internal M3 Threads **Thorlabs Catalog #MSA4/M**
- Mini-Post Right-Angle Post Clamp Fixed 90° Adapter **Thorlabs Catalog #MSRA90**
- Rotating Clamp for Ø1/2 Posts 360° Continuously Adjustable 5 mm Hex **Thorlabs Catalog #SWC/M**
- Mini-Series Optical Post Ø6 mm L = 1.5 **Thorlabs Catalog #MS1.5R**
- Mini-Post Swivel Post Clamp 360° Continuously Adjustable **Thorlabs Catalog #MSWC**

The palate bar was obtained from Harvard Apparatus hardware:
https://www.harvardapparatus.com/standard-u-frame-stereotaxic-instrument-for-mouse-and-neonatal-rat.html

2



Our custom stereotax.

Suction Pipets Fabrication

3 Clean the workstation near a bunsen burner, removing any flammable items from the area. Ensure no loose clothing, body parts, or hair would fall into the flame.

Safety information

Avoid wearing gloves to prevent potential melting while handling items near the open flame.

4 Turn on the bunsen burner and ignite. Hold a single Pasteur pipet over the open flame at the tapered portion of the glass.

Corning 5.75 inch Pasteur Pipets Disposable Bulk Pack Non-Sterile Unplugged **Merck MilliporeSigma (Sigma-Aldrich) Catalog #CLS7095D5X-1000EA**

5 While steadily holding the Pasteur pipet, gently torque each end in opposing directions while applying inward pressure.

6 As soon as the glass begins to become malleable, remove the Pasteur pipet from the flame and immediately pull each end outward in opposing directions until glass capillary extends to the desired width. Allow the pulled pipet to briefly cool, taking care not to touch the hot portion of the glass.

Note

We recommend pulling several vacuums of varying widths. The diameter of the glass determines the rate of tissue removal during bilateral hemispherectomy.

Suction pipets by diameter:

	A	B
	Suction Pipet	**Opening diameter**
	Large	1.5 mm
	Medium	1 mm
	Small	0.5 mm

7 Gently hold the pulled pipet at the base parallel to the bench, and submerge the elongated portion in the top of the flame at a 45-90° (based on preference) so that the glass again becomes malleable and the end drops to a ~90° angle from the base.

8 Set the pipet on the bench, with the new pulled suction end resting on the bench surface. Use a folded piece of Sandpaper **Amazon Catalog #B07JFDHXV5** (grain side out) to create a knick in the angled end at the desired end length of your vacuum tip.

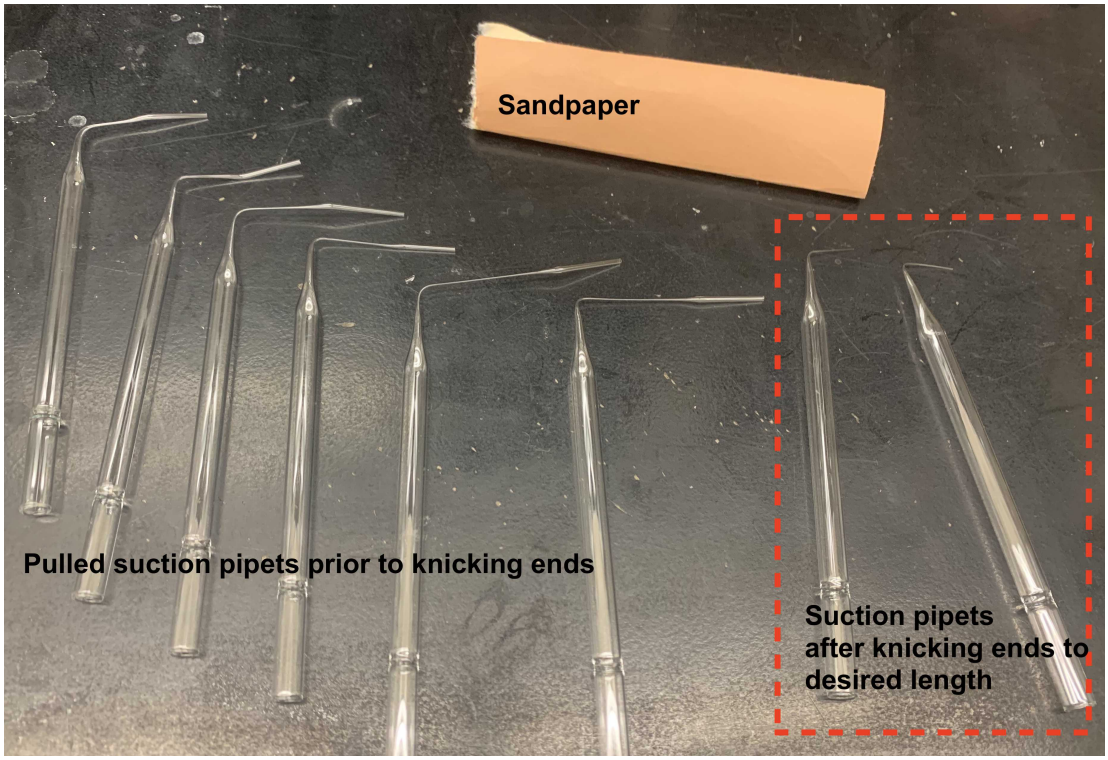
Safety information

Wear safety glasses when sanding and breaking the vacuum to protect your eyes from potential glass shards.

8.1 For larger vacuums, you may need to knick both sides to create a weak point.

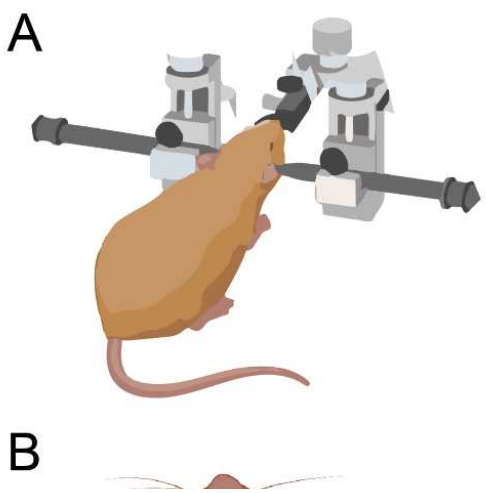
9 Once knicked, apply tension from the tip of the knicked end until the glass breaks neatly to the desired vacuum length.

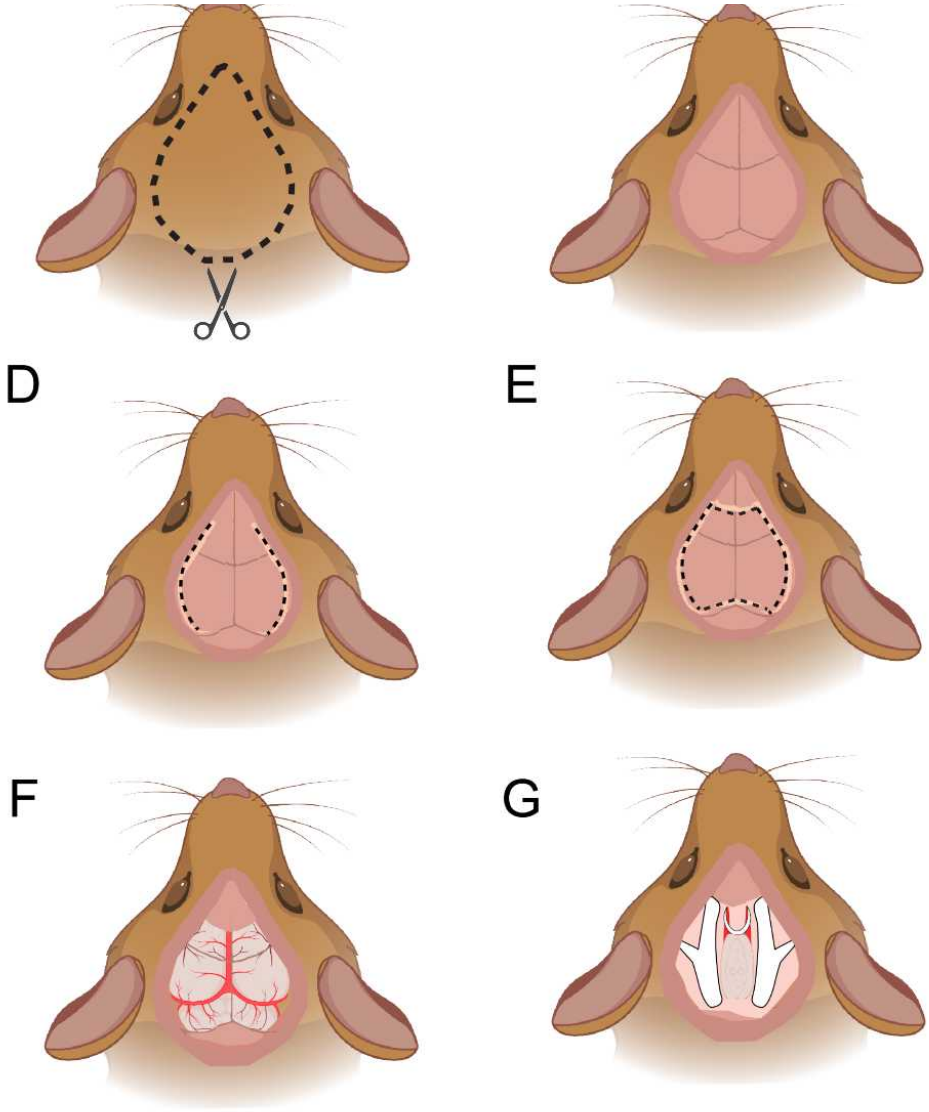
> **Note**
>
> If the top breaks unevenly, sandpaper can be used to gently smooth the rough edges.



Procedure Overview

10





Overview of Bilateral Hemispherectomy Procedure. Step-by-step description follows below.

A. Mouse must be anesthetized and secured in the stereotaxic apparatus.

B. The scalp is removed to enable access to the skull.

C. The skull is fully exposed with all overlying residual tissue removed.

D. The lateral edges of the skull are drilled in a pear shape, taking care not to puncture the underlying brain.

E. The causal and rostral midline skull is cut, to release the skull cap.

F. **See procotol for complete details.** Briefly, the skull cap is removed to create a window and fully expose the dorsal view of the brain. The superficial cortex is removed around the edge and then middle of the window. Brain tissue is removed from the anterior portion of the window to expose the rostral portion of the trigeminal ganglia. Remove the brain tissue over the midline. Avoid the caudal midline to prevent damage to brainstem. Finish removing brain from over the TGs.

G. After clotting and clot removal the TG can be visualized for imaging.

Positioning the mouse in the stereotax

11 Induce anesthesia using isofluorane according to your approved animal protocol.

Note

Mouse is adequately anesthetized when the righting reflex is gone.

We deliver anesthesia at 4% using the Somnosuite, but other vaporizers would be sufficient. We provide description of our methods. Please modify these variables as needed for alternative isoflurane units.

Equipment

Somnosuite Starter Kit for Mice Includes (2) 5mL glass syringes, low profile anesthesia mask, 0.5L induction chamber, 8-pack of waste anesthesia gas canisters	NAME
Anesthesia	TYPE
Kent Scientific	BRAND
SOMNO-MSEKIT	SKU

12 Remove mouse from induction chamber and change the anesthesia flow to the nose cone (2% isofluorane, flow rate 125mL/min). Parameters may need to be adjusted depending on mouse size and breathing.

Note

We prevent the nose cone from displacing by cutting a small hole near the outer edge of the nose cone that fits the top incisors. When placing the nose cone on, open the mouth and position the top incisors through the hole.

Note

Larger mice tend to require additional isoflurane percent and/or flow rate.

Note

After mice are in the maintenance phase of anesthesia following this step, breathing must be steady without gasping.

13 Position incisors (through the nose cone) above the palate bar hole. Tighten the top clamp down onto the mouse snout with nose cone to prevent nose cone displacement and secure the position of the mouth.

Note

Make sure that as the clamp tightens down, the nose stays straight and both eyes are level and parallel to the ground.

14 Position ear bars in the ear canals so that head is immobilized with the top of the head as flat as possible. A line through bregma to lambda should also be parallel to the ground.

Note

Ear canals tend to be lower and near eye level. Start by fastening the first ear bar lightly against the ear, approximately where you expect the canal to be. Next, insert the opposite ear bar and search until you catch the tip in the canal, so that slight vertical displacement of the ear bar steadily controls head movement in the same direction; fasten the ear bar to hold this position. Repeat with the opposite side so that the head is immobilized. Next, slowly inch up each side with the ear bars until the head is raised so that the front legs are almost entirely extended upwards, with the feet still contacting the platform. Ensure both ears are parallel so that the top the head is as flat as possible. It is helpful to adjust the palette bar as necessary while raising and flattening the head.

15 Wet eyes with artificial tears to prevent drying.

Ophthalmic Ointment **Fisher Scientific Catalog #NC0490117**

Bilateral Hemispherectomy and Exposure of Trigeminal Gan..

16 Warm 1X PBS to 37 °C using a standard laboratory hot plate.

1X PBS (Phosphate-buffered saline)

Equipment

Hot plate	NAME
ONiLAB 5 inch LED Digital Hotplate Magnetic Stirre	BRAND
B07H8H5XM1	SKU

We use a Pasteur pipet with a 1mL bulb to pipet PBS during the procedure

Corning 5.75 inch Pasteur Pipets Disposable Bulk Pack Non-Sterile Unplugged **Merck MilliporeSigma (Sigma-Aldrich) Catalog #CLS7095D5X-1000EA**

Latex 1 mL bulb **Fisher Scientific Catalog #0344821**

17 Trim whiskers and wet fur on top of the head with PBS.

18 Hold skin up with curved forceps. Cut and remove skin above the skull and carefully remove overlying connective tissue (scalp).

Note

Removing additional skin 1-2mm flanking the border of the skull will help prevent excess skin from getting caught in your drill.

Equipment

Fine Scissors - Straight/11.5cm	NAME
Surgical Tools	TYPE
Fine Science Tools	BRAND
14060-11	SKU
https://www.finescience.com/en-US/Products/Scissors/Standard-Scissors/Fine-Scissors-Sharp/14060-11	LINK

Equipment

Student Dumont #7 Forceps	NAME
Surgical Tools	TYPE
Fine Science Tools	BRAND
91197-00	SKU
https://www.finescience.com/en-US/Products/Student-Instruments/Student-Forceps/Student-Dumont-7-Forceps	LINK

19 Use curved forceps to displace residual thin membrane from the top of skull, so the skull is clear of any tissue.

Equipment

Student Dumont #7 Forceps	NAME
Surgical Tools	TYPE
Fine Science Tools	BRAND
91197-00	SKU
https://www.finescience.com/en-US/Products/Student-Instruments/Student-Forceps/Student-Dumont-7-Forceps	LINK

20 Connect prepared large (1.5 mm) suction pipet 2 to vacuum tubing and vacuum trap. Turn on the vacuum valve.

Note

We connect the vacuum trap to the vacuum valve using high pressure tubing to prevent tubing collapse.

21 Use a small dental bur (#35 carbide bur) and gentle cutting to create a shallow outline on the skull in a broad pear shape (as shown in D). Start laterally and cut the medial parts in front and back last because the midline contains large blood vessels. Irrigate any bleeding with warmed 1X PBS and suction with the suction pipet.

Note

Once the midline vessels are ruptured, either accidentally or deliberately, excessive bleeding will occur. It is essential to move through the following steps as quickly as possible to prevent death.

DynaCut FG #35 Inverted Cone 1.0mm Diameter Carbide Bur 100/Pk **Contributed by users Catalog #FG 35**

Equipment

Drill	NAME
Surgical Drill	TYPE
Amazon	BRAND
B07Q5F3BZC	SKU
https://www.amazon.com/Rechargeable-Lumcrissy-Professional-Portable-Scamander/dp/B07Q5F3BZC/ref=sr_1_44?crid=1O1DYCW4ROK4P&keywords=micro%252Bmotor%252Bdrill%252Bmanicure%252B35%252C000&qid=1642691640&sprefix=micromotor%252Bdrill%252Bmanicure%252B35%252C0	LINK
Drill must have high speed up to 35000 RPM	SPECIFICATIONS

22 Gently lift off detached skull with curved forceps. Assume that bleeding will now begin.

Equipment

Student Dumont #7 Forceps	NAME
Surgical Tools	TYPE
Fine Science Tools	BRAND
91197-00	SKU
https://www.finescience.com/en-US/Products/Student-Instruments/Student-Forceps/Student-Dumont-7-Forceps	LINK

23 Upon removing the skull cap, reduce isofluorane concentration by 1/4th of the total percentage (e.g., if at 2%, lower to 1.5%).

24 Begin to suction out the cortex with the large suction pipet, starting anteriorly and traveling around the edge of the window on the left and right sides. Blood may pool as portions of brain are removed. Continue to irrigate, and suction to remove pooling blood. Use small spring scissors to cut any remaining dura (possibly near anterior midline).

Note

If brain is harshly lifted at any time by the suction pipet during the procedure, switch to a smaller suction pipet.

Do NOT suction in a back-and-forth motion, rather usr a rapid blotting motion (blotting = up and down) while regularly irrigating with 37°C 1X PBS

There is usually a lot of brain hiding underneath the skull; angle the pipet tip to get brain flush against the skull on the sides.

Equipment

Student Vannas Spring Scissors	NAME
Surgical Tools	TYPE
Fine Science Tools	BRAND
15000-03	SKU
https://www.finescience.com/en-US/Products/Scissors/Spring-Scissors/Vannas-Spring-Scissors-2mm-Cutting-Edge/15000-03	LINK

25 Once the cortex within the window is removed, the underlying hippocampus will be fully exposed. At this point, suction away remaining brain at the anterior aspect of the window. Do not cross the midline. Continue to use a rapid blotting motion and regular irrigation.

26 As you remove deeper layers, avoid the optic chiasm. Continue focus on removing the anterolateral regions until the trigeminal ganglia (TG) are only visible at the rostral portion.

Note

There is a thin membrane between the brain and TG that may be present depending on how gently you suction. This membrane must be punctured and removed as you continue to remove brain to visualize the TGs.

27 Once the TGs are visible, switch to a medium suction pipet (1 mm) and gently suction at the midline to remove brain tissue above the optic chiasm. Ideally, the optic chiasm will be fully exposed and intact, with the brain above it removed.

Note

Take care not to pull on the optic nerves or chiasm; there two large blood vessels that run adjacent and medial to the TG. These must not be ruptured.

28 Before continuing to exposing the caudal portions of the TGs, suction the portions above them to remove additional brain without exposing them yet. Avoid the brain at the caudal midline which lies over the brainstem.

Note

Keeping the TG covered as much as possible throughout the procedure until the end prevents blood clots from drying on top of the TG, which will be challenging to remove later.

29 Once the majority of the brain is removed, except for a thin layer directly covering the ventral portions of the TGs, remove the remaining brain obstructing view of the TGs.

Note

Remove any blood that covers the TG while exposing the TGs. Try to suction away this blood so it does not adhere to the TG.

30 Fill the skull with 37°C 1X PBS and reduce isofluorane an additional 1/4th of the starting concentration (e.g., if starting at 2%, the isofluorane should be reduced to 1.5% during the procedure, and 1% once the brain is removed).

31 Now allow large blood clot to form over TG for 3-5 minutes.

Optional step: While waiting, we recommend replenishing fluids by giving the mouse an IP

injection of sterile saline heated to 37°C. (For males inject 0.5mL, for females, 0.25mL).

1/2 CC Syringes **Fisher Scientific Catalog #1482679**

32 Optional step:
Depending on the stimulation being performed during imaging, additional stabilization of the head may be needed to prevent shifting of the focal plane. We are happy to discuss additional methods for immobilization.

Krazy glue **Amazon Catalog #KG94548R**

33 Within the skull, you should now observe a blood clot with a gelatin-like consistency. Begin removing the clot by suctioning from the sides of the skull, and dragging the clot across the TG to uncover it. Remove all of the clot, but do not touch the TGs with the suction pipet.

Note

If the blood clot is not thick, refill the skull with PBS and wait 3-5 more minutes for clot to form.

34 TGs should now be fully exposed. Well done. If some bleeding continues, fill the skull with PBS and suction away. Repeat 2x and bleeding will typically subside.

If you encounter excessive bleeding return to go to step #33

35 Optional step:

If a clot cannot be removed from the TG surface, flow a shallow layer of PBS above the TG and use the large end of fine paper point saturated in PBS to mechanically dislodge the clot on the surface of the TG. We do not recommend physically contacting the TGs except as a last resort (i.e., here)

XX-Fine Absorbent Paper Points **Contributed by users Catalog #P.61-PP200-XXF**

36 TG are exposed and are now ready for imaging.

Note

Adjust the height of the stabilization points of the head to flatten the TG and increase the neurons in the imaging field and plane.

We find that lowering the height of the opposite earbar is very effective.