

2004

APPLICATION FOR APPROVAL TO USE VERTEBRATE ANIMALS IN RESEARCH and TEACHING NEW SUBMISSION

Institutional Animal Care and Use Committee
University of Pittsburgh, Suite 200, Hieber Bldg.

Instructions for Filling out this form

1. All teaching and research protocols utilizing living vertebrate animals must be tendered as New Submissions for complete evaluation by the Institutional Animal Care and Use Committee (IACUC). Applications must also be submitted when utilizing dead vertebrate animals or animal parts if the animal was both sacrificed and procured solely and specifically for the research or teaching purpose. All approved protocols must be re-submitted as a new protocol for review every three years.
2. All grants to be funded by the National Institutes of Health (NIH) must be reviewed *prior to* the release of funding to assure that all procedures on animals are included in an approved IACUC protocol. For this purpose, grant PIs must complete the "GRANT APPLICATION REVIEW FORM" and submit the form to the IACUC Office along with the grant application. The "GRANT APPLICATION REVIEW FORM" asks the PI to identify the IACUC protocols that correspond to the grant application animal work. A review of the grant submission and IACUC protocols will be performed to verify that all animal work has been described and approved by the IACUC. The Office of Research must certify that this review has been conducted before NIH will release grant funding. Therefore, all PIs receiving notice of a fundable score from NIH should contact the IACUC Office as soon as possible to initiate a review.
3. Download this Word and Excel based form at www.iacuc.pitt.edu and complete the entire application. Submit the completed application via email to iacuc@pitt.edu. Hardcopy applications will not be accepted. You will receive electronic confirmation of receipt within two days. Your application will be assigned to an appropriate subcommittee for review, and you may be asked to address reviewers' concerns. Following approval from all subcommittee members, you will receive an assurance letter. Should you wish to address the entire IACUC, please contact Dr. Ed Kennah at ekennah@pitt.edu or 383-2014. A minimum turn around time for protocol review is approximately 30 days. There are no expedited reviews since all protocols are required by regulation to go through the same review process.

4. All applications supported by an internal (Departmental) or corporate funding source must be accompanied by certification from the appropriate Dean, Department Head, or Division Chief that the protocol has been reviewed for scientific merit.
5. Postdoctoral fellows, graduate students, and undergraduate students submitting applications as principal investigators must also include sponsorship by an appropriate academic advisor. Please include the sponsor's name and contact information on the certification page of this application.
6. Protocols will not be approved for Principal Investigators who have not completed the required training programs. Contact Marilee Rose, Training Coordinator, at 578-3459 or mrose+@pitt.edu to arrange species-specific training. In addition, all personnel listed as participants in the study must complete the required training. Completion of Modules 1 and 3 is required training for all personnel listed as participants in the study. This can be accessed on the web at <http://www.health.pitt.edu/rpf/>.
7. Work involving rDNA must be approved by the Institutional Biosafety Committee for rDNA (Call 578-3799 for information and complete ~~Form 4~~ the Institutional Biosafety (Recombinant DNA) Application found at www.rcco.pitt.edu/rdna/).
8. If you place any of your animals in Pain and Distress Classification E, an explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on Attachment 1.
9. Work involving radiation must be approved by the Radiation Safety Office (Call 624-2728 for information and complete Attachment 2 of this form).
10. A Registration Workbook for Use of Biological and Chemical Agents must be completed for each IACUC application and approved by the Department of Environmental Health and Safety (see Attachment 3). IACUC protocols will not be reviewed until this Workbook is completed.
11. Investigators wishing to introduce any cell/tumor lines or bodily fluids of human or animal origin into animals in the DLAR, must first receive written permission from the Director of the DLAR or his designee before the first animal is injected. Attachment 4 must be completed.
12. Investigators requesting a dispensation from the nonhuman primate enrichment plan must complete Attachment 5. For information regarding enrichment techniques or any part of the enrichment plan, please contact Sarah Greene at serst28@pitt.edu , (412) 383-7892 or (724) 327-4607.

13. According to University guidelines, any persons receiving commercial funding must complete Module 4 on the Research Practice Fundamentals website - www.health.pitt.edu/rpf/. Investigators also need to complete Attachment 6 – Declaration of Conflict of Interest.

14. All Principal Investigators with approved protocols involving physical, chemical, and/or biological hazards must schedule a pre-study strategy meeting with the Division of Laboratory Animal Resources (DLAR) (648-8950) prior to ordering animals or initiating the project.

15. In an effort to limit the frequency of changes to IACUC submission forms, a single form will be used for each calendar year. Verify that you are using the current application form by checking the date at the top of this page. If changes are required during the year, the changes can be found under the "Additional Addendum" heading of the IACUC web site www.iacuc.pitt.edu/ Before submitting a protocol, check to ensure you are using the current form and that additional addendums (if required) are completed and attached.

16. Please type your response to the questions in the white space to the right of the question. (Do not type your response in the gray shaded areas.)

Consultation with a veterinarian from the DLAR prior to application submission is recommended. IACUC approval does not assure DLAR space availability.

A. Project Administration - Personnel:

1.	PI Name (First Name Last Name):	David Bush
2.	Co-PI 1 (First Name Last Name):	
3.	Co-PI 2 (First Name Last Name):	
4.	Co-PI 3 (First Name Last Name):	
5.	PI Faculty Title:	Professor
6.	PI Department:	Esoteric Medicine
7.	PI's Department Chair: (List Dean or Div. Chief if no Chair)	Dr. Barbara Bush
8.	PI Mailing Address:	100 BST-75
9.	PI Office Phone:	412-647-0001
10.	PI Lab Phone:	412-647-0002
11.	PI Fax:	412-647-0003
12.	PI E-mail Address:	davidbush@upmc.edu
13.	Emergency Contact (PI - 24 hr phone/pager):	412-123-4567 (home)
14.	Secondary Contact (C0 – PI or Staff - 24 hr phone/pager):	412-000-1111 (pager)

B. Project Information:

15.	Title:	Dementia in Baboons			
16.	Source of Funding:	NIH			
17.	Total Project Period:	9/01 to 8/05			
18.	Grant Title and Number:	NIH: Dementia in Baboons, R01-NS-00001			
19.	Is the grant administered through the University of Pittsburgh?	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO			
20.	If No, what institution is administering the grant?				
21.	Administering Institution's Protocol Approval # and Expiration Date:				
22.	Administering Institution's USDA Registration #:				
23.	Contact Person (name, phone, e-mail):				
24.	Will any aspect of the animal experimentation be performed at another institution (including custom antibody production)?	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO			
If Yes, provide the following: (See www.aphis.usda.gov/ac/ Policy #10 for more information.)					
25.	Institution Name:				
26.	Institution's IACUC Approval # and Expiration Date:				
27.	USDA Registration #:				
28.	AAALAC Accreditation Status:				
29.	Contact Person (name, phone, e-mail):				
<i>License and accreditation info available from the Office of Research at appropriate institution.</i>					
30.	Is this submission a major modification or a renewal of a currently active protocol?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No			
31.	If yes, please list the protocol #:	0103777			
32.	Please list the protocol's title:	Dementia in Baboons			

C. Research Staff (List all individuals handling live/dead animals or animal tissues, including PI)

33.	NAME	SOCIAL SEC # or PITT ID #	POSITION TITLE	*PROJECT ROLE	E-MAIL	PHONE/PAGE R #
	David Bush	001-22-3333	Professor	PI	davidbush@upmc.edu	412-647-0001
	Julian Cheney	002-33-4444	Nuclear Med Tech	Animal Tech; anesthesia and PET imaging	cheney@upmc.edu	412-647-0005
	Wonderful Scientist	003-44-5555	Assist. Professor	Co-PI	wonderful@upmc.edu	412-647-0007

*List the duties will this person perform relating to the animal studies.

D. Animal Usage:

Animals must be obtained from DLAR approved sources/vendors only. Quarantine may be required for certain animals based on their origin before they enter Institutional Animal

Housing Space(s). Contact the DLAR (648-8950 or dlar@pitt.edu) for further information. Approximate weight range and age range of experimental subjects at time of arrival should be provided.

34. SPECIES	STRAIN	AGE	WGT.	SEX	Number projected for 3 Years*
Baboon	Papis	5-15 years	20-45 kg	M	2 (the same two animals will be utilized over the 3 year investigational period covered by this IACUC protocol)

*Only the number of individual animals of each species needs to be denoted here, not the number of individual animals of each strain.

E. Animal Housing And Use Sites:

Housing:

35. SPECIES	HOUSING LOCATION (Building, Room/Floor -if known)	HOUSING DURATION (Days)	MAXIMUM NUMBER ANIMALS HOUSED IN AN APPROVED FACILITY AT ONE TIME
Baboon	BST-75 Animal Facility	365 days/yr	2

36. Rodent Caging Requirements: (Complete only for Rodent protocols):

- Microisolator caging is required.
- Solid bottom caging is requested.
- Standard wire bottom caging is requested (see below.)
- Wire bottom caging is necessary, and I am requesting an exemption from the IACUC policy regarding use of this caging. (Provide justification below.)

*IACUC policy states that animals exceeding 500 grams or maintained for > 9 months cannot be housed in wire-bottom caging unless a specific exemption is granted (See policy at www.iacuc.pitt.edu).

If necessary, please provide scientific justification:

F. Use Sites and Transportation Methods:

<p>37. Are animals transported from the holding room through approved animal facility space to another location/lab? [x] YES [] NO (Approved animal facility space is defined as DLAR operated facilities as well as other animal housing spaces approved by the IACUC.)</p>
<p>38. If yes, indicate building(s) and room(s) of destination (If using hospital areas, see “Use of Experimental Animals in UPMC Hospital/Patient Areas policy at www.iacuc.pitt.edu/. PET imaging performed in X-666 PUH</p>
<p>39. Provide rationale for the need to remove animals from dedicated facilities and justify why such work cannot be performed in dedicated, approved animal facility space? Non-mobile PET scanner located in X-666 PUH and use of short-lived radioactivity requires proximity to site of production, scanner, and radiometabolite analysis laboratory.</p>
<p>40. Will vertebrate animals be housed in these alternate locations for greater than 12 hours? Yes [x] No [] If yes, these sites must undergo additional evaluation and approval (contact the DLAR director at dlar@pitt.edu for further information).</p>
<p>41. Describe animal transportation route: Delivery of non-sedated baboon to Scaife Hall loading dock by CAF personnel from BST-75 Animal Facility in covered squeeze cage and transport to 3rd floor X-wing PUH via X-wing service elevator located adjacent to Scaife Hall loading dock. The baboon will be returned to BST-75 using the same route and will not be sedated for the transport. The baboon will be taken to the Scaife loading dock only upon receiving confirmation that the Animal Facility truck is waiting at the Scaife dock. The baboons are brought to PET Facility on the day of the study and kept overnight in a covered transport cage at the PET Facility in Room X-666 PUH while awaiting the Animal Facility truck. The transport cage is covered with a heavy tarp, and PET Facility personnel will ensure that the pathway between the PET Facility holding area and the 3rd floor X-wing elevators is clear prior to moving the animals.</p>
<p>42. Describe animal containment & transport mechanism: Covered squeeze cage. (See Animal Transportation SOP at www.iacuc.pitt.edu/.)</p>
<p>43. Indicate laboratory sites (if different than above) where hazardous tissues (e.g., nonhuman primate or tissues from studies involving the use of radiation, residual hazardous chemicals, or BSL-2 or greater containment) are to be taken: blood analysis for radiolabeled metabolites performed in Rm X-777, PUH</p>
<p>Note that if the tissue(s) being transferred will be handled by investigators not listed on this protocol, you are required to inform that person(s) of any inherent dangers associated with the tissues. Your sign off on the certification Section Q verifies you have provided this information.</p>
<p>44. Describe tissue containment & transport mechanism: blood transported for analysis in sealed syringes contained on ice in a secondary transport container</p>

G. Study Summary:

<p>45. Briefly summarize (in less than three sentences) the aim(s) of the study, and why the study is important to human or animal health, the advancement of knowledge, or the good of society.</p>
<p><i>Summarize here:</i> The goals of these studies are to determine the in vivo properties in non-human primates of several radioligands as candidates for future human studies of brain amyloid mapping. The brain uptake, brain distribution, and pharmacokinetics of these radiopharmaceuticals will be imaged using positron emission tomography (PET) at the UPMC PET Facility.</p>

H. Animal Welfare Act Required Information:

46. Explain the rationale for animal use, including why non-animal models cannot be used:

Explain here: There are no alternative procedures for the in vivo PET imaging studies proposed. The information sought can only be obtained in the whole animal preparation as it involves investigations of complex physical and metabolic interactions of different organ systems in competition with brain uptake and in vivo clearance of the radioligand to mimic the (living) human subject goal. These in vivo studies can not be adequately duplicated using tissue culture methods or by computer simulation procedures.

47. Justify appropriateness of the species selected and why a phylogenetically lower species could not be used?

Justify here:

A non-human primate model is required to fully evaluate promising imaging agents prior to human use, as in vitro models and rodent models are used to initially screen a host of potential agents prior to non-human primate testing. Issues unique to non-human primates include pharmacokinetics similar to humans (and often much different than in rodents) and the production of radiolabeled metabolic species similar to humans (and sometimes different than in rodents) which are critical to assess prior to human imaging studies. Baboons are required because of their relatively large brain size (compared to monkeys) and the limited spatial resolution (~6 mm FWHM) of state-of-the-art PET cameras. We are interested in quantifying the behavior of many of the radiotracers in brain sub-structures, such as the caudate-putamen, sub-cortical white matter, pons, and the cortical ribbon throughout the brain.

48. Number of experimental/control groups*: 1 group of normal baboons

49. Number of animals per group*: 2 normal baboons

*Number of Experimental/Control Groups X Number of Animals per group should equal the total number of animals delineated in the responses to Question 34.

50. How was sample size determined? (Answer by placing an X in the appropriate bracket(s))
(The number of animals requested should be the minimum required for statistically valid results.)*

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Numbers and Species of Animals and <ftp://ftp.grants.nih.gov/IACUC/GuideBook.pdf> Fundamental Issues, Criteria)

Pilot study. No report of a similar study exists. The proposed study will utilize a small number of animals (typically less than 10) to obtain preliminary data to justify the performance of a larger study. Each compound will be studied only once or twice in both baboons, and these could be considered "pilot studies" given the low number of imaging studies per compound. However, it is not anticipated that a larger group of baboons will be studied. These studies are not meant to be definitive in the sense of highly significant results, but rather are intended to be representative of typical non-human uptake, retention, and metabolism patterns as a necessary step towards future human studies.

Previous experience by this PI (if checked, answer question 51)

Studies cited in the literature (If checked, answer question 51)

51. If previous experience and/or literature review was used to determine sample size, a power analysis should be described if possible, citing type of statistical analysis used. If the experimental approach is not amenable to a power analysis, a thorough justification of the number of animals required must be provided.

Describe and Justify here:

I. Drug Administration

Anesthesia, Analgesia, Tranquilizing, and Paralytic Drugs (Specify dosage in mg/kg)

52. SPECIES	DRUG	DOSE	ROUTE	ADMIN. FREQUENCY
Baboon	ketamine	15mg/kg	im	Once at start of per study
Baboon	atropine	0.5 mg total/ administration	im and iv	Once at start of study and once at end of study
Baboon	pancuronium bromide	0-0.06 mg/kg/h	iv	Throughout imaging study (~3 h)
Baboon	isoflurane	0.2-1.5%	ventilator	Throughout imaging study (~3 h)
Baboon	neostigmine	2 mg	iv	Once at end of study

For drugs listed above, please supply the duration of treatment and method of monitoring efficacy:

53. DRUG	Duration of Treatment	Method of Monitoring Efficacy
ketamine	Once at start of study	Visual observation
atropine	Once at start of study and once at end of study	Visual observation
pancuronium bromide (p.b.)	Throughout imaging study (approx. 3 h)	Monitoring muscle twitch response to electrical stimulation (4 responses per 4 electrical stimulations indicates the need for increased p.b. while 0 to 1 twitches per 4 electrical stimulations indicates that p.b. should be interrupted or slowed)
isoflurane	Throughout imaging study (~3 h)	Endtidal pCO ₂ maintained at 35-40 mm partial pressure. Blood pressure and heart rate continuously monitored.
neostigmine	Once at end of study	Visual observation of reversal of p.b. and subsequent removal from ventilator

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> -Minimization of Pain & Distress)

54. Are Paralytic Drugs Used: Yes No

If Yes, paralytic drug used: [pancuronium bromide]

Describe Health Monitoring Procedures: Monitoring muscle twitch response to electrical stimulation (4 responses per 4 electrical stimulations indicates the need for increased pb while 0 to 1 twitches per 4 electrical stimulations indicates that pb should be interrupted or slowed). In addition, two blood pressure measurements (arterial line and pediatric cuff) will be continuously monitored throughout the study along with continuous heart rate monitoring. Endtidal pCO₂ continuously monitored and maintained at 35-40 mm partial pressure.

- Justification for use of the paralytic agent in this study. Paralytic agent is required to guarantee absence of movement in brain imaging studies.
- Provide information and/or data that demonstrate the proposed anesthetic regimen is adequate without the paralytic agent. This can be based on a) literature citations specifically using the same regime, b) documented experience of the PI with this anesthetic regime without paralytic inclusion or c) the previous documented performance of a "sham" procedure utilizing the regime without paralytic agent use. n/a
- Provide a plan to titrate the amount of anesthetic to establish a verifiably adequate plane of anesthesia in each animal prior to inducing paralysis. Monitoring muscle twitch response to electrical stimulation (4 responses per 4 electrical stimulations indicates the need for increased p.b. while 0 to 1 twitches per 4 electrical stimulations indicates that p.b. should be interrupted or slowed). In addition, two blood pressure measurements (arterial line and pediatric cuff) will be continuously monitored throughout the

study along with continuous heart rate monitoring. Endtidal pCO₂ continuously monitored and maintained at 35-40 mm partial pressure.

- d. Describe the proposed anesthetic monitoring procedures in paralyzed animals (e.g. electroencephalogram, electrocardiogram, blood pressure, etc.) Please note if heart rate or blood pressure is monitored, elevations or depressions of 15-20% from baseline prior to adding paralytics may be indicative of need for alteration of anesthetic levels. Monitoring muscle twitch response to electrical stimulation (4 responses per 4 electrical stimulations indicates the need for increased p.b. while 0 to 1 twitches per 4 electrical stimulations indicates that p.b. should be interrupted or slowed). In addition, two blood pressure measurements (arterial line and pediatric cuff) will be continuously monitored throughout the study along with continuous heart rate monitoring. Endtidal pCO₂ continuously monitored and maintained at 35-40 mm partial pressure.

55. Drugs Administered for Therapeutic Purposes (excluding anesthetics, analgesics, and tranquilizers) --- none

Will pharmaceutical agents be introduced to the living and/or dead animals? [x] YES [] NO
If Yes,

SPECIES	AGENTS	DOSE	ROUTE	EFFECT
Baboon	C-11- and F-18-labeled amyloid radiotracers	Cold: <10 micrograms per injection Hot: 2-10 mCi	iv	None - tracer doses only with no pharmacological or physiological effects

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable: The drugs (PET radiopharmaceuticals) are injected in tracer doses (< 10 micrograms) and at these doses there are no adverse effects associated with these agents.

56. Are any *non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) regulated species (vertebrate animals excluding rats, mice and birds)? [] YES [x] NO

*(Please note: As per specific Animal Welfare Act directive, "Non-pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. **Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in regulated animals.**")*

(See policy at www.pitt.edu/policies.htm)

***Non-pharmaceutical agent** – An agent that was not specifically prepared for injection into animal or human in its current form.

If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.

Describe:

57. Are non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) non-regulated species (rats, mice or birds) for the purpose of veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics)? [] YES [x] NO

(Please note: As per University of Pittsburgh IACUC approved policy, all compounds used in rats, mice or birds for veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics) must be pharmaceutical grade (when available) unless scientifically justified. Cost savings alone are not considered by the IACUC an adequate justification for using non-pharmaceutical grade drugs for veterinary medical care or to relieve pain and distress in these species.)

(See policy at www.pitt.edu/policies.htm)

If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.

Describe:

J. Euthanasia

58. Will animals be euthanized?	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
59. If NO, what is final disposition of animals?	The final disposition of the baboons will be that 1) they will be sold to other qualified University of Pittsburgh investigators or 2) returned to the vendor (Southwest) or 3) given away to zoos (all of which have been accomplished previously with CAF's approval and assistance with other baboons over the past 8 years).
60. If YES, provide drug and dose or method used.	
See http://www.avma.org/resources/euthanasia.pdf for approved euthanasia methods (Adobe Acrobat software required)	

K. Animal Exposures

Radioactive Agents (Prior to any study involving the administration of radioisotopes to animals, the Radiation Safety Office must review and approve the use.)		
61. Will radioactive substances be administered in live animals? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO If Yes, complete Attachment 2 and the table below.		
SPECIES	ISOTOPE	ACTIVITY
Baboon	C-11 or F-18	2-10 mCi
62. Will X-Ray equipment, accelerators, CT scanners, irradiators, or external beam radiation be used? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, complete the table below. (It is <u>not</u> necessary to complete Attachment 2 for a Yes response to this question.)		
EQUIPMENT	LOCATION OF USE	DOSE*

*Dose not applicable to diagnostic imaging studies

Biological Agents (complete Attachment 3)				
63. Will Biological Agents be used in living and/or dead animals? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes,				
SPECIES	BIOLOGICALS	DOSE	ROUTE	EFFECT
Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring				

and alleviating these effects if applicable:

64. Chemical Agents (Do not include drugs used for therapeutic purposes) ?

Will chemical agents be introduced to the living and/or dead animals? [] YES [x] NO

If Yes,

SPECIES	CHEMICALS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

65. Cells/Tumor Lines/Bodily Fluids (complete Attachment 4)

Will cells, tumor lines, or bodily fluids be introduced to the live and/or dead animals?

[] YES [x] NO

If Yes,

SPECIES	AGENTS	DOSE	ROUTE	EFFECT

L. Description of Experimental Design and Animal Procedures:

66. Describe the specific experimental manipulations and treatments of the animals in terms that are intelligible to non-specialists. This description should allow the IACUC reviewer to understand exactly what will be done to all the animals from entry into the study to the endpoint of the study. It would be helpful to provide a flow chart or table illustrating your experimental design and include animal numbers to be used. The experimental endpoint that is being measured must be indicated for each procedure. (Please do not insert the Methods Section from the grant application. The IACUC is only required to review procedures that are done to animals.)

Describe Experimental Design, Animal Procedures, and Surgical Procedures here:

A. Overview: As part of our continuing efforts to develop amyloid radiopharmaceuticals for positron emission tomography (PET) imaging studies in human subjects, animal studies in rodents and non-human primates are required to validate the usefulness of these agents prior to human use. We plan to evaluate the in vivo properties of 3-8 agents in non-human primates each year, and these agents have been shown previously to be promising compounds in both in vitro binding assays and in vivo rodent studies. These studies in baboons are aimed at assessing the following properties: 1) brain uptake and retention of radioactivity following intravenous injection of a positron-labeled agent; 2) regional brain localization of radioactivity; 3) clearance of radioactivity from brain regions; 4) appropriate brain time courses (kinetics) to match the half-life of the radionuclide used (either 20 min ¹¹C or 110 min ¹⁸F); 5) determination of the time course of radioactivity in arterial blood (input function determination); 6) quantitation of radiolabeled metabolites in the blood over the course of the study for accurate pharmacokinetic modeling; and 7) achievement of an accurate kinetic model capable of taking the metabolite-corrected input function and regional brain radioactivity measurements over time. As the baboons will

have little amyloid in their brains, behavior of the agents is expected to be similar to that in normal control human subjects. There is no good non-human primate model of amyloid deposition, and evaluation of the properties of our test compounds in baboons is as far as we can take these studies short of human experiments in Alzheimer's disease patients.

It is the ultimate goal of this work to be able to reliably determine regional amyloid densities non-invasively using PET imaging techniques following administration of a radiotracer which selectively binds to the amyloid plaques. The specific activities of the injected radiopharmaceuticals are typically between 500 - 2000 Ci/mmol. This means that each injection of 10 mCi of radiopharmaceutical will result in an injection of between 1.5 and 6 micrograms of the drug. At these tracer levels, no pharmacological effects will be experienced by the baboon.

The proposed studies will subject the baboons to minimal risk and discomfort. The baboons will be imaged once per month (at maximum) and no more than twelve times per year. The proposed studies are non-terminal in nature and involve the placement of one femoral artery and one venous catheter for arterial blood withdrawal and iv injection of radiotracer (respectively), while the animal is under anesthesia. The imaging studies will last 2-4 hours and a complete description of the experimental procedure is given below. We anticipate imaging the same baboons over the course of 3 years, therefore every effort will be made to ensure appropriate animal handling, catheter placement, and recovery from anesthesia for repeat PET imaging studies.

After an overnight fast with access to water, the baboon will be transported from BST-75 Animal Facility in a covered, portable squeeze cage by CAF personnel using the CAF animal transportation vehicle to the Scaife Hall loading dock of PUH. PET Facility personnel will transport the baboon in the covered cage to the PET Facility (X-666, PUH) using the X-wing service elevators located in the PUH loading dock area. Using the squeeze cage to immobilize the baboon, the animal will be injected in the rear flank (im) with a solution containing ketamine and atropine (15 mg/kg and 0.5 mg, respectively) for initial anesthesia and to control heart rate. When unconscious, a venous catheter will be placed in the baboon's antecubital vein and iv fluids started at 10 ml/kg/h with 0.9% sodium chloride. The baboon will be intubated with a large volume, low pressure cuffed endotracheal tube (4-6 mm ID). The baboon will be mechanically ventilated after paralysis with pancuronium bromide 0.06 mg/kg/h. The femoral-inguinal areas will be shaved and prepared for a sterile percutaneous placement of a femoral artery catheter to monitor arterial blood pressure and heart rate. A pediatric blood pressure cuff will be attached to one arm, and the two b.p. measurements will be routinely compared throughout the PET imaging study. Anesthetic isoflurane gas (0.2-1.5%) will be blended with a 40% oxygen/medical air ventilation mixture and administered to the baboon using the mechanical ventilator. Endtidal pCO₂ will be continuously monitored (maintained at 35-40 mm partial pressure). Pancuronium bromide will be added to the iv drip, and the iv administration rate of pancuronium bromide (between 0 mg/kg/h and 0.06 mg/kg/h) will be determined throughout the PET imaging study by monitoring muscle twitch responses to electrical stimulation (4 responses per 4 electrical stimulations indicates the need for increased pancuronium bromide and 0 or 1 twitches per 4 electrical stimulations indicates that pancuronium bromide administration should be interrupted or slowed). The baboon will be positioned supine on a sliding table in the PET scanner. An iv injection of 2-10 mCi of a sterile, pyrogen-free ¹⁸F- or ¹¹C-labeled radiotracer will be made in 10 mL of sterile, isotonic saline solution and the distribution of radioactivity in the brain monitored for the next 2-4 h using PET imaging techniques. Approximately 20 mL of arterial blood will be withdrawn through the femoral artery catheter over 2-4 hours post injection to determine the arterial radioactivity input function and blood metabolites. Arterial blood pressure will be continuously monitored, between occasional arterial blood sampling, using the same arterial catheter. Following the imaging study, the baboon will be removed from the PET scanner, the arterial catheter will be removed, and the iv drip will be stopped. Recovery from muscle relaxant will be accomplished with neostigmine and heart rate will be controlled with atropine, 2.0 mg and 0.5 mg respectively. The baboon will remain connected to the ventilator and pCO₂ monitor, and ventilated with room air. When the baboon begins to resist the mechanical ventilation, the iv catheter will be removed and the endotracheal tube cuff will be deflated. The endotracheal tube will be removed when active resistance becomes apparent. The baboon will be returned to BST-75 Animal Facility using the transport cage and the CAF transportation vehicle when it can breathe freely and is fully recovered from anesthesia (conscious and freely moving in the cage).

67. Describe Experimental Endpoints Here: (At what point is the experiment completed on animals?)
 The experiment will be completed at the end of the 2-4 h imaging study. The same baboons will be used for many imaging studies, with a frequency of no greater than once per month per animal.

For each <i>Non-surgical Procedure</i> for each species: Specifically address the following:
68. Will blood sampling be conducted? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
69. If Yes, provide rationale, method, site, volume, & frequency: Arterial blood sampling is required to determine the input function (for pharmacokinetic modeling) and quantify radiolabeled metabolites. The total volume of blood taken per study will be no more than 20 mL. Blood sampling will occur only when animals are anesthetized.
70. Will food scheduling or restriction (other than standard pre-operative fasting) be conducted? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
71. Provide rationale, method, frequency, & duration:
72. Will water scheduling or restriction (other than standard pre-operative fasting) be conducted? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
73. If Yes, provide rationale, method, frequency, & duration:
74. Will restraint methods be utilized? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No Baboon will be anesthetized, immobilized with pancuronium bromide, and ventilated
75. If Yes, provide rationale, method, frequency, & duration*:
*Note: Provide a detailed description of non-human primate handling methods (other than chemical immobilization) and justify the use of alternative methods and provide assurances of personnel safety.
76. Will stress paradigms be utilized? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
77. If Yes, provide rationale, type, frequency, & duration:

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Methodology)

<p>For Each <i>Surgical Procedure</i> for each species, specifically address the following (required): (The Surgical Guidelines are available at www.iacuc.pitt.edu under “Policies.”)</p>
<p>78. Will non-survival surgery be performed? Yes [] No [x] no surgery, percutaneous arterial line and iv catheter placements only</p>
<p>79. Will survival surgery be performed? Yes [] No [x]</p>
<p>80. Will multiple survival surgeries be performed on one animal? Yes [] No [x] (See www.aphis.usda.gov/ac/ -Policy #14.)</p>
<p>81. If yes to question 80, please justify: <i>Enter justification here:</i></p>
<p>82. Surgical Site (Building, Room Number): n/a, no surgeries performed</p>
<p>83. Names of Surgeon(s):</p>
<p>84. Has the surgeon(s) performed this procedure on the species requested? Yes [] No []</p>
<p>85. If no to question 84, please provide training plan:</p>
<p>86. Postoperative Plan (Methods to assess/alleviate pain/distress, Recovery Criteria, Monitoring Criteria - See http://grants.nih.gov/grants/olaw/GuideBook.pdf): <i>Enter plans for monitoring animals as well as alleviating pain and distress here:</i> Visual observation of mobility and return of normal function following recovery from anesthesia will be closely monitored prior to returning the animal to BST-75 Animal Facility. Analgesia will be administered by CAF personnel if determined necessary by CAF veterinarian up to 48 hrs post study.</p>

M. Anticipated Effects on Experimental Animals

<p>87. Changes to Health and Well-Being Please describe all signs of morbidity (e.g., decreased food and/or water consumption, weakness, infection neoplasia, neurological effects, etc.) expected to occur in the experimental subjects as a result of the procedures performed in this protocol (If mortality is expected please note here and see http://www.iacuc.pitt.edu/sop/Pain_%20Indicators.htm.) No morbidity expected as a result of these studies,</p>
<p>88. Describe the expected frequency of the complications noted in question 87 and how you will deal with these complications: <i>Describe here:</i> n/a</p>
<p>Please complete the following two questions for all procedures.</p>
<p>89. Methods and Frequency of Monitoring Health/Well-Being Changes Describe in detail the species specific procedures that the investigator will use to monitor the experimental animals’ health and well-being, monitor pain and distress, alleviate pain and distress, and the frequency with which these procedures will be performed: Visual observation of mobility and return of normal function following recovery from anesthesia will be closely monitored prior to returning the animal to PST-75 Animal Facility. Analgesia will be administered by BST-75 Animal Facility personnel if determined necessary by CAF veterinarian up to 48 hrs post study.</p>
<p>90. Criteria for Premature Removal of Animals from Study (This section applies to acute and chronic studies)</p>

Describe in detail the criteria utilized to determine that an experimental animal must be removed from a study: n/a

N. Pain or Distress Classification

Please note that this section applies to USDA and Public Health Service (NIH) regulated species. This includes all species of animals and is a change from an earlier application.

Pain or Distress Classification

91.	SPECIES (Common Name)	USDA CLASSIFICATION* % of Animals Classified			
		B	C	D	E
	Baboon	%	%	100%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%

* **USDA Classifications** (see www.aphis.usda.gov/ac/ -Policy #11 for more information)

Classification B: Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery, but not yet used for such purposes.

Classification C: Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, no distress, or no use of pain-relieving drugs. Euthanasia must precede any invasive procedure (i.e. tissue harvesting) to be in Classification C.

Classification D: Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used. Acute or terminal surgery is considered a painful procedure, which is alleviated by anesthesia.

Classification E: Animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests and/or animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted resulting in permanent physiological impairment that may lead to chronic pain or distress.

NOTE REGARDING CLASSIFICATION E: An explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on **Attachment 1**. This information is required to be

reported to the USDA, will be available from the USDA under the Freedom of Information Act, and may be publicly available through the Internet via USDA's website.

**O. Consideration of Alternatives To Painful/Distressful Procedures –
(Complete only if you are using vertebrate species other than purpose-bred
birds, mice of the genus *Mus*, and rats of the genus *Rattus*)**

This section applies to all USDA regulated species. Reference Librarian, Alice Kuller is available for consultation in performing literature searches (648-1971).

If any procedures fall into USDA's Classification D or E, causing more than momentary or slight pain or distress to the animals, USDA Policy 12 requires that you must address **all three** of the following issues:

- 1) You have refined potential pain-producing methods as much as possible to minimize distress.
- 2) You have reduced animal use as much as possible without jeopardizing statistical validity.
- 3) You have considered replacing potential pain-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

As part of the response, you must provide written narratives that will convince the IACUC reviewers you have made a good faith effort to substantively address **each** of these three issues. You must also provide **two sources** of information to show that you have addressed **each** of the three issues. At least one of these sources of information should be a search of a scientific database. For each search executed, you must specify the **database** considered, the **date** of the search, **period** covered by the database searched, and the **keywords** used. If you elect to search two databases to satisfy the consideration of alternatives, please note that both Medline and PUBMED are search engines that consider the same database, the National Library of Medicine, and thus employing both of these search engines is considered to be a single database search. **Note: the investigator should retain all searches, as they are reviewable by federal agencies.**

An alternate method of consideration for alternatives is consulting with an expert (e.g., DLAR veterinarian, another PI with extensive experience with the issue in question) or attending conferences or colloquia that provide relevant and up-to-date information directly related to the issue. When sources other than database searches are included, sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge on the availability of alternatives in the specific field of study.

For more information, see www.aphis.usda.gov/ac/ -Policy #12.

Instructions:

For each section below, provide at least 2 database searches, or 1 database search + an expert consultation, then provide a written narrative.

92. Refinement - Provide evidence that you have refined potential pain or distress-producing procedures as much as possible to minimize distress.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
3/11/04	Medline	1966-3/2004	Papio, disease models, animal models, amyloid imaging, amyloid beta-protein, radioligand assay, radiopharmaceuticals, radionuclide imaging, congo red, thioflavin-T, thioflavin-S, emission computed tomography, brain, blood sampl\$ or femoral arterial line or intravenous or iv infusion or intubate or intubation, refin\$ technique\$ or method\$ or procedure\$
3/11/04	Biosis	1969-3/2004	Same as above

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

Medline key words search produced 102510 references that described studies in papio baboons or animal models of disease, 52147 references that described studies of amyloid imaging, radioligand assay, radiopharmaceuticals, or radionuclide imaging, 1360 references that described studies of congo red or thioflavin-T or -S, 157212 studies that described studies of emission computed tomography or brain, and 165 studies that described a relevant combination of the above studies. A search of these studies to refine potential pain or distress-producing procedures provided 1 reference – Bush et al. J Med Chem 2003 (one of my papers). A similar search of Biosis produced 2 references, Bush et al. J Med Chem 2003 and Bush et al. J Neurosci 2002 (both are my papers). Neither paper was helpful in defining procedures to refine potential pain or distress-producing procedures as they described methods utilized in this IACUC application. To reduce pain and distress to the baboons we use the fewest numbers of animals possible (2) to obtain meaningful pharmacokinetic data. We did not identify any method to refine pain and distress in the two literature searches, and we attempt to cause as little pain and distress as possible by performing the studies under anesthesia.

93. Reduction - Provide evidence that you have reduced animal use as much as possible without jeopardizing statistical validity.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
3/11/04	Medline	1966-3/2004	Papio, disease models, animal models, amyloid imaging, amyloid beta-protein, radioligand assay, radiopharmaceuticals, radionuclide imaging, congo red, thioflavin-T, thioflavin-S, emission

			computed tomography, brain, blood sampl\$ or femoral arterial line or intravenous or iv infusion or intubate or intubation, baboon\$ or papio\$ reduce\$ or fewer, number\$ or laboratory or research
3/11/04	Biosis	1969-3/2004	Same as above

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

A search of the Medline data base provided no results to reduce animal use and the Biosis search provide 1 reference (Westlind-Danielson et al., Biochemistry 2001) that was not relevant in providing a method to reduce animal use.

We use a total of 2 baboons for these experiments, which we believe is the minimum number required to demonstrate in vivo pharmacokinetics of new amyloid imaging agents in a non-human primate model.

94. Replacement - Provide evidence that you have considered replacing potential pain or distress-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used
3/11/2004	Medline	1966-3/2004	Computer simulation, computer model, experimental models anatomic, models statistical, models theoretical, power analysis, in vitro, models biological, animal testing alternatives, animal use alternatives.
3/11/2004	Biosis	1969-3/2004	Same as above

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

A search of the Medline data base provided 219 citations to replace potential pain or distress-producing methods with other techniques, but none of these were relevant to the studies described in this IACUC application. A similar search of the Biosis data base provided 1 citation, but it was not relevant to the studies described here. At this time, there is no substitute for the intact whole non-human primate to provide time-varying brain uptake and clearance data and blood metabolite data following the injection of radiolabeled amyloid imaging agents.

P. Exemption from Environmental Enrichment For Nonhuman Primates or Exercise Program for Dogs

<p>95. For nonhuman primates, are you seeking an exemption from the institution’s plan for environmental enrichment? (see Plan to Promote the Psychological Well-Being of Nonhuman Primates at www.iacuc.pitt.edu/policies.htm) <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, complete Attachment5</p>
<p>96. For dogs, are you seeking an exemption from the institution’s plan for exercise (see www.iacuc.pitt.edu/policies.htm)? <input type="checkbox"/> YES <input type="checkbox"/> NO If Yes, justify below:</p>
<p><i>Type Justification Here</i></p>

Q. Principal Investigator Certifications

- I certify that I have determined that the research proposed herein is not unnecessarily duplicative.
- I certify that I will notify the IACUC before initiating any significant changes in this protocol.
- I certify that I will notify the IACUC regarding any unexpected results that impact animals. Any unanticipated pain, distress, morbidity or mortality will be reported to the attending veterinarian.
- I understand that if I cannot be contacted in the event that animals in this project show evidence of distress, illness or pain, emergency care, including euthanasia if necessary, will be administered at the discretion of the veterinary medical staff.
- For all USDA Classification D and E proposals (see Section N): I certify that I have reviewed the pertinent scientific literature and the sources and/or databases as noted in Section O and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
- **For any animal body fluids or tissues, derived from the studies described in this application and transferred to persons not listed in Section C, I certify I will notified the recipient of all hazards associated with these materials and instructed them to register with the EHS Department (624-9505)**
- I certify that I will share a copy of the approved protocol with all personnel identified in Section C and they will read and understand all elements described for the study.
- I certify that all surgeons involved in this project have read the Surgical Guidelines. (The Surgical Guidelines are available at www.iacuc.pitt.edu under “Policies.”)

R. Inclusions*: (Please respond by placing an X in the appropriate bracket):

- 97.** This protocol involves animals in Pain or Distress Classification E: YES NO
 If yes, has Attachment 1 been completed? YES NO

98. This protocol involves radiation usage: YES NO
 If yes, is this protocol registered with the Radiation Safety Office (Attachment 2)?
 YES NO
99. This protocol involves hazardous chemicals or biologicals: YES NO
 If yes, is this protocol registered with the Health and Safety Office (Attachment 3)?
 YES NO
100. This protocol introduces cells/tumor lines or bodily fluids into animals: YES NO
 If yes, is this protocol registered with the DLAR (Attachment 4)? YES NO
101. This protocol seeks an exemption from the institution's environmental enrichment plan for nonhuman primates: YES NO
 If yes, has a dispensation from the University of Pittsburgh's Nonhuman Primate Enrichment Plan (Attachment 5) has been requested? YES NO
102. This protocol involves private or commercial funding: YES NO
 If yes, has the Conflict of Interest Form has been completed (Attachment 6)?
 YES NO
103. This protocol involves rDNA: YES NO
 If yes, has registration been submitted to the IBC? YES NO
 If the rDNA is already registered, has the IBC been notified to add this protocol to your registration? YES NO

***Note – Please delete all Attachments not valid to your protocol before returning this completed form to the IACUC office.**

Principal Investigator Certification: (First Name Last Name):

David Bush

Date Submitted:

3/12/04

If Applicable, Post-Doctorate and Graduate Student Sponsor (Name, phone #, e-mail address):

As a sponsor, I agree to take responsibility for all experimental studies performed pertaining to this protocol and agree to comply with all federal regulations applicable to this research YES NO

Date: 3/8/04

USE OF RADIOACTIVITY IN ANIMAL STUDIES (FORM RSO-AN1)
Approval must be obtained from the IACUC before this study may be performed.

Prior to any study involving the administration of radioisotopes to animals, the Radiation Safety Office must review and approve the use. Please complete this form and submit to the RSO in G-7 Parran Hall. Allow two weeks for approval.

AUTHORIZED USER NAME:		David Bush
Protocol No(s): 7777		
Animal: Baboon	Weight (in grams): 20,000-45,000	
Animal:	Weight (in grams):	
Animal:	Weight (in grams):	
Animal:	Weight (in grams):	
Number of animals per study: 1		
Number of experiments per study: 1		
Project start date: ongoing		
Period of time over which experiments will be performed: 3/04-3/07		
Radio-nuclide: C-11 and F-18	Compound: amyloid imaging radiotracers	
Activity to be administered per animal: 2-10 mCi		
Route of Administration: intravenous		
Location where administration will occur:	Room#: X-666	Building: PUH
Location where radio-labeled animals will be housed: scanned in X-666 and after scanning will be kept until ready for transport in X-666	Lab: X-666, PUH	CAF Room: BST-75 Animal Facility
Period of time before animal will be sacrificed:	Non-terminal experiments	
Are pathogenic/infectious agents to be administered also? no		

REQUIREMENTS:

UNLESS AN EXEMPTION IS GRANTED BY THE RSO, THE FOLLOWING REQUIREMENTS ARE MANDATORY:

1. For animals housed in CAF facilities, all cages must be labeled with a "Caution Radioactive Material" label stating the isotope and amount per animal, the date of administration, and the authorized users name.
2. Animal cages must be properly surveyed for contamination before being returned to the CAF cage washer.
3. Animal carcasses that are disposed of as radioactive waste must be packaged separately. Do not include any other materials such as pads, tubing, needles, instruments, etc. with the carcass.
4. The RSO should be notified each time a study is to be undertaken.

Submitted by: David Bush	Date: 3/8/04
Reviewed by: Kevin Bohner	Date: 3/22/04
SPECIAL CONDITIONS AND REQUIREMENTS:	

Revised 9/96