THE
GRADUATE
SCHOOL
UNIVERSITY OF WISCONSIN–MADISON

September 23, 2004

Jean Barnes
Primate Freedom Project
P.O. Box 1623
Fayetteville, GA 30214

Dear Ms. Barnes:

I have enclosed material that is responsive to your Open Records request of July 21, 2004.

You will note that we have redacted certain information from these records, including identification of specific individuals on this campus and elsewhere who are involved in animal research and the locations of same. We have also redacted information relating to any specific intellectual property of the university researchers in question.

We have redacted the identification of these individuals and the location of their research in order to protect their safety and the safety of the university's students, employees and facilities.

We have also redacted minimal information relating to the specific thoughts, hypotheses and intellectual processes of the scientists in question. We have done so in an effort to prevent the misappropriation of these valuable intellectual assets.

In making these redactions, we have concluded that the public benefit in opening these records to examination is outweighed by the harm to the public interest that would result from such disclosure. We also believe that the redacted information should be sufficient to give the public a basic understanding of some of the research that is being done at this university.

To the extent that this response may amount to a denial of your request, it may be subject to review by mandamus under s. 19.37(1), Wis. Stats., or upon application to the Attorney General or District Attorney.

Sincerely,

R. Timothy Mulcahy

R. Timothy Mulcahy, Ph.D.
Associate Vice Chancellor for Research Policy

Encl.

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History for animal: r97082

Abstract

ID: r97082 Sex: f Location: ab167 0005 s
Dam: r90103 Sire: r91010
Origin: cen Status: alive Availability: ab Hold Code:
Weight: 6.470 (weighed on 2004-07-07 10:00)
Birth: 1997-09-18 Death:
Arrive: Depart:
TB: 2004-07-07
Medical:

Birth

Sex: f Location: ab108 0017 gm
Birth: 1997-09-18 Conception: 1997-04-06
Dam: r90103 Sire: r91010
Origin: cen Birth type: n
Weight: 0.460 kg. (weighed on 1997-10-07 11:30)
Remark:

Offspring (r97082 as dam)

ID: r01038 Sex: f Location: ab118 0006 gm
Birth: 2001-06-19 Conception: 2001-01-02
Dam: r97082 Sire: r92064
Origin: cen Birth type: n
Weight:
Remark:

ID: r02119 Sex: m Location: ab163 0005 gm
Birth: 2002-10-19 Conception: 2002-05-06
Dam: r97082 Sire: r96027
Origin: cen Birth type: n
Weight: 0.565 kg. (weighed on 2002-11-04 09:15)
Remark:

ID: r04010 Sex: Location: ab167 0005 gm
Birth: 2004-01-27 Conception: 2003-08-15
Dam: r97082 Sire: r96027
Origin: cen Birth type: n
Weight:
Remark:

Historical Records

HOUS 1997-09-18 Room: ab108/0017 Cond: gm Out: 1998-07-16 07:45
CLIN 1997-10-07 11:30 Drug: m-55930 (tattoo)
Amount: 0.0000 Route: car Time:
CLIN Remark: pe within normal limits
WT Weight: 0.460 kg
WT 1997-11-20 11:30 Weight: 0.620 kg
WT 1998-02-12 11:00 Weight: 0.930 kg
ASGN 1998-04-02 Project: 19960702 Released: 1998-04-02 Inves:
Title: dna profiling of primates used in biomedical research
BLD Project: 19960702 Quantity: 2.00 By: For:
P/S: A/V: Code: bh10673 Caretaker: y
WT 1998-04-02 09:30 Weight: 1.080 kg
WT 1998-06-05 07:45 Weight: 1.430 kg
HOUS 1998-07-16 07:45 Room: ab120/n002 Cond: s Out: 1998-08-15 08:50
TB 1998-07-23 Lot: Dilution: Eye: r Reaction: -
WT 1998-07-23 08:10 Weight: 1.520 kg
HOUS 1998-08-15 08:50 Room: ab120/n002 Cond: p Out: 1998-08-15 09:05
HOUS 1998-08-15 09:05 Room: ab120/n002 Cond: s Out: 1998-09-14 13:01
HOUS 1998-09-14 13:01 Room: ab119/0040 Cond: g Out: 1998-09-15 12:40
HOUS 1998-09-15 12:40 Room: ab119/0039 Cond: g Out: 1998-09-21 09:20
HOUS 1998-09-21 09:20 Room: ab119/0040 Cond: g Out: 1998-09-24 12:40
WT 1998-09-22 09:30 Weight: 2.040 kg
HOUS 1998-09-24 12:40 Room: ab110/0001 Cond: g Out: 1998-12-01 12:45
CLIN 1998-11-04 09:00 Remark: vomit
WT 1998-11-17 09:30 Weight: 2.455 kg
HOUS 1998-12-01 12:45 Room: ab110/0002 Cond: p Out: 1998-12-10 11:20
ASGN 1998-12-03 Project: 00300901 Released: 1998-12-03 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 6.00 By: For:
P/S: A/V: Code: Caretaker: y
CHEM Account:
Glucose: 103.0 MG/DL SGPT(ALT): 30.0 MU/ML
BUN: 34.0 MG/DL Total Protein: 6.0 GM/DL
Creat.: 0.7 MG/DL Albumin: 3.7 GM/DL
CK(CPK): 1228.0 MU/ML ALK Phosphatase: 687.0 MU/ML
Cholest.: 159.0 MG/DL Calcium: 9.9 MG/DL
Triglyc.: 90.0 MG/DL Phosphorus: 6.2 MG/DL
SGOT(AST): 47.0 MU/ML Iron: 95.0 UG/DL
LDH: 381.0 MU/ML Sodium: 144.0 MMOL/L
Tot. Bili: 0.1 MG/DL Potassium: 3.8 MMOL/L
GGT: 84.0 MU/ML Chloride: 108.0 MMOL/L
HEMA Account:
WBC: 10.8 ths/ul MCV: 69.0 fl RDW: 12.1 %

RBC: 5.60 mil/ul MCH: 22.5 pg PLT: 589 ths/ul
HGB: 12.6 g/dl MCHC: 32.6 % MPV: 8.9 fl
HCT: 38.6 % PCV: 39.0 %
N: 19.0 % L: 79.0 % M: 1.0 % E: 1.0 % B: 0.0 %
Bands: 0.0 % Metamyelocytes: 0.0 % Myelocytes: 0.0 %
Total Protein: 6.3 g/dl Reticulocytes: 0.0 %
CLIN 1998-12-03 12:45 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 30.0000 mg Route: im Time: 11:45
CLIN Drug: m-55930 (tattoo)
Amount: 0.0000 Route: thigh Time:
CLIN Remark: tattooing
HOUS 1998-12-10 11:20 Room: ab110/0001 Cond: g Out: 1999-01-07 12:00
BACT 1999-01-07 Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-52020 s (sulfamethoxazole/trimethoprim)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-52530 s (gentamicin,gentamycin)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-52800 s (chloramphenicol nos)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-53120 s (cefazolin)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-53140 s (cephalothin)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-53560 s (ceftriaxone)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-54630 s (amoxicillin/clavulanic acid)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-55000 s (tetracycline)
BACT Account:
Source: t-59600 (rectum, nos)

Result: @e-16524 (shigella flexneri, type 4)
c-55710 s (ciprofloxacin)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-d1507 s (enrofloxacin)
BACT Account:
Source: t-59600 (rectum, nos)
Result: @e-16524 (shigella flexneri, type 4)
c-d4275 s (naxcel)
HOUS 1999-01-07 12:00 Room: ab110/0002 Cond: s Out: 1999-01-10 08:40
CLIN 1999-01-08 14:45 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-09 09:45 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-10 08:20 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
HOUS 1999-01-10 08:40 Room: ab110/0001 Cond: g Out: 1999-03-30 11:30
CLIN 1999-01-11 09:00 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: im Time:
CLIN 1999-01-12 08:30 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-13 08:30 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
WT 1999-01-13 10:00 Weight: 2.240 kg
CLIN 1999-01-14 08:45 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-15 08:20 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-16 09:10 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-17 08:45 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
CLIN 1999-01-18 09:15 Drug: c-d1507 (enrofloxacin)
Amount: 11.4000 mg Route: oral Time:
BACT 1999-01-19 Account:
Source: t-59600 (rectum, nos)
Result: w-10001 (negative for salmonella, shigella, and campylobacter)
CLIN 1999-01-19 12:10 Remark: no diarrhea present. bright, alert, responsive
and hydrated; repeat rectal culture this week - by
TB 1999-02-23 Lot: 403 Dilution: 1:2 Eye: l Reaction: 0 0 0
WT 1999-02-23 10:00 Weight: 2.300 kg
CLIN 1999-02-23 12:00 Drug: c-67771 (atropine sulfate)
Amount: 0.0800 mg Route: im Time:

CLIN Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 30.0000 mg Route: im Time:
CLIN Remark: tb test and pe.
HOUS 1999-03-30 11:30 Room: c435/pen1 Cond: g Out: 1999-07-09 12:00
WT 1999-05-12 10:00 Weight: 2.500 kg
HOUS 1999-07-09 12:00 Room: c435/pen2 Cond: g Out: 1999-07-12 14:00
HOUS 1999-07-12 14:00 Room: c435/pen1 Cond: g Out: 2000-01-06 11:00
WT 1999-07-15 13:00 Weight: 2.880 kg
TB 1999-08-18 Lot: 408 Dilution: 1:2 Eye: r Reaction: 1 1 0
WT 1999-08-18 10:00 Weight: 2.760 kg
CLIN 1999-08-18 10:30 Drug: c-67771 (atropine sulfate)
Amount: 0.0800 mg Route: im Time: 10:30
CLIN Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 30.0000 mg Route: im Time: 10:30
CLIN Remark: pe and tb testing
CLIN Remark: pe within normal limits
WT 1999-11-26 10:00 Weight: 3.460 kg
ASGN 1999-12-27 Project: 00300901 Released: 1999-12-27 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 2.00 By: For:
P/S: A/V: Code: Caretaker: y
VSER Account:
Virus: srv Result: negative
HOUS 2000-01-06 11:00 Room: c435/pen2 Cond: g Out: 2000-02-07 08:00
HOUS 2000-02-07 08:00 Room: c435/pen1 Cond: g Out: 2000-09-19 08:30
ASGN 2000-02-09 Project: 00300901 Released: 2000-02-09 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 7.00 By: For:
P/S: A/V: Code: Caretaker: y
TB Lot: 408 Dilution: 1:1 Eye: l Reaction: 1 1 0
VSER Account:
Virus: b virus Result: positive
Virus: srv by eia (UC-Davis) Result: negative
Virus: srv by pcr (UC-Davis) Result: pbl negative
Virus: stlv-1 Result: negative
Virus: srv Result: negative
Virus: siv Result: negative
WT 2000-02-09 09:30 Weight: 3.720 kg
CLIN 2000-02-09 12:00 Drug: c-67771 (atropine sulfate)
Amount: 0.1200 mg Route: im Time: 12:00
CLIN Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 40.0000 mg Route: im Time: 12:00
CLIN Remark: pe and tb test
CLIN 2000-02-09 12:30 Remark: pe within normal limits
WT 2000-04-26 15:00 Weight: 4.080 kg
ASGN 2000-05-09 Project: 00300901 Released: 2000-05-09 Inves: clinical

Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 4.00 By: For:
P/S: A/V: Code: Caretaker: y
VSER Account:
Virus: srv eia (UC-Davis) Result: negative
Virus: srv pcr (UC-Davis) Result: negative
WT 2000-07-14 14:00 Weight: 4.580 kg
ASGN 2000-08-08 Project: 00300901 Released: 2000-08-08 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 3.50 By: jb For: ce
P/S: A/V: Code: Caretaker: y
TB Lot: 411a Dilution: Eye: r Reaction: 1 0 0
VSER Account:
Virus: b virus Result: positive
Virus: stlv-1 Result: negative
Virus: srv Result: negative
Virus: siv Result: negative
CLIN 2000-08-08 08:30 Drug: c-67771 (atropine sulfate)
Amount: 0.1600 mg Route: im Time: 8:30
CLIN Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 50.0000 mg Route: im Time: 8:30
CLIN Remark: pe and tb test
CLIN 2000-08-08 10:00 Remark: sparse hair coat
WT 2000-08-08 10:30 Weight: 4.500 kg
ASGN 2000-08-28 Project: 19960101 Released: (current) Inves:
Title: breeding colony
HOUS 2000-09-19 08:30 Room: ab118/0013 Cond: p Out: 2000-12-29 09:15
WT Weight: 4.675 kg
CLIN 2000-09-26 08:00 Remark: mens.
WT 2000-11-15 09:00 Weight: 5.680 kg
CLIN 2000-12-20 08:00 Remark: Mens.
HOUS 2000-12-29 09:15 Room: ab108/0009 Cond: b Out: 2001-01-05 11:15
HOUS 2001-01-05 11:15 Room: ab118/0013 Cond: p Out: 2001-03-07 12:30
WT 2001-01-11 08:45 Weight: 5.350 kg
CLIN 2001-01-24 08:00 Remark: mens.
CLIN 2001-01-25 13:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound-pregnant < 30 days; sire is r92064
WT Weight: 5.700 kg
TB 2001-02-05 Lot: 413a Dilution: Eye: l Reaction: 1 1 0
WT 2001-02-05 09:00 Weight: 5.360 kg
CLIN 2001-02-05 09:30 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 60.0000 mg Route: im Time:
CLIN Remark: pe and tb testing

CLIN 2001-02-05 09:35 Remark: cleaned gingiva with chlorhe
xidine solution
CLIN Remark: sex skin; tb test bleeding-p
ossible bruising
WT 2001-02-27 11:00 Weight: 5.720 kg
HOUS 2001-03-07 12:30 Room: ab118/0013 Cond: s Out: 2001-03-13 12:35
HOUS 2001-03-13 12:35 Room: ab118/0013 Cond: p Out: 2001-05-07 13:10
WT 2001-03-20 08:50 Weight: 6.230 kg
WT 2001-04-18 08:00 Weight: 6.470 kg
HOUS 2001-05-07 13:10 Room: ab118/0006 Cond: p Out: 2001-05-21 11:45
WT Weight: 6.800 kg
HOUS 2001-05-21 11:45 Room: ab118/0007 Cond: s Out: 2001-05-29 13:00
WT 2001-05-22 11:30 Weight: 7.210 kg
HOUS 2001-05-29 13:00 Room: ab118/0006 Cond: p Out: 2001-06-19 07:00
WT 2001-06-06 11:30 Weight: 7.330 kg
HOUS 2001-06-19 07:00 Room: ab118/0006 Cond: g Out: 2001-06-21 08:50
CLIN 2001-06-20 08:00 Remark: mens.
HOUS 2001-06-21 08:50 Room: ab118/0006 Cond: p Out: 2001-06-21 09:05
HOUS 2001-06-21 09:05 Room: ab118/0006 Cond: g Out: 2001-07-20 09:55
CLIN 2001-06-27 08:00 Remark: mens.
ASGN 2001-07-02 Project: 00300901 Released: 2001-07-02 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 3.00 By: For:
P/S: A/V: Code: Caretaker: y
TB Lot: 415a Dilution: Eye: r Reaction: 0 0 0
VSER Account:
Virus: srv Result: negative
Virus: b virus Result: positive
Virus: stlv-1 Result: negative
WT 2001-07-02 10:15 Weight: 6.370 kg
CLIN 2001-07-02 10:55 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 25.0000 mg Route: iv Time: 10:55
CLIN Drug: c-90287 (chlorhexidine)
Amount: Route: topically Time: 11:00
CLIN Remark: post partum exam; tb test; u
terus is involuted;
no vaginal discharge - by c.e.
HOUS 2001-07-20 09:55 Room: ab118/0006 Cond: p Out: 2001-07-20 10:10
HOUS 2001-07-20 10:10 Room: ab118/0006 Cond: g Out: 2001-09-05 09:00
WT 2001-08-28 11:10 Weight: 5.000 kg
HOUS 2001-09-05 09:00 Room: ab163/0005 Cond: g Out: 2001-10-28 09:45
WT 2001-10-24 08:00 Weight: 5.610 kg
HOUS 2001-10-28 09:45 Room: ab163/0005 Cond: p Out: 2001-11-17 08:45
HOUS 2001-11-17 08:45 Room: ab163/0005 Cond: g Out: 2001-12-06 11:40
HOUS 2001-12-06 11:40 Room: ab163/0005 Cond: p Out: 2001-12-27 11:30
WT 2001-12-20 12:00 Weight: 5.790 kg

HOUS 2001-12-27 11:30 Room: ab163/0005 Cond: g Out: 2002-01-04 13:30
HOUS 2002-01-04 13:30 Room: ab163/0005 Cond: p Out: 2002-01-15 14:00
HOUS 2002-01-15 14:00 Room: ab163/0005 Cond: g Out: 2002-03-13 11:30
ASGN 2002-01-18 Project: 00300901 Released: 2002-01-18 Inves: clinical
Title: clinical blood or surgery
BLD Project: 00300901 Quantity: 3.50 By: For:
P/S: A/V: Code: Caretaker: y
TB Lot: 415a Dilution: Eye: l Reaction: 0 0 0
CLIN 2002-01-18 08:00 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 60.0000 mg Route: im Time:
CLIN Remark: pe and tb test
WT Weight: 5.880 kg
CLIN 2002-01-18 08:30 Remark: pe within normal limits
CLIN 2002-01-27 07:00 Remark: mens.
CLIN 2002-02-20 07:00 Remark: mens.
HOUS 2002-03-13 11:30 Room: ab163/0005 Cond: p Out: 2002-04-29 14:15
WT Weight: 5.690 kg
CLIN 2002-04-20 08:00 Remark: mens.
CLIN 2002-04-24 10:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and
retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound pregnant; ~18 day
s
HOUS 2002-04-29 14:15 Room: ab142/0005 Cond: b Out: 2002-04-29 14:45
HOUS 2002-04-29 14:45 Room: ab163/0005 Cond: p Out: 2002-04-30 13:00
HOUS 2002-04-30 13:00 Room: ab142/0005 Cond: b Out: 2002-05-09 12:45
CLIN 2002-05-01 08:50 Remark: caretaker reports a sore on
left knee; 1cm skin
swelling with bruise around noted at rounds; using leg to
locomote and is alert/responsive and hydrated; no
treatment at this time - by a.u.
CLIN 2002-05-02 10:00 Remark: sore on left knee; apparentl
y improved over last
24 hours; should resolve without incident; no action
indicated - by.
HOUS 2002-05-09 12:45 Room: ab163/0005 Cond: p Out: 2002-06-22 07:00
WT 2002-05-10 12:30 Weight: 6.070 kg
WT 2002-05-20 08:30 Weight: 5.990 kg
CLIN 2002-05-22 09:30 Drug: p5-bb320 (diagnostic ultrasound of abdomen and
retroperitoneum, nos)
Amount: Route: Time:
CLIN Drug: p5-bb320 (diagnostic ultrasound of abdomen and
retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound pregnant; very ea
rly; ~ 16 days

(recheck); sire = 96027
CLIN Remark: ultrasound-not pregnant
WT Weight: 6.055 kg
CLIN 2002-05-23 10:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound pregnant; early
CLIN 2002-05-24 07:00 Remark: mens.
WT 2002-06-13 Weight: 6.040 kg
HOUS 2002-06-22 07:00 Room: ab163/0005 Cond: g Out: 2003-05-07 11:30
WT 2002-07-08 13:30 Weight: 6.480 kg
ASGN 2002-07-19 Project: 20011002 Released: 2002-07-26 Inves:
Title: dna profiling of primates used in biomedical research
BLD 2002-07-22 Project: 20011002 Quantity: 2.00 By: For:
P/S: A/V: Code: Caretaker: y
TB Lot: 415a Dilution: Eye: r Reaction: 0 0 0
WT 2002-07-22 08:00 Weight: 6.580 kg
CLIN 2002-07-22 09:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: pregnant; ultrasound positiv
e fetal heartbeat;
~77 days; tb and pe within normal limits;
CLIN 2002-07-22 09:30 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 70.0000 mg Route: im Time:
CLIN Remark: pe and tb test
WT 2002-08-16 10:00 Weight: 7.290 kg
WT 2002-09-17 13:00 Weight: 8.150 kg
WT 2002-09-30 10:30 Weight: 8.130 kg
WT 2002-10-10 13:15 Weight: 8.290 kg
CLIN 2002-10-21 07:00 Remark: mens.
CLIN 2002-10-21 10:00 Remark: delivered infant on 10-19 wi
thout incident;
estimated due date of 10-18; no evidence of problem - by

CLIN 2002-11-04 09:07 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 30.0000 mg Route: iv Time:
CLIN Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: 16 day post partum pe-unrema
rkable - by
WT 2002-11-04 09:15 Weight: 6.910 kg
WT 2003-01-03 09:30 Weight: 6.590 kg
CLIN 2003-01-08 Remark: "purina chow" rhesus diet re

placed with "teklad
2050" diet
TB 2003-01-22 Lot: 416x Dilution: Eye: l Reaction: 1 0 0
CLIN 2003-01-22 08:58 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 70.0000 mg Route: im Time:
CLIN Remark: pe and tb test
CLIN 2003-01-22 09:00 Remark: pe within normal limits
WT 2003-01-22 10:00 Weight: 6.360 kg
CLIN 2003-02-14 10:15 Remark: small cut on lateral aspect of d2 right hand; no
bleeding or swelling at rounds; bright, alert, responsive and hydrated; using hand/finger; no treatment indicated - by
CLIN 2003-04-22 07:00 Remark: mens.
WT 2003-04-25 10:30 Weight: 6.230 kg
CLIN 2003-05-07 07:00 Remark: infant weaned
HOUS 2003-05-07 11:30 Room: ab163/0005 Cond: p Out: 2003-06-05 11:30
WT Weight: 6.240 kg
BLD 2003-06-04 Project: 00300901 Quantity: 0.40 By: For:
P/S: A/V: Code: Caretaker: n
BLD Project: 00300901 Quantity: 2.50 By: jb For: jk
P/S: A/V: Code: Caretaker: n
CHEM Account:
Glucose: MG/DL SGPT(ALT): MU/ML
BUN: MG/DL Total Protein: GM/DL
Creat.: MG/DL Albumin: GM/DL
CK(CPK): MU/ML ALK Phosphatase: MU/ML
Cholest.: MG/DL Calcium: MG/DL
Triglyc.: MG/DL Phosphorus: MG/DL
SGOT(AST): MU/ML Iron: UG/DL
LDH: MU/ML Sodium: MMOL/L
Tot. Bili: MG/DL Potassium: MMOL/L
GGT: MU/ML Chloride: MMOL/L
Test: APTT 29.9 SEC
Test: INR 1.0
Test: PROTIME 14.1 SEC
CLIN 2003-06-04 09:10 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 30.0000 mg Route: im Time:
CLIN Remark: sedated for blood draw for c oagulated blood
testing - by j.b.
HOUS 2003-06-05 11:30 Room: ab163/0005 Cond: s Out: 2003-06-16 13:30
HOUS 2003-06-16 13:30 Room: ab163/0005 Cond: p Out: 2003-07-03 14:30
CLIN 2003-06-25 07:00 Remark: mens.
HOUS 2003-07-03 14:30 Room: ab142/0005 Cond: b Out: 2003-07-16 09:45
BLD 2003-07-08 Project: 00300901 Quantity: 3.50 By: au For: jk

P/S: A/V: Code: Caretaker: y
TB Lot: 416x Dilution: Eye: r Reaction: 0 0 0
CLIN 2003-07-08 08:55 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 70.0000 mg Route: im Time:
CLIN Remark: tb test/pe
CLIN 2003-07-08 10:00 Remark: tb and physical exam within normal limits;
WT Weight: 6.280 kg
VSER 2003-07-10 Account:
Virus: B Virus Result: positive
Virus: SRV Result: negative
Virus: SIV Result: negative
Virus: STLV-1 Result: negative
HOUS 2003-07-16 09:45 Room: ab163/0005 Cond: p Out: 2003-08-08 11:00
CLIN 2003-07-28 09:30 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound-not pregnant; men s., too early; redo later
CLIN 2003-08-01 07:00 Remark: mens.
CLIN 2003-08-01 11:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound not pregnant
CLIN 2003-08-04 13:20 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound-not pregnant
HOUS 2003-08-08 11:00 Room: ab142/0005 Cond: b Out: 2003-08-13 15:00
WT Weight: 6.280 kg
HOUS 2003-08-13 15:00 Room: ab142/0005 Cond: s Out: 2003-08-14 13:00
HOUS 2003-08-14 13:00 Room: ab142/0005 Cond: b Out: 2003-08-21 10:50
HOUS 2003-08-21 10:50 Room: ab163/0005 Cond: p Out: 2003-11-06 07:00
CLIN 2003-09-05 08:40 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound-pregnant < 25 day s; sire is r96027
WT Weight: 6.420 kg
CLIN 2003-09-13 07:00 Remark: mens.
CLIN 2003-09-15 07:00 Remark: mens.
CLIN 2003-09-16 11:00 Drug: p5-bb320 (diagnostic ultrasound of abdomen and retroperitoneum, nos)
Amount: Route: Time:
CLIN Remark: ultrasound-pregnant, ok (red

one)
WT Weight: 6.260 kg
WT 2003-10-15 10:00 Weight: 6.300 kg
HOUS 2003-11-06 07:00 Room: ab163/0005 Cond: g Out: 2003-12-02 09:30
WT 2003-11-13 09:30 Weight: 6.530 kg
HOUS 2003-12-02 09:30 Room: ab167/0005 Cond: g Out: 2004-03-22 11:00
WT 2003-12-09 11:00 Weight: 7.230 kg
WT 2003-12-22 10:45 Weight: 7.460 kg
WT 2004-01-07 11:20 Weight: 7.840 kg
TB 2004-01-16 Lot: 417a Dilution: Eye: l Reaction: 1 0 0
CLIN 2004-01-16 09:00 Remark: pregnant with positive fetal heartbeat; tb and
physical exam within normal limits;
CLIN 2004-01-16 10:00 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 80.0000 mg Route: im Time:
CLIN Remark: pe and tb test
WT Weight: 7.670 kg
CLIN 2004-01-27 07:00 Remark: newborn infant
CLIN 2004-02-02 07:00 Remark: mens.
WT 2004-02-05 09:00 Weight: 6.600 kg
CLIN 2004-02-05 09:32 Drug: c-64095 (medetomidine (domitor))
Amount: 0.2300 mg Route: im Time: 9:32
CLIN Drug: c-684c1 (atipamezole)
Amount: 1.1500 mg Route: iv Time: 9:49
CLIN Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 38.0000 mg Route: im Time: 9:32
CLIN Remark: s/o: anesthetized for post p artum exam; 9 days
out; uterus involuted; no post partum bleeding; good anesthetic recovery; a: pe within normal limits; p: no action indicated - by m.h.
CLIN 2004-02-08 07:00 Remark: mens.
HOUS 2004-03-22 11:00 Room: ab140/tmp1 Cond: g Out: 2004-03-29 09:40
HOUS 2004-03-29 09:40 Room: ab167/0005 Cond: g Out: 2004-07-07 08:30
WT 2004-05-07 Weight: 6.750 kg
TB 2004-07-07 Lot: 418x Dilution: Eye: r Reaction: 0 0 0
CLIN 2004-07-07 08:25 Drug: c-6a157 (ketamine hcl, vetalar)
Amount: 70.0000 mg Route: im Time:
HOUS 2004-07-07 08:30 Room: ab167/0005 Cond: p Out: 2004-07-07 10:00
HOUS 2004-07-07 10:00 Room: ab167/0005 Cond: g Out: 2004-07-08 08:25
WT Weight: 6.470 kg
CLIN 2004-07-07 10:30 Remark: s/o: physical exam is within normal limits. MH
a: p:
HOUS 2004-07-08 08:25 Room: ab167/0005 Cond: p Out: 2004-07-19 11:10
HOUS 2004-07-19 11:10 Room: ab167/0005 Cond: g Out: 2004-08-05 11:30

HOUS 2004-08-05 11:30 Room: ab167/0005 Cond: p Out: 2004-08-11 11:25
CLIN 2004-08-07 07:00 Remark: bruise on right knee
HOUS 2004-08-11 11:25 Room: ab167/0005 Cond: s Out: 2004-08-26 09:30
HOUS 2004-08-26 09:30 Room: ab167/0005 Cond: p Out: 2004-09-10 08:00
HOUS 2004-09-10 08:00 Room: ab167/0005 Cond: s Out:

"G" 2nd Revision CONFIDENTIAL

OCT 29 2003
emailed w/
RESEARCH ANIMAL
RESOURCES CENTER
orig 7.28.03
1st 10.01.03

Revision 11/99

Protocol Code: A-55-1000-L00336-3-07-03

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

elp 10.9.03

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only
Committee
Action:_____

Veterinarian Signature _____ Date: _____

Chairperson Signature: _____ Date: _____

Type of Protocol Procedure _____

Type of Surgical Procedure: _____

✓ Survival Surgery ____ Non-Survival Surgery ____ Rodent Surgery ✓ Non-rodent Surgery
____ Multiple Major Survival Surgery ____ Exercise Exemption ____ Paralytic Agents ✓ Restraint
____ Critical Veterinary Care ____ Fluid/Food Restrictions ____ Nonstandard Housing ____ Nonstandard Husbandry
✓ Occupational Health/Personnel Safety ____ Class B Dog/Cat ____ Biohazards ____ Radiation
____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted]

   Telephone Numbers: Office: [redacted] Lab: [redacted] Animal Emergency: [redacted]

   Home: [redacted] Fax: E-mail Address: [redacted]

If Investigator is Unavailable for Emergency

   Alternate to Contact With Authority to Act in Investigator's Absence: [redacted]

   Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Psychiatric Institute. Office Address: [redacted]

   Unit & Division Number (UDDS): A – 55 - 1000

3. Is this protocol a: NEW **RENEWAL** AMENDMENT application? (Circle appropriate category)

   If Renewal or Amendment application, please give current protocol code: **A-55-1000-L00336-4-07-00**

4. Is this protocol for: **TEACHING or RESEARCH: (indicate research type) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER** _____**(SPECIFY)**
Circle **all** that apply.

5. **Title of this project: The Effects of Psychological Stress on Healing in Non-human Primates.**

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) **1 2 3 4 5**

7. Will ANY surgery be performed on any of the animals? **YES** **NO** If yes, you must fill out questions 24-30.
Will you be working with wild-caught animals? **YES** **NO** If yes, you must fill out questions 31-34.
Will you be using non-human primates? **YES** **NO** If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs or will animals be housed outside of their normal animal housing areas? **YES** **NO** If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s):

Animals will be removed from their normal animal housing area for procedures and testing only. Animals will be tested in [redacted] for no longer than 1.5 hours per test period. Animals will be returned to their home cage following all procedures and tests. See flow chart.

9. NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.

a) Species of Animal: Total Number For **3** yr.: Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
(#1) Rhesus Monkeys (*M. mulatta*).
(#2) A maximum of 30 animals will be biopsied per year. (90 over 3 years)
(#3) The subjects will be obtained from the National Primate Research Center and Harlow Center for Biological Research.

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) **YES** **NO**

c) Have any of the animals from above been part of any other protocols. **YES** **NO** If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research.

These studies will involve animals that are naive to the healing protocol. Animals that are understudy in our lesioning protocol may also be subjects. All animals will be naïve to the healing protocol. No more than 20 per year will have been part of lesioning studies. Our initial results demonstrate an effective method to assess the molecular mechanisms underlying wound healing and the effects of stress on this process in primates. In subsequent work, we would like to capitalize on using our lesioned monkeys, as this will provide insight into the brain mechanisms that underlie these effects on wound healing. This will maximize the use of these animals from the standpoint of data that is medically relevant to humans. The wound healing studies are identical and have no different risks whether they are performed in intact or lesioned animals. The animal's history records will be evaluated by the principle investigator and senior research staff to determine that previous use will not compromise the animal's health or proposed research.

Animals that have been used in studies with the same or similar stress models would be excluded, as well as, animals that are currently not healthy and have significant medical illness.

If naïve animals are not available we will have to use animals that have been assigned to other protocols. These animals will be evaluated based on their individual history for inclusion into this study.

10. Building(s) or facility where the animals will be housed (normal housing):

[redacted]

11.

a) Outline the specific scientific goal(s) and significance of this research in straight-forward, non-medical, non-technical language that would be understandable to a **layperson**.

The goal of this research is to establish a skin biopsy healing model in non-human primates in order to investigate the effects of psychological stress on various aspects of the healing process. Several recent studies in humans and rodents have indicated that stress can significantly delay healing and suppress the function of the immune system. Delayed wound healing can have many deleterious health consequences such as increased risk of bleeding and infection in patients who have sustained trauma or undergone surgery.

Our laboratory is currently investigating the mechanism of stress and anxiety in primates. Very little is known about the mechanisms that mediate fear and anxiety in humans. These studies can increase our knowledge about these mechanisms and open ways of understanding the causes of common psychiatric disorders like anxiety disorders and mood disorders.

We will do punch skin biopsies on the monkeys and using serial photographs monitor the effects of psychological stress on the healing process. Blood samples will be collected from the animals on a regular basis to measure several hematological, immunological and endocrinological parameters.

We have initial data from these studies that demonstrates an effect of stress exposure on healing in primates. Initally, we established the parameters needed to study wound healing in primates and the effects of stress on wound healing. Our findings are pointing to molecular mechanisms that may mediate the effects of stress on delayed healing. For example, the findings indicate that the expression of 2 genes that are involved with the inflammatory response and white blood cell migration are affected. Our ongoing studies in intact monkeys will continue to address these issues which will be very informative in understanding the mechanism underlying the effects of healing in humans. Furthermore, by using these procedures in our amygdala and prefrontal cortex lesioned animals, we will be able to assess the role of specific brain regions in mediating these effects in the skin. This is crtical to understanding how stress, as it is processed by the brain, actually alters wound healing in humans.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.

This protocol has been used in humans and rodents. However, because of species differences, we need to perform non-human primate studies to link and to inform the basic studies in rodents with the clinical human studies. Because of species differences it is very difficult to link the results of rodent studies to humans. With the controlled laboratory stress models that we will use with primates we are much better able to inform the basic laboratory studies in rodents with the human studies. As mentioned above we are studying the mechanism of anxiety and fear in primates in our laboratory. After establishing a healing model in primates we are going to use this model to investigate the effects the various changes in the limbic system, such as orbitofrontal cortex and amygdala inactivation, have on the immune system. Therefore these studies can not be done in humans. Because of the immense complexity of these biological systems, methods like computer simulation or in vitro systems are not possible.

c) Provide justification for why you have chosen the species cited in 9-A for your work.

These studies must be done in primates because of their important resemblance to humans. In other animal species, like rodents, the structures of the limbic system and its multiple connections to other brain regions are very different from humans. Non-human primates will be used because they provide the best model to study human emotionality

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.

We will use the lowest number of animals we can in these studies and estimate that no more than 30 subjects per year will be needed to answer our questions. To obtain statistically reliable results when measuring skin healing and the various immunological parameters we estimate that 15 animals will be needed in both the study group and control group or a total of 30 animals. Depending on the results of this study we are planning to use this model in other animals in subsequent years. Based on the effect size from our initial data collected under this protocol we estimate we will need 15 animals per group. We are looking at the expression of multiple genes.

13. Indicate any current or pending funding for this project:

Funding Source (1): NIH — Grant Number (1): MH46729
Title of Grant (1): Development and Regulation of Emotion in Primates.

Funding Source (2): — Grant Number (2)
Title of Grant (2):

Funding Source (3): — Grant Number (3):
Title of Grant (3):

14. Identify the person(s) or unit responsible for **daily** animal care:

[redacted] Care Staff will care for all primates.

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	27 yrs. Primate Research Experience
		38 yrs. Animal Research, 32 yrs w/Primates
		1 yr Primate Research Experience
		21 yrs. Primate Research Experience
		2 yrs Primate Research Experience
		1 yr. Primate Research Experience.
		1 yr. Primate Research Experience.
		1 yr. Primate Research Experience.
		1 yr. Primate Research Experience.
		1 yr. Primate Research Experience.
		Student in training
		Student in training

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.

Use of recent medical journals, topical Medline searches, books, and information obtained from colleagues. Medline search was done in May 2000 covering the years from 1966-2000 using the keywords wound healing, psychological stress and animal research. An updated expanded literature search was completed in July 2003. It incorporated the above keywords and also included bacterial/wound infection, immunosuppression, skin biopsy, and healing models. The search covered a period from 1966 to the present. We are in close collaboration with Drs. John F. Sheridan, Phillip T. Marucha and David A. Padgett at Ohio State University who are pioneers in this area of research.

b) Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.

N/A

Category	Specific Material(s)

_____ Recombinant DNA

_____ Genetically Altered Materials

_____ Infectious Agents

_____ Bacteria

_____ Virus

_____ Prion

_____ Carcinogen or Mutagen

_____ Toxic Agent

Status of OBS-2: (circle one) APPROVED PENDING

_____ Radioactive Material

Status of 99-A: (circle one) APPROVED PENDING

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.

No employee is allowed access to animal housing areas or allowed any interaction with animals until they have successfully completed the following:
RARC Animal Care and Use Course
RARC Primate Handling Course
UW Radiation Safety Course
Herpes B Quiz
[redacted] Tour and Orientation.

After completion of all orientation and training, personnel will be issued keys for animal area access. When doing skin biopsies and handling primates we will use all appropriate precautions and protections. All personnel working with animals or in animal housing and testing areas are required to wear a lab coat or appropriate apparel, latex gloves, eye protection and a surgical mask. Shoe coverings or designated shoes are also required. All personnel are trained to dispose of animal waste and biological sampling supplies in the properly labeled receptacles.

To ensure animals from the [redacted] will be kept separate from each other to avoid the possibilities of transmission of disease we will transport them in transports, which will be placed inside a rigid secondary leak proof container, from their home facility. Any apparatus involved in the study will be sanitized with disinfectant to ensure no contact between animals from each facility. All staff are well versed in the PPE standards.

You must address questions 17 separately for each species.

17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all

drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary).

To establish a healing model we will do punch skin biopsies on rhesus monkeys. The animals will be anesthetized with 15 milligram/kilogram Ketamine intramuscularly. A sterile 3.5 millimeters full-thickness skin biopsy will be obtained from a shaved area on the back of the animal between the scapula, a site that animals cannot reach with their hands. This is a very safe and common procedure that is used routinely in awake humans in every dermatology clinic. Each animal will have a maximum of six biopsies done over three years. We then will use a 6.0 millimeter skin biopsy kit to obtain a second tissue sample. -The second biopsy will sample at the initial biopsy site. This will allow for the assessment of the process of healing. Differing intervals will be used between the 2 biopsies ranging from 4 hrs- 2wks.

To measure the healing process, skin biopsy areas will be photographed approximately every 2 days until healed. The digitized photographs will be analyzed by a computer. Human studies have shown that healing of punch skin biopsies takes 35 – 40 days. We have observed that surgical incisions in rhesus monkeys heal in approximately 12 –22 days. We expect that healing from punch biopsies will be comparable.

Half of the animals will be put under psychological stress for eight consecutive days. We will use four different stressors and animals will be exposed to each of them twice during the eight days. These stressors are:

1. Chair restraint for 1.5 hours/day on two separate days.
2. Visual exposure to a significantly older and bigger animal for 1.5 hours/day on two separate days.
3. Administration of 12 brief blocks of random air blasts over a period of 1.5 hours/day on two separate days.
4. Chair restraint with visual exposure to older and bigger animal for 1.5 hours/day on two separate days.

On day four after stress begins, a punch skin biopsy will be obtained from all the animals.

Blood samples (7 milliliters or less of venous blood from an arm vein) will be obtained by saphenous venipuncture every four to eight days from all the animals for measurements of various hematological, endocrinological and immunological parameters such as blood cell count, serum cortisol, IL-1, IL-6, TNF-alpha and natural killer cell activity. Each animal will receive a maximum of ten blood samples. Clearly no more than 40 milliliters of blood will be drawn over a 2 week period. Blood volumes will not exceed the recommended 10% of 60 mililiters per kilogram or no more than 6 mililiters per kilogram in any 2 week period. Two weeks after healing is complete we will repeat the process crossing over the groups. See flow charts.

Flow Chart

I.

	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10	Day11	Day12
	BD		BD	P		BD/P		BD/P		P		P
M-1	Chair	Rand A	C + Big M	Big M+Bx	Chair	Rand A	C + Big M	Big M				
M-2	Big M	Chair	Rand A	C+Big M+Bx	Big M	Chair	Rand A	C + Big M				
M-3				Bx								
M-4				Bx								

	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24

		BD/P		P		P		P		BD/P		P
M-1												
M-2												
M-3												
M-4												

Will continue for approximately 35-40 days.

M-1 = Monkey 1
BD = Blood draw
Chair = Chair restraint (1.5 hours)
Big M = Visual access to bigger monkey (1.5 hours)
Rand A = Random air blasts (1.5 hours)
C + Big M = Chair restraint + visual access to bigger monkey (1.5 hours)
Photo = Photographing of wounds
Bx = Skin biopsy

b) Do any animals undergo any type of restraint beyond normal housing methods? **YES** **NO** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable---available on the web at: www.rarc.wisc.edu

Each animal in the experiment group will be placed in a restraining chair for four 1.5 hour periods over eight days. In other protocols we have been approved to restrain primates for extended periods of time with successive doubling of restraint time. However, in this protocol the initial short adaptation period is being used as a stressor. The animals will be monitored continuously during the chairing periods.

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.) **YES** **NO** If YES, indicated nonstandard husbandry required and justification for this practice.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

We do not anticipate that the animals will experience more than momentary slight pain or discomfort from the procedures. As previously mentioned, this procedure is frequently done in awake humans in routine medical practice and is not known to cause any significant pain or discomfort. For the skin biopsy procedures, 15 milligrams/kilogram Ketamine will be administered intramuscularly to provide anesthetic (general-dissociative). If there are any signs of discomfort after the animal is awake and alert, Acetaminophen (6 milligrams / kilogram) will be administered orally. The veterinary staff will be contacted if a more aggressive treatment is needed. However, based on our earlier experience with the biopsy technique we have found that no analgesia post-anesthesia is necessary. The animals show no evidence of discomfort.
The stressors will elevate stress hormones acutely but will not cause any physical pain. Based on early work, we do not expect the stressors to have any significant adverse effects on the animals' health.

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

Animals will be monitored as always, daily, for signs or symptoms of ill health by checking fecal material, food intake,

behavior, and general appearance. Animals will be weighed monthly and their weights reported to [redacted] records. In the case of unanticipated illness or injury the veterinarians will be contacted and if necessary euthanasia will be considered under veterinary consultation. The biopsy areas will be monitored closely as part of the investigation and the animals will be monitored continuously during chairing.

20. If experiments could induce chronic disease, tumors or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.

Acute stress will resolve over a short time period and based on our prior work they will not affect the health of the animals. No chronic illness is anticipated.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

If an unanticipated illness or injury occurs during this study the subject, under the supervision of the attending Veterinarian and Pathologist, will be anesthetized using Ketamine HCL (15 milligram per kilogram) intramuscularly. The animal will be humanely euthanized with an overdose of sodium pentobarbitol greater than 50 milligrams per kilogram intravenously and then perfused with heparanized saline and 4% paraformaldehyde. This is a standard method of euthanization and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?

Animals will be returned to [redacted]

23. Will any animal products be used for human consumption? **YES** NO

If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____.

Attending Veterinarian:_____

(SVM ONLY)

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?:

Last name, first	Type and length of **SURGICAL** training/experience
[redacted]	M.D. training. 26 years of primate surgical experience.

[redacted] M.D. training.

25. Where will surgery be performed? Room(s): [redacted] Building: [redacted]

26. How many animals listed in question nine will undergo surgery?

A maximum of 30 animals per year.

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.

A standard dose of Ketamine, 15 milligram/kilogram-intramuscularly, will be given before obtaining the skin biopsies. Animals will be monitored continuously until they no longer support their body weight and do not respond to stimuli. After the 5 minute procedure is completed the animals will be visually monitored every 15 minutes for regular respiration until awake, alert, sitting up and mobile.

a) Are any paralytic agents being used **YES** **NO** If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. **Surgical Procedures** (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies.

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be used for all survival surgery. (Append additional page if necessary).

Under ketamine anesthesia, the hair on the back of the animal will be clipped and the skin prepared with iodine and alcohol. We will use the same standard sterile equipment for taking the biopsy as is used in humans. With a sterile disposable skin biopsy kit a 3.5 millimeters full-thickness skin biopsy will be obtained from each animal. The whole procedure takes approximately ten minutes. The actual sizes of biopsies will be 4 millimeters in diameter (actual size represented by dashes) (---) and 1.5 millimeters deep (--).

We will later use a 6.0 millimeter skin biopsy kit to obtain tissue samples from the same biopsy site for histological research. The actual sizes of the second biopsies will be 6.5 millimeters in diameter (actual size represented by dashes) (-----) and 1.5 millimeters deep (--).

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

Hair will be clipped and the skin area will be prepared with iodine and alcohol. Lab coats or surgical scrubs, sterile gloves, and surgical masks will be worn during the procedure. A single use sterile biopsy kit will be used for obtaining the biopsies.

29. Will the animals be allowed to recover from surgery? **YES** **NO**

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

Post biopsy monitoring will be done visually no less often than every 15 minutes until the animal is sitting up. We will carefully monitor the animals' for any signs of pain or infection. As mentioned in the protocol, this procedure is commonly done in humans without causing any significant pain. We do not expect the animals to suffer more than very slight, if any, discomfort from the procedure. In the very unlikely event an animal suffers pain post biopsy buprenorphine (0.01 milligram per kilogram intramuscularly) or Acetaminophen (6 milligrams / kilogram) will be administered either orally. Analgesics are

almost never prescribed for humans undergoing this procedure.

We will keep the skin area as clean as possible. The animals will not be able to touch the biopsy site, die to its location. They will be single caged during the healing period in order to prevent access and manipulation of the biopsy site from other animals.

The biopsy site will not receive any suture. When these biopsies are done in humans, no sutures are needed. Sutures would interfere with monitoring the healing procedure.

30. Will any animal(s) be allowed to recover from **more than one major operative procedure**? **YES** **NO**
(A major operative procedure is defined as any surgical intervention that penetrates **and** exposes a body cavity **or** any procedure which produces permanent impairment of physical or physiological functions.)

N/A.

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals:

b) What is minimum length of time between the operative procedures?

N/A.

QUESTIONS FOR PROJECTS USING WILD-CAUGHT ANIMALS

It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild?

N/A.

YES **NO, Observation only**

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked.

N/A.

33. a) Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

N/A.

b) If animals will be release back to the wild, justify why this release will not result in disease exposure to wild population

N/A.

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released.

N/A.

QUESTIONS FOR PROJECTS USING NON-HUMAN PRIMATES

35.

a) If non-human primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

The animals will be individually housed for approximately eight weeks. This period encompasses the three week period of adaptation and a maximum of five weeks for healing. We are concerned that the animals will touch and pick the others biopsy areas while grooming and that may affect the healing process. However, as soon as healing is complete each animal will be returned to pair housing.

b) Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.

There will be no restrictions to environmental enrichment in this study

Revision 11/99

2nd Rewrite "L"

CONFIDENTIAL

email Hlle
JUL 30 2004
Orig 5.25.04
RESEARCH ANIMAL RESOURCES CENTER
1st Rew 6.25.04

Protocol Code: ___-___-___-___-G00382-3-10-01

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only
Committee Action: **AMENDMENT APPROVAL**

Veterinarian Signature _____ 7.30.04 **Date** [redacted] **Veterinarian**

Chairperson Signature: _____ Date: [redacted]

Type of Protocol Procedure: _____ 7.30.04 **Date** **Chair**

Type of Surgical Procedure: _____

_____ Survival Surgery _____ Non-Survival Surgery _____ Rodent Surgery _____ Non-rodent Surgery

_____ Multiple Major Survival Surgery _____ Exercise Exemption _____ Paralytic Agents _____ Restraint

_____ Critical Veterinary Care _____ Fluid/Food Restrictions _____ Nonstandard Housing _____ Nonstandard Husbandry

_____ Occupational Health/Personnel Safety _____ Class B Dog/Cat _____ Biohazards _____ Radiation

_____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted] C

   Telephone Numbers: Office: [redacted] Lab: [redacted] Animal Emergency: Unlisted, call hospital paging

   Home: Unlisted, call hospital paging Fax: [redacted] E-mail Address: [redacted]

If Investigator is Unavailable for Emergency

Alternate to Contact With Authority to Act in Investigator's Absence: [redacted] C

Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Department of Psychiatry

   Office Address: Dept. of Psychiatry, [redacted]

   Unit & Division Number (UDDS): Unit #A, Division #55

3. Is this protocol a: **NEW** **RENEWAL** **AMENDMENT** application? (Circle appropriate category)

   If Renewal or Amendment application, please give current protocol code: **A-55-1000-G00382**

4. Is this protocol for: **TEACHING or RESEARCH**: **(indicate research type) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER** _____ **(SPECIFY)**
   Circle **all** that apply.

5. Title of this Project: **In Vivo Examination of Brain Systems Related to Emotional Expression in Primates.**

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) 1 2 [3] 4 5

7. Will ANY surgery be performed on any of the animals? [YES] NO If yes, you must fill out questions 24-30.
Will you be working with wild-caught animals? YES [NO] If yes, you must fill out questions 31-34.
Will you be using non-human primates? [YES] NO If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs or will animals be housed outside of their normal animal housing areas? YES If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s:

MRI scan procedures are done at [redacted] PET scan procedures are done at [redacted]) and the [redacted]. Animals will be away from their normal housing areas for approximately 3 hours.

9. **NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.**

a) Species of Animal:	Total Number For 3 yr.:	Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
(#1) Rhesus Monkeys (M. mulatta)	Maximum 220 animals.	WI National Primate Center, and Harlow Center for Biological Psychology
(#2)Northern Pine Snakes (P. Melanolencus)	1	Commercial vendor.

The experimental animals in Experiments 1, 2, and 3 will have received surgery as part of another protocol.

Experiment 1: Amygdala Lesion(N=24)
12 Lesion
12 Control

Experiment 2: Orbitofrontal Bilateral Lesion(N=24)
12 Lesion
12 Control

Experiment 3: Unilateral Lesion (OFC)(N=48)
12 Right lesion
12 Left lesion
12 Bilateral lesion
12 Control

These animals will be obtained from the primate colony after animal histories are evaluated on a case-by-case basis to determine suitability for this project.

Experiment 4: Exposure to Stimuli(N=64)
4a: Behavior Adaptation Test (n=16)
4b: Snake Exposure (n=16)
4c: Novel Animal (n=16)
4d: Learning/conditioning (n=16)

Experiment 5: Individual Differences in the Behavioral Adaptation test (n=60)

Total Animals 220

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) YES NO [N/A]

c) Have any of the animals from above been part of any other protocols. [YES] NO If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research.

Not all animals assigned to this protocol will have been part of other protocols. Animal histories will be evaluated on a case-by-case basis to determine suitability for this project. All lesion animals will have had surgery while assigned to protocol G00181.

10. Building(s) or facility where the animals will be housed (normal housing).

[redacted]

11.

a) Outline the specific scientific goal(s) and significance of this research in straight-forward, non-medical, non-technical language that would be understandable to a **layperson**.

This research is important because it will allow us to understand the brain systems that mediate anxiety and depression in humans. Using non-invasive positron emission tomography (PET), we intend to monitor regional cerebral glucose metabolism, blood flow, changes in neuroreceptor density and the synthesis rate of dopamine, serotonin, CRH and other brain systems in appropriate regions of the brain in monkeys We also intend to monitor changes in these parameters in monkeys that have had brain regions inactivated as part of another protocol. These PET imaging studies will use radiolabeled tracers to assess regional brain activity to elucidate our understanding of how the brain works.

This protocol will serve as our standard operating procedure for PET scans, which will use animals from other protocols, as well as animals assigned only to this protocol.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.

Our work and other studies in the field have clearly demonstrated the importance of using animal models to develop a better understanding of the factors involved in anxiety and depression. These studies have the potential to ultimately result in the development of new treatments for patients suffering from anxiety and depressive disorders. Animals are used for these studies because many of the novel tracers being developed have not been approved for human use. Furthermore, these studies involve neuronal inactivation that cannot be done in humans. The only other alternative to the PET studies would be to perform autoradiography. Autoradiography can only be done once and requires euthanizing the animals, while PET is a relatively non-invasive procedure that can be done repeatedly. PET imaging has been done extensively in rhesus monkeys, and a useful database exists which will facilitate these studies.

c) Provide justification for why you have chosen the species cited in 9-A for your work.

Rhesus monkeys provide the best animal models to study mechanisms underlying severe mental illnesses. They also are critical for the development of new treatments for these disorders. Rhesus monkeys have been chosen for this work because of the similarity of their brain function and anatomy to that of humans. Their brain is also large enough to see regions of interest on the PET images that are too small to be seen in many other species. Rhesus monkeys provide a unique opportunity to study functioning brain systems.

It is necessary to use a live Northern Pine Snake because previous data from our laboratory are based on this model. Furthermore, we have shown that the response to an artificial snake is less robust than the response to a live snake.

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.

Previous experience with the variance found in similar data sets (amygdala studies) and a power analysis based on these data sets have been used in determining the minimum number of subjects required for these experiments. The effect size of the difference between the experimental and control groups is d=1.02. The power to detect a difference of 0.05 in the predicted direction for this effect size with an N of 10/group is 0.70. With recent experience , it is clear that up to 16 animals maybe required. We do not know the effect size for each of these tracers until we have gained experience with them. For those scans that will not require arterial access monkeys maybe scanned up to 12 times at weekly intervals. Weekly weights will document that they will not have a decrease of weight greater than 5%. For those scans requiring arterial access only 2 will be done per animal.

The Northern Pine snake will only be used as a stimulus and will not be tested.

13. Indicate any current or pending funding for this project:

Funding Source (1): Psychiatric Institute Grant Number (1): MH46729

Title of Grant (1): Development & Regulation of Emotions in Primates

Funding Source (2): NIH Grant Number (2): MH69315

Title of Grant (2): Affective Style: Social And Biological Substrates

14. Identify the person(s) or unit responsible for **daily** animal care:

[redacted] animal care staff.

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	23 yrs. Primate Research Experience
		38 yrs. Animal Research, 35 yrs w/Primates
		21 yrs. Primate Research Experience
		16 years, PET scanning of monkey brain
		16 years, PET Scanning of monkey brain
		2 yr Primate research experience
		26 Months Primate research experience
		22 Months primate research experience
		21 Months primate research experience
		8 Months primate research experience
		Student in training
		Student in training
		Student in training
		Student in training
		Student in training
		Student in training

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.

We consult current journals, topical searches of the Primate Information Center (Washington Regional Primate Research Center, Seattle) on-line data base, Medline, Index Medicus, Psych. Abstracts, and other publications and books for alternatives to procedures that might cause pain or discomfort to research animals, to assure that work is not redundant, and to explore other potential experimental models. The following Medline & Biological Abstracts keyword searches were updated in 4/04: medetomidine and ketamine. In 5/04 we searched Primates, benzodiazepines, and valium. In 6/04 we searched the following keywords: PET, EMG, EEG, EKG, ACTH, Cortisol, CSF, MRI, Anxiety, Stress, Fluothane, Acetaminophen, Xylazine, MRI, and Fluorodeoxy-glucose (FDG) and primates. The data bases have been searched as far back as the database extends. We receive neuroscience publications on a bi-weekly basis, along with such journals as Nature and Science. We attend numerous conferences where we present our own relevant data and meet with and attend lectures by experts in our area of research. In addition we regularly invite colleagues to our department to present their research.

b) Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.

Category	Specific Material(s)
_____ Recombinant DNA	The following tracers will be administered by IV or by inhalation at a sub-pharmacologic tracer dose of 0.6 milligrams per kilogram or less.
_____ Genetically Altered Material	
_____ Infectious Agents	18F-Fallypride (dopamine D2 receptor) 11C-WAY-100635 (serotonin 5-HT1a receptor) 18F-MPPF (serotonin 5-HT1a receptor) 11C-Raclopride (dopamine D2 receptor 11C-SCH 23390 (dopamine D1 receptor) 11C-Diprenorphine (opiate receptor) 11C-Flumazenil (benzodiazepine receptor) 11C-CFT
_____ Bacteria	
_____ Virus	

_____ Prion

_____ Carcinogen or Mutagen

_____ Toxic Agent

Status of OBS-2: (circle one) APPROVED/PENDING

__X___ Radioactive Material

Status of 99-A: (circle one) APPROVED PENDING

18F-CFT (dopamine transporters)
18F-L-DOPA
18F-6-FMT
18F-FDG
18F-F-DOPA
18F-FMPT
18F-FMTrp
18F-Deoxyglucose analogs
15-0-H20
15-0-CO2
15-0-CO
15-0-O2
14-0-H20
14-0-CO2
14-0-CO
14-0-O2
10C-CO2
17F-CH3F
CRH Analogs

None of these agents have a 1/2 life that exceeds 110 minutes; most are significantly shorter.

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.

Radiation Safety will approve personnel to work with radioactive compounds. All personnel exposed to radioligands will have been trained by taking the radiation safety course. Exposure to radiation will be monitored for all personnel. All personnel involved in the use of radioactive tracers operate under procedures approved by the UW Safety Department, and are monitored regularly for radiation dose with badge and when appropriate, ring dosimeters. These short lived tracers decay quickly to background levels, and pose no risk to personnel following the studies. Whenever possible, exposure will be limited by decreasing time of exposure and increasing distance from the radiation source. Areas where radiation is in use will be properly marked until background levels of radiation have been achieved by decay (usually overnight). Any contaminated materials will be isolated until the radiation has decayed to background levels before being properly disposed of.

You must address questions 17 separately for each species.

17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

MRI:

Animals that have received surgery while assigned to protocol G00181 will be imaged with MRI pre and post surgery. These MRIs which will also be used to co-localize with PET and to image the lesions at two time points following surgery. Animals from experiments 4 and 5 will undergo MRI for co-localization of PET scans.

Monkeys are transferred to [redacted] for an MRI which is used to co-localize regions of interest defined by PET with MRI defined anatomical structures. Under anesthesia with ketamine (15 mg/kg, as needed) or a mixture of ketamine hydrochloride (5 mg/kg) and medetomidine (30 μg/kg) intramuscularly, the brain is imaged with the monkey positioned in a stereotaxic head holder. If medetomidine is used, it can be reversed with atipamezole (150 μg/kg) administered (IM or IV) at the end of the procedure. Atropine (< .027 mg/kg, IM) may also be administered. Acquiring the MRI takes approximately one hour

not including set up and travel time. Each animals will receive a maximum of 4 MRI scanning sessions at least one week apart. In most cases, animals will only be scanned twice. However, we may need to scan four times because of developmental brain changes as well as to take advantage of new technological advances in imaging equipment. After the MRI is acquired the monkey is returned to its home cage in [redacted]

PET:

For those scans that will not require arterial access monkeys maybe scanned up to 12 times at weekly intervals. Weekly weights will document that they will not have a decrease of weight greater than 5%. For those scans requiring arterial access only 2 will be done per animal.

All animals will be scanned with Positron Emission Tomography (PET). Animals will be adapted to catheter placement and radiotracer administration for up to 21 days. This will involve transporting animals from their home cage to the restraint table where we will simulate catheter placement (while not actually placing a catheter) and shaving of the calf area (although we will shave on the last day of adaptation). Animals will be treated with pharmacological agents that affect the benzodiazepine system, specifically Valium and Xanax, at doses not to exceed 100mg/kg and Flumazenil and betacarboline as well as an appropriate vehicle in the same dose ranges. These drugs will be given during uptake of radioligands. During this time EEG will be sampled while animals are hand restrained as another measure of brain activity. Animals will be hand restrained while awake by the arms and legs in the prone position for a maximum of 1 1/2 hours. These drugs are short-lived and will not have any long-term effects on the monkeys.

The animals are anesthetized with ketamine (15 mg/kg, as needed) or a mixture of ketamine hydrochloride (5 mg/kg) and medetomidine (30 μg/kg) intramuscularly to permit the transfer of the animal to [redacted] and also to allow placement of in-dwelling saphenous catheters for the administration of a radiotracer (<10.0 milliCurie). If medetomidine is used, it can be reversed with atipamezole (150 μg/kg) administered (IM or IV) at the end of the procedure. Atropine (< .027 mg/kg, IM) may also be administered. When necessary, arterial lines will be inserted percutaneously or via cutdown in order to take blood samples to be used to follow blood tracer kinetics and allow blood analysis to follow the time course of tracer metabolism. A maximum of 7 ml of blood will be collected during each PET study. During the PET study, anesthesia will be maintained with up to 5% isoflurane for a maximum of 3 hours. After the PET study the arterial and venous lines will be removed and bleeding controlled by pressure as required. The animal will be transported from the PET scanner to a room that has been pre-approved for radioisotope use at [redacted] until the injected radioactivity decays to background levels. The imaging agents will be labeled with accelerator-produced short-lived positron emitters such as F-18 or C-11. None of these agents have a half-life which exceeds 110 minutes; most are significantly shorter. A minimum of 7 days will be allowed for animals to recuperate after a PET scanning session. No more than 12 scanning sessions will be done per monkey per year. All scans will involve administration of a tracer. PET procedures are routinely done in humans with no permanent adverse sequellae. Our research group at the UW has more than 15 years experience using PET to study monkey brain activity.

For the PET imaging the following tracers will be administered intravenously or by inhalation at a sub-pharmacologic tracer dose of 0.6 milligrams per kilogram or less. These compounds are given in minute amounts and have no harmful effects. As stated in question 12, animals will receive approximately 2 different tracers, none given simultaneously. Animals may receive multiple scanning sessions using these tracers. The tracers with short half-lives, such as oxygen, are typically given by inhalation. Tracers with a longer half-life are administered IV (intravenous). Each of these 27 radio-labeled compounds has been developed to trace different substances in the brain with varying temporal resolution. These tracers are administered in sub pharmacological doses which means they have no effects. While we list 27 compounds we are only using 6 different radiotracers. A radiotracer is the radioactive chemical that is attached to another chemical to form a compound (e.g. for 18-FDG, Flourine-18 (18.5) is the radiotracer and F-18 deoxyglucose (18-FDG) is the compound). The six radiotracers we plan to use are 18F, 11C, 15-O, 14-O, 10-C and 17-F. 18F-FDOPA and 18F-FMTrp will be administered at a mass dose of 0.6 milligrams per kilogram. All other tracers will be administered in doses of .2 milligrams per kilogram.

18F-Fallypride (dopamine D2 receptor)
11C-WAY-100635 (serotonin 5-HT1a receptor)
18F-MPPF (serotonin 5-HT1a receptor)
11C-Raclopride (dopamine D2 receptor
11C-SCH 23390 (dopamine D1 receptor)
11C-Diprenorphine (opiate receptor)
11C-Flumazenil (benzodiazepine receptor)
11C-CFT
18F-CFT (dopamine transporters)
18F-L-DOPA
18F-6-FMT
18F-FDG
18F-F-DOPA
18F-FMPT
18F-FMTrp
18F-Deoxyglucose analogs
15-0-H20
15-0-CO2

15-0-CO
15-0-O2
14-0-H20
14-0-CO2
14-0-CO
14-0-O2
10C-CO2
17F-CH3F
CRH Analogs

Animals will be exposed to the following tests during tracer uptake PET scanning.

Behavioral Adaptation Test

This test is used to assess the animal's behavioral response to the presence of a human making and avoiding eye contact. Behavior is recorded for a maximum of one hour each time, once per week on no more than nine occasions.

Learning Tests and Snake Exposure

These standard learning tests are used to evaluate the brain areas involved in different aspects of learning. After the animals are adapted to the test situation they will be tested on matching-to-sample, spatial and non-spatial reversals, oddity learning, reward devaluation and their response to a snake. The animals are tested for their ability to move the correct object to uncover a treat or the time it takes to remove a treat from the top of a clear plastic box that encloses and protects the snake or object. Most of these tasks have significant enrichment value, providing cognitive stimulation and positive reinforcement. Animals will be tested every week a minimum of five days per week for a maximum of one hour and 30 minutes each day, for as many weeks as it takes to complete the task. Some of these tests may take several months to complete. Animals will receive treats ~~D~~ during these tests. In addition they will be given their normal daily food ration following testing. Testing will end daily before 4 pm which provides animals with enough time to finish eating their daily food ration before the lights go off. Animals almost always eat their daily ration by the next morning making it unnecessary to remove left over food. Animals will always have access to water. In the rare instance an animal does not perform, despite being offered several different treats, normal daily food rations will be withheld for one day in an attempt to increase motivation. If the animal is pair-housed, the cage-mate will be fed while the subject is being tested. No animal will be deprived for more than one day each week for up to one month. During this brief food deprivation period animals will be weighed once a week. In the unlikely event a greater than 5% weight loss is observed, food deprivation will cease.

Response to Conspecifics Test

Test subjects will be presented with novel monkeys to evaluate social interaction. The animal's behavior will be videotaped while they are separated by a clear partition and then while they are together in a double cage. Behavior will be tested and recorded for a maximum of 2 hours for up to five times per week for three months. While there is always a risk of injury, our laboratory has had extensive experience with this test. Animals will be continuously monitored and if any signs of physical aggression occur, the tester will intervene by separating the animals and the test will be discontinued. This test also provides social enrichment.

Heart Rate Conditioning

We will study the role of the amygdala and orbitofrontal cortex on emotional processing using classical conditioning of heart rate. In this test, two different tones will be used as the conditioned stimuli (CS+ and CS-). For negative conditioning, the unconditioned stimulus (US) will be a rapid onset one second delivery of white noise paired with a puff of air randomly delivered to the left or right side of the monkey's face. The strength of the air puff can be compared to a person blowing hard on their hand from about three inches from their mouth. We will also test the role of these brain regions in a positive conditioning test. For this, the positive tone (CS+) will be paired with the delivery of a treat which will serve as the unconditioned stimuli (US). Unanesthetized animals will be adapted to a primate chair by exposing them to the test environment one hour a day, for four days. Following adaptation, subjects will be negatively conditioned, in the primate chair. The negative conditioning task will last for four test days and the positive conditioning task will continue until they have learned to respond only during the CS+ presentation. On each day, of both positive and negative conditioning, the animal will be presented 18 CS+ and 18 CS- trials. Testing is accomplished within three hours. For each test, heart rate (EKG) will be recorded from surface electrodes. These studies also provide cognitive stimulation and positive reinforcement.

b) Do any animals undergo any type of restraint beyond normal housing methods? **NO** **YES** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

Animals will be adapted to the primate chair and test environment for one hour a day, for four days. The study design requires the animal to remain in the chair for a maximum of three hours per day. The animals will typically be tested for two hours each

day but may on occassion remain in the chair for a maximum of three hours in case of technical problems.

The primate chair is being used because of the limitations of telemetry systems currently available. These systems must be surgically implanted and they would have to be surgically removed for Magnetic Resonance Imaging (MRI) procedures because they contain ferrous metals that are incompatible with MRI. Furthermore, implantable telemetry systems have a very limited signal range and the transmitted signal is often positional and lost. Previous experience by our research group has shown that obtaining data with surface electrodes is the least stressful and least invasive technique available for obtaining this data.

While restrained each animal will be continuously monitored.

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

To prevent aspiration, while animals are anesthetized, normal daily food rations will be withheld 12-24 hours and fluids no more than 3 hours prior to anesthesia.

In the rare instance an animal does not perform, normal daily food rations will be withheld for one day in an attempt to increase their motivation. If the animal is pair-housed, the cage-mate will be fed while the subject is being tested. No animal will be deprived for more than one day each week for up to one month. During this brief food deprivation period, animals will be weighed once a week. In the unlikely event a greater than 5% weight loss is observed, food deprivation will cease.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)
YES **NO** If YES, indicated nonstandard husbandry required and justification for this practice.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

The animals will be anesthetized from before the cut down procedure until the end of the scanning session. Post-operative analgesics will be given (buprenorphine, .01mg/kg intramuscularly) just before extubation with a second dose determined in consultation with the veterinarian.

To minimize discomfort during MRIs, PET scans, and catheter placement, animals will be anesthetized using either ketamine (15 mg/kg, as needed) or a mixture of ketamine hydrochloride (5 mg/kg) and medetomidine (30 μg/kg) intramuscularly. If medetomidine is used, it can be reversed with atipamezole (150 μg/kg) administered (IM or IV) at the end of the procedure. Additionally, animals will be anesthetized with up to 5% isoflurane during PET scans. Animals will be monitored until recovered from anesthesia.

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

After any procedure requiring anesthesia, animals will be monitored at least every 15 minutes until they are awake. They will be monitored daily thereafter. If complications arise the animal will be monitored more frequently and a treatment plan will be developed in consultation with the veterinary staff tailored to the complication. For example, in the event the animal should develop an infection, the veterinary staff will be contacted, a CBC will be taken and, if possible, the infected site will be cultured. Appropriate antibiotics will be given. In the event of an unexpected injury or illness, the veterinary staff will be contacted and appropriate treatment given. The veterinarian will determine when euthanasia is necessary.

20. If experiments could induce chronic disease, tumors or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.

Some animals assigned to this protocol will have been lesioned as part of another protocol. There always exists a possibility of complications during any surgical procedure which would compromise the health of the animal. This has been addressed in that protocol (G00181). It is unlikely there will be any chronic sequelae to be dealt with after the surgery is over and the animals are assigned to this protocol. Therefore, we do not anticipate any chronic problems from this or any other experiments outlined in this protocol.

The amount and type of radiotracer used for PET scans is extremely unlikely to produce adverse sequelae. Our laboratory established PET scanning of primates on this campus and in the intervening decade we have not observed any long-term effects of these radiotracers. Moreover, similar scans are routinely done in humans.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other

acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

No animals will be euthanized as part of this project. In case of severe, unanticipated illness or injury, euthanasia will be considered in consultation with a veterinarian. The subjects will be anesthetized using Ketamine HCL (15 milligrams per kilogram) intramuscularly under the supervision of the attending Veterinarian and Pathologist. They will be humanely euthanized with an overdose of pentobarbitol greater than 50 milligrams per kilogram intravenously and then perfused with heparinized saline and 4% paraformaldehyde. This is a standard method of euthanasia and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If the animal was part of another protocol (G00181 or G00343) perfusions will be done to obtain histological information.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?
All animals will be returned to [redacted]

23. Will any animal products be used for human consumption? **YES** [NO]
If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____.

Attending Veterinarian:_____

(SVM ONLY)

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?:

Last name, first	Type and length of **SURGICAL** training/experience
[redacted]	23 yrs. Primate surgical experience, MD training 38 yrs. surgical experience, 35 yrs with Primates 2 year primate experience

25. Where will surgery be performed? Room(s): [redacted]

26. How many animals listed in question nine will undergo surgery?
A maximum of 40 animals will receive no more than two arterial cut-down procedures for arterial sampling for tracer kinetics. This procedure isn't necessary for each tracer. Other tracers will only employ PET procedures without frequent arterial sampling which necessitates the cut-down procedure. The lesioned animals will be those from protocol G00181.

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.

The animals are initially anesthetized with ketamine (15 mg/kg, as needed) or a mixture of ketamine hydrochloride (5 mg/kg) and medetomidine (30 μg/kg) intramuscularly. After the initial dose, only ketamine (15 mg/kg) intramuscularly will be given every 30 minutes or as needed during the MRI and for catheter placement. During the PET study, anesthesia will be maintained with up to 5% isoflourane administered through an endotracheal tube. Heart rate, respiration, and percent oxygen saturation will be used to monitor animal status and anesthesia level. The animal's body temperature will be maintained using a warm air blanket. If medetomidine is used, it can be reversed with atipamezole (150 μg/kg) administered (IM or IV) at the end of the procedure.

a) Are any paralytic agents being used **YES** [NO] If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. **Surgical Procedures** (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be

used for all survival surgery. (Append additional page if necessary)

Under aseptic conditions a 3 cm long skin incision is made in the inner side of the upper leg and the tibial artery is dissected. After the artery is ligated a small incision is made in the arterial wall and a sterilized polyethylene catheter is introduced into the tibial artery and ligated around the arterial wall with silk thread. A sterile blunt needle will be inserted into the opposite end of the polyethylene tubing and capped with a sterile PRN adapter. The tubing will be coiled and taped within a gauze pad for protection during transport to the PET scanner. This will allow for arterial blood sampling during the PET procedure. After the animal has been scanned the catheter will be removed from the artery, the arterial wall and skin sutured using Vicryl® dissolving sutures or another suitable absorbable suture and antibiotic ointment will be applied to the incision site. An arterial cut down will only be done to determine the uptake of designated isotope. No more than two cut downs will be performed on each animal.

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

All instruments used for the procedure are autoclaved or gas sterilized. The site is prepped with betadine and alcohol, and covered with sterile drapes. Sterile surgical gloves and gown will be worn during the procedure, as well as standard laboratory clothing.

29. Will the animals be allowed to recover from surgery? **YES** **NO**
If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

Animals are continuously monitored by the research and operating room staff until they recover from anesthetic; thereafter they will be monitored daily, and if the respondent has a problem, the veterinarian will be consulted and appropriate action taken. There is a chance for some discomfort from surgical incisions. This will be evaluated in conjunction with the veterinary staff for behavioral signs such as slouched posture, not eating, abnormal movement. Maximum number, frequency, and amount of post-procedural analgesic doses are determined and administered under the direction of the veterinarian. Buprenorphine, (0.01 milligrams per kilogram intramuscularly or subcutaneously) or Ketofen (5 milligrams per kilogram-intramuscularly) will be administered. Sutures will be removed within 14 days post surgery.

30. Will any animal(s) be allowed to recover from **more than one major operative procedure**? **YES** **NO**
(A major operative procedure is defined as any surgical intervention that penetrates **and** exposes a body cavity **or** any procedure which produces permanent impairment of physical or physiological functions.)

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals: N/A

b) What is minimum length of time between the operative procedures? N/A

QUESTIONS FOR PROJECTS USING WILD-CAUGHT ANIMALS

It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild?

YES **NO, Observation only**

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked

N/A

33.

a) Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

N/A

b) If animals will be release back to the wild, justify why this release will not result in disease exposure to wild population

N/A

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released.

N/A

QUESTIONS FOR PROJECTS USING NON-HUMAN PRIMATES

35.

a) If non-human primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

Animals will only be housed individually during anesthesia recovery and decay of radioactive tracers. This will sometimes be overnight.

b) Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.

Because these radioactive tracers are short lived and decay to background levels quickly it is not necessary to remove environmental enrichment manipulanda from the animals home cage because by the time the animals are returned to their home cages they pose no risk to personnel.

Revision 11/99

2nd Revision 12-7-01 orig sign on top of file

[redacted] RECEIVED emailed DEC - 6 2001 orig 10-31-01 RESEARCH ANIMAL RESOURCES CENTER

Protocol Code: A-55-1000-MO1132-1-10-01

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only
Committee Action: _____

Veterinarian Signature _____ Date: _____

Chairperson Signature: _____ Date: _____

Type of Protocol Procedure: _____

Type of Surgical Procedure: _____

_____ Survival Surgery _____ Non-Survival Surgery _____ Rodent Surgery _____ Non-rodent Surgery

_____ Multiple Major Survival Surgery _____ Exercise Exemption _____ Paralytic Agents _____ Restraint

_____ Critical Veterinary Care _____ Fluid/Food Restrictions _X_ Nonstandard Housing _____ Nonstandard Husbandry

_____ Occupational Health/Personnel Safety _____ Class B Dog/Cat _____ Biohazards _____ Radiation

_____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted] C Telephone Numbers: Office: [redacted]

   Lab: [redacted] Animal Emergency: [redacted]

   Home: [redacted] Fax: [redacted] E-mail Address: [redacted]

If Investigator is Unavailable for Emergency

Alternate to Contact With Authority to Act in Investigator's Absence: [redacted] C

Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Department of Psychiatry

   Office Address: Dept. of Psychiatry, [redacted]

   Unit & Division Number (UDDS): Unit #A, Division #55

3. Is this protocol a: NEW **RENEWAL** AMENDMENT application? (Circle appropriate category)

   If Renewal or Amendment application, please give current protocol code: MO1132

4. Is this protocol for: TEACHING or **RESEARCH**: (indicate research type) **BIOMEDICAL**; **BEHAVIORAL**; **OBSERVATIONAL**; AGRICULTURAL; **FIELD**; OTHER _____ (SPECIFY)
   Circle **all** that apply.

5. Title of this Project: **Effects of Stress and Mood on Disease Progression and Mortality**

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) [1] 2 3 4 5

7. Will ANY surgery be performed on any of the animals? YES [NO] If yes, you must fill out questions 24-30.
Will you be working with wild-caught animals? YES [NO] If yes, you must fill out questions 31-34.
Will you be using non-human primates? [YES] NO If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs or will animals be housed outside of their normal animal housing areas? [YES] NO If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s):
They will be behaviorally tested for approximately two hours in the laboratory space available on the island. For their safety, animals will be held overnight in cages in a holding area on the island designated for this purpose.

9. **NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.**

a) Species of Animal:
Rhesus Monkeys
Total Number For 3 yr.:
Maximum total of 200 animals. Year 1: 120 animals, Year 2: 140* animals, Year 3: 140* animals
*approximately 20-30 monkeys that have transferred from their natal troop for 1-2 years will be dropped and replaced with younger animals
Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
[redacted]

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) YES NO [N/A]

c) Have any of the animals from above been part of any other protocols. [YES] NO If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research.

Most animals assigned to this protocol will not have been part of other protocols. Animal histories will be evaluated on a case-by-case basis to determine suitability for this project.

10. Building(s) or facility where the animals will be housed (normal housing).

[redacted]

11.

a) Outline the specific scientific goal(s) and significance of this research in straight-forward, non-medical, non-technical language that would be understandable to a **layperson**.

In humans, social stress is ubiquitous and often leads to psychological and physical disability, however little is known about the factors that increase or decrease an individual's vulnerability. The monkeys [redacted] have been demonstrated to show marked individual differences in their response to naturalistically occurring social stress. In fact, some of the animals [redacted] die prematurely when they attempt to join a new social group. We believe this phenomenon is relevant to humans and may be important in understanding stress-induced physical demise in vulnerable human populations (i.e. an increased death rate in geriatric patients when they are transferred to a nursing home). To understand the biology underlying this phenomena, we will sample animals [redacted] and assess their levels of stress hormones, indices of immune function, and neurotransmitters.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.

It is not possible to simulate many of the questions being investigated under this protocol, because of the complexity of the questions. No other methods allow for the assessment of the effects of naturalistic social stressors in the life course of an animal. The ethical and technical problems make this work impossible to accomplish in humans. Therefore, we propose to study free-ranging monkeys.

11. (cont.)

c) Provide justification for why you have chosen the species cited in 9-A for your work.

Laboratory housed rhesus monkeys have been routinely used to model human stress in attempts to understand mechanisms underlying physiological and behavioral responses to stress and their negative consequences. Rhesus monkeys provide a good model because their neuroendocrine systems are regulated similarly to humans and they exhibit complex social behavior. In addition, studies in rhesus monkeys, using social stressors, have provided some of the most relevant models of human psychopathology. Furthermore, rhesus monkeys provide relevant models to study human social behavior and stress, to understand why social stress can be so severe for some individuals. Studies in laboratory-housed rhesus monkeys do not allow for an assessment of the effects of naturalistic social stressors on the life course of the animal. However, [redacted] where this research will be conducted, adolescent male rhesus monkeys leave their natal group and attempt to transfer to another group and the stress associated with transfer can be intense and even lethal with a 20% mortality rate. In sons of high-ranking females this mortality rate increases to 40% during the first transfer.

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.

Previous experience with the variance found in preliminary data obtained from [redacted] colony and similar data sets, as well as power analyses, have been used to determine the minimum number of subjects for these experiments

13. Indicate any current or pending funding for this project:

Funding Source (1): Medical School and Private Foundation — Grant Number (1): MH 61083

Title of Grant (1): Emotion Interface – Social Stress in Primates: Vulnerability and Resilience

Funding Source (2): NIH — Grant Number (2): 133-AC75

Title of Grant (2): Mechanisms of Mind Body Interactions

14. Identify the person(s) or unit responsible for **daily** animal care:

[redacted] Animal Care Staff.

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	22 yrs. Primate Research Experience
		36 yrs. Animal Research, 32 yrs w/Primates
		20 yrs. Primate Research Experience
		3 yrs. Primate Research Experience
		4 yrs. Primate Research Experience
		1.5 yrs. Primate Research [redacted]
		1 year Primate Research Experience
		2.5 years Primate Research Experience

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.

We consult current journals, topical searches of the Primate Information Center (Washington Regional Primate Research Center, Seattle) on-line data base, Medline, Index Medicus, Psych. Abstracts, and other publications and books for alternatives to procedures that might cause pain or discomfort to research animals, to assure that work is not redundant, and to explore other potential experimental models. Key word searches have included EEG, EKG, ECG, ACTH, Cortisol, CSF, and Ketamine. We receive neuroscience publications on a bi-weekly basis, along with such journals as *Nature* and *Science*. We attend numerous conferences where we present our own relevant data and meet with and attend lectures by experts in our area of research. In addition we regularly invite colleagues to our department to present their research.

16. (cont.)
b) **Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.**

Category	Specific Material(s)

_____ Recombinant DNA

_____ Genetically Altered Materials

_____ Infectious Agents

_____ Bacteria

_____ Virus

_____ Prion

_____ Carcinogen or Mutagen

_____ Toxic Agent

Status of OBS-2: (circle one) APPROVED PENDING

_____ Radioactive Material

Status of 99-A: (circle one) APPROVED PENDING

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.

You must address questions 17 separately for each species.

17. **Experimental Protocol**
a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

As part of the normal husbandry routine [redacted] the animals will be captured in a feeding corral and anesthetized with Ketamine HCl (10 milligrams per kilogram IM). A maximum of 9 milliliters per kilogram of blood and 7 milliliters of CSF will be collected. Blood will be sampled by venipuncture of the femoral vein and CSF will be sampled by percutaneous puncture of the cisterna magna. Animals are weighed, and testicular volume will be measured. A dose of 0.1 milliliters (1/5 the human dose) of killed influenza vaccine will be given intramuscularly. As with humans this is noninfectious, and will simply generate an antibody response to the viral proteins. These are well-established and safe techniques. The animals will be released into their resident troop after they have fully recovered from the anesthesia. Animals will be retrapped a minimum of one week later and held overnight. The following morning they will be behaviorally tested using the standard human intruder paradigm that records the animal's response to the presence of a human looking at them and avoiding eye contact with them. EEG recording will follow this with electrodes placed on the surface of the scalp while animals are manually restrained. The use of anesthesia would alter the EEG measurements, therefore EEG must not be recorded while animals are awake. Our staff has had years of experience with these techniques without serious incident. Furthermore, we only measure EEG on smaller animals that can be safely handled. Each of these procedures will be accomplished in less than one hour. At the end of EEG recording a 9-milliliter blood sample will be obtained by venipuncture. The animals will then be released.

b) Do any animals undergo any type of restraint beyond normal housing methods? **YES** [NO] If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

c) Are any animals subjected to fluid or food restriction? **YES** [NO] If YES, discuss level of restriction, expected consequences, and justification for such restrictions

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.) **YES** [NO] If YES, indicated nonstandard husbandry required and justification for this practice.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

Discomfort, distress, pain and injury are unlikely. For CSF sampling 15 milligrams/kilogram of Ketamine will be administered intramuscularly every thirty minutes, or as needed, to provide anesthetic (general-dissociative).

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

Animals will be monitored while being held in cages overnight for signs or symptoms of ill health by checking fecal material, food intake, behavior, and appearance. Since this is a free ranging colony, criteria for euthanasia, or removal from the colony, are determined by [redacted] staff.

20. If experiments could induce chronic disease, tumors or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.

N/A

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

Under the supervision of the attending Veterinarian the subjects will be anesthetized using Ketamine HCL (15 milligram per kilogram) intramuscularly. They will be humanely euthanized with an overdose of pentobarbital greater than 50 milligrams per kilogram intravenously. This is a standard method of sacrifice and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?

They will remain [redacted] for further behavioral study.

23. Will any animal products be used for human consumption? **YES** [NO]

If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____

Attending Veterinarian:_____
(SVM ONLY)

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?: N/A

Last name, first N/A Type and length of **SURGICAL** training/experience N/A

25. Where will surgery be performed? Room(s): N/A Building: N/A

26. How many animals listed in question nine will undergo surgery? N/A

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.

N/A

a) Are any paralytic agents being used **YES NO** [N/A] If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. **Surgical Procedures** (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be used for all survival surgery. (Append additional page if necessary)

N/A

28. (cont.)

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

N/A

29. Will the animals be allowed to recover from surgery? [N/A] **YES** **NO**

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

30. Will any animal(s) be allowed to recover from **more than one major operative procedure**? [N/A] **YES** **NO**
(A major operative procedure is defined as any surgical intervention that penetrates **and** exposes a body cavity **or** any procedure which produces permanent impairment of physical or physiological functions.)

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals:

N/A

b) What is minimum length of time between the operative procedures? N/A

QUESTIONS FOR PROJECTS USING WILD-CAUGHT ANIMALS

It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild?

YES [NO], **Observation only**

The primates [redacted] are not considered wild animals. [redacted] in which the animals are in the presence of researchers and animal care staff daily.

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked

Animals are caught within 2 feeding corrals, and anesthetized with 15 milligrams/kilogram Ketamine by the animal care staff.

33.

a) Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

[redacted] is a closed monkey colony meaning that new animals are never introduced; therefore, no quarantine procedures are necessary. All researchers, students, and animal care staff wear standard protective clothing including gloves and eye protection when handling monkeys

b) If animals will be released back to the wild, justify why this release will not result in disease exposure to wild population

The primates [redacted] are not considered wild animals. [redacted] in which the animals are in the presence of researchers and animal care staff daily.

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released.

These are free ranging, not wild animals. Animals are anesthetized using Ketamine HCl at 15 milligrams per kilogram intramuscularly and are continually monitored until they have recovered. They must be eating, drinking and locomoting before being reintroduced into their resident troop.

QUESTIONS FOR PROJECTS USING NON-HUMAN PRIMATES

35.

a) If non-human primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

Non-human primates will be used in these studies. The subjects normally live in a free-ranging environment. For their protection, they will be single cage housed overnight and the next day released into their environment. This is standard operating procedure as part of animal husbandry practices when animals are trapped for a variety of purposes.

b) Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.

These animals live in a highly enriched naturalistic environment.

Revision 11/99

Rewrite 6

emailed HM
AUG 10 2004
orig 5.7.04

Protocol Code: A-55-1000-L00355-3-05-04

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

1st 6.9.04
2nd 6.25.04
3rd 6.28.04
4th 7.16.04
5th 7.30.04

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only
Committee Action: _____

Veterinarian Signature _____ Date: _____

Chairperson Signature: _____ Date: _____

Type of Protocol Procedure: _____

Type of Surgical Procedure: _____

_____ Survival Surgery _____ Non-Survival Surgery _____ Rodent Surgery _____ Non-rodent Surgery

_____ Multiple Major Survival Surgery _____ Exercise Exemption _____ Paralytic Agents _____ Restraint

_____ Critical Veterinary Care _____ Fluid/Food Restrictions _____ Nonstandard Housing _____ Nonstandard Husbandry

_____ Occupational Health/Personnel Safety _____ Class B Dog/Cat _____ Biohazards _____ Radiation

_____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted]

   Telephone Numbers: Office: [redacted] Lab: [redacted] Animal Emergency: [redacted]
   Home: Unlisted Fax: [redacted] E-mail Address: [redacted]

If Investigator is Unavailable for Emergency

   Alternate to Contact With Authority to Act in Investigator's Absence: [redacted] C

   Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Psychiatric Institute Office Address: [redacted]

   Unit & Division Number (UDDS): A-55-1000

3. Is this protocol a: NEW RENEWAL AMENDMENT application? (Circle appropriate category)

   If Renewal or Amendment application, please give current protocol code:

4. Is this protocol for: **TEACHING or RESEARCH: (indicate research type) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER** _____ **(SPECIFY)**
   Circle **all** that apply.

5. **Title of this Project: Characterization of the Anxious Endophenotype**

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) 1 2 3 4 5

7. Will ANY surgery be performed on any of the animals? **YES** **NO** If yes, you must fill out questions 24-30.
Will you be working with wild-caught animals? **YES** **NO** If yes, you must fill out questions 31-34.
Will you be using non-human primates? **YES** **NO** If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs or will animals be housed outside of their normal animal housing areas? **YES** **NO** If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s):

[redacted]

Animals will be removed from their normal animal housing area for procedures and testing only. Lengths of tests may vary and have been defined in question 17. Animals will be returned to their home cage following all procedures and tests.

9. **NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.**

a) Species of Animal:	Total Number For 3 yr.:	Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
(#1)Rhesus Monkeys (M. mulatta)	150	Harlow Center for Biological Psychology and the Wisconsin National Primate Research Center primate colonies
(#2)Northern Pine Snakes (P. Melanolencus)	1	Commercial vendor.

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) **YES** **NO**

c) Have any of the animals from above been part of any other protocols. **YES** **NO** If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research.

All animals considered for assignment to this protocol will be screened using colony health and research records. Only healthy animals will be assigned to this protocol. The Northern Pine snake is used as a stimulus.

10. Building(s) or facility where the animals will be housed (normal housing).

Rhesus Monkeys: Harlow Center for Biological Psychology and the National Primate Research Center.
Northern Pine Snake: Harlow Center for Biological Psychology

The Northern Pine snake's cage is cleaned once every two weeks and it is given water ad libitum and fed a dead rat obtained from a commercial vendor approximately every 2 weeks.

11.

a) Outline the specific scientific goal(s) and significance of this research in straightforward, non-medical, non-technical language that would be understandable to a **layperson**.

Very little is known about the biological mechanisms underlying human emotional problems such as anxiety and depression. While some studies have been attempted in humans, the data generated are merely correlational and cannot be used to understand the actual causes of psychiatric illness. A large number of studies that have been performed in rats have been useful in generating hypotheses for understanding human emotional problems. However, the generalizability of these studies to humans is highly questionable because of differences between rodents and primates in brain structure and function, as well as differences in the factors that elicit these problems. For example, the frontal cortex that has been implicated in the expression of emotion is much larger in monkeys and humans than it is in rats. It is for these reasons that systematic studies of the brain structures thought to mediate emotion in humans are more relevant when performed in nonhuman primates.

Previous studies from our laboratory characterized a fearful/anxious endophenotype in rhesus monkeys that is highly relevant to the understanding of human emotion and psychopathology, such as anxiety and depression. These findings are particularly relevant to understanding the development of human psychopathology, since children with extremely inhibited temperament and similar biological features are at increased risk to develop anxiety disorders. It is likely that different aspects of the anxious endophenotype are mediated by the interactions of the limbic, brain stem, and cortical regions. We will define three groups of animals according to their anxiety levels (high, medium, and low) which we will determine by observing the animals in the behavioral adaptation paradigm. We will then determine from our battery of tests what other characteristics are shared by each group. While our laboratory has implicated the amygdala in mediating acute fear-related responses, the data suggested that the

amygdala may not play a major role in mediating the stable biological and behavioral traits associated with the anxious endophenotype. The prefrontal cortex is also part of the neural circuitry involved in emotion processing, and studies are underway in our lab to examine the role of the orbitofrontal cortex. Additionally, the LPFC has been implicated in cognitive processes such as attention, anticipation, and working memory. Recent studies suggest the LPFC may be involved in integrating emotional and cognitive information important in directing goal-related behavior. We plan to systematically evaluate the contributions of the LPFC in emotion processing by utilizing Positron Emission Tomography (PET) in conjunction with behavioral and physiological tests. Our hypothesis is that high levels of anxiety will be associated with more right LPFC activity, and that compared to less anxious animals, monkeys at the extreme end of the anxious endophenotype continuum will have increased anticipatory anxiety, will be hypervigilant, and will have impaired spatial working memory. It is important to understand how these brain structures normally mediate emotion before we can understand how they underlie psychopathology, such as anxiety and depression.

Anxiety and depression are often associated with sleep disturbances. In addition, it is known that insomnia patients ~~also~~ show abnormal responses to stressors during wakefulness, suggesting that underlying abnormalities in stress response and mood regulatory systems may be involved in insomnia and explain the robust correlation between insomnia and anxiety/depression. However, little is known about the pathophysiological mechanisms involved in human insomnia. Insomnia in humans has been strongly correlated with a large number of negative health outcomes, including psychiatric illness, increased medical morbidity and mortality, increased accidents, greater absenteeism, decreased productivity, disability and greater utilization of health care services. At least 15% of the general population suffers from significant insomnia, making it one of the most prevalent medical complaints. Studies in human insomnia patients have suggested that they suffer from hyperarousal. They also have objective abnormalities in sleep EEG activity and brain activation patterns as determined by functional imaging studies. Studies to definitively identify brain mechanisms and regions involved in insomnia are best performed in primates, since primates provide the best model for the cognitive and emotional interactions involved in the genesis of human insomnia. These sleep studies provide important data from a precious resource, since the opportunity to gather sleep data in the context of the wealth of other behavioral data that is being obtained from these subjects is not likely to occur again. The strength of this study is our ability to correlate sleeping behavior with waking behavior and to study mechanisms for both. Current insomnia research has focused on studying waking responses to stressors and correlating them with sleep disturbance. In humans, however, it is not easy to do more than demonstrate these correlations.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.

These studies cannot be performed in humans or in vitro because they require the ability to perform mechanistic work. Before you can create a computer model of the human brain, you must understand how it works, and since we do not understand how the human brain works or affects other systems, we cannot create an adequate model of it. Non-human primates will be used because they provide the best model to study human emotionality. The numerous similarities in lateral prefrontal cortex between the two species are critical. The social and mental processes of non-human primates are unparalleled elsewhere in the animal world, making them the best research models for emotional and psychiatric disorders.

Use of a live Northern Pine Snake is necessary, because previous data from our laboratory has shown that real snakes are a more potent stimulus than artificial snakes, and therefore individual differences are more easily discerned.

c) Provide justification for why you have chosen the species cited in 9-A for your work.

Rhesus monkeys have been selected as the subjects because of similarities to humans in brain structure, hormone levels, social behavior, development, and emotional response. In addition, a vast body of knowledge has been collected in this primate species that has direct relevance to these studies. Based on our own prior work and archival data, we have proposed to use the smallest number of animals necessary to adequately answer the questions posed.

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.

We have shown that the presence of a human avoiding eye contact with a monkey elicits freezing, a period of risk-assessment, which is analogous to human anxiety. Our objective is to find three groups that exhibit different levels of freezing behavior: high (approximately 1 SD above the mean), medium (mean), and low (approximately 1 SD below the mean). By utilizing our archival database, we've determined that, out of approximately 150 animals, we will be able to obtain a group of 18 animals that freeze for a long time. We hope to be able to define our three groups of 18 animals by testing fewer animals, but have proposed the maximum number we might have to test. We will also need animals to serve as stimulus animals for some behavioral testing. Stimulus animals and the snake are not tested but are used to elicit a response from the experimental animals.

13. Indicate any current or pending funding for this project:

Funding Source (1): NIH Grant Number (1): MH46729

Title of Grant (1): Development and Regulation of Emotion in Primates.

Funding Source (2): NIH Grant Number (2): MH69315

Title of Grant (2): Affective Style: Neural and Behavioral Substrates

Funding Source (3): Grant Number (3):

Title of Grant (3):

14. Identify the person(s) or unit responsible for **daily** animal care:

[redacted] Animal Care Staff will care for all primates. The research staff will provide care for the Northern Pine snake.

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	24 yrs. Primate research experience
		39 yrs. Animal research, 32 yrs w/primates
		22 yrs. Primate research experience
		2 yrs. Primate research experience
		2 yrs. Primate research experience
		22 Months primate research experience
		21 Months primate research experience
		Student in training.
		Student in training.
		Student in training.
		Student in training.
		Student in training.
		Student in training
		Student in training

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.

We have searched for all articles published on the lateral prefrontal cortex. We update our searches several times a year and whenever we are writing research papers or grant proposals. Numerous sources are searched, including Medline and pre-Medline sources. Key words used to search data bases in April 2004 were: lateral prefrontal cortex, fear-potentiated startle, PET, MRI, amygdala, lesion verification, vagal tone, heart rate variability, ketamine, and many other related terms. A keyword search in June 2004 included: Primates and EKG, EEG, EMG, Micro PET, stress and anxiety. To investigate any new innovations in MRI, EEG and EKG technology a recent update of our literature searches included the keywords EEG, EKG, MRI, telemetry and primates. The databases are searched for all years. We receive neuroscience publications on a bi-weekly basis, along with such preeminent journals as *Nature* and *Science*. We attend numerous conferences where we present our own research and meet with and attend lectures by experts in our area of research. In addition, we invite colleagues to our department to present their research on a regular basis, and we host the HealthEmotions Symposium annually.

b) **Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.**

Category	Specific Material(s)
_____ Recombinant DNA	

_____ Genetically Altered Materials

_____ Infectious Agents

_____ Bacteria

_____ Virus

_____ Prion

_____ Carcinogen or Mutagen

_____ Toxic Agent

Status of OBS-2: (circle one) APPROVED PENDING

___X___ Radioactive Material — The radioactivetracer, 18-FDG (less than 7 mCi), will be administered intravenously in sub-physiological doses.

Status of 99-A: (circle one) APPROVED PENDING

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.

Extensive training and orientation is required before any employee is allowed access to animal housing areas. This includes a tour of the facilities, as well as, successfully completing:
RARC Animal Care and Use Course
Primate Handling Course
UW Radiation Safety Course
Herpes B Quiz

After completion of orientation and training, personnel are issued keys for animal area access. All personnel working with animals or in animal housing and testing areas are required to wear a lab coat, latex gloves, eye protection, a surgical mask, head covering and shoe coverings or designated shoes. Personnel will follow the [redacted] standard operating procedure (SOP 2.01) when entering primate areas in [redacted] All personnel are trained to properly dispose of animal waste and biological sampling supplies. Lab personnel are required to wear personal dosimeters at all times when using radiotracers. In addition, they are taught to limit their exposure by increasing the distance and decreasing the time in proximity to the radioactive source.

Animals will be hand restrained by the arms and legs in the prone position during collection of EEG data. In addition to taking the primate handling course mentioned above, all staff are trained by experienced personnel in our laboratory and approved by a supervisor before they are allowed to perform this procedure. All personnel will be trained by observing the procedure while each step is explained by a supervisor. After they have observed the procedure numerous times, they will be given their first experience by restraining the animal's legs. Following this experience, they will be taught to hold the animal's arms. Manual restraint will be achieved by initially grasping an animal's arms while the animal is immobilized with a restraint table. One person will hold the animal's arms above the elbows behind the animal's back, while a second person controls the legs. The animal will then be placed on a padded table while EEG is being recorded. A similar procedure will be used to prepare the animal for EKG recording.

You must address questions 17 separately for each species.

17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated.

Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

EXPERIMENTAL PLAN

Experiment 1: Screening for Group Formation (N=150)

No more than 150 juvenile male and female rhesus monkeys will be tested.

After the monkey is moved to a test cage for 10 minutes, it's freezing duration in response to a human entering the room and avoiding eye contact for another ten minutes will be assessed. Each animal will be tested on two separate occasions at least one week apart. Based on freezing durations, we will select 18 monkeys that are approximately 1 SD below the mean, 18 monkeys that are around the mean, and 18 monkeys that are approximately 1 SD above the mean to define three groups of monkeys that have low, middle, and high levels of anxiety.

Experiment 2: Assessment of Endophenotype (N=54 of 150 in Experiment 1)

The three groups (n=18/group) obtained in the first experiment will then be assessed utilizing behavioral and physiological measures. The behavioral measures include behavioral adaptation, response to conspecifics, learning, and conditioning. The physiological measures include hormonal sampling, Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), Electroencephalography (EEG), Electrocardiogram (EKG), startle, and sleep recording.

During these experiments, experimental animals will be housed with other experimental animals. In the case that an experimental animal is incompatible with his assigned cage mate, an alternative cage mate will be introduced that will be taken from the pool of animals originally screened. These cage mates will not be tested beyond the original screening. A total of six stimulus animals will be used across all experiments.

Behavioral Tests

To investigate the role of the lateral prefrontal cortex in emotion and learning, we will administer the following behavioral and cognitive tests.

Behavioral Adaptation Test

To assess freezing behavior and other defensive responses in monkeys, we developed the behavioral adaptation test. This paradigm is a reliable method to evaluate the effects of different types of ethologically relevant threatening situations on anxiety related behaviors. By assessing changes in monkey's defensive behaviors in relation to different environmental contexts, the behavioral adaptation test also allows for the study of emotion regulation. This test is used to assess the animal's behavioral response to the presence of a human making and avoiding eye contact. Behavior is recorded for less than one hour in a special cage, no more than once per week on no more than eight occasions.

Response to Conspecifics Test

Test subjects will be presented with novel monkeys to evaluate social interaction. The animal's behavior will be videotaped while they are separated for one hour by a clear plastic divider and then while they are together in a special double cage. Behavior will be recorded for less than 2 hours for up to five times per week for three months. When two animals are together there is always the risk of one animal injuring the other; however, our laboratory has had extensive experience with this test. Animals will be continuously monitored and if any signs of physical aggression occur, the tester will intervene by separating the animals and the test will be discontinued. This test also provides social enrichment.

Learning Tests

These standard learning tests are used to evaluate the brain areas involved in different aspects of learning. After the animals are adapted to the test situation (special apparatus and special cage) they will be tested on matching-to-sample, spatial and non-spatial reversals, oddity learning, reward devaluation and their response to a snake. The animals are tested for their ability to move the correct object to uncover a food treat or for the time it takes to remove a treat from above an object or from a clear plastic box that encloses and protects the snake. Most of these tasks have significant enrichment value, providing cognitive stimulation and positive reinforcement. Animals will be tested as often as every day until the test is completed. For some individual animals, some tests may take several months to learn. During this period, animals will receive normal weekly food rations following testing, as outlined in Nutrient Requirements of Nonhuman Primates (National Research Council of The National Academy of Sciences) and will always have access to water. Weekly food rations will be determined, utilizing the above-mentioned guidelines, taking into account the animal's developmental stage. We will provide 2-4% body weight in

food, daily, as recommended by the chow manufacturer. Additionally, during the learning tests, food rewards will be given, which will contribute to the daily caloric intake. If the animal is pair-housed, the cage-mate will be fed while the subject is being tested. Animals will be weighed once a week, and if a greater than 5% weight loss is observed, animals will be supplemented accordingly.

Heart Rate Conditioning

We will study emotional processing using classical conditioning of heart rate. In this test, two different tones will be used as the conditioned stimuli (CS+ and CS-). For negative conditioning, the unconditioned stimulus (US) will be a rapid onset one second delivery of white noise paired with a puff of air randomly delivered to the left or right side of the monkey's face. The strength of the air puff can be compared to a person blowing hard on their hand about three inches away. We will also test the subjects in a positive conditioning test. For this, the positive tone (CS+) will be paired with the delivery of a food reward, which will serve as the unconditioned stimuli (US). Animals will be adapted to a primate chair (special apparatus) by exposing them to the test environment and chair one hour a day, for a minimum of four days. Following adaptation, subjects will be conditioned every other day, for up to six days. The positive conditioning task will continue until they have learned to respond only during the CS+ presentation. On each day, of both positive and negative conditioning, the animal will be presented with no more than 60 trials. Testing is generally accomplished in approximately two hours. For each test, heart rate (EKG) and muscle activity using electromyogram (EMG) will be recorded from surface electrodes placed on shaved areas. These studies also provide cognitive stimulation and positive reinforcement.

Physiological Tests

Hormonal Sampling

Hormonal sampling will be carried out under two conditions. The first, acute hormonal response, will evaluate the animal's hormonal response to a novel test environment. Ten milliliters or less of blood will be sampled before and after relocation to a novel environment (special cage). Sampling for acute hormonal response will be done no more than once per week for four weeks.

The second condition will assess the time course of the hormonal response to relocation, which in primates is known to elevate the hormones of interest. A total of 25 milliliters of blood (2 mL taken at each time point over the course of 24 hours) will be sampled from an indwelling venous catheter. As an example, a two milliliter sample will be drawn at approximately 0 (baseline-one hour after catheter placement), +5, +10, +20, +30, +60, +90, +120 minutes, +4 hours, +8 hours, +12 hours, and +24 hours. The animal will be placed in a primate chair and the IV catheter will be inserted percutaneously one hour before sampling begins and removed approximately three hours later following the 120 minute blood draw. To avoid chairing animals for an extended period of time, they will be returned to their home cages after the 120 minute blood draw. There is not enough time between blood draws to catch the animals and since repeatedly catching them will elevate the hormones we are measuring, they must be restrained in the primate chair for the first 120 minutes. After 120 minutes, blood draws will be obtained after animals are removed from their home cage. Sampling for time course of the hormonal response will be done no more than a total of four times, at least one month apart. In compliance with RARC blood collection guidelines, 25 mL will only be drawn from animals weighing 5 kg or more. The blood volume sampled from animals weighing less than 5 kg will be reduced in accordance with these guidelines. Animals will be closely monitored while they are restrained.

After hormonal sampling, we will obtain 5 milliliters or less of cisternal cerebrospinal fluid (CSF). The sample will be obtained while the monkeys are anesthetized with 15 mg/kg of ketamine administered intramuscularly. If needed, a supplemental dose of 5-10 mg/kg ketamine will be administered intramuscularly, as recommended by the veterinary staff. The sample site will be shaved and prepped with alcohol. The sample is obtained by percutaneous puncture into the cisterna magna while the animal is held in the lateral decubitus position. No more than three cisternal punctures will be made to obtain CSF. We have obtained hundreds of CSF samples without ever having observed any signs of discomfort (see question 18); however, in the unlikely event that we see any signs of discomfort after CSF sampling, aspirin (20 milligrams per kilogram-orally) will be provided as recommended by the veterinarian staff. This procedure will be done no more than 8 times and no more often than one time per week.

These tests must be run more than once to minimize the variability of the hormonal measures.

Startle

The startle response will be assessed to test the role of the lateral prefrontal cortex in emotional processing. Procedures, which will differentiate the response to specific cues associated with conditioned stimuli, will be used. In the potentiated startle procedure, animals will be placed in a primate chair (special apparatus to which they have previously been adapted) for less than two hours per day. While in the chair, they will be trained for a minimum of four days by exposing them to conditioned stimuli (CS+) of either tones or lights. The CS+ will be paired with the US, a 1 second air puff paired with white noise. The presentation of the CS+ is used to enhance the startle response to an unexpected sound. Animals will be presented with the startle test for no more than two hours on each test day for a maximum of six days. Startle will be measured by whole body movement using an accelerometer attached to the chair. Eye blinks and EMG will also be measured from surface electrodes.

Imaging

To investigate the role of the lateral prefrontal cortex in processing emotion, PET will be used to monitor changes in regional cerebral glucose metabolism. This will at times be done in conjunction with a behavioral or physiological test. Animals will be adapted to catheter placement and radiotracer administration for up to 21 days. This will involve transporting animals from their home cage to the restraint table where we will simulate catheter placement (while not actually placing a catheter) and shaving (although we will shave on the last day of adaptation). Animals will be adapted to the procedure of catheter placement to minimize stress during the early uptake period. The resultant PET image is biased to activity occurring during the early uptake period, and we are attempting to image animals in a baseline or unperturbed state while in their home cage. A less than 7.0 milliCurie dose of 18 Fluoro-deoxy glucose (FDG) radiotracer will be administered intravenously. The animals will be anesthetized with ketamine (15 mg/kg), as needed, to allow safe transfer of the animal to [redacted] and placement of an endotracheal tube. During scanning they will be placed in a special apparatus in the microPET scanner. During these procedures, a maximum of 25 milliliters of blood will be sampled (0.5 mL each draw) simultaneously from an artery and a vein over the course of 3 hours to monitor hormonal changes and tracer kinetics. In compliance with RARC blood collection guidelines, 25 mL will only be drawn from animals weighing 5 kg or more. The blood volume sampled from animals weighing less than 5 kg will be reduced in accordance with these guidelines. Animals will be anesthetized with up to 5% isoflurane. Heart rate, respiration rate, and oxygen saturation will be monitored throughout the scanning procedure, and the animal's body temperature will be maintained using a Bair hugger (warm air blanket). Following the procedure, which typically lasts for two hours, animals will be returned to their home cage. All housing areas and test rooms used at [redacted] have been approved by the Radiation Safety Department for this use. PET procedures are routinely done in humans with no adverse effects. Animals will be scanned no more than once per week on no more than ten occasions. Scans in which tracer kinetics are monitored via arterial blood sampling will be performed no more than once a month, on no more than two occasions.

Additionally, MRIs will be obtained on no more than two occasions, no less than one week apart for MRI co-registration of the PET images. Animals will be anesthetized with ketamine (15 mg/kg, as needed) or a mixture of ketamine hydrochloride (5 mg/kg) and medetomidine (30 μg/kg) intramuscularly, as needed for imaging and safe transfer to [redacted]. If medetomidine is used, it can be reversed with atipamezole (150 μg/kg) administered (IM or IV) at the end of the procedure. Atropine (0.5cc, IM) may also be administered. Monkeys will be placed in a special apparatus during imaging. Following the imaging procedure, animals will be returned to their home cage.

Frontal Electroencephalogram (EEG) Asymmetry and Electrocardiogram (EKG)

EEG is a measurement of brain electrical activity. It has been shown in humans that there is a difference between the electrical activity in the front of one side of the brain compared to the electrical activity in the front of the other side of the brain. This lateralized frontal brain activity can be used to sort out differences in temperament, as well as susceptibilities to psychiatric problems such as depression and anxiety disorders. We will measure EEG laterality in monkeys to establish their temperamental style, as well as the role of the lateral prefrontal areas in defining this electrical brain asymmetry.

EEG and EKG will be collected from surface electrodes for approximately one hour while the animals are manually restrained. Animals will be hand restrained by the arms and legs in the prone position during collection of EEG data. We are attempting to model human EEG laterality which has been defined in awake subjects. Anesthesia dramatically changes EEG patterns and therefore cannot be used. All EEG laterality data collected in our laboratory has been obtained from animals while they were manually restrained and it would require additional studies to determine if EEG data collected from animals restrained in a primate chair would be comparable. In addition, since EEG is collected during a relatively short period it would require more time to restrain an animal by other means than to simply manually restrain the animal. Additionally, it is necessary to manually restrain animals in order to place the leads in preparation for recording of EKG.

Sleep Recording

Because the lateral prefrontal region is involved in emotional responses and Rapid Eye Movement (REM) sleep, it is likely that they are involved in emotional aspects of dreaming. These brain regions may also mediate the effects of stress on sleep. Insomnia is strongly associated with exposure to stress, and patients with psychiatric disorders commonly have sleep disturbances. In addition, those with mood disorders often have abnormalities of REM sleep expression. The mechanisms for sleep abnormalities in psychiatric disorders and insomnia are largely unknown, but could likely involve these brain regions.

In order to achieve a better understanding of the role of these brain regions in normal sleep and sleep disturbances, sleep measures will be recorded. Animals will be gradually adapted to a chair (special apparatus) by increasing the time they spend in it each day from one to eight hours over a maximum two week period. Generally animals are placed in a primate chair for two hours on day one, four hours on day two, six hours on day three and eight hours on day four of the adaptation period. If an animal continues to struggle, showing no adaptation to the chair, it will be removed from the study. For each recording session, the monkey will be placed in a primate chair at 1600h for setup and equipment calibration. Recording will begin at 1700 h and continue until 0700h. After the data collection period, it will take approximately 1 hour for electrode removal and removal of the animal from the chair. Therefore, the animal will have been in the primate chair for a total of 16 hours. Animals will be continuously monitored by research staff using infrared cameras, video monitors and computers. In addition to time of set up and testing times, we will record comments on changes in sleep and behavior as well as any abnormal

events. EEG will be recorded during sleep no more than one time by attaching surface electrodes to the scalp and to the outer canthus of each eye for recording eye movement (EOG). Heart rate will be monitored using surface electrodes attached to the chest and flank. Ketamine HCL will be administered (15 mg/kg IM, as needed) at the end of EEG recording to safely remove the electrodes. If a sleep study is interrupted and an animal must be re-tested, there will be a minimum of one week between sleep testing sessions.

b) Do any animals undergo any type of restraint beyond normal housing methods? **YES** **NO** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

The laboratory environment is a 12 hour day/night cycle, with lights off at 1800 hours. Primates typically sleep during most of the 12h dark period and the entire sleep period needs to be measured to make any conclusions regarding sleep architecture and overall sleep amount. This is comparable to clinical and research studies in humans, which must be conducted for the entire sleep period in order to be valid. To study sleep in the laboratory it is necessary to have the animal prepared for sleep recording before the lights go off (1800H). One of the important measures used in sleep research is the time it takes to fall asleep; like humans, sleep latency will be affected by stressors immediately prior to trying to initiate sleep, so it is necessary to apply recording electrodes and then allow the animal to settle prior to starting the sleep recording. We also need to gather EEG data during quiet wakefulness immediately prior to sleep onset and following awakening, since one of the aims of the study is to compare laterality of brain activation between waking and specific stages of sleep. Since we are trying to model human insomnia and we do not know how the brain regions we are studying affect sleep, it is important to characterize how we are affecting the entire sleep/wake cycle. Human insomnia can vary, ranging from primary difficulties falling asleep, episodes of wakefulness during sleep, and/or early morning awakening. Insomnia has been shown to be highly correlated with depression and anxiety disorders in humans, and is highly predictive of individuals who are at risk to develop major depressive disorder. Gaining a better understanding of brain mechanisms for anxiety/depression and insomnia are critical for understanding the potential causal links between sleep and psychiatric illness as well as guide development for treatment and prevention of these disorders. One of the unique strengths of this study is that sleep parameters can be correlated with a large number of waking measures of anxiety and temperament.

Animals will gradually be adapted to the primate chairs as previously described under 'sleep recording'. After adaptation, monkeys will be placed in the primate chair for sleep recording on only one occasion for approximately 16 hours. Even though it is a novel sleeping environment, some animals sleep more than 75% of the night in the chair, similar to what would be expected of a human sleeping in a novel environment. Since chairing will occur during what is normally a period of sleep and limited activity for rhesus monkeys, they will not be deprived of normal exercise or enrichment. Removing the animal from the chair after 12 hours (0400h) for exercise would disturb their sleep cycle and compromise the sleep data.

The use of a primate chair is necessary because of the limitations of telemetry systems currently available. These systems must be surgically implanted and because they contain ferrous metals, would have to be surgically removed for the Magnetic Resonance Imaging (MRI) procedures. Furthermore, implantable telemetry systems have a very limited range and the transmitted signal is lost in some areas and positions within the cage. Implantable telemetry systems that record the requisite number of channels for electroencephalograph (EEG), eye movement (EOG) and heart rate (EKG) are not available. Even if such a system were available to cover the desired range with a sufficient number of channels, given current technology, it would be too large to implant in a monkey. Previous experience by our research group has shown that chairing animals is the least stressful and least invasive technique available for collecting continuous data, which is critical for staging sleep. Each animal's EEG, EKG, and behavior will be continuously monitored using video and computers.

During EEG data collection, animals will be hand restrained by the arms and legs in the prone position for approximately one hour. They will also be restrained in this manner for lead placement in preparation for recording of EKG for approximately 20 minutes.

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

During learning testing, animals will receive normal weekly food rations following testing, as outlined in Nutrient Requirements of Nonhuman Primates (National Research Council of The National Academy of Sciences) and will always have access to water. Weekly food rations will be determined, utilizing the above-mentioned guidelines, taking into account the animal's developmental stage. We will provide 2-4% body weight in food, daily, as recommended by the chow manufacturer. Additionally, during the learning tests, food rewards will be given, which will contribute to the daily caloric intake. If the animal is pair-housed, the cage-mate will be fed while the subject is being tested. Animals will be weighed once a week, and if a

greater than 5% weight loss is observed, animals will be supplemented accordingly. In order to prevent aspiration, food will be withheld for at least 12 hours but no more than 24 hours before any prolonged procedures (over 1/2 hour) that require anesthesia.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)
YES **NO** If YES, indicated nonstandard husbandry required and justification for this practice.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

We do not expect any of these procedures to cause pain. CSF sampling has been known to cause headaches in humans, however research has shown that this is from pressure changes resulting from leakage of CSF following this procedure, which in humans is done using a very large (16-18 gauge) needle. We will be using a very small (25 gauge) needle to minimize CSF leakage. CSF is sampled from humans while they are awake, and we will be sampling while the monkeys are anesthetized with ketamine hydrochloride (15 mg/kg) administered intramuscularly as needed to provide anesthetic (general-dissociative). In the unlikely event that we see any signs of discomfort after CSF sampling, aspirin (20 milligrams per kilogram-orally) will be provided as recommended by the veterinarian staff. As we have tested numerous animals without adverse effects, we do not expect any discomfort as a result of restraint. As previously mentioned, animals will be asleep most of the time they are restrained.

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

Animals will be monitored for signs or symptoms of ill health by checking fecal matter, food intake, behavior, and general appearance. Weight changes will also be monitored regularly. In the case of unanticipated illness or injury the veterinarians will be contacted. Veterinary consultation will determine if euthanasia is necessary.

20. If experiments could induce chronic disease, tumors or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.

We do not anticipate these experiments to induce chronic disease, tumors or radiation sickness.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

The subjects will be anesthetized using Ketamine HCL (15 milligram per kilogram) intramuscularly. They will be humanely euthanized with an overdose of sodium pentobarbitol greater than 50 milligrams per kilogram intravenously. This is a standard method of euthanization and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?

Animals not euthanized will be returned to [redacted] primate colonies.

23. Will any animal products be used for human consumption? **YES** **NO**

If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____.

Attending Veterinarian:_____
(SVM ONLY)

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?:

Last name, first — Type and length of **SURGICAL** training/experience

25. Where will surgery be performed?

26. How many animals listed in question nine will undergo surgery?

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.

a) Are any paralytic agents being used **YES NO** If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. Surgical Procedures (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be used for all survival surgery. (Append additional page if necessary)

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

29. Will the animals be allowed to recover from surgery? YES NO

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

30. Will any animal(s) be allowed to recover from more than one major operative procedure? YES NO
(A major operative procedure is defined as any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions.)

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals:

b) What is minimum length of time between the operative procedures?

QUESTIONS FOR PROJECTS USING WILD-CAUGHT ANIMALS

It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild?

YES NO, Observation only

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked

Does not apply

33.

a) Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

Does not apply

b) If animals will be release back to the wild, justify why this release will not result in disease exposure to wild population

Does not apply

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released.

Does not apply

QUESTIONS FOR PROJECTS USING NON-HUMAN PRIMATES

35.

a) If non-human primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

If aggressive behavior is observed between cage mates, they will be separated and singly housed for their own safety. Following separation the Animal Care Staff will make the determination of whether it is safe to attempt to find another cage mate.

b) Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.

Environmental enrichment will not be restricted, as many of the tests serve as cognitive enrichment. The normal enrichment schedule will be followed but delayed until after testing.

36.

In response to concerns raised during our last laboratory animal care and use accreditation site visit, the Medical School Animal Care and Use Committee now requires responses to the following for each protocol submission:

1. Does this protocol involve the in vivo use of infectious microorganisms, cultured cells or tissues, human-derived tissues or substances, toxic or carcinogenic/mutagenic material, recombinant DNA, or radionuclides? If "yes", answer the following:

Yes

a. Specify the name and type of agent (e.g. infectious organism, toxin, etc.). Radiotracers
b. Has the use of this agent been reviewed by the appropriate safety committee? Yes
c. Provide any special precautions that should be followed by your lab personnel, animal care workers or any other personnel entering areas where experiments are conducted or animals housed. Include any special practices required for handling of animal waste, animal carcasses, cages, or equipment.

Lab personnel are required to wear personal dosimeters at all times when using radiotracers. Personnel are taught to limit their exposure by increasing the distance and decreasing the time in proximity to the radioactive source.

Revision 11/99 Rewrite "L"

CONFIDENTIAL emailed JUN 23 2004 aug 5.25.04

Protocol Code: __-___-_____-G00181-4-09-02

RESEARCH ANIMAL RESOURCES CENTER

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only

Committee Action: **AMENDMENT APPROVAL**

Veterinarian Signature [redacted] Date: 7-16-04

Veterinarian / **Date**

Chairperson Signature: [redacted] Date: 7-16-04

Chair / **Date**

Type of Protocol Procedure: _____

Type of Surgical Procedure: _____

_____ Survival Surgery _____ Non-Survival Surgery _____ Rodent Surgery _____ Non-rodent Surgery

_____ Multiple Major Survival Surgery _____ Exercise Exemption _____ Paralytic Agents _____ Restraint

_____ Critical Veterinary Care _____ Fluid/Food Restrictions _____ Nonstandard Housing _____ Nonstandard Husbandry

_____ Occupational Health/Personnel Safety _____ Class B Dog/Cat _____ Biohazards _____ Radiation

_____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted] C

   Telephone Numbers: Office: [redacted] Lab: [redacted] Animal Emergency: call Lab [redacted]

   Home: Unlisted Fax: [redacted] E-mail Address: [redacted]

If Investigator is Unavailable for Emergency

   Alternate to Contact With Authority to Act in Investigator's Absence: [redacted] C

   Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Psychiatric Institute Office Address: [redacted]

   Unit & Division Number (UDDS): A-55-1000

3. Is this protocol a: NEW RENEWAL **AMENDMENT** application? (Circle appropriate category)

   If Renewal or Amendment application, please give current protocol code: **A-55-1000-G00181-3**

4. Is this protocol for: **TEACHING** or **RESEARCH:** **(indicate research type) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER** _____ **(SPECIFY)**
   Circle **all** that apply.

5. **Title of this Project:** Neural Circuitry of Emotion

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) **1 2 3 4 5**

7. Will ANY surgery be performed on any of the animals? **YES** NO If yes, you must fill out questions 24-30.
Will you be working with wild-caught animals? YES **NO** If yes, you must fill out questions 31-34.
Will you be using non-human primates? **YES** NO If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs or will animals be housed outside of their normal animal housing areas? **YES** NO If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s):

[redacted]

Animals will be removed from their normal animal housing area for procedures and testing only. Lengths of tests may vary and have been defined in question 17. Animals will be returned to their home cage following all procedures and tests.

9. **NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.**

a) Species of Animal:	Total Number For 3 yr.:	Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
(#1)Rhesus Monkeys (M. mulatta)	142	Harlow Center for Biological Psychology and the National Primate Research Center-UW Madison primate colonies
(#2)Northern Pine Snakes (P. Melanolencus)	1	Commercial vendor.

Experimental subjects (receive surgery) = 64
Control subjects (no surgery) = 54 We are requesting an additional 12 control subjects because animals were assigned to this protocol but for practical reasons couldn't be used.
Cage mates for experiment 3 = 18 (These will not receive any tests)
Stimulus animals = 6

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) **YES** NO

c) Have any of the animals from above been part of any other protocols. **YES** NO If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research.

All animals considered for assignment will be screened using colony health and research records. Only healthy animals will be assigned to this protocol. The Northern Pine snake is used as a stimulus animal only.

10. Building(s) or facility where the animals will be housed (normal housing).

Rhesus Monkeys: [redacted]
Northern Pine Snake: [redacted]

11.

a) Outline the specific scientific goal(s) and significance of this research in straight-forward, non-medical, non-technical language that would be understandable to a **layperson**.

Very little is known about the biological mechanisms underlying human emotional problems such as anxiety and depression. While some studies have been attempted in humans, the data generated are merely correlational and cannot be used to understand the actual causes of psychiatric illness. A large number of studies that have been performed in rats have been useful in generating hypotheses for the understanding of human emotional problems. However, the generalizability of these studies to humans is highly questionable because of differences between rodents and primates in brain structure and function, as well as differences in the factors that are important in eliciting these problems. For example, the part of the cortex that is critical for thinking and understanding emotion is present in monkeys and humans, but not in rats. For these reasons, systematic studies of the brain structures thought to mediate negative emotion in humans must be performed in nonhuman primates. Earlier attempts to study nonhuman primates have been made. Unfortunately, these studies were unable to capitalize on newer technologies allowing for highly selective inactivation of brain cells, or neurons, and state-of-the-art functional imaging techniques. Data from the earlier studies is now thought to be unreliable. We will perform selective inactivation of the orbitofrontal cortex and amygdala, and by doing so will establish the role of these structures in the processing of emotion.

Previous studies from our laboratory characterized fearful/anxious temperament factors in rhesus monkeys that are relevant to understanding human emotional problems such as anxiety and depression. We plan to continue our work to inactivate neurons in specific brain regions, such as the amygdala and orbitofrontal cortex. We plan to use sophisticated behavioral, imaging, and hormonal measures to address mechanisms underlying emotional processing in primates. We demonstrated that the amygdala plays an important role in the processing of acutely fearful stimuli. However, early indications suggest a lack of amygdala involvement in mediating the dispositional behavioral, emotional, and physiological characteristics associated with fearful/anxious temperament. Orbitofrontal regions are bi-directionally linked with the amygdala. The proposed studies will examine the role of orbitofrontal cortex in emotional processing, as well as the functional interaction between the amygdala and orbitofrontal cortex using ibotenic acid to specifically inactivate neuronal cells and positron emission tomography (PET), with the glucose tracer 18-Fluoro-deoxy glucose (FDG) and the serotonin tracer (18F-MPPF) which has just become available. Understanding of the serotonin system is important because serotonin has been implicated in the expression of emotion and psychopathology. We will explore the hypothesis that right orbitofrontal regions are primarily involved in mediating the behavioral and emotional responses associated with fearful/anxious temperament. The findings from these studies will be highly relevant to humans, addressing the role of amygdalar-orbitofrontal interactions in mediating normal emotion and mental illness. We expect that monkeys with an inactive orbitofrontal cortex will have subtle behavioral changes, expressing a failure to activate physiological systems in anticipation of making decisions that have emotional consequences. In essence, they will not have the ability to make decisions and guide their behavior based upon the learned positive or negative consequences of their actions. These animals may have a change in affect and are more likely to perseverate.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.

These studies cannot be performed in humans or in test tubes because they require the ability to perform mechanistic work. No petri dish or computer model can adequately mimic the complexity of a living creature. Non-human primates will be used because they provide the best model to study human emotionality. The numerous similarities in orbitofrontal cortex between the two species is critical. The social and mental processes of non-human primates are unparalleled elsewhere in the animal world, making them valuable research models for emotional and psychiatric disorders

Use of a live Northern Pine Snake is important as previous data from our laboratory has shown that the response to an artificial snake is not as potent as the response to a live snake.

c) Provide justification for why you have chosen the species cited in 9-A for your work.

Rhesus monkeys have been selected as the subjects because of similarities to humans in brain structure, social behavior, and emotional response. In addition, a vast body of knowledge has been collected in this primate species that has direct relevance to these studies. Based on our own prior work, we have proposed to use the smallest number of animals necessary to adequately answer the questions posed.

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.

The number of animals selected has been based on prior work in these types of experiments, as well as power analyses. Previous experience with the variance found in similar data sets and a power analysis based on these data sets have been used in determining the minimum number of subjects required for these experiments. The effect size of the difference between the experimental and control groups is d=1.02. The power to detect a difference of 0.05 in the predicted direction for this effect size with an N of 10/group is 0.70. Control animals for each experiment are necessary to account for individual differences in all measures. The cagemates are for pair housing purposes and will not be tested. Sixty four animals will be needed for surgery, the first 4 being pilot surgeries. Pilot surgeries) are necessary to refine surgical techniques prior to starting the experiments. To achieve an n of 10 good experimental subjects per group it will require 12 surgeries. Over the course of the protocol 60 experimental subjects will be needed to form 5 groups. Stimulus animals and the snake are not tested but are used to elicit a reaction from the subjects being tested.

13. Indicate any current or pending funding for this project:

Funding Source (1): NIH Grant Number (1): MH46729

Title of Grant (1): Development and Regulation of Emotion in Primates.

Funding Source (2): NIH Grant Number (2): MH69315

Title of Grant (2): Affective Style: Neural and Behavioral Substrates

Funding Source (3): Grant Number (3):

Title of Grant (3):

14. Identify the person(s) or unit responsible for **daily** animal care:

[redacted] Animal Care Staff will care for all primates. The research staff will provide care for the Northern Pine snake daily.

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	24 yrs. Primate Research Experience
		39 yrs. Animal Research, 32 yrs w/Primates
		22 yrs. Primate Research Experience
		17 yrs. PET scanning of monkey brain
		2 yrs, Primate research experience
		26 Months Primate research experience
		22 Months primate research experience
		21 Months primate research experience
		8 Months primate research experience
		Student in training
		Student in training
		Student in training
		Student in training
		Student in training
		Student in training

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.

We have searched for all articles published on the amygdala and orbitofrontal cortex. We update these searches several times a year whenever we research literature to write research papers or grant proposals. Numerous sources are used, including Medline and pre-Medline sources. Key words used to search data bases are: Amygdala, learning, sleep, stress, PET, primates, emotion, fear, hormones, CRH and many other related terms. The following Medline & Biological Abstracts keyword searches were updated in 5/04: Primates, benzodiazepines, and valium, animal models of depression, animal modeling, snake fear in primates and fear potentiated startle. We also updated the following searches in 5/04: EEG electrode placement, MRI and histology, and sleep in primates. New literature searches include MRI and brain lesion verification (4/04), medetomidine and ketamine (4/04), caloric restriction in primates (5/04) and alternatives to neurosurgery in primates (5/04). The data bases have been searched as far back as the data base extends. We receive neuroscience publications on a bi-weekly basis, along with such journals as *Nature* and *Science*. We attend numerous conferences where we present our own relevant data and meet with and attend lectures by experts in our area of research. In addition we invite colleagues to our department to present their research on a regular basis.

b) **Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.**

Category	Specific Material(s)
_____ Recombinant DNA	
_____ Genetically Altered Materials	
_____ Infectious Agents	
_____ Bacteria	
_____ Virus	
_____ Prion	
Carcinogen or Mutagen	

___X___ Toxic Agent

The toxic agent Ibotenic acid is kept in a vial in powder form until mixed to a liquid state under a hood while wearing eye and hand protection. The liquid form is contained in this vial or in a syringe used to deliver the solution (1 milligram per 100 microliters).

Status of OBS-2: (circle one) APPROVED PENDING

___X___ Radioactive Material

The following tracer will be administered by IV injection at a sub-pharmacologic tracer dose of 0.6 milligrams per kilogram or less. 18F-FDG and 18F-MPPF

Status of 99-A: (circle one) APPROVED PENDING

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.

No employee is allowed access to animal housing areas or allowed any interaction with animals until they have successfully completed the following:
RARC Animal Care and Use Course
RARC Primate Handling Course
UW Radiation Safety Course
Herpes B Quiz
[redacted] Tour and Orientation.

After completion of all orientation and training, personnel will be issued keys for animal area access. All personnel working with animals or in animal housing and testing areas are required to wear a lab coat, latex gloves, eye protection and a surgical mask. Shoe coverings or designated shoes are also required. All personnel are trained to dispose of animal waste and biological sampling supplies in the properly labeled receptacles.

You must address questions 17 separately for each species.

17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

EXPERIMENTAL PLAN

Experiment 1: Effects of Amygdala Inactivation on Temperament, Positive Emotion, and Prefrontal Activity

Subjects will comprise two groups of young adult male rhesus monkeys (control and experimental; n=12/group).

We will collect the same behavioral and physiological measures prior to and after surgery, in order to design a between and within subjects study. The behavioral measures include: behavioral adaptation, response to conspecifics, learning, and conditioning, startle, and tactile habituation (see below for detailed descriptions). The physiological measures are hormonal sampling, PET, EEG, EKG, and sleep recording.

Experiment 2: Effects of Bilateral Orbitofrontal Inactivation

To establish and refine the technique for reliable orbitofrontal inactivation, no more than four animals will be used in a pilot

study and euthanized shortly after surgery to histologically analyze the results. After piloting, the subjects will comprise two groups of young adult male rhesus monkeys (control and experimental; n=12/group). Animals will receive the same behavioral and physiological tests as described in Experiment 1.

Experiment 3: The Role of the Right Compared to Left Orbitofrontal Cortex in Mediating Emotion

Subjects will be right, left and bilateral orbitofrontal inactivated experimentals and their controls (n=12/group). Behavioral and physiological assessments will be identical to those described above. To effectively understand the effect of unilateral lesions, a bilateral comparison group and an unoperated control group must be included. In addition to being critical to the interpretation of this experiment, the bilateral group will allow us to replicate findings from experiment two. Because a variety of variables may influence the outcome of our dependent measures, the bilateral group used in experiment two cannot be directly compared to the unilateral groups used in experiment three.

For each of the experiments described above, the control animals will be cage mates with the experimental animals, except in Experiment 3 where one half of experimental animals will live with controls, and the other half of experimental animals will live with other cage mates. A total of six stimulus animals will be used across all experiments.

Behavioral Tests

To investigate the role of the amygdala and orbitofrontal cortex in emotion and learning, we will administer the following behavioral and cognitive tests.

Behavioral Adaptation Test

To assess freezing behavior and other defensive responses in monkeys, we developed the behavioral adaptation test. This paradigm is a reliable method used to evaluate the effects of different types of ethologically relevant threatening situations on anxiety related behaviors. By assessing changes in monkey's defensive behaviors in relation to different environmental contexts, the behavioral adaptation test also allows for the study of emotion regulation. This test is used to assess the animal's behavioral response to the presence of a human making and avoiding eye contact. Behavior is recorded for less than one hour in a special cage, once per week for no more than two months.

Response to Conspecifics Test

Test subjects will be presented with novel monkeys to evaluate social interaction. The animal's behavior will be videotaped while they are separated for one hour by a clear plastic divider and then while they are together in a special double cage. Behavior will be recorded for less than 2 hours for up to five times per week for three months. When two animals are together there is always the risk of one animal injuring the other; however, our laboratory has had extensive experience with this test. Animals will be continuously monitored and if any signs of physical aggression occur, the tester will intervene by separating the animals and the test will be discontinued. This test also provides social enrichment.

Learning Tests

These standard learning tests are used to evaluate the brain areas involved in different aspects of learning. After the animals are adapted to the test situation (special apparatus and special cage) they will be tested on matching-to-sample, spatial and non-spatial reversals, oddity learning, reward devaluation and their response to a snake. The animals are tested for their ability to move the correct object to uncover a food treat or the time it takes to remove a treat from above an object or snake. Most of these tasks have significant enrichment value, providing cognitive stimulation and positive reinforcement. Animals will be tested five days per week for one hour until the task is completed. Some of these tests may take several months to complete. During these tests, animals will receive their total normal daily food rations following testing and will always have access to water. In the rare instance an animal does not perform after five days of testing, despite being offered several different food treats, food will be withheld for one day in an attempt to increase motivation. If the animal is pair-housed, the cage-mate will be fed while the subject is being tested. No animal will be deprived for more than one day each week for up to one month. During this brief food deprivation period animals will be weighed once a week. In the unlikely event a greater than 5% weight loss is observed, food deprivation will cease.

Heart Rate Conditioning

We will study the role of the amygdala and orbitofrontal cortex on emotional processing using classical conditioning of heart rate. In this test, two different tones will be used as the conditioned stimuli (CS+ and CS-). For negative conditioning, the unconditioned stimulus (US) will be a rapid onset one second delivery of white noise paired with a puff of air randomly delivered to the left or right side of the monkey's face. The strength of the air puff can be compared to a person blowing hard on their hand from about three inches from their mouth. We will also test the role of these brain regions in a positive conditioning test. For this, the positive tone (CS+) will be paired with the delivery of a food reward which will serve as the unconditioned stimuli (US). Animals will be adapted to a primate chair (special apparatus) by exposing them to the test environment and chair one hour a day, for a minimum of four days. Following adaptation, subjects will be tested for negative conditioning every other day, for four days. The positive conditioning task will continue for up to 30 days or until they have learned to respond only during the CS+ presentation. If an animal does not learn the appropriate response in 30 days or less, a veterinarian will be consulted for any testing extensions. Testing is accomplished in less than two hours. For each test, heart rate (EKG) will be recorded from surface electrodes. These studies also provide cognitive stimulation and positive reinforcement.

Tactile Habituation

To investigate the interaction between the amygdala and orbitofrontal cortex a tactile habituation test will be used. Tactile habituation is thought to be mediated by the limbic system. Animals will be assessed for habituation to a light tactile stimulus (a feather, a cotton ball, and a soft brush) across the fur. Objects will be attached to a stick and gently brushed along the side of the animal's face, under its chin and over its head and neck. Animals will be tested in a special cage once for approximately five minutes. The procedure will be videotaped for later analysis.

Physiological Tests

Hormonal Sampling

To evaluate the animal's hormonal response to a novel test environment, 7 milliliters or less of blood will be sampled immediately before and after relocation to a novel environment (special cage). When animals are chaired, a total of 25 milliliters of blood will be sampled from an indwelling venous catheter, for the assessment of the time course of the hormonal response. As an example, a two milliliter sample will be drawn at approximately 0 (baseline), +5, +10, +20, +30, +60, +90, +120 minutes, +4 hours, 8 hours, 12 hours, and 24 hours. The cathetor will be inserted percutaneously one hour before sampling begins and removed approximately three hours later. To avoid chairing animals for an extended period of time they will be returned to their home cages after the 120 minute blood draw with subsequent samples taken using a vacutainer. Five milliliters, or less, cisternal cerebrospinal fluid (CSF) sample will be obtained while the monkeys are anesthetized using 15 milligrams per kilogram of Ketamine HCL given intramuscularly. The site is shaved and prepped with alcohol. The sample is obtained by percutaneous puncture into the cisterna magna while the animal is held in the lateral decubitus position. No more than three cisternal punctures will be made to obtain CSF. If there are any signs of discomfort after CSF sampling, analgesia will be provided as recommended by the veterinarian staff. This procedure will be done no more than four times, with at least a one week interval between sampling.

Startle

To test the role of the orbitofrontal cortex and amygdala in emotional processing the startle response will be assessed. Procedures, which will differentiate the response to specific cues associated with conditioned stimuli, will be used. In the potentiated startle procedure animals will be placed in a primate chair (special apparatus) for less than two hours per day. While in the chairs they will be trained for four days. Training will consist of exposure to conditioned stimuli (CS+) of either tones or lights. The CS+ will be paired with the unconditioned stimuli (US), which is a one second air puff paired with white noise. The presentation of the CS+ is used to enhance the startle response to an unexpected sound. Animals will be presented with the startle test for no more than 2 hours on each test day for a maximum of six days. Startle will be measured by whole body movement using an accelerometer attached to the chair. Eye blinks and electromyogram (EMG) will be measured from surface electrodes.

Positron Emission Tomography (PET) Imaging

To investigate the role of the amygdala and orbitofrontal cortex in processing emotion, positron emission tomography will be used to monitor changes in regional cerebral glucose metabolism. This will occasionally be done in conjunction with a behavioral or physiological test. Animals will be adapted to catheter placement for up to 21 days. This will involve transporting animals from their home cage to the restraint table where we will simulate catheter placement (while not actually placing the catheter) and shaving (although we will shave only on the last day of adaptation). A less than 10 milliCurie dose of 18 Fluoro-deoxy glucose (FDG) or 4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-[18F] fluorobenzamido]ethylpiperazine (p-[18F]-MPPF, 2) (18F-MPPF) radiotracer will be administered intravenously. The animals will be anesthetized with a 15 milligram/kilogram dose of Ketamine HCL intramusculary to allow placement of an endotracheal tube and safe transfer of the animal to [redacted] and placed in a special apparatus. A 0.5 milliliter or .27 milligram dose of atropine will be given intramuscularly to depress salivary secretion. During transportation animals will be closely monitored and Ketamine HCL will be readily available. During the PET scan or test a maximum of 25 milliliters of blood will be collected from arterial and venous lines to monitor hormonal changes and tracer kinetics. Animals will be anesthetized with 5% or less isoflurane and heart rate, respiration and oxygen saturation, will be monitored throughout the procedure. The animal's body temperature will be maintained with a warm air blanket. All housing areas and test rooms used at [redacted] have been approved by the Radiation Safety Department for this use. Following the procedure, which typically lasts for two hours, animals will be returned to their home cage. PET procedures are routinely done in humans with no adverse effects. Animals will undergo no more than 12 PET scanning sessions no more than once per week.

Frontal Electroencephlogram (EEG) Asymmetry and Electrocardiogram (EKG)

Electroencephalogram is a measurement of brain electrical activity. It has been shown in humans that there is a difference between the electrical activity in the front of one side of the brain compared to the electrical activity in the front of the other side of the brain. This lateralized frontal brain activity can be used to sort out different types of temperament, as well as susceptibilities to psychiatric problems such as depression and anxiety disorders. We will measure EEG laterality in monkeys to establish their temperamental style, as well as the role of the amygdala and orbitofrontal areas in defining this electrical brain asymmetry.

EEG and EKG will be collected from surface electrodes. Animals will be held by hand during EEG data collection. We are attempting to model Human EEG laterality which has been defined in awake subjects. Anesthesia dramatically changes EEG and therefore cannot be used. All EEG laterality data collected in our laboratory has been obtained from animals while they were manually restrained. Since EEG is collected over a relatively short period it is more efficient and therefore less stressful to manually restrain an animal than to restrain it by other means. Manual restraint is necessary as part of EKG to prepare the animals for the test. When necessary, because animals are too big to manually restrain, EEG data will be collected while subjects are seated in a primate chair (special apparatus) to which they have been adapted for at least four days. The EEG data collection procedure will take approximately one hour not including equipment calibration, electrode placement and electrode removal. We have handled hundreds of animals in this manner for collection of EEG and EKG data without any problems for animals or technicians. We employ numerous safety measures for handlers who hold animals for these tests, in addition to approved laboratory clothing and eye protection. Monkeys are handled by a minimum of two people who have had extensive experience with rhesus monkeys.

Sleep Recording

Because the orbitofrontal region and amygdala are involved in emotional responses and Rapid Eye Movement (REM) sleep, it is likely that they are involved in emotional aspects of dreaming. These brain regions may also mediate the effects of stress on sleep. Insomnia is strongly associated with exposure to stress, and patients with psychiatric disorders commonly have sleep disturbances. In addition, those with mood disorders often have abnormalities of REM sleep expression. The mechanisms for sleep abnormalities in psychiatric disorders and insomnia are largely unknown, but could likely involve the amygdala and/or orbitofrontal cortex.

In order to achieve a better understanding of the role of these brain regions in normal sleep and sleep disturbances, sleep measures will be recorded. Animals will be gradually adapted to a chair (special apparatus) by increasing the time they spend in it each day from one to eight hours over a maximum two week period. Generally animals are placed in a primate chair for two hours on day one, four hours on day two, six hours on day three and eight hours on day four of the adaptation period. If an animal continues to struggle, showing no adaptation to the chair, it will be removed from the study. For each recording session, each monkey will be placed in a primate chair at 1600h and will remain in the chair until 0800h. Animals will be continuously monitored by research staff using infrared cameras, video monitors and computers. EEG will be recorded no more often than one night per week for no more than 6 consecutive weeks by attaching surface electrodes to the scalp and to the outer canthus of each eye for recording eye movement (EOG). Heart rate will be monitored using surface electrodes attached to the chest and flank. Ketamine HCL will be administered (15 or less milligrams per kilogram-intramuscularly) at the end of EEG recording to safely remove electrodes.

The Northern Pine snake is housed in an aquarium that contains bark, rocks, and a tree limb to aid in the shedding process. The aquarium, and its contents, are cleaned every two weeks. The snake is fed a large dead mouse every 2 weeks and given water "ad libitum". Feeder mice are obtained from commercial vendors.

b) Do any animals undergo any type of restraint beyond normal housing methods? **YES** **NO** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

Animals will gradually be adapted to the primate chairs as defined in this protocol. The study design requires chairing for a maximum of 16 hours to monitor sleep. Most animals sleep during the laboratory night that lasts from 1800 until 0600. Before EEG recording begins one hour is required to place the animal in and adjust the chair, attach the electrodes, and calibrate the equipment. Thereafter, another hour is required for awake EEG recording before the lights out period. In the morning, after the lights turn on, we will record for one hour of wake EEG. This will be followed by an hour for removal of the electrodes and returning the subject to his home cage. Monkeys will be chaired nightly for a maximum of one week following two weeks of adaptation. Since chairing will occur during what is normally a period of limited activity for rhesus monkeys, they will not be deprived of normal exercise.

The use of a primate chair is necessary because of the limitations of telemetry systems currently available. These systems must be surgically implanted and would have to be surgically removed for Magnetic Resonance Imaging (MRI) procedures because they contain ferrous metals. Furthermore, implantable telemetry systems have a very limited range and the transmitted signal is lost in some areas and positions within the cage. Continuous data collection is critical for staging sleep. Implantable telemetry systems that record the requisite number of channels for electroencephalograph (EEG), eye movement (EOG) and heart rate (EKG) are not available. Even if such a system were available to cover the desired range with a sufficient number of channels, given current technology, it would be too large to implant in a monkey. Previous experience by our research group has shown that chairing animals is the least stressful and least invasive technique available for obtaining this data.

Each animal's EEG, EKG, and behavior will be continuously monitored using video and computers.

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

During the learning test paradigm animals will be given their total normal daily food rations once a day following testing and will have access to water ad libitium. In the rare instance an animal does not perform, despite being offered several different food rewards over five days, food will be withheld for one day in an attempt to increase motivation to perform. If the animal is pair housed its cage mate will be fed while it is being tested. No animal will be deprived of food for more than one day each week for four weeks. Animals that have this brief food deprivation will be weighed once a week. If an animal loses 5% of its weight or more it will not be food deprived. In order to prevent aspiration, food will be withheld the evening before any procedures which require long-term anesthesia.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.) **YES** **NO** If YES, indicated nonstandard husbandry required and justification for this practice.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

Working closely with veterinarians, we will make every effort to reduce pain in the animals. The anesthesia used for the surgery is similar to that used for human procedures. After surgery, the veterinary staff, the animal care staff, and members from our research team closely monitor the animals. They are provided with extra fluids and receive special food supplementation. They are also provided with warming lamps to maintain their body temperature. Our pain management protocol involves routine monitoring for signs of any physical distress and the regular administration of pain medication. After veterinary staff approval, animals are moved from the recovery area back to their home cages.

Animals will be monitored continuously until they recover from anesthetic; thereafter they will be monitored daily and treated with 20 milligrams per kilogram Cefazolin given intramuscularly twice daily for five days. If the respondent has a problem, the veterinarian will be consulted and appropriate action taken. Discomfort, distress, pain or injury will be minimized by the appropriate use of anesthetics and analgesics, which will be administered under the direction and supervision of the veterinarian on an as needed basis. The analgesic of choice is Buprenorphine, which is usually administered at 0.01 –0.03 milligram per kilogram intramuscularly or subcutaneously. Acetaminophen (20 milligrams / kilogram) administered either orally, or via nasogastric intubation or Ketoprofen (2.1 milligrams/kilogram) intramuscularly is given as required. Analgesia is provided for at least 48 hours post surgery.

For CSF sampling and surgical procedures, 15 milligrams/kilogram Ketamine HCL will be administered intramuscularly every thirty minutes, or as needed, to provide anesthetic (general-dissociative). If there are any signs of discomfort after CSF sampling, analgesics will be provided as recommended by the veterinary staff.

Animals will be receive Magnetic Resonance Imaging (MRI's) while anesthetized with up to 15 milligrams/kilogram Ketamine HCL intramuscularly as needed. When muscle relaxation is necessary a mixture of Ketamine HCL (5 milligram per kilogram) and medetomidine (30 microgram per kilogram) intramuscularly. This mixture is reversible with 150 micrograms/kilogram dose of atipamezole given intramuscularly. Additional injections of Ketamine HCL will be given approximately every 30 minutes or as needed. Atropine (0.5 milliliters or 0.27 milligrams) will be given intramuscularly to depress salivary secretion.

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

Post surgical complete blood counts (CBC's) will be taken 48 hours after antibiotic treatment has finished to monitor for possible infections or other complications. Animals will be monitored as always, daily, for signs or symptoms of ill health by checking fecal material, food intake, behavior, and general appearance. Weight changes will also be monitored at least once a month. In the case of unanticipated illness or injury the veterinarians will be contacted and if necessary euthanasia will be considered under veterinary consultation.

20. If experiments could induce chronic disease, tumors or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.

All animals will be monitored for any post surgical problems including any signs of stroke, seizure and bacterial encephalitis. Behavior will be closely observed for signs of inactivity, listlessness or muscle weakness that extend beyond immediate post surgical recovery. Any change in food intake causing weight loss of more than 5% of the animal's body weight will be reported

to the veterinary staff. If an animal's pain or distress is not alleviated by analgesics, sedatives, or other treatments it will be humanely euthanized in consultation with the veterinary staff.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

Under the supervision of the attending Veterinarian and Pathologist the subjects will be anesthetized using Ketamine HCL (15 milligram per kilogram) intramuscularly. They will be humanely euthanized with an overdose of sodium pentobarbitol greater than 50 milligrams per kilogram intravenously and then perfused with heparanized saline and 4% paraformaldehyde. This is a standard method of euthanization and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. In order to interpret the data from the tests it is necessary to know the extent of the neural inactivation. The only way we can accomplish this is by staining the cells of the brain areas involved.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?

Animals not euthanized will be returned to [redacted] primate colonies.

23. Will any animal products be used for human consumption? **YES** **NO**

If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____

Attending Veterinarian: _____
(SVM ONLY)

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?:

Last name, first	Type and length of **SURGICAL** training/experience
[redacted]	24 years primate surgical experience, MD training
	39 years of surgical experience, 35 years with primates
	2 years primate experience

25. Where will surgery be performed? Room(s): [redacted] Building: [redacted]

26. How many animals listed in question nine will undergo surgery?

64 (See question #17)

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.

For surgical procedures, animals will be premedicated with up to 15 milligrams per kilogram Ketamine HCL intramuscularly When muscle relaxation is necessary a mixture of Ketamine HCL (5 milligram per kilogram) and medetomidine (30 microgram per kilogram) intramuscularly. This mixture is reversible with 150 micrograms/kilogram dose of atipamezole given intramuscularly. An endotracheal tube will be inserted and animals will be anesthetized with 5% or less isoflurane based on individual response. Heart rate, rectal temperature, oxygen saturation and respiration will be monitored throughout the surgical procedure and the animal's body temperature will be maintained with a warm air blanket.

a) Are any paralytic agents being used **YES** **NO** If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. Surgical Procedures (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be used for all survival surgery. (Append additional page if necessary)

To inactivate neurons in the amygdala, landmarks will first be established. Two 3-millimeter glass beads filled with a 3% solution of copper sulfate (which is hyperintense in T1-weighted MRI images), will be stereotactically implanted into shallow indentations in the skull under strict aseptic conditions and deep anesthesia (~~1-4~~ 5% or less isofluorane). The beads will be placed 11 millimeters lateral to the midline coordinate (AP +9, 15 millimeters from the interaural line), approximately at the mid-anteroposterior level of the amygdala, and fastened in place with dental acrylic. The animal will then be allowed to recuperate from the procedure for at least two weeks. After recovery, the animal will be brought to the MRI. Animals will be imaged while anesthetized with up to 15 milligrams/kilogram Ketamine HCL intramuscularly as needed. When muscle relaxation is necessary a mixture of Ketamine HCL (5 milligram per kilogram) and medetomidine (30 microgram per kilogram) intramuscularly. This mixture is reversible with 150 micrograms/kilogram dose of atipamezole given intramuscularly. Atropine (0.5 milliliters or 0.27 milligrams) will be given intramuscularly to depress salivary secretion. After the initial does of anesthesia the monkey will be placed in a plastic replica of a Kopf stereotaxic apparatus that will be positioned in the head coil and scanned for approximately one hour.

Prophylactic doses of antibiotics, Cefazolin 20 milligram/kilogram intravenously or intramuscularly, will be given just before surgery. After the animals are pre-anesthetized with Ketamine HCl (15 milligram/kilogram), and fitted with an endotracheal tube, they will be anesthetized with 5% or less isofluorane gas anesthesia using a ventilator when available. Atropine (0.5 milliliters or 0.27 milligrams) will be given intramuscularly to depress salivary secretion. Heart rate, rectal temperature, oxygen saturation, and respiration will be monitored throughout the surgical procedure. Using standard aseptic surgical techniques, the animal will be mounted in a Kopf ® stereotaxic apparatus. Three milliliters Lidocane HCL 2% with epinephrine will be injected subcutaneously along the proposed incision site to control bleeding. The skull will be exposed, and a 1.5-centimeter in diameter skull opening will be made above the intended site as determined from the MRI procedure. The glass beads and dental acrylic will be removed.

Neurons within specific subnuclei of the amygdala will be permanently inactivated bilaterally using an array of approximately 24, one microliter ibotenic (10 microliter/milliliter of phosphate buffered saline) infusions in three rows, forming a matrix within the targeted area approximately two millimeters apart in all three dimensions. Injections will be made simultaneously bilaterally at a rate of 0.2 microliter/minute. Each injection will inactivate neurons in an area roughly the size of a period. During surgery mannitol (1.5-2.0 gram per kilogram) will be administered intravenously over 30 minutes to control brain swelling. Post surgical brain swelling will be controlled with Dexamethasone (2 milligrams per kilogram-intramuscularly) given twice daily and tapered by half for each day following surgery for three days as prescribed by the veterinary staff.
For orbitofrontal neuronal inactivation a craniotomy will be performed. The dura will be reflected, and the cortex lying medial to the lateral orbital sulcus, extending medially to the rostral sulcus, will be removed by aspiration. The caudal boundary of the area of inactivation is roughly five millimeters rostral to the junction of the frontal lobe and temporal lobe, and the rostral boundary of the inactivation is an imaginary line joining the rostral tips of the lateral and medial orbital sulci. This will involve orbitofrontal regions (areas 11, 12m and 121, 13a and 13b, and 14r and 14c).

After the neurons have been inactivated or removed, the incision will be sutured and the animals will be allowed to recover from anesthesia.

Magnetic resonance images will be used to plan the surgical procedure. Since these scans are used for surgical positioning they will not be repeated any more frequently than weekly. Pre and post surgical MRIs will be obtained to determine specificity of the neural inactivation and for coregistration of PET images.

Catheter Placement Surgery
Under aseptic conditions a three centimeter long skin incision is made in the inner side of the upper leg and the tibial artery is dissected. After the artery is ligated a small incision is made in the arterial wall and a sterilized polyethylene catheter is introduced into the tibial artery and ligated around the arterial wall with silk thread. A sterile blunt needle will be inserted into the opposite end of the polyethylene tubing and capped with a sterile PRN adapter. The tubing will be coiled and taped within a gauze pad for protection during transport to [redacted] This will allow for arterial blood sampling during the PET procedure. After the animal has been scanned the catheter will be removed from the artery, the arterial wall and skin sutured, and antibiotic ointment will be applied to the incision site. An arterial cut-down will only be done when necessary to determine the uptake of a designated isotope. No more than two cut-downs will ever be performed on any animal.

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

For aseptic surgical procedures the incision site is shaved and prepped with betadine, and then covered with a disposable sterile adhesive drape. The surgeon wears disposable shoe covers, a sterile surgical gown, sterile gloves, eye goggles and a splash-resistant faceshield or facemask. Stainless steel surgical instruments are autoclaved for sterilization. Other, non-metal surgical equipment, such as polyethylene tubing, is gas sterilized.

29. Will the animals be allowed to recover from surgery? YES NO

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

Animals will be monitored by staff continuously until they recover from anesthetic (sitting up and locomoting); thereafter they will be monitored daily and treated with 20 milligrams per kilogram Cefazolin given intramuscularly twice daily for five days. Body temperature will be maintained with a warm air blanket, isothermal pads or infrared lights depending on the animals'condition. If the respondent has a problem, the veterinarian will be consulted and appropriate action taken. Discomfort, distress, pain or injury will be minimized by the appropriate use of anesthetics and analgesics, which will be administered under the direction and supervision of the veterinarian on an as needed basis. The analgesic of choice is buprenorphine, which is usually administered at 0.01 – 0.03 milligram per kilogram intramuscularly or subcutaneously. Acetaminophen (20 milligrams / kilogram) is also administered either orally, or via nasogastric intubation or Ketoprofen (2.1 milligrams/kilogram given intramuscularly as required. Analgesia is provided for at least 48 hours post surgery. Individual animal treatment records are maintained in our log books, as well as [redacted] computerized records. All drugs, drug dosages, food and water consumption, as well as the animals health condition will be noted when the animal is treated.

30. Will any animal(s) be allowed to recover from more than one major operative procedure? YES NO
(A major operative procedure is defined as any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions.)

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals:

b) What is minimum length of time between the operative procedures?

One Month.

QUESTIONS FOR PROJECTS USING WILD-CAUGHT ANIMALS

It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild?

YES NO, Observation only

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked

Does not apply

33.

a) Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

Does not apply

b) If animals will be release back to the wild, justify why this release will not result in disease exposure to wild population

Does not apply

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released.

Does not apply

QUESTIONS FOR PROJECTS USING NON-HUMAN PRIMATES

35.

a) If non-human primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

Animals will be singly housed during surgical procedures and for about one week post-surgery for recovery and treatment purposes. At all other times, they will be pair housed. If aggressive behavior is observed between cagemates, they will be separated and singly housed for their own safety. Following separation the Animal Care Staff will make the determination of whether it is safe to attempt to find another cage mate.

b) Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.

Environmental enrichment will not be restricted, as many of the tests serve as cognitive enrichment.

36.

In response to concerns raised during our last laboratory animal care and use accreditation site visit, the Medical School Animal Care and Use Committee now requires responses to the following for each protocol submission:

1. Does this protocol involve the in vivo use of infectious microorganisms, cultured cells or tissues, human-derived tissues or substances, toxic or carcinogenic/mutagenic material, recombinant DNA, or radionuclides? If "yes", answer the following:

Yes

a. Specify the name and type of agent (e.g. infectious organism, toxin, etc.). Radiotracers
b. Has the use of this agent been reviewed by the appropriate safety committee? Yes
c. Provide any special precautions that should be followed by your lab personnel, animal care workers or any other personnel entering areas where experiments are conducted or animals housed. Include any special practices required for handling of animal waste, animal carcasses, cages, or equipment.

Lab personnel are required to wear personal dosimeters at all times when working in radioactive areas and to limit their exposure to the radioactive source. Animal waste and cages/equipment are isolated in a well labeled, radioactivity approved area until radioactivity levels have decayed to background levels, which takes about 24 hours.

2nd Rewrite

CONFIDENTIAL

Revision 11/99

M00155·4·05·02

JUL 12 2004
e-mailed D
RESEARCH ANIMAL
RESOURCES CENTER

Protocol Code [redacted]

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

orig 4.22.04
2nd 6.25.04

Forms should be typed or in computer-printed format. IBM & Macintosh word processing diskettes are available through Research Animal Resources Center (RARC) or the form can be downloaded via the RARC homepage: http://rarc.wisc.edu/

Return Completed Forms to RARC, 396 Enzyme Institute, 1710 University Ave, Madison, WI 53705

Office Use Only

AMENDMENT APPROVAL

Committee Action: _____

Veterinarian Signature _____ [redacted] Date: _____

7.21.04 Date — Veterinarian [redacted]

Chairperson Signature: _____

Type of Protocol Procedure: _____

7.21.04 Date — Chair [redacted]

Type of Surgical Procedure: _____

_____ Survival Surgery _____ Non-Survival Surgery _____ Rodent Surgery _____ Non-rodent Surgery

_____ Multiple Major Survival Surgery _____ Exercise Exemption _____ Paralytic Agents _____ Restraint

_____ Critical Veterinary Care _____ Fluid/Food Restrictions _____ Nonstandard Housing _____ Nonstandard Husbandry

_____ Occupational Health/Personnel Safety _____ Class B Dog/Cat _____ Biohazards _____ Radiation

_____ Enrichment Exemption

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. Principal Investigator/Project Director: [redacted] C
   Telephone Numbers: Office: [redacted] Lab: [redacted] Animal Emergency: [redacted]
   Home: [redacted] Fax: [redacted] E-mail Address: [redacted]

   If Investigator is Unavailable for Emergency
   Alternate to Contact With Authority to Act in Investigator's Absence: [redacted] C
   Alternate Office Phone: [redacted] Alternate Emergency Phone: [redacted]

2. University Department (of PI): Psychiatry Office Address: [redacted]
   Unit & Division Number (UDDS): A – 55 – 1000

3. Is this protocol a: **NEW** **RENEWAL** **AMENDMENT** application? (Circle appropriate category)
   If Renewal or Amendment application, please give current protocol code: A - 55- 1000 - M00155 - 3 - 05 - 02

4. Is this protocol for: **TEACHING or RESEARCH: (indicate research type) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER** _____ **(SPECIFY)**
   Circle **all** that apply.

5. **Title of this Project:** Stress and Regulation of CRH Systems & CRH-binding Protein: A Regulator of CRH in Stress

6. Classification of Research Animal Use (See attached schedule; **circle** highest category applicable) 1 (2) 3 4 5

7. Will ANY surgery be performed on any of the animals? (YES) NO If yes, you must fill out questions 24-30.
   Will you be working with wild-caught animals? YES (NO) If yes, you must fill out questions 31-34.
   Will you be using non-human primates? YES (NO) If yes, you must fill out questions 35 & 36.

8. Except for surgery (see question 25) will any procedures (e.g. blood collection, injections, euthanasia, etc.) be conducted in labs

or will animals be housed outside of their normal animal housing areas? (YES) NO If YES, indicate building and room numbers and anticipated length of time away from normal housing area(s):

[redacted], infusions (length of time: less than 2 hours).

9. NOTE: NUMBERS OF ANIMALS REFERS TO THE TOTAL NUMBER OF ANIMALS THAT ARE ANTICIPATED TO BE USED DURING THE ENTIRE THREE YEAR LIFE OF THIS PROTOCOL.

a) Species of Animal:	Total Number For 3 yr.:	Source of Animals (e.g. commercial, U.W. breeding colony, or list other):
(#1) rat	1800	commercial
(#2) ferret	12	commercial or from another UW research protocol
(#3)		

b) Will any of the animals be obtained from Class B dealers? (dogs & cats only) **YES** **NO**

c) Have any of the animals from above been part of any other protocols? (YES) **NO** If YES, how have you determined that the previous use will not compromise the animal's health and proposed current research?
Two ferrets were used for a single day for practice intubation which would leave them in perfect health. They were previously under protocol [redacted].

10. Building(s) or facility where the animals will be housed (normal housing).
[redacted]

11.

a) Outline the specific scientific goal(s) and significance of this research in straight-forward, non-medical, non-technical language that would be understandable to a **layperson**.
It is thought that abnormal responses to stress and dysregulation of the brain systems that mediate stress are critical factors in the etiology of psychiatric illnesses such as anxiety disorders and depression. Brain corticotropin-releasing hormone (CRH) systems are hypothesized to be the primary neurobiological mechanism for integrating an organism's physiological and behavioral response to stress. Thus, the goal of this research is to understand the relationship between CRH systems and stress. This will be done by examining the effects of stress on the expression of various components of the CRH system in brain tissue and also by assessing the behavioral effects of CRH system manipulations. To alter CRH systems, we will administer CRH, CRH antagonist, CRH antiserum, or oligodeoxynucleotides intracerebroventricularly (i.c.v.) or in specific brain nuclei.

b) Provide a justification for the use of animals for this research. Indicate why it is imperative to use animals for this research and explain why alternatives such as computer simulation or in vitro systems are not possible.
Because of the invasive methods required to study CRH function in brain as well as the need to carry out behavioral analyses of stress responses, we have chosen the rat as our model species. Therefore, the aims of this research preclude the use of computer simulation or in vitro systems.

c) Provide justification for why you have chosen the species cited in 9-A for your work.
The wide majority of the existing literature in this field utilizes rats; so it is additionally important for us to use similar animal models. For experiments involving the predator stress paradigm, ferrets will be used because this species is a natural predator of the rat, and has been shown in previous studies to elicit a potent stress response in rats. A total of six ferrets are needed because on a given test day, multiple rats undergo this stress procedure, and ferret approach behaviors towards the rat cage diminish significantly after the ferret has sequentially been exposed to 3 rats. We expose up to 18 rats to the predator stress paradigm on a given test day, thereby requiring 6 ferrets (each ferret is exposed to 3 rats) to carry out this procedure with high, constant approach levels from the ferrets towards the rat cages.

12. Explain how the number of animals required was determined and justify that need. Include control animals in this discussion.
We will use approximately 600 rats per year. In order to collect enough brain tissue for measurement of mRNA as well as to have sufficient numbers of subjects for reliable behavioral assessments, we need a minimum of 10 rats per condition (based on previous data; control group plus 2 treatment groups). We typically run up to 3 conditions per experiment and 20 experiments per year (10 x 3 x 20 = 600 / year).
We would like to use up to 50 rats to perform the additional defensive burying study and controls (apparatus control and home cage control). This number is included in the 1800 rats previously approved for the protocol over the 3-year span. We have increased the number of ferrets needed from 6 to 12 because the original 6 were becoming less interactive with the rats as they aged. Four of the original 6 ferrets were adopted out and 2 had to be euthanized due to development of adrenal tumors.

13. Indicate any current or pending funding for this project:
Funding Source (1): NIMH Grant Number (1): MH40855
Title of Grant (1): Role of Central CRH in Stress-Induced Behavior
Funding Source (2): NIMH Grant Number (2): MH65109
Title of Grant (2): CRH-binding Protein: A Regulator of CRH in Stress

14. Identify the person(s) or unit responsible for **daily** animal care:

RARC, [redacted]

15. Personnel working with animals: Everyone must take the "Responsible Use and Care of Laboratory Animals" exam or course. Protocols cannot be processed until PI and all personnel are certified. For information, call RARC 262-1238.

Last name, first name	Phone Number	Type and length of training/experience for animal use
[redacted]	[redacted]	Rat and monkey surgical experience and supervisory experience for 30 years
[redacted]	[redacted]	Monkey surgical experience and supervisory experience for 25 years
[redacted]	[redacted]	Rat experience including injections and decapitations and supervisory experience for 18 years
[redacted]	[redacted]	Rat surgical experience and supervisory experience for 15 years
[redacted]	[redacted]	Rat handling and surgical experience for 6 years
[redacted]	[redacted]	Rat and mouse handling and surgical experience for 3 years
[redacted]	[redacted]	Rat and mouse handling and surgical experience for 1 year
[redacted]	[redacted]	Rat handling and surgical experience for 1 year

16.

a) Give a brief summary of the methods and sources you use to keep current with pertinent information in your field in order to assure that alternatives to the use of animals have been considered, this work is not duplicating existing knowledge, and that the procedures are the least stressful to animals. If electronic databases are utilized, include sources, date of search, years covered by the search, and key words and/or search strategy used. This information for electronic databases is required by USDA Policy #12. Full text of USDA Policy #12 may be viewed at: http://www/aphis.usda.gov/ac/policy12.html.
PubMed electronic database; 1966 – present; "stress", "CRH", "CRF"
Medline electronic database; 1966 – present; "stress", "CRH", "CRF"

b) **Radiation or Biohazard Material Usage In Animals: mark all that apply, indicate specific material used, and show status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.**

Category	Specific Material(s)
_____ Recombinant DNA	
_____ Genetically Altered Materials	
_____ Infectious Agents	
_____ Bacteria	
___X_Virus	rAAV-2 viral vector; replication-deficient
_____ Prion	
_____ Carcinogen or Mutagen	
_____ Toxic Agent	

Status of OBS-2: (circle one) (APPROVED) PENDING

_____ Radioactive Material

Status of 99-A: (circle one) APPROVED PENDING

Occupational Health & Safety: If you are using any agent that could be hazardous to humans or animals please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such

requirements as: masks or respirators, eye protection, lab coats, gloves, and disposal methods.
Lab coat and gloves should be worn at all times when handling rats. Dental cement should be mixed under the hood and a respirator mask should be worn while cementing.

You must address questions 17 separately for each species.

17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary.)

HOUSING

All rats are housed in groups of two to three in clear plastic cages. Occasionally, for the purpose of the experimental question, animals will be housed individually (still in the clear plastic cages). Finally, for some experiments, the influence of an enriched housing environment (rats housed in groups of 5-8 in large plastic cages with plastic toys and running wheels inside) will be evaluated. All rats will receive food and water ad libitum except in a few cases where mild food or water restriction is necessary for the behavioral test (ie—measuring ingestive behavior; see Section III, F). At no time will food or water be withheld for more than 20 hours; rats' weights will be taken to ensure that dehydration or sickness does not occur at any point during the experiment. A 20 hour food or water restriction period has been used routinely in the literature by this and other labs over the last 40 years to elicit a reliable ingestive behavior baseline for testing. Rats are always allowed unlimited access to food and water for a period of at least 4 hours after any food or water restriction period. Ferrets are housed in large metal cages, with 2 ferrets per cage. Ferrets have food and water available ad libitum.

SECTION I – STRESSORS

A. Restraint Stress

The rat is placed in a clear Plexiglas container (8" x 3.5" x 2.5") that restricts extensive movement. Containers have airholes and allow for comfortable respiration. Rats will be restrained for no more than 2h at a time.

B. Shock

The rat is placed in a chamber and exposed to three 1-second foot shocks of mild intensity (0.2 to 1.5 mA). The behavior of the animals is observed by the experimenter before and after the shocks are delivered.

C. Swim Stress

The rat is placed for 90 seconds in a plastic bucket containing tap water. The water is approximately 30-35 cm deep and is kept at room temperature (21-23°C). Rats are gently towel dried when removed from the bucket.

D. Social Isolation

Rats are housed singly in clear plastic cages after weaning.

E. Resident Intruder

A dominant rat and a subordinate rat are placed in a clear Plexiglas chamber with an attached safety chamber. The subordinate rat is allowed to escape voluntarily into the safety chamber following the first display of aggression by the dominant rat. The subordinate rat stays in the safety chamber for the remainder of the test.

F. Partial Sleep Deprivation

The rat is placed on a 9cm circular platform surrounded by water no deeper than 8cm (flower pot method) for up to 120 hours (five days). The extra day is to conform with our other protocol for sleep restriction and wound healing (#M01478). Upon entering REM sleep, muscle atonia causes the rat's head to dip into the water, preventing the maintenance of REM sleep. Food and water are accessible at all times. This procedure allows for all stages of sleep except REM sleep to occur normally; thus, rats are subjected to only partial sleep deprivation. Rats are monitored for general body condition several times per day, and are weighed once a day in order to monitor any weight loss. Weight loss is not expected to be more than 5% during the study. Animals who appear sick or who have weight loss greater than 5% are removed from the study immediately. We will have an apparatus control group that is placed in bigger flower pots and therefore their sleep will not be materially changed.

G. Maternal Separation

Pups are separated from dams briefly (3 hours per day) during the 3 weeks between birth and weaning. Pups are placed as a group in an incubator maintained at 30-32°C, which allows normal thermoregulation. Nutritional deprivation does not occur with this procedure (dams in the wild are away from pups for similar brief periods of time to forage for food) and separated animals gain weight normally.

H. Predator Stress

Rats are placed individually in a metal protective cage that is placed inside of the homecage of a ferret. The animals can thus see, hear, and smell each other, but there is no possibility of physical contact between the species. The rat's cage is allowed to remain in the ferret's cage for 5-30 min. The rat cage is then removed along with the rat. At no point is any invasive procedure carried out on any of the ferrets. During the 5-30 min test, an experimenter sits outside of the test

room and monitors the test.

I. Hotplate/Analgesia

To test for analgesic effects, a rat is placed on a 50 C plate and removed as soon as it licks its hind paws. No rat will remain on the plate longer than 60 seconds (at which time a rat is automatically removed if it does not lick its paws). The latency to paw lick is recorded by the experimenter as a reliable measure of analgesia. The hot plate test can be considered a stressor because it is used to demonstrate pain threshold to allow for interpretation of studies using sensory stimuli (e.g., shock-induced freezing).

J. Defensive burying

Burying behavior is a rodent specific defense reaction to aversive stimulation and is an active strategy of a rodent to cope with a stressor. In this paradigm, rats are shocked once from an electrified probe and subsequently push the bedding material of the chamber toward or over the stationary probe (Treit et al, 1981). The expression of defensive burying behavior has been considered to reflect the rat's emotional level in a direct way, i.e., low defensive burying equals diminished experimental anxiety. On the contrary, high burying behavior indicates increased experimental anxiety. Shock-induced freezing evaluates inhibition of locomotor activity while burying behavior measures the increase of locomotor activity.

Testing is performed in a transparent, Plexiglas chamber, the floor of which is covered with wood shavings (height 3.5cm). A removable, electrified probe, is placed through a small hole, 2 cm above the bedding material, in one of the long walls of the cage. The aversive stimulus is generated by a shock generator-scrambler and delivered through the shock probe whenever the animal touches the probe with a forepaw or the snout. The rat receives a 1.5 mA shock for the duration of contact with the probe (less than 1 s). The behavior is recorded with a videocamera for 15 min.

Subjects are placed in the test chambers individually, for 45-60 min habituation sessions on consecutive days before the testing day. The bedding material is changed and the chamber cleaned between each habituation and testing trial.

Treit D, Pinel JP, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 15:619-26, 1981

Treit D, Pinel JPJ, Terlecki LJ. Shock intensity and conditioned defensive burying in rats. Bull Psychonom Soc 16:5-7, 1980

General information:

As most experiments are concerned with the effects of *acute* stressors, animals in these studies will undergo only one of the aforementioned procedures once. Some animals will undergo sleep restriction up to 5 days and be tested for their reaction to other stressors before, during, and after the period of sleep restriction. This will enable us to study the effects of acute REM sleep deprivation on physiologic responses to other stressors. In other experiments the effects of chronic or repeated stress will be studied by presenting rats with either a chronic mild stressor (partial sleep deprivation or social isolation) or repeated presentation of one of the other stressors (ie--repeated restraint). Chronic or repeated stress is important to study because it may alter the CRH system in a long-term fashion and may thus be involved in the development of depression. In some experiments, the ability of an antianxiety compound (such as CRH antagonists) to prevent stressor-induced effects will be examined in order to learn which brain regions are important in the control and alleviation of stress. There is a need for 2 different paradigms using shock exposure in order to measure different behavioral responses to the shock. Shock-induced freezing measures anxiety as behavioral *inhibition* whereas shock-induced burying (defensive burying) measures anxiety as behavioral *activation.* We need to distinguish whether sleep deprivation has primary effects on anxiety vs. behavioral activation. *No rat will receive both tests using shock.* Overall, only one type of stressor will be used for a set of rats, and rats will receive no more than 14 days of stressor exposure. The health of all animals undergoing chronic/repeated stress will be monitored daily. Rats will immediately be removed from the study or euthanized if they become ill. All equipment that the animal comes in contact with will be cleaned thoroughly between uses. All procedures in this section are routinely and widely used by many other labs around the world; the present protocol adheres to the well-accepted standards that are reported in the literature.

SECTION II – BEHAVIORAL TESTS

These tests are noninvasive and are essentially observations of different aspects of the animals' normal behavioral repertoire.

A. Plus Maze

The plus maze is a black Plexiglas apparatus that is arranged in a "+" shape, with 2 enclosed arms (high walls) and 2 open arms. The rat is placed in the center of the maze, and the amount of time that it spends in the various arms is rated by an experimenter.

B. Freezing

Freezing is a naturalistic defensive behavior that is exhibited by many organisms in response to feeling stressed. A measure of stress is the latency and duration of freezing in response to a stressful situation, a state in which rats are hypervigilant and voluntarily inhibit their body movements except for respiration. Thus, freezing behavior is rated by an experimenter after exposing a rat to one of the stressors described in Section I, usually mild footshock.

C. Open Field

An open field is a large (1 m^2) square enclosure with high walls. In certain experiments, a smaller open-ended black Plexiglas box (12 x 12 x 9 inches) is placed inside of the open field. A rat is placed in the open field and is allowed to freely explore this environment while its behavior is scored by an experimenter. The pattern of exploratory behaviors as well as the time spent in the smaller chamber within this arena is rated.

D. Social Interaction

Five days before testing, rats normally housed together are housed separately in clear plastic cages. For the test, two rats

are placed in an open field arena and their social interaction is rated.

E. Startle Chamber

A rat is placed in a Plexiglas chamber that is housed within a large, ventilated and lit chamber. It is then presented with a series of noises (65 – 120 decibels), and the startle response to the noise bursts is recorded.

F. Behavioral Observation

After mild food or water deprivation (20 h), rats are allowed free access to food and water, and exploratory and ingestive behaviors are rated by an experimenter.

G. Locomotor Activity

Rats are placed in clear plastic cages and their pattern of general activity is monitored.

General information:

Usually, these behavioral tests take place after presentation of a stressor (as in Section I) or the administration of CRH or related peptides (0-5 μg/ 3 μl). In other experiments, the ability of CRH antagonists, CRH system oligonucleotides (0-5nmol/μl), or CRH system DNA enzymes (0-**100** μg/ μl) to block the effects of stressors will be examined. CRH antagonists include α-helical CRH (0-20 μg/3 μl), d-phe CRH (0-20 μg/3 μl), and novel experimental nonpeptide CRH antagonists (because these compounds are proprietary, we cannot reveal their names or dosages, however, dosing will conform to the suggested guidelines of the pharmaceutical company and will not approach toxic ranges; if any animals become sick from these treatments, they will immediately be removed from the study and euthanized). Finally, in some experiments the effects of anxiety-reducing compounds such as benzodiazepines (ie--diazepam, alprazolam, midazolam or adinazolam, 0-10 mg/kg) will be evaluated. Compounds will be administered either systemically (ip, sc or orally) or directly into the brain (sterile injectors are lowered through indwelling guide cannulae aimed at a particular brain region and compounds are delivered into that region of interest).

All experiments in this protocol will follow one of these 2 outlines:

Type 1 (roughly 200 animals per year) – stressor (either acute or repeated/chronic) followed by behavioral test(s) (see Section II), followed by euthanasia. *Example*: rats receive 1 hr. of restraint stress, are tested in a plus-maze, and are then euthanized.

Type 2 (roughly 400 animals per year) – surgery (one of the procedures in Question 28) followed a stressor (one procedure from Section I) and/or administration of CRH-related compounds followed by behavioral test(s) (from Section II).

Example: rats have stereotaxic surgery for cannulae implantation, (after post-operative recovery) receive an infusion of CRH antagonist, undergo swim stress, and are then observed in an open field.

Please note that these are approximate estimates of the number of animals in each type of study. Actual numbers will be determined by the outcomes of the studies, which will dictate the subsequent experiments. Experiments on average will last for one to two months from the time the animals arrive to when they are euthanized.

b) Do any animals undergo any type of restraint beyond normal housing method? **YES** **NO** If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

Please see response to question 17----Section IA, "restraint stress"

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions.

Brief food or water restriction (never exceeding **20** hours) is carried out for certain experiments in which ingestive behavior is studied.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)

YES **NO** If YES, indicate nonstandard husbandry required and justification for this practice.

During the flower pot sleep deprivation paradigm each rat has access to overhead food and a spigot for water using the same type of cage tops that are used in the animal's general housing. Every day the animals will be removed and the platforms will be scrubbed. The water bath will be replaced daily during the experiment. The tank will be cleaned every 2-3 days and disinfected every 5 days during the experiment and also between experiments.

18. For animals experiencing more than momentary or slight pain or discomfort as a result of your procedure(s), describe what you will do to relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research. Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. If you do not believe animals will experience any momentary or slight pain, provide explanation for that belief.

All invasive procedures (such as surgery) are carried out under complete anesthesia, therefore protracted pain and discomfort are not expected to occur. The procedures described in Question 17 under Section I are all momentary stressors, and do not entail induction of pain in a long-term fashion.

19. Describe how frequently and how you will monitor your animals to insure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury. Include criteria when euthanasia would be utilized for dealing with the unanticipated illness or injury not necessarily directly related to your research.

Undue pain is not expected to occur with any procedure (if lethargy, decrease in appetite, hyperalgesia or hypersensitivity to touch, redness or swelling are observed in any animal, a veterinarian will be contacted immediately). However, the discomfort associated with more invasive procedures such as surgery will be treated with analgesic measures such as the use of lidocaine (40 mg/mL, 0.1 mL, injected subcutaneously into the incision site at least 2 min before making the incision), a topical

anesthetic. All animals will be monitored closely for 10 consecutive days after a surgical procedure for postoperative recovery. In the event of undue pain, sickness, or infection, a veterinarian will be consulted immediately; if necessary, rats will be removed from the study and euthanized. All of the above procedures are well-accepted and widely used techniques that are well-documented in the literature. The present protocol adheres to the standards that are reported in the literature.

20. If experiments could induce chronic disease, tumors, or radiation sickness, describe the specific criteria for termination of the affected animals. This description should be detailed enough so as to indicate such things as tumor size, specific animal characteristics or behaviors, weight loss criteria, clinical signs, etc.
The outlined experiments are not expected to induce chronic disease, tumors, or radiation sickness.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation and provide necessary justification as necessary. Euthanasia methods must follow the Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (1993). "In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded; in sedated or unconscious animals when practical; when scientifically or clinically justified, and with Animal Care Committee approval. Physical methods **without** pre-anesthesia require scientific justification.

NOTE: Even if euthanasia of animals is not part of this project, complete this section to provide direction in cases of unanticipated illness or injury.

At the completion of the experiment, rats are overdosed in one of two ways: 2-5% O_2/isoflurane (1 L/min) administered in an inhalation chamber for 2.5 – 5 min, after which a cylinder is placed over the nose of the rat to allow continuous flow of O_2/isoflurane; or sodium pentobarbital (160 mg/kg, ip). Brain tissue is processed for histological procedures. Prior to removing the brain death is assured by transcardial perfusion with 4% paraformaldehyde followed by decapitation. In experiments where it is necessary to collect fresh tissue, rats are rapidly decapitated at the termination of the study. Decapitation, rapid extraction of the brain, and collection of trunk blood are necessary procedures for obtaining fresh samples for the accurate measurement of hormone and mRNA levels. Experiments in which the effects of stress on brain CRH systems is being assessed will require decapitations; these studies represent roughly 30% of the total number of rats that will be used in this protocol. Decapitations will be performed by [redacted] who has 18 years of experiences with decapitation of over 1000 rats. In addition, [redacted] will be doing some decapitations and has been trained by [redacted]. There are two guillotines in the lab and they will be sharpened once every four months. A log recording the sharpening dates will be kept in laboratory.

22. If the animals are **not** euthanized at the end of the study, what will happen to them?
Not applicable

23. Will any animal products be used for human consumption? **YES** **(NO)**
If YES, list any drugs to be given to the animals, and their withdrawal times before consumption:

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____

QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES

24. Which of the persons listed on question 15 will be performing surgery?:

Last name, first	Type and length of **SURGICAL** training/experience
[redacted]	Stereotaxic surgery; 3 years
	Stereotaxic surgery; 1 year
	Stereotaxic surgery; 1 year

25. Where will surgery be performed? Room(s): [redacted] Building: [redacted]

26. How many animals listed in question nine will undergo surgery? 1200

27. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation schedules. Include how anesthesia level is monitored.
Sodium pentobarbital (50 mg/kg), ip, supplementation: 0.05 – 0.10 mL, not more frequently than every 10 minutes.
Ketamine-xylazine HCl mixture (80 mg ketamine and 12 mg xylazine per mL of the mixture; given 1 mL/kg), ip, supplementation: 0.1 mL of ketamine alone (80 mg/ml), not more frequently than every 10 minutes.

Please note that only one of these anesthetics will be used in a given animal; they will not be used simultaneously. Both drugs are listed in the protocol because each one is ideal for different applications: ketamine/xylzine is preferred for short

procedures and pentobarbital is preferred for longer procedures and euthanasia. Anesthesia level is monitored by testing rat reflexes.

a) Are any paralytic agents being used YES (NO) If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. Surgical Procedures (see relevant Campus policies at www.rarc.wisc.edu or Medical School policies at www.acu.medsch.wisc.edu/Policies/policies

a) Describe the surgical procedure(s), including a narrative description(s) giving: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery. Note: aseptic procedures must be used for all survival surgery. (Append additional page if necessary.)

All surgical procedures are performed under anesthesia and will utilize aseptic rodent surgical techniques.

A. Stereotaxic surgery for cannulae implantation or discrete neuroanatomical lesions

Adult rats are anesthetized and are given 0.1 mL atropine sulfate (0.54 mg/mL, s.c.) in order to minimize respiratory complications from anesthesia. The heads of the rats are shaved, placed in a stereotaxic instrument, and swabbed well with ethanol. Eyes are protected with antibiotic eye ointment. A scalp incision is made, the skull is exposed, and small burr holes are drilled. For implantation of guide cannulae (sterile stainless steel tubes), cannulae are lowered through the burr holes and held in place with stainless steel jeweler's screws and dental cement. For making lesions, the sterile metal probe (electrode or tube for making electrolytic or excitotoxic lesions) is lowered through the burr holes and left in place until the lesion is made. After either procedure, the incision site is sutured. Antibiotic ointment is applied to the suture wound, and rats are given antibiotic (dual-cillin, 300000 units/mL, 0.1 ml, i.m.) at the end of surgery before waking from the anesthetic. The entire procedure takes roughly 40 minutes to complete.

B. ICV Infusions in Neonates

Rat pups are anesthetized by hypothermia (if less than 3 days old) or inhalant anesthesia, and the head is cleaned with ethanol. The skin at the site of infusion is clipped with small, sterile scissors (incision is less than 0.1 mm). CRH or a related peptide is delivered into the ventricle using a sterile Hamilton 10 µl syringe that is inserted through the incision site. The wound is then cleaned and treated with topical antibiotic. Animals are allowed to recover on sterile gauze strips placed on top of a warm heating pad and once awake, are immediately returned to the dam. The entire procedure is completed in 5 minutes. Infusions will take place once every 24 h for no more than 5 days. Pilot results from previous studies indicate that this regimen of infusions is well tolerated and does not produce infection or sickness.

C. Osmotic Minipump Implantation

In some adult rats, the effects of continuous infusion of CRH and related compounds will be studied. In order to continuously deliver compounds of interest into discrete neuroanatomical regions, implantation of osmotic minipumps will be carried out in conjunction with the cannulation surgery outlined in part A of this section. Thus, an osmotic minipump kept under sterile conditions before its use is implanted subcutaneously through a small incision (less than 2 cm) that is made between the shoulder blades. After subcutaneous placement of the pump, the pump is attached to the guide cannulae (see part A) via sterile flexible tubing. The incision site for the minipump is then closed with sutures and is treated with topical antibiotic. Pumps are generally smaller than 1 cm. Minipump implantation is completed in 5 minutes.

D. Catheterization for Blood Sampling

In order to obtain samples to assess plasma levels of stress-related hormones, some adult rats will be equipped with chronic jugular catheters (0.3 mm diameter Silastic catheter). Rats are anesthetized, and the neck and middle back are shaved and swabbed with alcohol. A small incision (1 cm) is made in the neck, and a catheter tube is inserted into the jugular vein and secured with a suture. The catheter tubing is passed through a subcutaneous channel, emerging through a small incision (3 cm) in the middle back. The portion of the catheter that is fixed to the animals' back is mounted on a square piece of sterilized surgical-grade mesh. The mesh is implanted subcutaneously, so that during healing, the subcutaneous tissues grow into the mesh and secure the catheter in place. The catheter is flushed with 0.1 to 0.2 ml of a mixture of saline, heparin (0.03 mL of 30 USP units/mL per mL of saline), and antibiotic (Timentin, 100 mg/mL). Dorsal and ventral incisions are flushed with a mixture of saline and antibiotic (gentamicin, 0.3 mL of 40 mg/mL gentamicin sulfate in 0.6 mL saline), closed with silk suture, and antibiotic ointment is applied. This procedure is completed in about 25 minutes. A small plastic catheter-linked infusion port protrudes from the dorsal incision; blood can be withdrawn through this port. This port is kept firmly capped when not in use. After the appropriate post-operative recovery, blood samples can be taken. Samples (300 µl) of blood will be taken every 24 hours over a period of 76 hours.

E. Stereotaxic Surgery for Targeting Brain CRH Systems

In order to examine the functional role of brain CRH system proteins in stress, gene-targeting techniques that result in a long-term up- or downregulation of CRH proteins will be used. Using standard stereotaxic techniques and aseptic surgical procedures, rats will receive infusions of replication-deficient viral vectors (rAAV-2) that contain either antisense or sense sequences for the targeted protein. Thus, after anesthesia, the heads of the rats are shaved, placed in a stereotaxic instrument, and swabbed well with ethanol. Eyes are protected with antibiotic eye ointment. A scalp incision is made, the skull is exposed, and small burr holes are drilled. A sterile metal tube containing the vector is lowered through the burr holes to the brain region of interest, and left in place while a small volume (1 – 10 µl) of the vector is delivered. In separate animals, the same procedure will be performed, but instead of viral vectors, an enzyme that selectively targets mRNA for the protein of interest will be infused. After either procedure, the incision site is sutured. Antibiotic ointment is applied to the suture wound, and rats are given antibiotic (dual-cillin, 300000 units/mL, 0.1 ml, i.m.) at the end of surgery before waking from the anesthetic. The entire procedure takes roughly 40 minutes to complete. All procedures, including surgery, involving the viral vector will be carried out under a biosafety hood (level II). Both of these techniques

are well-accepted procedures in the scientific literature for inducing a long-term increase or decrease in protein levels, and are necessary for studying the functional properties of the proteins over an extended period of time. After surgery, rats will be allowed 1 – 3 weeks of postoperative recovery, and then be tested in some of the behavioral paradigms outlined in the following section. Rats will be kept for up to 1 year after the surgery. At the end of the experiments, rats will be euthanized and their brain tissue will be collected for analysis of protein content.

General information:

Except for procedure C, animals will be anesthetized with either a ketamine/xylazine HCl mixture (80 mg ketamine and 12 mg xylazine per mL of the mixture) or with sodium pentobarbital (50 mg/kg); either compound will be delivered by an ip injection in a volume of 1 mL/kg body weight. (thus, doses are 80 mg/kg ketamine and 12 mg/kg xylazine). Rats will receive 0.1 ml of atropine sulfate (0.54 mg/mL, s.c.) in order to minimize respiratory complications from anesthesia, and will receive both topical (neosporin) and i.m. (dual-cillin, 300,000 units/mL, 0.1 mL) antibiotics. For all procedures, aseptic techniques are always employed. Analgesic measures such as the use of lidocaine (40 mg/mL, 0.1 mL injected subcutaneously into the incision site at least 2 min prior to making the incision), a topical anesthetic, will be employed. If animals display pain responses when grooming around the surgery site or when the area close to the surgery site is touched, they will be removed from the study and be given buprenorphine (0.1 mg/kg subcutaneously, every 6 to 12 hours) until pain responses are no longer detected, and then be reassigned to another experiment. All animals are monitored for 10 consecutive days after a surgical procedure for postoperative recovery. In the event of undue pain, sickness, or infection, a veterinarian will be consulted immediately; if necessary, rats will be removed from the study and euthanized. All of the above procedures are well-accepted and widely used techniques that are well-documented in the literature. The present protocol adheres to the standards that are reported in the literature.

b) Detail the aseptic procedures used for this survival surgery including incision site preparation, instrument sterilization, and clothing worn.

All instruments are sterilized by autoclaving prior to surgery. There is a bead sterilizer to use for instrument sterilization during surgery. Incision site is prepared by cleaning with ethanol and betadine. Surgeon wears gloves, mask, and labcoat.

29. Will the animals be allowed to recover from surgery? **YES** **NO**

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including all drugs and dosages, how body temperature will be maintained during recovery, who will do the monitoring, frequency/duration of monitoring, the parameters which will be evaluated, and method of maintaining written records of these examinations. Describe measures designed to alleviate post-operative discomfort. (For Medical School: see analgesic policy at: www.acu.medsch.wisc.edu/Policies/policies).

After surgery, rats are placed on circulating hot water pads to prevent hypothermia. Rats are checked daily for infection. Postoperative analgesia (lidocaine 40mg/mL, 0.1 mL applied topically to a square centimeter area) will be administered as needed during recovery. The systemic analgesic buprenorphine (0.1 mg/kg subcutaneously, every 6 to 12 hours) will be given at least once during the postoperative period. Any infection or illness arising from surgery will be treated under the supervision of a veterinarian.

30. Will any animal(s) be allowed to recover from **more than one major operative procedure**? **YES** **NO**

(A major operative procedure is defined as any surgical intervention that penetrates **and** exposes a body cavity **or** any procedure which produces permanent impairment of physical or physiological functions.)

a) If YES, provide scientific justification for performing these procedures and list the species and number of animals:

not applicable

b) What is minimum length of time between the operative procedures?

not applicable