

The following procedures may cause pain/distress:

1. Induction of anesthesia with Ketamine:

The only 'conscious' distress will be during the initial ketamine injection using the squeeze cage. The PI has recently attended a national meeting where several studies were presented that used non-human primates and this is currently the least painful method to induce anesthesia in this species.

2. Risk of headache after spinal puncture:

In humans, the risk of a post-dural puncture headache (PDPH) is dependent on several factors such as age, gender, amount of CSF drainage and spinal needle used<sup>8-11</sup>. PDPH is a well-known morbidity in association with spinal puncture for diagnostic purposes (i.e. myelography) or spinal anesthesia and is described as a severe headache (usually occipital in location) that may radiate to the neck, forehead or behind the eyes). Associated symptoms may include nausea, vomiting and dizziness. It typically starts during the first 24-48 hrs after dural punctures. The incidence in adult human varies between 1.5% and 30% dependent on the needle used<sup>9,12,13</sup>. The risk factor for developing PDPH is directly related to the diameter of the needle used. For example it is less frequent with a small-gauge (<25Ga) spinal needle, however, in many clinical papers it is proven that although the risk of PDPH is less with a small spinal needle, this technique is associated with higher failure rates and is not always recommended. Another factor involved is the design of the needle tip, for example a Sprotte needle which has its opening on the lateral aspect of the needle are better than those with a cutting configuration (Quincke)<sup>14</sup>. It is also important to orient the needle parallel to the dural fibers instead of perpendicular in order to avoid cutting them during the procedure. In children, the incidence of PDPH is 2-15% and apparently not age-dependent<sup>9</sup>. In other words, children are less likely to experience PDPH. Further, in children the configuration of the needle tip is less important because the dural lining is more 'elastic' and is thought to close more efficiently after a puncture compared to the adult. We have limited information on the risk of PDPH in baboons, however, given their size (10-30 Kg) we will expect the risks to be similar to that observed in human children. Further since we are not removing CSF, will limit CSF spill but replacing the needle stylet after insertion into the intrathecal space; and further we will insert the needle tip in a perpendicular direction to the dural fibers we will minimize the risk of causing a PDPH. However, we will follow closely the baboons over the ensuing 24-48hrs after the procedures to assure that there are no signs of headache (e.g. less activity, baboon lying down instead of sitting, less food or water intake, vomiting). If there are clear discomfort indicating the presence of PDPH we will treat it with conservative treatment (analgesics and increased hydration) since the natural history of PDPH is one of spontaneous resolution.

3. Risk of introducing an infective agent during the spinal intrathecal procedure:

According to the American Society of Anesthesiologists (ASA) Closed Claims Project Database, infection including meningitis or abscesses associated with spinal anesthesia procedures from 1980-1999 were extremely low (a total of 3 cases reported<sup>15,16</sup>). Thus, since we will be carrying out the intrathecal procedures with utmost sterility including iodine preparation of the skin, sterile draping and use sterile personal protection gear (face masks and sterile gowns and gloves) we do not expect to introduce infection intrathecally. Further, the <sup>18</sup>F injection solution is prepared in a sterile fashion according to good manufacturing practice (GMP) regulations; and therefore is cleared of pyrogens. In other words, we consider the risk of introducing a central nervous system infection to be extremely low.



#### 4. Risk of neurologic complications after spinal intrathecal procedure:

As with any invasive procedure in or near the spinal column there is always a risk of injuring the structures such as nerve roots and/or the spinal cord. There are several ways to modify procedures to minimize risk of neurologic complications. First, we plan to introduce the spinal needle at L2/L3 or L3/L4 which is below the medulla spinalis therefore minimizing the risk of injuring the spinal cord. Second, we will optimize the position of the baboon for lumbar puncture (lateral decubitus position with flexion of the lumbar spine so as to expose the intervertebral spaces optimally). Third, only two investigators experienced in intrathecal procedures (Drs. FOIA (b)(6) and FOIA (b)(6)) will be doing the procedure. Fourth, we will start with inserting the needle in the midline position which is the easiest and where the needle will pass through less sensitive structures. However, if we fail to obtain CSF using midline position we will attempt intrathecal access via the paramedian approach. We will not do more than 2 attempts per approach/interspace to avoid damage and/or bleeding. In summary taking these precautions should minimize the risk of neurological complications such as nerve injuries after the procedures.

#### 5. Risk of toxicity from $^{18}\text{F}$ introduced into the intrathecal space:

There is no data on the toxicity of intrathecally administered sodium [ $^{18}\text{F}$ ]fluoride and thus we are using toxicology data from oral doses of sodium fluoride for osteoporosis therapy (Murray et al., 1996) and the amount of [ $^{18}\text{F}$ ]fluoride from Cardinal Health specifications to estimate a safety margin. We also note that the major constituent of the solution is normal (0.9%) sodium chloride which is commonly used as a diluent for other intrathecally administered drugs for spinal anesthesia, pain management and chemotherapy.

We purchase sodium [ $^{18}\text{F}$ ]fluoride for injection from Cardinal Health (specifications are attached email from John Vernon, Cardinal Health 12/19/12). The sodium [ $^{18}\text{F}$ ]fluoride solution contains 0.001-0.002 mg/mL of [ $^{18}\text{F}$ ]fluoride ion. At most we would inject 0.5 mL  $\pm$  0.001 mg. The baboons weigh on average 15 Kg so that the dose of fluoride would be 0.000067 mg/Kg. Sodium fluoride has been used for the treatment of osteoporosis in humans at daily doses of 30-90 mg/day for two or more years (Murray et al., 1996). Assuming a 60 Kg person the dose would be 0.5-1.5 mg/Kg. Side effects reported depend on the dose and the formulation and are limited to the GI tract and to the musculoskeletal system. Assuming a 1.0 mg/Kg dose, there is a 15,000 fold safety margin relative to the amount of [ $^{18}\text{F}$ ]fluoride which will be administered intrathecally in this study.

Murray TM, Ste-Marie L-G, Fluoride therapy for osteoporosis, Can Med Assoc Journal 155 (7): 949954, 1996.

<b>Date of Search:</b>	12-30-2012
<b>Databases Searched:</b>	Medline, pubmed, google
<b>Years included:</b>	1980-Present
<b>Provide a narrative of Search Results</b> <i>When alternative procedures are discovered, you must identify them and justify why those procedures are not being considered:</i>	



The sterile intrathecal procedure is performed while the animal is anesthetized and should therefore not be associated with any distress. Further, we have actively pursued means to refine the procedures to be minimally invasive, minimally risky and minimally distressing. Specifically we have consulted with staff veterinarians (and FOIA (b)(6) who is a board-certified veterinarian has experience performing intrathecal injection in non-human primates and FOIA (b)(6) who is a board-certified anesthesiologists has more than 20 years of experience with intra-thecl injections in humans) and pediatric surgeons and anesthesiologists about the uses and administration intrathecal agents in small subjects. We have also consulted with colleagues at other institutions that perform similar procedures, and have read the literature on these procedures. As a general resource, we have consulted "Loco-regional Anesthetic Blocks for Small animal Patients" (edited by Lois Campoy and Matt Read, 2012).

The ultimate alternative to all of these procedures would be to not do experiments at all. We have searched the literature carefully (search terms: **pain, intra-thecl injection, cerebrospinal fluid flow, distress, minimizing pain and distress, anesthesia, respiratory drive, hemodynamics intrathecal procedures, children, small animals**) to investigate alternatives to anesthesia and intrathecal injections which are the most distressing procedures in our experiments; but were unable to find literature which support alternate non-invasive procedures to achieve our goals.

**E.3 Indicate how procedures have been refined to reduce the amount of potential pain, distress or morbidity.**

We have performed several modifications and refinements to our experimental protocol to reduce the amount of potential pain, distress and morbidity. First we do not intubate until we have i.v. lines established and initiate the deep anesthesia with propofol and remifentanyl; this avoids coughing during the intubation regimen and significantly reduces the risk for erroneous intubation. Second we have implemented a standard-of-care hydration regimen that will prevent hypotension is association with anesthesia and also be potentially preventive for Post-dural puncture headache. Third, for the intrathecal injections we have taken utmost precaution to prevent damage to the spinal cord (accessing the intrathecal space below the medulla spinalis), prevent infection (all procedures and injections are performed under sterile conditions) and the toxicity risk is minimal from the miniscule amount of  $^{18}\text{F}$  injected into the intrathecal space.

**E.4 Describe if animals are subjected to food/water deprivation or prolonged and/or unusual restraint and provide justification. Describe how animal health is monitored during deprivation.**

The animals will be fasted and not fed their morning meal as a standard precaution against aspiration during induction of general anesthesia

**E.5 Is death used as a study endpoint wherein animals must die without intervention such as pain relief and/or euthanasia? If yes, explain why an earlier end point is not acceptable.**

N/A

**F. ANIMAL CARE**

**F.1 Please indicate if animals will be housed (kept for more than 24 hours) at BNL in other than in the Brookhaven Laboratory Animal Facility (BLAF).**

N/A

**F.2 Describe additional requirements for other than routine animal care (e.g. housing, feeding, hazardous waste bedding disposal)** *Investigative staff must be responsible for feeding all animals, weighing the correct amount of food, logging each feeding and adjusting the ration as needed to maintain the animal at the desired weight. If food, equipment and/or other supplies are to be shipped from another institution's animal facility, a recent health report from the facility must be submitted to the BLAF Manager at least six weeks before the planned experiment.*

Diapers will be used during the PET experiments and during transport and recovery to prevent spillage of radioactive urine on equipment and investigators during work in accordance with the RWP.

**F.3 Scientifically justify if singly-housed rodents will not be provided with environmental enrichment.**

N/A

**F.4 List the building and room number(s) in which experimental procedures, surgery, and/or postoperative recovery will be performed on live animals (if known).**

BLAF

PET Building

## G. PROCEDURE SPECIFICS

**G.1 List all chemical agents (sedatives, analgesics, anesthetics, paralytics, euthanasia, study drugs, radiotracers) administered to the animals.** *For euthanasia involving CO<sub>2</sub>, please use 100% CO<sub>2</sub> at a 20% air replacement per minute rate. For ketamine anesthesia, please use intraperitoneal (ip) injections, not intramuscular (im). Ketamine/xylazine may be stored for up to 28 days after mixture.*

Type	Agent	Dose	Route	Frequency	Controlled Substance (Y/N)
Anesthesia	Ketamine	10mg/kg	i.m	1x-2x	Yes
	Propofol	80-400 µg/kg/min	i.v.	Continuous infusion	No
	Remifentanyl	0.01-0.3 µg/kg/min	i.v.	Continuous infusion	No
	Isoflurane	1-2%	inhalational	1x	no
Antimuscarinic	Glycopyrulate	0.02 mg/kg	1.m	1x	no
Fluid	Hextend	20cc/kg	i.v	10cc boluses	no
	Lactated Ringer	4cc/kg/hr	i.v.	1x	no



Radiotracer	Sodium [ <sup>18</sup> F]fluoride	Tracer: ~ 0.000067 mg/Kg	IV	1X-2X	Not applicable
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**G.1.a List the name(s) of the individual(s) administering the above agents:**

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**G.1.b Indicate building and room numbers where agents are stored and security procedures for controlled substance(s):**

PET Building Room #?

**G.1.c If paralytic agents are used in conjunction with surgical manipulations, indicate the means by which absence of pain is monitored and/or determined, and who is responsible:**

N/A. Paralytic agents are not used.

**G.2 Is surgery involved?** *If yes, indicate whether surgery is survival or non-survival.*

No.

**G.2.a Describe monitoring and supportive care provided during surgery (who, what and how will this be done?):**

N/A

**G.2.b Describe indications for analgesic therapy to be administered before, during, and/or following surgery:**

N/A

**G.2.c Describe post-operative and/or anesthetic monitoring and supportive care (who, what and how often): Please use Surgery and Recovery Record**

N/A

**G.2.d Who will maintain surgical and post-operative records and where will they be maintained? Please note: Records must be accessible for inspection**

N/A.

**G.3 Is anesthesia involved?**

Yes.

**G.3.a Describe monitoring and supportive care provided during anesthesia (who, what, and how will this be done?): Please use Surgery and Recovery Record**



All baboons will be monitored continuously during anesthesia with routine non-invasive monitors including non-invasive blood pressure, end-tidal CO<sub>2</sub>, body temperature, EKG, heart rate and respiratory rate. Hemodynamic stability will be ensured by modifying anesthetic depths when required in combination with adequate hydration. Mechanical ventilation will be adjusted to ensure normal ventilation and oxygenation. Finally, body temperature will be maintained by means of a bear hugger (heating blanket using convective air flow). This monitoring will be performed by FOIA (b)(6) and/or FOIA (b)(6) FOIA (b)(6)

**G.3.b Who will maintain anesthetic records and where will they maintained? Please note: Records must be accessible for inspection**

FOIA (b)(6) and FOIA (b)(6) Privacy. The records will be maintained at the PET building as well as in FOIA (b)(6)'s office at Stony Brook University (FOIA (b)(6) Privacy).

**G.4 Are animals to be used in more than one major surgical procedure from which they are allowed to recover? If yes, please describe and justify.**

N/A

**G.5 For euthanasia performed at BNL (including early euthanasia), what method and by whom will animals be euthanized and how will death be confirmed? If a chemical agent is used, please list in Section G.1. For euthanasia involving CO<sub>2</sub>, please use 100% CO<sub>2</sub> at a 20% air replacement per minute rate. Justification must be provided for any physical method, such as decapitation or cervical dislocation, without anesthesia.**

N/A.

**G.6 List criteria for intervention and/or removal of animals from study or early euthanasia.**

• Examples are severe ataxia; rapidly increased heartrate or respiratory rates; oral, nasal or vaginal discharge such as pus or blood; wound dehiscence; marked swelling, tumor(s) greater than 2 cm or ulcerating, ulcer greater than 10% of body surface area, inability to eat or drink, loss of weight, great discoloration in an appendage or surgical area; immobility.

• Unless otherwise noted 100% CO<sub>2</sub> at a 20% air replacement per minute rate will be used for early euthanasia for rodents.

This is a non-invasive imaging study and there is no euthanasia involved. We 'intervene' all the time to prevent hemodynamic incidents (we control pressure and hydrate the baboons continuously), hypoxia (we control ventilation and monitor the oxygen saturation continuously). Further if the animals appear sick (lack of activity, no appetite, no urination or bowel movements) we will not start experimentation and the animals will be care for by the veterinarian at BNL. If on conclusion of the imaging experiments the animals do not recover well from anesthesia/imaging we will immediately contact the veterinarian and intervene appropriately. Based on our previous experience we do not expect morbidity or mortality inassociation with our experiments.

**H. SPECIAL CONSIDERATIONS**

**H.1 Check materials that are hazardous to personnel being used in this study.**

<input type="checkbox"/> Human cells or fluid	<input type="checkbox"/> Microorganism	<input type="checkbox"/> Chemicals including fixatives	<input type="checkbox"/> Recombinant DNA
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<input type="checkbox"/> Nanoparticles	<input checked="" type="checkbox"/> Radioactivity (isotopes)	<input type="checkbox"/> Other (list)	<input type="checkbox"/> Irradiation
For each agent listed above, please ensure that it is covered under an approved ESR			

<b>H.2 Indicate if animals will be shipped from BNL.</b> <i>If yes, indicate that BNL's preferred shipping procedures will be followed. If other arrangements are necessary, please describe.</i>
N/A

<b>H.3 If not shipped from an approved vendor, detail how animals will be transported to BNL.</b>
N/A



## I. INVESTIGATOR ASSURANCE

I affirm to the best of my knowledge that all the above information is complete and accurate and agree to accept responsibility for this project in accordance with applicable Federal and State of New York regulations, USDA guidelines, and established BLAF policies and procedures. No changes will be made without prior approval from the IACUC.

In order to reduce risk to all personnel and laboratory animals, I agree to:

- Follow BNL procedures for aspects of the animal care and use such as preoperative care, anesthesia, surgical technique, postoperative care, sampling techniques, euthanasia, and disposal of contaminated carcasses and waste.
- Ensure that my instructions to laboratory personnel are implemented.
- Ensure that all project personnel comply with the required Occupational Health Program before handling animals.
- Instruct all personnel in my laboratory that they should inform me if they believe that the treatment of any research animal is inappropriate. If the situation is not resolved, the employee should contact the Attending Veterinarian, or the IACUC Chair and/or Institutional Official.

I am aware that all research outlined under this protocol must be carried out under approved Experimental Safety Review(s) (ESR). I am aware that it is my responsibility to ensure that all individuals working on this protocol have been listed on an appropriate ESR and that their training is up to date. I am aware that work cannot proceed without an approved ESR.

**PRINCIPAL INVESTIGATOR**

FOIA (b)(6) Privacy

**DATE**

December 30,  
2012

*Your Department Safety Coordinator will be notified of your IACUC approval.*

## J. APPROVALS

I attest that the following issues have been appropriately addressed: Scientific merit of project; Appropriateness of conducting the project at BNL; Adequacy of funding for the project; Appropriateness of the expertise and experience of the PI and project personnel; Appropriateness of training for the PI and project personnel, and; Adequacy of department resources to support this protocol.

**BNL DEPARTMENT CHAIR**

**DATE**

**PHARMACIST (or designee)**

**DATE**

*Required for Schedule I controlled substances*

### References

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for the U.S. Department of Energy

## Memo

DATE: April 16, 2013

TO: FOIA (b)(6)

FROM: FOIA (b)(6), Institutional Animal Care and Use Committee (IACUC)

SUBJECT: IACUC Protocol 459 "Glymphatic Pathways of the Baboon Brain Visualized by  $^{18}\text{F}$  PET"

FOIA  
(b)(6)  
Privacy

Digitally signed by FOIA  
(b)(6)  
Date: 2013.04.16 15:25:55  
-04'00'

As of 04/16/13, the initial application of the above protocol was approved.

This protocol is approved until 02/06/14. This approval is given only for the protocol submitted; any changes must be approved by the IACUC prior to being implemented.

You should be aware that all research outlined in this protocol must be carried out under approved Experimental Safety Review(s) (ESR) and that this application must contain the same information as that listed in the approved ESR(s). You must be aware that it is your responsibility to ensure that all individuals working on this protocol are listed on an appropriate ESR and that their training is up to date.

Please note: other approvals, such as Facility approval, may be necessary.

FOIA