

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 used⁸⁻¹¹. 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^{6,12,13}. 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 so-called Sprotte needle which has its opening on the lateral aspect of the needle are better than those with a cutting configuration (Quincke)¹⁴. 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-dependent⁹. 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^{15,16}). 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 ¹⁸F 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. Forth, 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}F introduced into the intrathecal space:

There is no data on the toxicity of intrathecally administered sodium [^{18}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}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}F]fluoride for injection from Cardinal Health (specifications are attached email from John Vernon, Cardinal Health 12/19/12). The sodium [^{18}F]fluoride solution contains 0.001-0.002 mg/mL of [^{18}F]fluoride ion. At most we would inject 0.5 mL or 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}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.

Date of Search:	12-30-2012
Databases Searched:	Medline, pubmed, google
Years included:	1980-Present

Provide a narrative of Search Results When alternative procedures are discovered, you must identify them and justify why those procedures are not being considered:

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 intrathecal 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-thecal 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 Postdural 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 18F 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 overnight 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 CO₂, please use 100% CO₂ 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 [¹⁸ 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:

FOIA (b)(6) Privacy

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

PET Building

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 surgery is involved in these experiments. The only invasive procedures for these experiments are: 1) insertion of two i.v.s., 2) anesthesia and intubation and 3) intrathecal injection of 18F.

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. No surgery is involved, however as mentioned in section E2 in case a post-dural puncture headache (PDPH) is suspected after recovery from anesthesia (the animals will be monitored closely over 24-48 hrs) it will be treated conservatively with non-steroidal analgesics and hydration as per recommendation of FOIA (b)(6). However, given the precautions we take and the literature and experts stating that the risk of PDPH in young children (and same size non-human primates) are low or non-existent we do not expect this to occur.

G.3 Is anesthesia involved?

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 noninvasive monitors including non-invasive blood pressure, end-tidal CO₂, 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).

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 in 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 CO₂, please use 100% CO₂ 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. Animals will be brought back to home environment at BLAF to live out their lives. Technicians observe the baboons daily.

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% CO₂ 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. Ifon 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 in associaton 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			

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

H.3 If not shipped from an approved vendor, detail how animals will be transported to BNL.
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.

FOIA (b)(6) Privacy	FOIA (b)(6) Privacy	DATE	1/2/13
PHARMACIST (or designee)		DATE	
Required for Schedule I controlled substances			

References

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BROOKHAVEN
NATIONAL LABORATORY

managed by Brookhaven Science Associates
for the U.S. Department of Energy

Memo

date: April 12, 2013

to: FOIA (b)(6)
P i FOIA (b)(6)
from: FOIA (b)(6) Privacy

subject: Modified Institutional Risk Committee Review of IACUC # 459

The Modified Institutional Risk Committee (M-IRC) reviewed IACUC Protocol #459 "Glymphatic Pathways of the Baboon Brain Visualized by ¹⁸F PET" on March 15, 2013.

Based on the M-IRC recommendation, this experiment is approved, contingent on the following:

1. Each animal's file will include documentation of their health prior to the experiment, and the planned six week recuperation period.
2. The ALD for Environmental, Biological and Computational Sciences will resolve funding issues.

As of April 10, 2013, funding for this experiment is in place, and can go forward.

Cc:

FOIA (b) IACUC Chair
FOIA (b)(6) IACUC Administrator
FOIA (b)(6) Privacy

FOIA (b)(6) Privacy

From: FOIA (b)(6)
Sent: Wednesday, March 20, 2013 12:02 PM
To: FOIA (b)(6) Privacy; FOIA (b)(6)
Cc: FOIA (b)(6) Privacy
Subject: RE: baboon studies

Dear FOIA (b)(6):

I got a message from FOIA saying that he wasn't going to approve the study until we had identified a source of funding for the studies. I am preparing a package to send to him for approval that would include keeping FOIA (b)(6) and FOIA (b)(6) long enough to do these studies (which makes them relatively inexpensive) although not just for these studies. I am not sure of the answer, but I think I have good justification for my requests. I just don't know if the Lab has the money.

As soon as I hear anything, I will pass the word along.

FOIA (b)(6)
FOIA (b)(6) Privacy
Brookhaven National Laboratory
(631) 344-FOI

From: FOIA (b)(6) [mailto:FOIA (b)(6)@stonybrookmedicine.edu]
Sent: Wednesday, March 20, 2013 11:05 AM
To: FOIA (b)(6)
Cc: FOIA (b)(6) Privacy
Subject: baboon studies

Hi FOIA (b)(6) – I just wanted to find out the time frame for doing the baboon studies (if ever...) now that we are approved by MIRC; just let me know if I need to do anything.

Best, FOIA (b)(6)

FOIA (b)(6) Privacy
Stony Brook Medicine
Stony Brook, NY 11768
631-FOIA (b)(6)

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FOIA (b)(6) Privacy

From: FOIA (b)(6) e [FOIA (b)(6)]@stonybrookmedicine.edu
Sent: Thursday, March 07, 2013 5:15 PM
To: FOIA (b)(6)
Cc: FOIA (b)(6) Privacy
Subject: FW: Modified Institutional Risk Committee (MIRC)

FYI FOIA. Too bad. FOIA

FOIA (b)(6) Privacy

Stony Brook Medicine
Stony Brook, NY 11768
631-FOIA (b)

From: FOIA (b)(6) [mailto:FOIA (b)@bnl.gov]
Sent: Thursday, March 07, 2013 5:14 PM
To: FOIA (b)(6) Privacy
Cc: FOIA (b)(6); FOIA (b)(6)
Subject: RE: Modified Institutional Risk Committee (MIRC)

Dear all:

I have been notified by FOIA (b) that it is not feasible to do the baboon studies. I am therefore alerting you all so as not to waste time arranging the meeting.

Thanks for considering these important studies. too bad that it is not possible.

Sincerely,

FOIA (b)(6)

From: FOIA (b)(6)
Sent: Tuesday, March 05, 2013 1:20 PM
To: FOIA (b)(6) Privacy
Subject: Modified Institutional Risk Committee (MIRC)
When: Friday, March 15, 2013 9:30 AM-10:30 AM.
Where: Buildign 460 - Conference Room

When: Friday, March 15, 2013 9:30 AM-10:30 AM (UTC-05:00) Eastern Time (US & Canada).
Where: Buildign 460 - Conference Room

Note: The GMT offset above does not reflect daylight saving time adjustments.

~~*~*~*~*~*~*~*~*

All,

The MIRC will meet to review and make a recommendation regarding the attached proposal – IACUC Protocol #459. The researcher, FOIA (b)(6) Privacy, FOIA (b)(6) Privacy at Stony Brook University will make a presentation, and FOIA (b)(6), FOIA, FOIA (b)(6) Privacy, Princeton University will be participating via telcon.

Thank you,

FOIA

FOIA (b)(6)
Privacy

Brookhaven National Laboratory

Upton, NY 11973

631-344-FOIA

FOIA @bnl.gov

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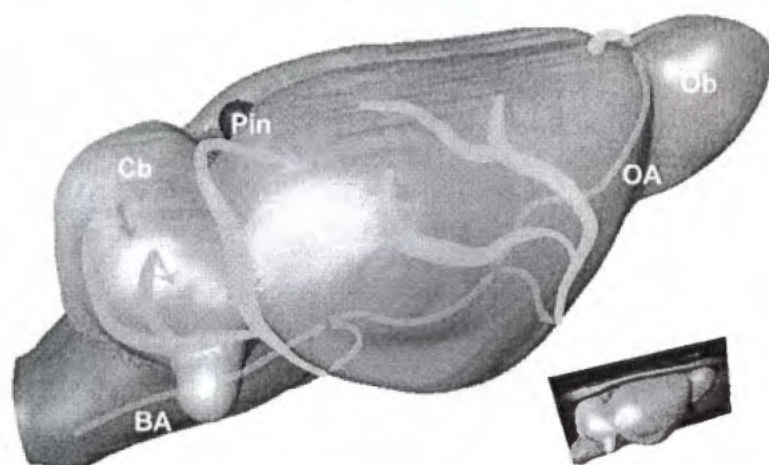
Title: Glymphatic Pathways of the Baboon Brain visualized by ^{18}F PET

PI: FOIA (b)(6) Privacy, Stony Brook University, FOIA (b)(6) Privacy

Funding Source: BNL

IACUC Protocol No.: 459; **IACUC Review Date:** February 7, 2013

The glymphatic system is a recently defined brain-wide pathway that facilitates efficient clearance of waste products including soluble amyloid from the brain (amyloid precipitated in 'plaques' is one of the key hallmarks of Alzheimer's Disease). The glymphatic pathway comprises an efficient exchange system between the cerebrospinal fluid (CSF) and interstitial fluid (ISF) surrounding the brain cells. These dynamic cleaning conduits enter the brain along para-arterial channels to exchange with ISF, which is in turn cleared from the brain along para-venous pathways. Because soluble amyloid β clearance depends on glymphatic pathway function, we proposed that failure of this clearance system contributes to amyloid plaque deposition and Alzheimer's disease progression. Recently we provided proof-of-concept that glymphatic pathway function can be measured using a clinically relevant imaging technique in the rodent brain (Iliff et al., Journal of Clinical Investigation, under second revision, 2012). Dynamic contrast-enhanced MRI was used to visualize CSF-ISF exchange across the rat brain following intrathecal (i.e. fluid compartment surrounding the spine and brain) contrast agent administration. Key features of glymphatic pathway function were confirmed, including visualization of para-arterial CSF influx and molecular size-dependent CSF-ISF exchange. Whole-brain imaging allowed the identification of two key influx nodes, at



the pituitary and pineal gland recesses. Here we propose to translate the recent findings in the rat brain to the non-human primate brain by developing methodology to visualize the brain-wide glymphatic pathways using PET imaging in combination with intrathecal administration of a molecular tracer such as ^{18}F because of its low molecular weight and size (the glymphatic system allows passage of molecules smaller than $\sim 20\text{nm}$). This new PET approach may provide the basis for a wholly new strategy to evaluating Alzheimer's disease susceptibility and progression in the live human brain.

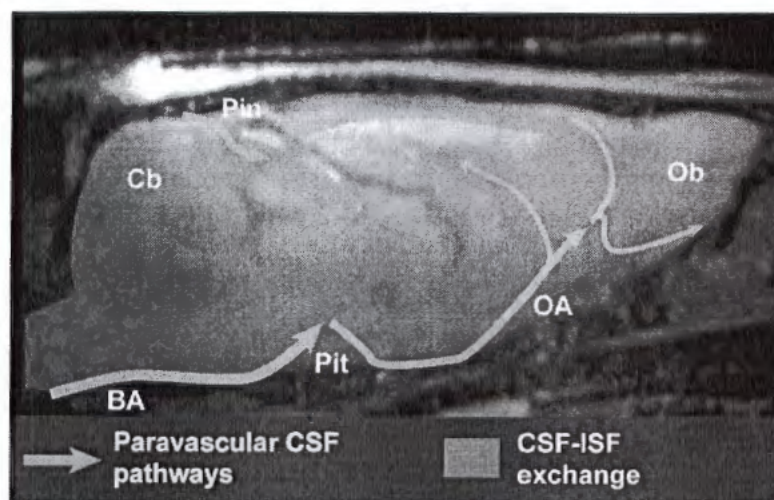


Figure 1. Brain-wide glymphatic pathways of CSF-ISF exchange assessed via contrast-enhanced MRI in the rat. Brain-wide glymphatic pathways of CSF-ISF exchange assessed by contrast-enhanced MRI in the rat. After injection into the subarachnoid space of the cisterna magna, contrast agent follows specific paravascular pathways (yellow arrows) to enter the brain parenchyma and exchange with the interstitial compartments (orange

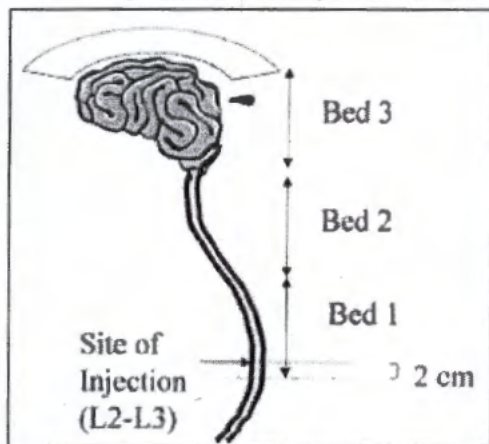
arrows and fields). Acquisition of dynamic image series identified key CSF influx nodes at the pineal (Pin) and pituitary (Pit) recesses and allowed simple kinetic parameters to be derived that deflect the extent and rate of glymphatic CSF-ISF exchange throughout the whole brain. Cb: cerebellum; Ob: olfactory bulb; BA: basilar artery; OA: olfactory artery.

Induction of Anesthesia. PET imaging requires that the baboons are anesthetized. Animals selected for scanning are not fed the morning of the scan to prevent vomiting in this species. The baboon will be injected with a mixture of 10mg/kg Ketamine and glycopurulate 0.02mg/kg, intramuscularly. The animal is removed from the cage and routine monitors will be placed which include non-invasive blood pressure monitoring, pulse oximetry, body temperature and electrocardiogram. Two 22-gauge intravenous catheters will be placed in a lower extremity vein for hydration maintenance and administration of drugs (see below). Intravenous hydration is provided using Hextend, (High Molecular Weight Hydroxyethyl Starch 6% (Hetastarch) in Buffered Electrolyte Dextrose Solution, 5-10cc/kg) administered in 10 cc boluses to a max of 20cc/kg. With careful attention to physiological parameters, a continuous infusion of propofol (Diprivan®, AstraZeneca, 120 µg/kg/min) and remifentanyl hydrochloride (ULTIVA, 0.1 µg/kg/min) is started using a micro infusion pump (Baxter, Model AS50) within 1-min intubation criteria are met and the animal is intubated via laryngoscope with a disposable 5.0-6.0 pediatric cuffed endotracheal tube (ETT) which is held in place with tape. Endotracheal intubation will be confirmed by auscultation and brief inflation of the lungs with an ambu bag and the presence of endtidal CO₂. Supplemental O₂ will be administered to the manually ventilated animal via an Ambu bag attached to the ETT.

Transport from BLAF to PET building. The animal is transported from BLAF to the PET building and during transport the animal will be manually ventilated via the Ambu bag connected to oxygen at a flow of >4l/min. Vital signs will be measured continuously during this time-period via a transport monitor. A qualified research nurse will be with the animal at all times and the body temperature will be maintained normal with thermo-shields. All transport of the baboons follows guidelines put forward in our current SOPs

Procedures to take place at the PET Facility:

Anesthesia (described above) and mechanical ventilation and physiological monitoring will continue during the PET study. **Intrathecal injection of ¹⁸F:** In preparation for the PET study an intrathecal injection of ¹⁸F at the



level of L2/L3 will be performed immediately prior to the PET scanning. Specifically, A 22-ga needle will be introduced at the level of L2/L3 under sterile condition and 0.5-1.0cc of CSF will be slowly aspirated into the syringe containing about 4.0-6.0mCi of ¹⁸F (will be passed through an anion exchange resin to remove other metal radionuclides from the solution and then through a Millipore filter to sterilize the resulting solution) dissolved in a sterile phosphate-buffered solution (initial volume ~0.5cc). The radioactive material + aspirated CSF will subsequently be injected into the subarachnoid space over 30 seconds. The spinal needle will be withdrawn and the baboon will be repositioned supine and dynamic PET images will be collected in frames over the entire CNS? field of view (Figure).

Figure: Schematic of imaging fields of view in relation to the baboon

spinal column (taken from McCarthy et al., 2002).

Dynamic PET imaging will continue to cover the transport of ¹⁸F from the site of injection to the brain, and continue to follow clearance from the brain over a period of 2-3 hrs. Specifically, based on other studies in the literature we expect that the spread towards the brain will be faster since the solution injected will have a density that is slightly lighter than plain CSF.

Recovery:

Following conclusion of the last scan the animal will be transported back to BLAF under the RWP and ESR guidelines. The anesthesia infusion will be discontinued, the i.v. disconnected and the stomach emptied with an orogastric tube prior to extubation. The animal will be monitored for at least 30-min to 1-hr after extubation to assure normal recovery.

Four baboons will be used for this study. This study uses standard procedures previously approved in other studies using baboons.

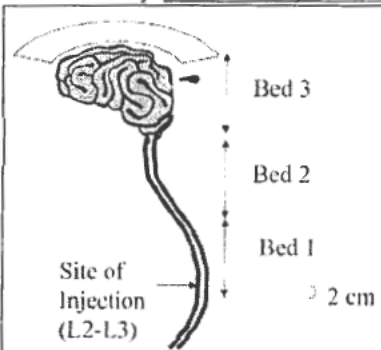
Procedures to take place at BLAF, Bldg. 490:

Induction of Anesthesia. PET imaging requires that the baboons are anesthetized. Animals selected for scanning are not fed the morning of the scan (6 - 8 hours) fast to prevent vomiting in this species. The baboon will be injected with a mixture of 10mg/kg Ketamine and glycopyrulate 0.02mg/kg, intramuscularly. The animal is removed from the cage and routine monitors will be placed which include non-invasive blood pressure monitoring, pulse oximetry, body temperature and electrocardiogram. Two 22-gauge intravenous catheters will be placed in a lower extremity vein for hydration maintenance and administration of drugs (see below). Intravenous hydration is provided using Hextend, (High Molecular Weight Hydroxyethyl Starch 6% (Hetastarch) in Buffered Electrolyte Dextrose Solution, 5-10cc/kg) administered in 10 cc boluses to a max of 20cc/kg. With careful attention to physiological parameters, a continuous infusion of propofol (Diprivan®, AstraZeneca, 120 µg/kg/min) and remifentanyl hydrochloride (ULTIVA, 0.1 µg/kg/min) is started using a micro infusion pump (Baxter, Model AS50) within 1-min intubation criteria are met and the animal is intubated via laryngoscope with a disposable 5.0-6.0 pediatric cuffed endotracheal tube (ETT) which is held in place with tape. Endotracheal intubation will be confirmed by auscultation and brief inflation of the lungs with an ambu bag and the presence of endtidal CO₂. Supplemental O₂ will be administered to the manually ventilated animal via an Ambu bag attached to the ETT. Supplemental O₂ will be administered to the manually ventilated animal via an Ambu bag attached to the ETT.

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Comment [TMG1]: Is this the same as subarachnoid space? In principle yes, but we do not really use this term clinically because the subarachnoid space can be tightly interwoven with subdural and pial membranes.

Figure: Schematic of imaging fields of view in relation to the baboon spinal column (taken from McCarthy et al., 2002²).

Dynamic PET imaging will continue to cover the transport of ¹⁸F from the site of injection to the brain, and continue to follow clearance from the brain over a period of 2-3 hrs. Specifically, based on other studies in the literature we expect that the spread towards the brain will be faster since the solution injected will have a density that is slightly lighter than plain CSF – referred to as 'hypobaric'.

FOIA (b)(6) Privacy

From: FOIA (b)(6)
Sent: Wednesday, January 02, 2013 11:13 AM
To: FOIA (b)(6) Privacy; FOIA (b)(6); FOIA (b)(6)
Subject: Re: IACUC protocol

yes lets do so

From: "FOIA (b)(6) Privacy" FOIA (b)(6) Privacy <@stonybrookmedicine.edu>
Date: Wed, 2 Jan 2013 11:10:03 -0500
To: FOIA (b)(6) Privacy <@bnl.gov>, FOIA (b)(6) Privacy <@bnl.gov>
Cc: "FOIA (b)(6) Privacy" <@bnl.gov>
Subject: RE: IACUC protocol

That would be great...

FOIA (b)(6) Privacy

Stony Brook Medicine
Stony Brook, NY 11768
631-FOIA (b)

From: FOIA (b)(6) [mailto:FOIA @bnl.gov]
Sent: Wednesday, January 02, 2013 11:09 AM
To: FOIA (b)(6)
Cc: FOIA (b)(6); FOIA @bnl.gov
Subject: Re: IACUC protocol

Can we use the NIAAA account? The cost of the radiotracer is ~\$350.00 and FOIA will do the baboon anesthesia etc.

FOIA (b)(6) Privacy

F

note below. As part of the review process, I need to ensure a funding source for any new protocols. Please advise when you get a chance. Thanks, F

From: FOIA (b)(6) FOIA (b)(6) Privacy
2013 10:34 AM
To: FOIA (b)(6)
Cc: FOIA (b)(6); nFOIA @nida.nih.gov
Subject: RE: IACUC protocol

FOIA or FOI will be able to provide this information;

Minutes of the BNL Institutional Animal Care and Use Committee
February 7, 2013

Present: FOIA , FOIA (b) , FOIA (b) , FOIA (b) , FOIA (b) (non-voting), FOIA (b) (non-voting), F
(6) (6) (6) (6) (6) (6) OI
Secretary: FOIA (b)(6) (non-voting) A
Guest: FOIA (b)(6)
Excused: FOIA (b)(6) , FOIA (b) , FOIA (b)
P i (6) (6) P i

The meeting was held in the 490D Conference Room in Building 490 and was called to order at 1:05 pm by FOIA (b) , Chair, with seven voting members present.
(6)

Old Business

The minutes of the January 3, 2013 IACUC meeting were approved as submitted.

Actions Taken Since the Previous Meeting

IACUC Protocol 431 "FOIA (b)(6) Privacy Non-Responsive

Non-Responsive

IACUC Protocol 352 "XXXXXXXXXXXXX Non-Responsive

Non-Responsive

IACUC Protocol 466 "XXXXXXXXXXXXX Non-Responsive

Non-Responsive

IACUC Protocol 406 "FOIA (b)(6) Privacy XXXXXXXXXXXXXXXX Non-Responsive

Non-Responsive

IACUC Protocol 455 "XXXXXXXXXXXXX Non-Responsive

Non-Responsive

IACUC Protocol 345 "XXXXXXXXXXXXX Non-Responsive

New Business

IACUC Protocol 459 "Glymphatic Pathways of the Baboon Brain Visualized by ¹⁸F PET", PI: FOIA (b)(6) . Initial application discussed. A member questioned how the investigators choose which baboon to use. It was stated that they rotate the animals so no one of them is used too often. The initial application was approved as submitted for one year by a vote of 6 yes, 0 no, 1 abstention (FOIA (b)(6) due to protocol involvement). Minor typos/grammar corrections will be made administratively. There was a general discussion of baboon retirement. The protocol will be forwarded to the MIRC for review.

IACUC Protocol 460 "XXXXXXXXXXXXX Non-Responsive

Non-Responsive

[REDACTED]

Non-
Responsive

[REDACTED]

Non-responsive

[REDACTED]

Non-responsive

[REDACTED]

Non-responsive

[REDACTED]

[REDACTED]

Non-responsive

[REDACTED] Non-responsive

Non-responsive

Non-responsive

Non-responsive

Non-responsive

There being no further business, the meeting adjourned at 2:45 pm and members went to inspect the BLAF and NSRL.

[REDACTED]
FOIA (b)(6) Privacy

cc: FOIA (b)(6)
Privacy

FOIA (b)(6)
P I [REDACTED]

(6) (6) (6) P I (6) P I (6)

(6) (6) FOIA (b) FOIA (b)
(6) (6) (6) P I (6)

The meeting was held in the 490D Conference Room in Building 490 and was called to order at 1:35 pm by FOIA (b)(6) Privacy, with seven voting members present.

Old Business

The minutes of the December 13, 2012 IACUC meeting were approved as submitted.

Actions Taken Since the Previous Meeting

Non-responsive

Non-responsive

Non-responsive

Non-responsive

New Business

Non-responsive

Non-responsive

Non-responsive

IACUC Meeting 01/03/13

[REDACTED]

Non-responsive

[REDACTED]

Non-responsive

[REDACTED]

[REDACTED]

Non-responsive

IACUC Protocol 459 "Glymphatic Pathways of the Baboon Brain Visualized by 18F PET", PI: H. Benveniste. Initial application tabled until next month to give IACUC members sufficient time to review.

There being no further business, the meeting adjourned at 3:30 pm.

Re [REDACTED]
FOIA (b)(6) Privacy

cc: FOIA (b)
FOIA
(b)(6)

[REDACTED]