

FOIA (b)(6) Privacy

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

Memo

* *

DATE: February 7, 2013

TO: FOIA (b)(6)

FOIA
(b)(6)
Privacy

Digitally signed by FOIA
(b)(6)
Date: 2013.02.07 16:52:32
-05'00'

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

SUBJECT: IACUC Protocol 459 "Glymphatic Pathways of the Baboon Brain Visualized by
¹⁸F PET"

As of 02/07/13, the initial application of the above protocol has been reviewed by the IACUC.

However, since the protocol involves a USDA covered species it has been forwarded to a Laboratory Director's advisory committee. Should that committee need additional information concerning your experiment and use of animals, you will be contacted. The Director will consider the recommendation of the committee and his approval is required before you may commence work.

FOIA

From: FOIA (b)(6) Privacy [FOIA (b)(6)]@stonybrookmedicine.edu]
Sent: Friday, February 08, 2013 9:41 AM
To: FOIA (b)(6)
Subject: Re: IACUC Protocol 459

Looks great. Thanks a lot. F

From: FOIA (b)(6) [mailto:FOIA (b)(6)]
Sent: Friday, February 08, 2013 09:01 AM
To: FOIA (b)(6)
Subject: IACUC Protocol 459

Attached is your protocol with grammar/spelling corrections. Please let me know if they are acceptable and I will finalize the protocol.

FOIA (b)(6)
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Brookhaven National Laboratory

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BROOKHAVEN NATIONAL LABORATORY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) ANIMAL USE PROTOCOL	BROOKHAVEN NATIONAL LABORATORY
<i>The protocol must be submitted in typed form and all applicable items must be answered. Answers must be written in English and in terms understandable to all IACUC members.</i>	

PROTOCOL #:	
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Title:	Glymphatic Pathways of the Baboon Brain visualized by ¹⁸ F PET		
Principal Investigator*:	FOIA (b)(6) Privacy, MD, PhD		
Institution:	Stony Brook University		
Address:	FOIA (b)(6) Privacy		
Phone:	631-FOIA (b) or 631-FOIA (b)		
Fax:	631-FOIA (b) (6)		
E-mail:	FOIA (b)(6) Privacy @stonybrookmedicine.edu		
Key Investigators*:	FOIA (b)(6) Privacy		
<small>* Note - if no investigators are BNL employees, please list a BNL employee contact:</small>			
Funding Source:		BNL Account Number:	
Submit animal methods section of grant	Internal		
Protocol Type (e.g. Research, Teaching, Other):	Research		
Home Institution IACUC Approval # and dates	N/A		

A. OVERVIEW
A.1 Please provide a brief description of the proposed studies in lay terms.

A.1 Please provide a brief description of the proposed studies in lay terms.

The glymphatic system is a recently defined brain-wide pathway for facilitates efficient clearance of waste products including soluble amyloid β from the brain¹ (amyloid β 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 cells¹. 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 small molecular tracer such as ^{18}F . 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

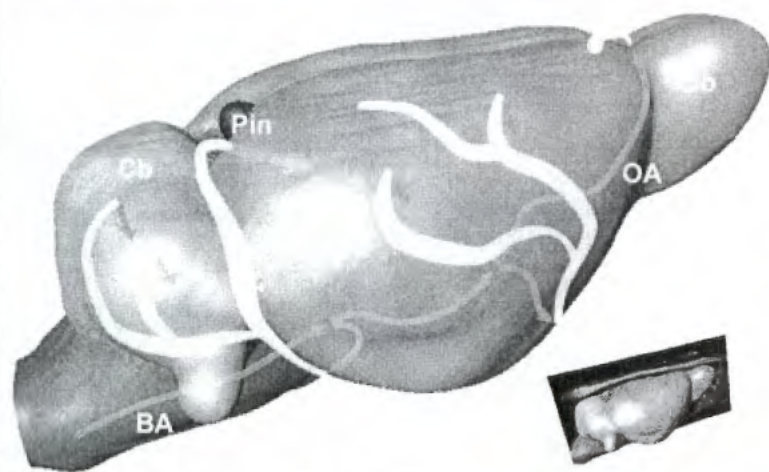
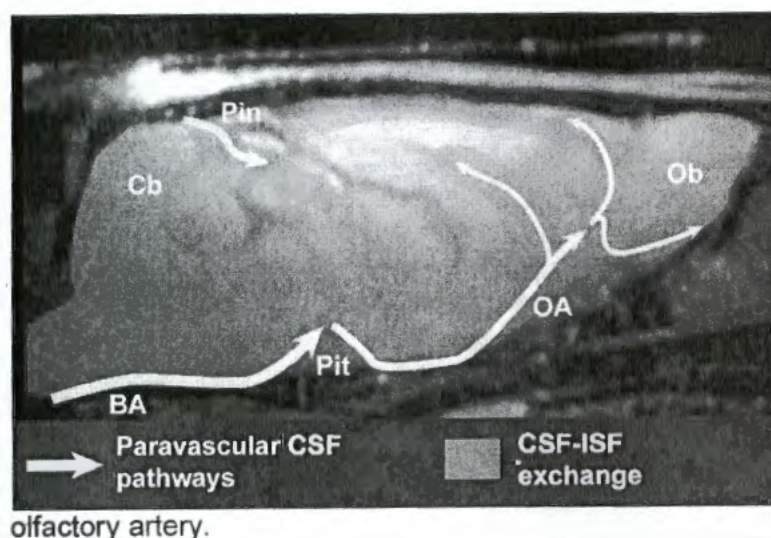


Figure 1. Brain-wide glymphatic pathways of CSF-ISF exchange assessed via contrast-enhanced MRI in the rat.



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 reflect the extent and rate of glymphatic CSF-ISF exchange throughout the whole brain. Cb: cerebellum; Ob: olfactory bulb; BA: basilar artery; OA:

B. PERSONNEL AND TRAINING

B.1 In each box, list all personnel working directly with animals and indicate number of years of experience for each procedure for each species. All BNL personnel will be put on the appropriate Occupational Medicine Protocol. Non-BNL employees working with primates will be put on the appropriate Occupational Medicine Protocol.

NAME	SPECIES	MONITORING & HANDLING	NONSURGICAL MANIPULATION	ANESTHESIA, SURGERY	BLOOD COLLECTION	EUTHANASIA
FOIA (b)(6) Privacy	Non-human Primate	10	10	10	10	N/A
FOIA (b)(6)	Non-human Primate	10	10	10	10	N/A
FOIA (b)(6)	Non-human Primate	10	10	10	10	N/A
FOIA (b)(6) P i	Non-human Primate	10	10	10	10	N/A
FOIA (b)(6) Privacy	Non-human Primate	10	10	10	10	N/A

Note: Any personnel with less than one year experience in any of the above categories must take the applicable training listed below.

B.2 Indicate which training courses apply to this protocol. Use A to indicate all personnel or put initials of those required to take the training. All courses are located at <http://www.bnl.gov/training>

Required	COURSE TITLE	PROCEDURES COVERED
A	Basic Overview of Laboratory Animal Care and Use	Overview required by all animal users
	Biomethodology of the Mouse	Restraint, handling, identification, sexing, husbandry, behavior of mice
	Biomethodology of the Rat	Restraint, handling, identification, sexing, husbandry, behavior of rats
	Experimental Techniques in Rodents	Injections, blood sampling, oral gavage, euthanasia
	Post-Procedure Care of Mice and Rats: Reducing Pain and Distress	Analgesia, pain & distress recognition and alleviation, post-operative care
	Survival Surgery in Rodents	Anesthesia, aseptic surgical techniques
A	Primate Safety	Covers safe handling of non-human primates
A	Controlled Substance Awareness and DEA background Check	Required if any controlled substances will be used
A	Regulated Medical Waste Management	Required if regulated medical waste (animal carcasses, needles, syringes) will be generated as a result of the work

C. PROCEDURES

C.1 Concisely describe all manipulations and experimental procedures, including surgeries, performed on the animals. *Everything done to the live animal at BNL must be detailed here. A short description of experimental procedures done elsewhere should be included. Include the end point of the experiment and timing of euthanasia, if applicable. Flow diagrams or charts are helpful. Materials and methods portion of grant applications or other detailed descriptions may be attached.*

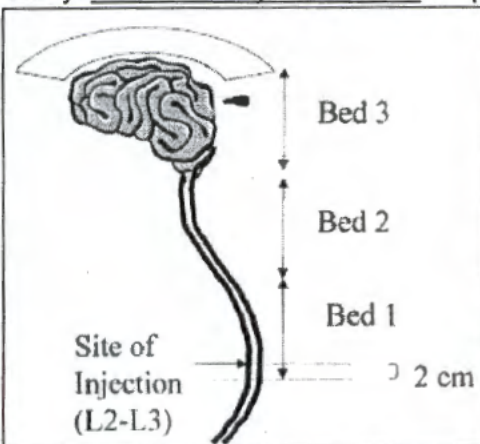
Procedures to take place at BLAF, Bldg. 490:

Induction of Anesthesia PET imaging requires that the monkeys are anesthetized. Animals selected for scanning are not fed the morning of the scan (6-8 hours) fast to prevent vomiting in this species of monkey. The baboon will be squeezed and injected with a mixture of 10mg/kg Ketamine and glycopyrrolate 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.

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 an oxygen at a flow of >4l/min. Vital signs will be measured continuously during this time period via a transport monitor. A skilled experimenter will be with the animal at all times and the animal will be kept normo-thermic with thermo-shields. All transport of the macaques follows guidelines put forward in our experimental safety review.

Procedures to take place at the PET Facility:

Monitors will be replaced to assure normal hemodynamics, oxygenation and temperature. 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 the other metal radionuclides from the solution and a Millipore filter the resulting solution to sterilize it) 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 CSN field of view (Figure).

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

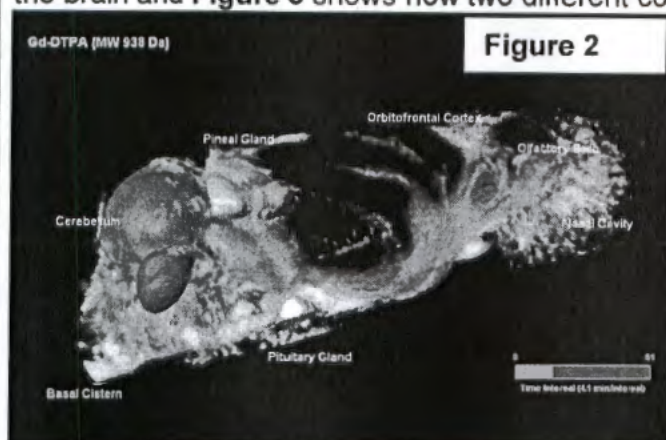
Dynamic PET imaging will continue to cover the transport of ¹⁸F from the site of injection to the brain, and continue to follow clearance from the brain over a period of 23 hrs. Specifically, based on other studies in the literature we expect that the spread towards the brain will be faster since the solution injected will be slightly hypobaric.

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 maternal stomach emptied with an orogastric tube prior to extubation. The animal will be monitored for a least 30 min to 1-hr after extubation to assure normal recovery.

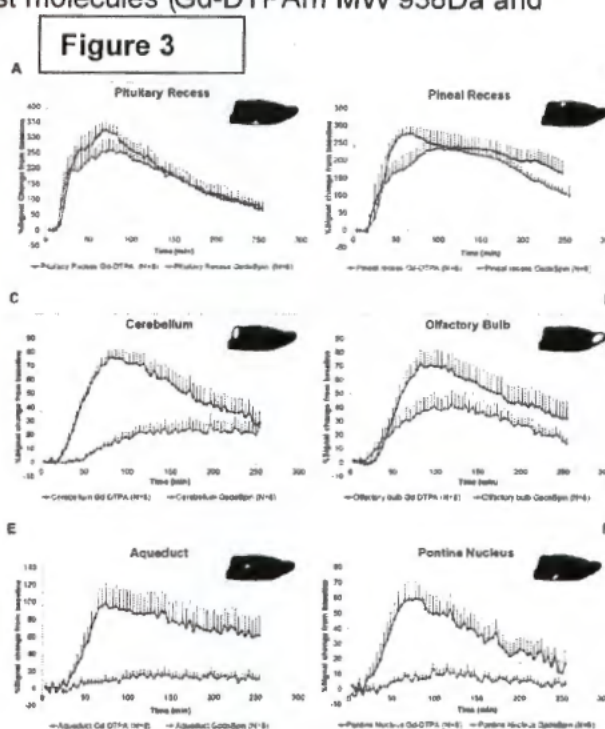
C.2 Does this work duplicate previous experiments/activities? If yes, justify.

No, the glymphatic brain-wide pathways have never been characterized in the nonhuman primate brain; and this step is crucial before human experiments can commence. The only study which have previously documented 'waste' clearance from the brain have been carried out in mice¹ and in rats ((Iliff & Benveniste et al. submitted, JCI 2012) using metabolically inert paramagnetic contrast agents (the paper documenting these experiments are currently under 2nd revision in Journal of Clinical Investigations). **Figure 2** (from Iliff & Benveniste et al. JCI 2013, under revision) shows how the paramagnetic contrast molecule (Gd-DTPA, MW=938 Da) when injected into the cisterna magna (intrathecal space close to the brain) distributes into the brain and **Figure 3** shows how two different contrast molecules (Gd-DTPAm MW 938Da and



GadoSpin, MW 200,000) enters and clears from different brain areas over time.

We expect ¹⁸F (MW 19Da) will be mimicking the smaller paramagnetic contrast molecule and will readily distribute throughout the brain of the Baboon after it travels along the spinal column to the base of the brain and enters via the para-arterial channels which comprise part of the Glymphatic pathways.



D. ANIMAL DESCRIPTION

D.1 Species:	Baboon (Papio)
D.2 Strain/Breed:	N/A
D.3 Sex:	Female
D.4 Age/Weight:	6-10 years / 30-80 lb
D.5 Supplier:	BLAF

If not a commercial vendor, a recent health report (no older than three months) from the animal facility must be submitted to the BLAF Manager at least six weeks before the planned experiment or shipment of animals. Please contact the BLAF Manager at 631 344-FOIA (b)(6) to make arrangements for the receipt of the animals.

D.6 Justify that the work is appropriate to be done in an animal model. Indicate why a computer or non-animal model is not a viable alternative.

The brain is unique among virtually all other body organs in its lack of a conventional lymphatic vasculature³⁻⁵. In the periphery, the lymphatic circulation facilitates the clearance of extracellular proteins and excess fluid from the interstitial space, a role critical to tissue homeostasis and function^{6,7}. Yet within the brain, despite its complex architecture, high metabolic activity, and sensitivity to changes in the extracellular environment, no specialized organ-wide anatomic structure has yet been identified that facilitates the efficient 'lymphatic' clearance of extracellular solutes and fluid. Recently, we identified a system in the rodent brain which we termed the 'glymphatic pathways' (because of its dependence on glia cells) which efficiently clears small and larger molecules from the interstitial space of the brain¹. We now need to document that this waste removal system which is responsible for clearing soluble amyloid and tau proteins from the brain is also present in the non-human primate. This step is crucial before we can commence experiments in humans. We are proposing that a technique to evaluate the efficiency of the 'glymphatic pathways' using an inert radioactive tracer such as ¹⁸F-fluoride ion (which does not cross the blood-brain-barrier but remains in the CSF) in combination with PET imaging will provide the basis for a wholly new prognostic strategy for evaluating Alzheimer's Disease susceptibility and disease progression in the future.

D.7 Justify species to be used and why a lower phylogenetic species cannot be used.

Non-human primates are essential as a next step before experiments in humans can be initiated.

D.8 Animal Numbers

D.8.a Total for first three years:	3-4
D.8.b Maximum housed at one time:	All baboons used for this protocol are permanently living at BLAF

D.9 Justify number of animals. Indicate design of study groups and statistical methods and include power calculations. Include steps taken to minimize the number of animals required.

This is a first step to characterize the glymphatic pathways in nonhuman primates; and we expect to use 1 baboon to work out the methodological aspects of the protocol (i.e. intrathecal injection, dynamic PET protocol) and another 2-3 baboons to document the pathways. In other words it is a descriptive study and we are planning to characterize how the radioactive tracer ¹⁸F enters, distributes and exits over time in the baboon brain when delivered intrathecally.

E. PAIN/DISTRESS

E.1 List total number of animals at applicable levels of stress/discomfort

Level A: No pain or distress: Animals will be euthanized without any treatments or manipulations or irradiation with unrestricted movement and without anesthesia and without anticipated subsequent effects at BNL.

Level B: Relieved or momentary pain or distress: Momentary pain or potential pain or distress relieved by pharmacologic, behavioral or other means, e.g., injection of any substance including anesthetics, post procedural analgesics, behavioral conditioning, restraint or minor pain/distress and medical treatment of disease states.

Level C: Unrelieved or sustained pain or distress: Any procedure that would cause more than momentary or slight pain or distress, e.g., chronic untreated disease states, pain research

Species	LEVEL A	LEVEL B	LEVEL C
Baboon		x	

Include scientific justification for any animals in Level C

E.2 For animals used in Level B or C, perform a literature search for alternatives to pain/distress.

Please note the Research Library Staff is available to assist with literature searches.

List procedures that may cause pain/distress (e.g. imaging, surgery, injection, behavioral testing, food restriction, etc) and perform a search using the procedures. Procedures that have pain eliminated by the use of anesthetics and/or analgesics are still considered painful even though the animal is not expected to experience any pain/distress.