

Great Ape Neuroscience Project

Brain Collection Protocol

(December 2020)

Animal Death

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graph TD; A[Animal Death] --> B[If brain can be collected within 12 hours of death]; A --> C[If brain is collected more than 12 hours after death];
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If brain can be collected within 12 hours of death

* Follow PMI < 12 hour procedure on page 2

If brain is collected more than 12 hours after death

* Follow PMI > 12 hour procedure on page 5

PMI < 12 Hours Procedure

Step 1: Photograph whole brain and weigh it

Step 2: Cut brain in half along the midsagittal plane

Step 2: Cut left hemisphere into ~ 2 cm-thick slabs in the coronal plane (see adjacent figure)

Step 3: Place each slab of the left hemisphere in separate plastic Ziploc bags; number them in order

Step 4: Flash freeze the left hemisphere slabs and store in a -80 degree freezer (see page 3 for instructions)

Step 5: Place right hemisphere in 10% formalin



Step 6: Ship the formalin fixed right hemisphere to William Hopkins: Ship frozen slabs on dry ice OVERNIGHT to Dr. Sherwood (contact information on page 6). Please contact Dr. Sherwood prior to shipping to make sure staff is available for pick up of the materials.

Step 7: MRI scan at 7T will be conducted on right hemisphere sent to Dr. Hopkins. A brief summary of any clinical abnormalities in gross morphology will be provided.

Step 8: After scanning, right hemisphere will be shipped to Dr. Sherwood for histological analyses. A report on any histological abnormalities will be provided to the ApeTag committee or participating zoos upon request. Tissue from any brain region can be provided to zoo authorities or research institutions upon request in order to conduct their own pathology analyses.

Freezing Methods for Brains

We realize that not all facilities will have the same capacity for freezing brain tissue, therefore we have outlined below three options (in preferred order):

1. **Isopentane/dry ice.** The preferred method of freezing of tissue slabs is a slurry of isopentane/dry ice at -30 to -40 C.
2. **Liquid nitrogen.** The second method of choice is freezing in liquid nitrogen by immersion.
3. **-80 C freezer.** The third method is freezing the samples on a tray in a -80 C degree freezer.

Place hemisphere on a horizontal surface with the medial side of the brain down and the lateral side facing up. If possible, take images with a digital camera before and during sectioning. Cut serial coronal slices of the hemisphere from the frontal pole and proceeding caudally at about 2 cm intervals. The number of slices per brain will vary depending on its size. Snap freeze all slices using one of the three freezing method described above and number sequentially with a letter designating the left (L) or right (R) hemisphere. Store each slice in its own individual Ziploc or vacuum plastic bag at -80 C.

Shipping Instruction

For Formalin Fixed Tissue:

- 1) Place in sealed container
- 2) Write “Biological samples – nonhazardous” on the label of the container (formalin will kill any pathogenic agent)
- 3) Ship to addresses on next page following instructions from previous pages

For Frozen Tissue:

- 1) Place in sealed container on dry ice, Please use enough dry ice to keep the brain tissue frozen for up to 48 hours in case of delay in shipping.
- 2) Write “Biological samples – nonhazardous” on the label of the container if the individual has no known pathogenic agent is present
- 3) Write “Biohazard materials” if there is a known pathogenic label.
- 4) Ship to addresses on next page following instructions from previous pages

PMI > 12 Hour Procedure

Step 1: Photograph whole brain and weigh it

Step 2: Place entire brain in 10% formalin

Step 3: Ship the formalin fixed whole brain to Dr. Hopkins (contact information on page 6)

Step 4: Samples can be shipped in a labeled box with a minimum amount of formalin. Shipping can take place any day of week except Friday (i.e., no weekend delivery). Please contact Dr. Hopkins (contact information provided on page 6) before shipping to coordinate pick up and tracking of the brain.

Step 5: MRI scan at 7T will be conducted on whole brain sent to William Hopkins. A brief summary of any clinical abnormalities in gross morphology will be provided.

Step 6 : Copies will be made available of all MRI scans upon request to any interested parties

Step 7: After scanning, whole brain will be shipped to Dr. Sherwood for histological analyses. A report on any histological abnormalities will be provided to the ApeTag committee or participating zoos upon request.

Step 8: Tissues from any brain region can be provided to zoo authorities or research institutions upon request in order to conduct their own pathology analyses.

Contact Information

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