



Necropsy SOP for harvesting live brain tissue

I. Personnel

a. A smooth necropsy typically requires at least 5-6 people:

- i. Two people help with preparation
 - ii. Two people conduct most of the necropsy procedures (e.g., craniotomy, thoracotomy, etc.)
 - iii. One-two people microdissect while a third places microdissected tissue in tubes
 - iv. One person flash freezes blocks
 - v. One person conducts large tissue dissection; this person should have intimate knowledge of primate gross anatomy (i.e., veterinarian)
 - vi. One person needs to monitor tissues collected and distributed to make sure no tissue is lost or given to the wrong person; this task can easily be done by one of the people already involved in the necropsy
- b. Typically, a technician or graduate student assists in the craniotomy and thoracotomy/perfusion. Once the brain is removed and microdissections begin, that individual generally moves the carcass to another table and begins the removal of organs and tissues.

i. Person A – tube prep, suite prep, collection of microdissected tissue, floater to assist with any additional needs

ii. Person B – tube prep, suite prep, animal prep, main procedures assistant, large tissue dissection

iii. Person C – animal prep, conducts main procedure, microdissects

iv. Person D – blocks and microdissects

v. Person E – flash freezing of blocks, floater to assist with any additional needs

vi. If tissue share is taking place, collaborators must send representatives for their desired tissue. It is the collaborators responsibility to supply specific reagents/buffers or liquid nitrogen for collection of their specific tissues.

c. The person who assists with craniotomy, thoracotomy, etc. should wear

Kevlar gloves during procedures

II. Necropsy preparation

a. Tubes

i. Determine what tissues will be collected (see sample collection sheet attached)

ii. Make labels for the microdissected tissue collected during necropsy

1. Use Cryo-Babies labels (1.28x0.5)

2. Use the Avery standard mini address label 2160 template in

Microsoft Word to create a sheet of labels

3. Type the animal number, necropsy date and the tissue collected for each label

iii. Gather enough cryotubes and affix labels for microdissection

iv. Number each tube to correspond with the appropriate number on the microdissection list

1. Write number on the cap of the tube so that it is visible when looking down on the tubes in the cryotube box

v. Arrange labeled cryotubes in numerical order in cryotube box

vi. Use larger labels and Falcon 15 -50 ml tubes with blue top for larger tissues collected (i.e., liver, heart, lung)

vii. Use larger labels and blood collection tubes if collecting blood during necropsy

viii. Print out a checklist of all microdissected tissues to be collected and all larger tissues to be collected during the necropsy (2 copies of each)

b. Necropsy suite

i. Main necropsy table:

1. Place instruments for anesthesia, craniotomy, perfusion,

and microdissection on a large cutting board close to where the animal's feet will lay; supplies for these procedures are listed on attached necropsy supply list

2. Place a small stack of gauze and paper towels and a squeeze bottle of saline close to where the animal's

head will lay during the procedure – gauze and paper towels will be used by assistant to clean rongeurs of bone chips.

3. Place the cryotube box with microdissection tubes on dry ice (or liquid nitrogen) in a small cooler next to the large cutting board; tape the microdissection checksheet above the cooler; keep a pencil close by to check off tissues once they are collected

4. Fill three stainless steel beakers halfway with isopentane, place them on dry ice in cooler, place the lid on the cooler and place the cooler on a countertop close to the main necropsy table

a. Place thermometer close to the cooler in order to gauge the temperature of the isopentane (see III.a for instructions on preparing isopentane for flash freezing)

5. Cover three potato mashers with aluminum foil so that the foil is spread very thin without any bumps

6. Tear 8-10 square pieces of aluminum foil to cover the brain blocks after flash freezing – these should be placed in the cooler of dry ice to cool them before wrapping the brain blocks

7. Prepare porous tape to secure angiocatheter

a. Create two pieces of tape 1½” in length from one by splitting it vertically

b. Cut one long piece of tape that will be long enough to go around the entire animal's leg

c. Hang tape off of necropsy table for easy access during catheterization

8. Tape a large biohazard bag to the side of the main necropsy table close to where the animal's head will lay

9. Fill plastic container or Tupperware container with wet ice and place brain block on ice. Place large glass Petri dish upside down on ice – brain will be placed on this for further blocking.

10. Remove brain blades from wrapping and mark the sharp end with a squiggly line along its entire length using a black marker. Place blades back in wrapping until use

11. Put blades on scalpels

a. Open scalpel wrapping only enough to reveal the end that attaches to the handle

b. Place blade on handle and pull back wrapping so that it covers the entire blade; leave blades in wrapping until use

ii. Large tissue collection table:

1. Place instruments for large tissue collection on a smaller cutting board on a second table out of the way of the main table; supplies for these procedures are listed on attached necropsy supply list

2. Cover a large stainless steel tray with a large biohazard bag and place on table

3. Get out a second large biohazard bag so that it is ready for double-bagging during animal disposal

4. Place the tubes, pencil and check sheet for large tissue collection close by

c. Animal

i. Sedate animal with ketamine (15 mg/kg i.m.) – pull up sodium pentobarbital (20-35 mg/kg) in syringe and have available

ii. Remove collar

iii. Remove hair from head and neck beginning at the brow ridge, around each ear and down to the 3rd or 4th cervical vertebra

iv. Remove hair from the backs of the calves to enable catheter access and the fronts of the thighs if collecting the quadriceps

v. Remove hair from the abdominal area if it is particularly dense or long

vi. Weigh the animal and make note for sodium pentobarbital administration

vii. Transport animal to the necropsy suite

viii. Collect CSF from foramen magnum using 25g 1½ inch needle.

III. Bring animal to a deep surgical plane of anesthesia

a. At the onset of anesthesia, the isopentane temperature should be brought down to -35°C to -40°C by placing stainless steel beakers on dry ice. This takes approximately 20-25 minutes.

i. Need to stir isopentane to get accurate temperature

ii. Closely monitor temperature to be sure it doesn't get below -40°C;

the brain risks cracking at lower temperatures

1. If the isopentane temperature is too low, remove from dry ice momentarily

2. Liquid nitrogen can be used to flash freeze tubes containing microdissected tissue.

b. Insert angiocatheter into saphenous vein and attach saline syringe and prime t-connector.

c. Apply benzoin using swab around the catheter site (this will help tape adhere)

d. Secure angiocatheter with tape by slipping tape sticky side up underneath the catheter tip and criss crossing

tape across top of catheter towards knee. Secure catheter and tape with another piece of tape approximately 1 ½ inches long placed lengthways. Then secure this assembly by taping around the ankle including the t-connector.

i. Draw back to confirm the patency of the angiocatheter

e. Remove saline syringe and connect syringe with sodium pentobarbital (20-35 mg/kg i.v.)

i. Administer sodium pentobarbital slowly to effect (see f. below)

ii. Flush with equal amounts of saline until animal is at desired plane of anesthesia

f. Test for an adequate plane of anesthesia with corneal, palpebral and withdrawal reflexes

i. Corneal reflex – lightly touching the cornea should produce an eye blink in a lightly anesthetized animal

ii. Palpebral reflex – tapping the skin at the medial canthus of the eye or running a finger along the eyelashes should produce an eye blink in a lightly anesthetized animal

iii. Withdrawal reflex – pinching the bottom of the animal's foot with the hemostats should produce a withdrawal of the foot in a lightly anesthetized animals

iv. Observing a response means that the animal is not under an adequate plane of anesthesia

1. Be sure you have waited long enough for the sodium pentobarbital to take effect before administering a larger dose

v. Confirm the absence of reflexes before moving forward with the necropsy

g. Monitor reflexes throughout necropsy procedures

h. It is important to closely monitor cardiac and respiratory function once the pentobarbital has been administered; personnel should be

prepared to move hastily in case of respiratory failure due to acute sensitivity to pentobarbital

IV. Craniotomy

a. At the onset of the craniotomy, the carboy containing chilled perfusate should be hung 3-4 feet above the necropsy table and assembled ready for perfusion

i. Requires carboy, perfusate, tubing, large bore cannula, and Babcock atraumatic hemostat for clamping cannula in place

b. Make sure isopentane is at appropriate temperature at this time and that aluminum foil squares are chilled on dry ice

c. Assistant should hold free skin/muscle taut during incisions and reflection throughout this procedure using curved hemostats

d. Make a rostral to caudal incision along the sagittal suture with a scalpel and forceps with teeth beginning at the bregma and extending caudally beyond the foramen magnum to approximately the 3rd cervical vertebrae

e. Make a second incision lateral to medial along the coronal suture perpendicular to the first incision

f. Reflect the temporal, frontal and occipital muscles bilaterally to expose the skull

i. Make a rostral to caudal incision on each side of the sagittal suture following the line of the muscle (crescent-shaped)

ii. Reflect the muscle laterally and ventrally with bone chisel and tissue scoop

iii. Reflect tissue from the sagittal suture using rongeurs or hemostats to grip and then pull back removing the tissue off the skull

g. Reflect the occipital muscle beyond the occipital ridge

i. Make a lateral to medial incision just ventral to the occipital ridge ii. Use the bone chisel and tissue scoop to remove the muscle from this area

h. If previous attempt at collecting CSF is unsuccessful, a second attempt to collect CSF can be made at this time if necessary from foramen magnum using a 25 gauge 1½" needle

i. Clean and dry the skull with 70% ethanol and gauze pads

j. Remove a small piece of bone (approximately 1 cm²) from the right parietal bone of the skull using a cordless drill/dremel and skull bit

i. Go slowly, taking extra care not to puncture through the skull during this portion of the procedure

ii. Use saline to clean the area and reduce aerosolization of bone dust during drilling

iii. Remove the piece of bone with forceps

k. To enlarge the opening, use small-tipped rongeurs to chip and remove small fragments of the skull

i. Take care to pull bone fragments away from the brain during this portion of the procedure

- ii. Continue to remove bone so that the skull rostral to the occipital ridge can be removed and the brain exposed
- iii. Use the periosteal elevator frequently to separate the dura mater from the bone while working
- iv. Use caution when crossing over the midsagittal line as there is a large superficial vascular source here that will bleed if disturbed
- v. Use larger double-action rongeurs when applicable
- l. Once the bone is detached, remove calvaria from the dura mater using the periosteal elevator; discard the bone
- m. Remove the occipital ridge using rongeurs
 - i. Use caution when removing the occipital ridge as there is a large vascular source here (cynomologus macaques) that will bleed if disturbed. Gauge bleeding at this time and prepare to move hastily if bleeding is excessive
 - ii. Do not remove bone any further than the occipital ridge
- n. Gently cover the exposed brain with saline-soaked gauze
- V. Thoracotomy & transcardial perfusion
 - a. Turn the animal over on its back making sure that the brain remains covered with saline-soaked gauze – the gauze should support the head at the occipital bone without placing undue pressure on the brain
 - b. Using forceps and a scalpel, begin to open the abdomen along the midline by making an incision through the skin only from the clavicles to the lower pelvic region - Assistant should hold free skin/muscle taught during incisions and reflection throughout this procedure using curved hemostats
 - c. Using the forceps and a scalpel reflect the skin from the abdominal muscle and make two incisions just below the rib cage perpendicular to the first incision
 - i. Use caution making sure not to puncture the diaphragm
 - d. Make a similar midline incision through the abdominal muscle beginning just below the ribcage and ending at the lower pelvic region
 - e. Make two more incisions through the abdominal muscle along the bottom of the ribcage in a lateral direction– this allows blood and perfusate to drain from the abdomen/thorax
 - f. Collect blood from the inferior vena cava at this point if necessary
 - i. Insert butterfly needle attached to syringe
 - ii. Pull back on syringe to collect blood
 - g. Use the scalpel to cut through the muscle (pectoral) laying over the ribcage, making the incisions at a 45 degree angle from bottom of ribs to just below each clavicle
 - h. Cut the diaphragm away from the ribcage using a scalpel
 - i. Use heavy duty scissors to cut through the ribcage along the same line where the muscle was cut earlier (see i. above)
 - j. The assistant should pull back the ribcage with a hemostat clamped at the xiphoid process to expose the heart
 - k. Open the pericardium surrounding the heart using small, curved, blunt-ended scissors and forceps with teeth and expose heart
 - l. To drop blood pressure, cut the right atrium with small curved blunt-ended scissors and forceps with teeth
 - m. Remove the apex of the left ventricle with small, curved, blunt-ended scissors and insert a large bore cannula (16 gauge or larger; a 1 cc tuberculin syringe with both ends cut off will work) into the ascending aorta for perfusion; clamp in place with Babcock atraumatic hemostat
 - n. Clamp the descending aorta in the lower thorax prior to perfusion with a curved hemostat
 - o. Allow gravity to perfuse the animal with oxygenated buffer for approximately 1.5 minutes; amount of perfusate used during perfusion is approximately 1.5 L
 - i. Buffer contains 124 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 23 NaHCO₃, 3 NaH₂PO₄, 10 glucose (pH 7.4, osmolarity 290-300 mOsm) oxygenated with 95% O₂:5% CO₂.
 - p. Use fingers to drain blood and perfusate from animal by putting a small amount of weight on one side of the animal's body close to incisions made along the ribcage
 - q. Remove any tissues from the thoracic and abdominal cavities (i.e., liver, lung, etc.) during the perfusion as time allows and as is necessary
- VI. Brain extraction and microdissection
 - a. Following perfusion immediately turn the animal over
 - b. Remove the remaining occipital bone with rongeurs

- c. Cut the dura mater at the posterior-most extent of the occipital lobes and along the midsagittal line and reflect using small, curved, blunt-ended scissors and small forceps with teeth
 - i. If the dura has already been pierced during craniotomy, use this as a starting point to cut
- d. Remove the falx cerebri and tentorium cerebelli using small, curved, blunt-ended scissors and small forceps with teeth
 - i. Assistant should elevate the occipital lobes using the blunt end of a pair of forceps
- e. Excise the cervical spinal cord at approximately C2-C3 using a scalpel
- f. Use the flat end of the scalpel to bluntly dissect the cranial nerves from the brain and remove the brain from skull in a caudal to rostral fashion
- g. Rinse brain mold with perfusate once blunt dissection begins
- h. Immediately place the brain in the brain mold and rinse with cold perfusate (OHSU – place brain in dish with cold ACSF in order to isolate brainstem/ olivary nuclei from cerebellum. Resect anterior and posterior cerebellar lobes from vermis under cold ACSF).
- i. Transfer animal to tray covered with biohazard bag on side table for large tissue dissection at this time (skip to step VI for further instruction)
- j. Block the brain using brain blades
 - i. MRI coordinates can be useful for determining where to make blocks
- k. Hand off blocks for flash freezing to assistant with the isopentane (see step n of this section for flash freezing instructions)
- l. Microdissect the brain using small, curved, blunt-ended scissors and small, curved forceps for placing the tissue into cryotubes (OHSU – place brain

in brain matrix, block the frontal lobes at the anterior tips of the temporal poles and microdissect the cortical fields and freeze in tubes. Using brain knives placed every 4 mm, cut the remainder of the brain from rostral to caudal direction. Structural MRIs will be used to place knives in particular position in order to collect specific brain regions).

- m. Return cryotubes (in cryotube box) to dry ice following microdissection n. Freeze the blocked portions of brain using stainless steel potato mashers covered with aluminum foil
 - i. Place each potato masher into a stainless steel beaker filled with isopentane
 - 1. Beakers should be kept on dry ice at a temperature of -35°C to -40°C (see III.a for instructions on how this should be prepared)
 - ii. Remove blocks of tissue from isopentane and place directly on dry ice for a few minutes to allow the isopentane to evaporate
 - iii. Wrap frozen blocks in cooled aluminum foil and place in a cooler on dry ice (or liquid nitrogen)
 - iv. Move blocks to cryotube box and keep on dry ice once microdissection has been completed

VII. Pituitary & large tissue removal

- a. Transfer small-tipped rongeurs and periosteal elevator from main necropsy table to side table for pituitary removal
- b. Use rongeurs to remove the pituitary by pulling back each of the “four corners” of the sella turcica that surround it
 - i. Once exposed, use the periosteal elevator and small forceps with teeth to carefully remove the pituitary and place it in a labeled cryotube
 - c. If desired, separate the anterior pituitary from the posterior using a scalpel and forceps
- d. Remove large tissues (i.e., heart, liver, lung, kidney, etc.) using small, curved, blunt-ended scissors and forceps with teeth
- e. Place each dissected tissue in the respective tubes and place in cooler on dry ice

VIII. Animal disposal

- a. Be sure that all tissues for collection have been checked off of the checklist before disposing of animal
 - b. Collect all dirty gauze, tissue, bone and other biohazardous materials and place in tray covered with biohazard bag
 - c. Close the animal in the biohazard bag by removing the bag from the tray and tying it in a knot
 - d. Double bag the animal before placing it in the appropriate disposal area (i.e., biohazard box in necropsy cooler)
- #### IX. Clean up
- a. Transfer all collected tissue from the cooler with dry ice to the appropriate storage unit (i.e., -80°C freezer)
 - b. Used instruments

- i. Soak all used instruments in soap (e.g., Sparkleen soap)
 - ii. Scrub instruments to remove blood and tissue remnants
 - iii. Rinse instruments with water
 - iv. Lay instruments out on paper towels
 - v. Spray instruments with disinfectant
 - vi. Hand dry with paper towels
 - vii. Return dry, disinfected instruments to storage cabinet
- c. Necropsy table, tray, cutting boards, etc.
- i. Rinse with water
 - ii. Scrub with soap
 - iii. Spray with disinfectant
 - iv. Wipe dry
- d. Disinfect any items that were touched or could have come into contact with blood or animal tissue during necropsy (this includes walls that may receive blood spatter, door handles, cabinets, etc.) using MB10 or other appropriate cleanser. Rinse necropsy tools with water after disinfecting in order to avoid rusting
- e. Pick up any loose bone particles from sink and floor drains and dispose of in biohazard bag
- f. Spray and squeegee floor