NATIONAL INSTITUTES OF MENTAL HEALTH
ANIMAL STUDY PROPOSAL
(Revised NIH 2014, NIMH 2014)
(See NIH PM 3040-2, 2014)

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PROPOSAL # LN 20

APPROVAL DATE 12/16/16
12/16/19

A. ADMINISTRATIVE DATA:

APPROVED
ACUC

Institute or Center: NIMH

Principal Investigator Elisabeth A. Murray

Building/Room location Email murraye@mail.nih.gov Telephone 301-443-7401 FAX 301-402-0046
Emergency Treatment and Animal Care instructions shall be provided on the attached form at the end of this document.

Division, Laboratory, or Branch Laboratory of Neuropsychology

Project Title Neural Substrates of Sensory Memory, Reward, and Emotion

Initial Submission [ ] Renewal [X] or Modification [ ] of Proposal Number LN 20 (13)
List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., Co-investigator(s)): A brief summary of the training and/or experience for procedures each individual will be expected to perform in this ASP must be documented and available to the ACUC. The name(s) of the supervisor, mentor, or trainer who will provide assurance each individual is/has achieved proficiency in those procedures shall be included in that documentation.

Name: Elisabeth A. Murray Degree: Ph.D. Position title, Affiliation: Chief, Section on Neurobiol. Learn & Mem, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-443-7401 Email address: murrayе@mail.nih.gov

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary personnel phone # Email address: secondary personnel

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: secondary personnel phone # Email address secondary personnel

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary personnel phone # Email address: secondary personnel

Name: secondary personnel Degree: B.S. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary personnel phone # Email address: secondary personnel

Name: secondary personnel Degree: B.S. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary personnel Degree: B.S. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301phone # Email address: secondary personnel

Name: secondary personnel Degree: B.S. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:

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Name secondary Degree: B.S. Position title, Affiliation: secondary personnel
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary personnel Degree: BA/MBA Position title, Affiliation: secondary personnel
• fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-phone # Email address: secondary personnel

Name: secondary personnel Degree: Ph.D. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-secondary personnel phone # Email address: secondary personnel

Name: secondary personnel Degree: D.Phil Position title, Affiliation: secondary personnel
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: 301-secondary personnel phone # Email address: secondary personnel

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Name: secondary personnel Degree: B.S. Position title, Affiliation: secondary personnel
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301 phone # Email address: secondary personnel

Name: secondary Degree: B.S. Position title, Affiliation: secondary personnel
fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone secondary personnel phone # secondary personnel

NOTE: Approval of an animal study proposal by an ACUC does not ensure it acceptance by an animal holding facility. Acceptance by a facility is dependent on available space, personnel and resources to support the ASP. Allocation of animal holding space is the role and responsibility of the IC Scientific Director.

B. ANIMAL REQUIREMENTS:

Species: *Macaca mulatta* Age/Weight/Size: 3.0 kg - adult Sex: M / F

Stock or Strain: N / A Source(s): NIH Approved Sources and/or the NIH NHP Recycling Program

Holding Location(s): location location Animal Procedure Location(s): location location

Estimated Number of New and Existing Animals Required to Support this Proposal:

		Year 1	Year 2	Year 3	Total
1	**Estimated number of new animals required** (Include all future purchases, births, and transfers)	20	20	20	60
2	**Number of existing naïve animals to be transferred into this ASP (any age)**	4	N/A		4
3	**Number of existing animals previously used in any experiment to be transferred into this ASP***	85	N/A		85
	YEARLY TOTALS	109	**20**	**20**	149

*If animals have been in a previous study, briefly describe how animals were used, e.g. any surgeries, procedures that caused pain and or distress (relieved or unrelieved):

There are a total of 89 animals being transferred onto this protocol from the preceding approved version of this protocol. Of these, 4 are naïve and 85 have been studied in the preceding version of this ASP. For the animals engaged in studies, the experimental design, methods, and survival surgeries were the same as described below in Sections F and G.

The 4 naïve monkeys will begin behavioral testing and take part in lesion studies on visual learning and memory.

The 85 monkeys that have been used in the previous version of this ASP are as follows:

9 monkeys are being tested on a social cognition task. They currently all have headposts. A subset of this cohort will receive medial prefrontal cortex lesions once they reach behavioral criterion.

8 monkeys are being trained on a spatial memory task. Half will undergo fornix transection when they reach criterion.

8 monkeys are being trained on an appetitive Pavlovian task to assess autonomic responses. Of these, 4 have received excitotoxic lesions of the entire orbitofrontal cortex (lateral plus medial) and 4 are unoperated.

4 monkeys with bilateral lesions of the ventrolateral prefrontal cortex are being trained on an automated task assessing learning of probabilistic stimulus-reward associations. These monkeys will complete training in late 2016. At the end of the study these animals will be euthanized and the brains removed for histological processing and analysis.

20 monkeys are being tested, or are waiting to be tested, on behavioral tasks to assess their ability to learn object-reward associations. 15 of these are operated (4 with crossed lesions of amygdala and orbital frontal cortex, 4 with crossed lesions of

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the amygdala and prelimbic cortex, 7 monkeys with crossed lesions of the amygdala and premotor cortex) and 5 are unoperated controls.

3 monkeys with bilateral lesions of the ventral striatum (nucleus accumbens) are being trained on automated tasks to assess stimulus-reward and action-reward reversal learning

6 monkeys are being trained on automated tests of attentional capture. Two of these have bilateral excitotoxic lesions of the amygdala and 4 are unoperated.

4 monkeys are being assessed for their ability to link reward with abstract concepts (same-different). All have headposts, chambers, and craniotomies to allow infusion of agents into frontal cortex.

7 monkeys are participating in fMRI studies of stimulus-reward association. All have headposts. In addition 3 of the 7 have chambers and craniotomies to allow infusion of compounds into the amygdala.

2 monkeys with bilateral excitotoxic lesions of the amygdala are being assessed for stimulus familiarity judgements.

9 monkeys are on rest pending euthanasia. 2 have bilateral excitotoxic lesions of the lateral orbitofrontal cortex, 2 have bilateral excitotoxic lesions of the medial orbitofrontal cortex, 3 have lesions of the caudal orbitofrontal cortex, 1 monkey has a lesion of the anterior cingulate cortex, and 1 monkey has a lesion of the orbitofrontal cortex.

5 unoperated controls will be assigned to other projects. One of these has a headpost.

At the end of the study animals with lesions will be euthanized and the brains removed for histological processing and analysis.

C. TRANSPORTATION:

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within [location redacted] also include the route and elevator(s) to be utilized.

Monkeys will be transported from monkey home cages to [location redacted] and back [location redacted] [location redacted] following the approved transportation SOP for this facility. Anesthetized monkeys will be transported in covered transportation carts, and awake monkeys will be transported in transportation chairs. Awake monkeys will be covered and restrained with a minimum of two forms of restraint when being transported through public areas. In addition to the restraint provided by the chair itself, a chain attached to the chair will also be hooked to the monkey's collar.

The animals are transported from [location redacted] and back using an approved opaque transport box and following the approved transportation SOP. For CT imaging, There will be two people with the monkey at all times. Anesthetized monkeys will be transported in an approved, specifically designed, covered, opaque transport box. We will enter [location redacted] and proceed to [location redacted] until the locked door at [location redacted] We will then call for the CT technician to meet us and escort us through the locked corridor that houses the [location redacted]

All [location redacted] and NIH Transportation Guidelines will be followed.

D. STUDY OBJECTIVES:

Provide no more than a 300 word summary of the objectives of this work. Why is this work important/interesting? How might this work benefit humans and/or animals? This should be written so that a non-scientist can easily understand it. Please eliminate or minimize abbreviations, technical terms, and jargon. Where they are necessary, they should be defined.

Earlier work from this laboratory has indicated that the medial temporal lobe limbic structures, the amygdala, hippocampus and rhinal cortex, are critical for information storage in the brain. Specifically, these structures are known to underlie the ability of organisms to recognize previously experienced sensory events, to associate these events with one another, and to associate these events with reward and with motor acts. The proposed study will build on these earlier findings in two ways: 1) by contrasting the specific contributions to memory made by the limbic structures with those of other brain structures implicated in memory processes, and 2) by examining the interaction of medial temporal lobe structures with neocortical or thalamic processing areas in the storage/recall of sensory memory, stimulus-reward associations, and stimulus-action associations. These goals will be achieved by examining the effects of selective brain lesions, reversible brain lesions, or pharmacological manipulations on the acquisition and flexible use of different kinds of memory.

By determining the neural substrates of memory in monkeys, we hope to uncover information relevant to the treatment of human amnesic disorders. Identifying the neural substrates of memory in monkeys will indirectly benefit the treatment of human amnesic disorders by promoting the use of other investigative tools or approaches (e.g. electrophysiological, neurochemical) in specific mnemonic processing areas; findings from such studies may lead to greater understanding of the cellular events underlying information storage that will be important in

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treating patients with Alzheimer's disease, semantic dementia, Parkinson's disease, or Huntington's disease, brain disorders which are characterized, in part, by progressive memory impairment. Moreover, the neural substrates of stimulus-reward association and emotional expression and regulation are relevant to understanding the etiology and pathophysiology of mood and anxiety disorders.

E. RATIONALE FOR ANIMAL USE: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used. (Use additional sheets if necessary)

1) Animals must be used to investigate the neural substrates of memory because the same kind of information cannot be gleaned from human studies or from use of alternative techniques. Brain damage in humans is rarely either selective or complete for a given structure. Consequently it is impossible in most instances to draw conclusions regarding the neural basis for an identified memory deficit. Animals, however, can be used for the behavioral evaluation of the effects of selective brain lesions.

2) Nonhuman primates are required for these studies because they are the only readily available animals that can sufficiently master the kinds of cognitive problem-solving tasks being employed. *M. mulatta* is the preferred species. There is a long history of anatomical and physiological study of both limbic and neocortical structures in rhesus monkeys, thus providing a basis for comparison across studies. In addition, rhesus monkeys adapt readily to the laboratory environment. Furthermore, the organization of the brains of these animals is similar to that of humans, so any findings concerning the neural bases of behavior may apply to humans as well.

3) In most cases the control and experimental groups will be comprised of 4 animals each; this is the minimum number of subjects that will yield statistically meaningful results. Each year, I have the resources to conduct one new main study using 12 monkeys and another smaller one using 8 monkeys. The larger study would be comprised of two experimental groups and one control group (N = 12). The smaller one would consist of one experimental group and one control group (N=8). Therefore, I will need 3 X 20 monkeys, or a total of 60 monkeys, over the three-year period. In all cases, I will use the procedures as described in Section F, and would have the general experimental goals outlined in Section D.

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following: (Use additional sheets if necessary.)

Injections, Inoculations or Instillations (substances, e.g., infectious agents, adjuvants, medications, drugs, etc.; dose, sites, volume, route, diluent, and schedules). All substances administered to an animal must be pharmaceutical grade unless otherwise justified in accordance with the NIIH ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals

Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)

Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)

Radiation (dosage and schedule)

Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)

Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)

Other Procedures (e.g., survival studies, tail biopsies, etc.)

Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain or distress, ascites production, etc.) For Column E studies provide: 1) a description of the procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress cannot be relieved.

Resultant Effects, if any, that an animal(s) are expected to experience or exhibit (e.g., circling, abdominal distension, weight loss, etc.)

Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.

Breeding Paradigms (Include the maximum age of breeders, use of permanent breeding pairs or trios, weaning age, maximum number of animals in a cage, fate of pups not carrying the required gene, collection of tissues and/or body fluids for genetic testing, etc.)

Past Experience with Identical or Similar Experimental Treatments, Procedures, etc. (e.g. direct experience or the published experience of others related to the well-being of animals undergoing identical or similar experimental procedures, etc.).

Most experiments involve: 1) creating a permanent brain lesion using either subpial aspiration or injection of excitotoxins, OR manipulating neuronal activity using drugs; 2) measuring the effects of these manipulations on behavior; and 3) euthanizing the animal to examine the brain. Descriptions of the different procedures involved in these experiments are provided below.

Identification of animals: Animals will be identified by chest tattoos, subcutaneous transponder, and by the tags attached to their home cage.

Behavioral testing: Monkeys will be trained daily on behavioral tasks administered either in a modified Wisconsin General Testing Apparatus (WGTA) or in an automated (computer-run) test apparatus. In general, the monkeys learn to displace objects or make responses to pictures presented on a color monitor fitted with a touch-sensitive screen to earn food or fluid (juice or water) rewards. Monkeys trained in the WGTA will be held in a transport/testing cage for approximately 20 - 120 minutes per day, whereas monkeys trained in automated apparatuses will be restrained in either nonhuman primate chairs or a testing cage from approximately 30 minutes to 3 hours. In addition, monkeys will have their diet or their fluid intake manipulated in order to achieve prompt responding in the test situation. During training all monkeys will receive food or liquid rewards (e.g. banana pellets, peanuts, fruit juice, water). During training the animals' food / water may be controlled. All food / water control will be in strict adherence to the NIH Guidelines for Diet Control in Behavioral Studies. Daily records of food / water intake will be kept and all monkeys will be weighed weekly. The NIMH veterinarian will be consulted if any problems develop.

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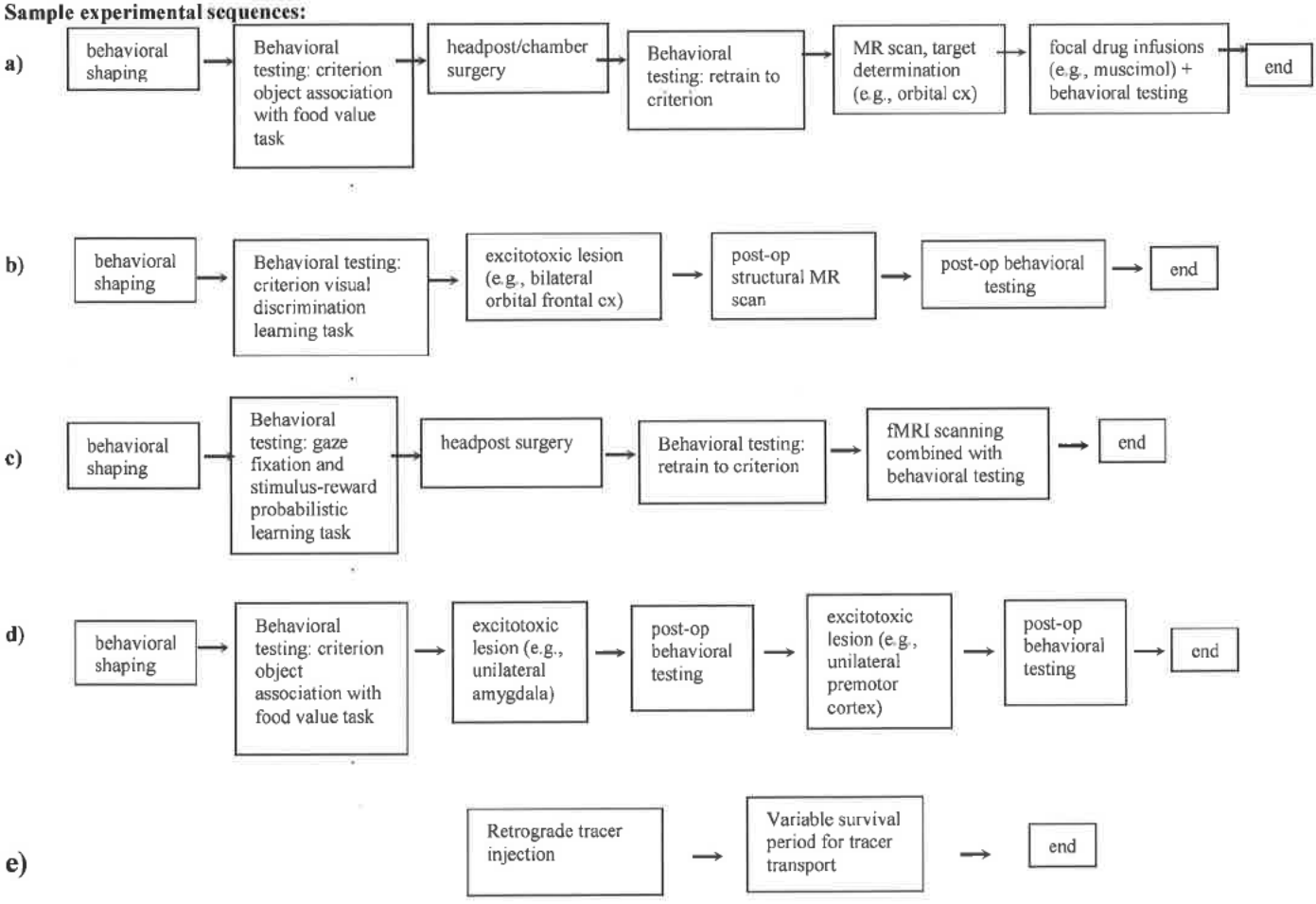
Monkeys will be evaluated for their visual learning and memory abilities. This is carried out using a variety of specific tasks, each involving the animal's selection of objects (manual test apparatus), images (automated test apparatus) or locations (manual and automated apparatuses) that leads to delivery of food or fluid rewards. In addition, monkeys will be evaluated for their emotional responses to objects and to unfamiliar humans. We use two different procedures, each of which involves exposing the monkey to a potentially emotionally-charged stimulus and measuring the animal's behavioral responses. One procedure involves exposing the monkeys to several neutral objects and to a fake (rubber) snake and rubber spider. The latter two objects evoke an innate fear response. The emotional response is mild, in that it typically involves freezing behavior, eye and head aversion, and withdrawal (i.e., moving away from the object), and dissipates over time. The exposures will be limited to 30 sec and will occur only a handful of times in the course of the experiment. A second procedure involves exposure to an unfamiliar "human intruder" for roughly 10 minutes. Responses range from submission (e.g. lip smacking, moving away) to aggression (e.g. cage shaking) to defensive behaviors (e.g. freezing). Although these procedures may induce mild fear and anxiety for brief periods of time which are momentary and transient, they are necessary for evaluating the effects of brain lesions on monkeys' innate behavioral repertoire. These are widely used experimental procedures that have no identified long-lasting effects on behavior or on the health of the animal.

In some experiments we will measure monkeys' autonomic responses, including pupil diameter, skin conductance and heart rate, during performance of a behavioral task. The measures can all be obtained using noninvasive procedures. Monkeys first receive extensive training to acclimate them to accepting placement of electrodes on the palm of the hand (for skin conductance responses or SCR) and back (for heart rate). Both SCR electrodes and heart rate electrodes have conductive gel between them and the skin. To obtain reliable SCR, we apply vetwrap around hand to hold the electrode in place. In addition, it is necessary to restrict the movement of one or both arms. An arm is restrained with either a soft Velcro strap or a ring of aluminum, steel or plastic, sometimes in combination with a loosely fitting tube surrounding the lower arm and hand. Pupil diameter is measured with a camera. To obtain reliable measures of pupil diameter the monkey's head must be immobilized. The pupil diameter is measured using an infrared sensitive camera placed a fixed distance from the monkey's head.

All monkeys assigned to this protocol will not necessarily undergo the same procedures, nor will any monkey undergo all of the procedures described below. The following sample flow charts demonstrate several examples of how individual animals might be used.

Sample experimental sequences:



Surgical lesions: Most experiments will examine the effects of bilaterally symmetrical lesions intended to achieve a permanent loss of neurons in a specific brain region. For example, a monkey might be trained on a behavioral task, receive a lesion of either the amygdaloid complex, the

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hippocampal formation, the rhinal cortex, caudate nucleus, medial thalamus, prefrontal or premotor cortex, or other related structures, and then be retested on the behavioral task. After training on some additional behavioral tasks, the operated monkeys would be euthanized so that we can perform histological examination of the brains to document the extent of the lesions.

Some experiments will employ a "disconnection" technique to test the hypothesis that interaction of two different, but anatomically connected, structures (e.g. a limbic structure and a neocortical area) is critical for the storage/retrieval of the same kind of memories examined after the symmetrical lesions. The disconnection will be achieved by removing one structure in one hemisphere in one stage, and the other structure in the other hemisphere in a second stage, and transecting the forebrain commissures (anterior commissure and corpus callosum) in a third stage, not necessarily in that order. To assess the effects of the individual lesions, training will intervene between each surgical stage. (A positive result would yield a behavioral effect only following the third and final surgical stage, the one that completes the disconnection of the two structures being examined.) The disconnection procedure can only be achieved using multiple survival surgery. In this type of experiment, for example, a monkey would receive a unilateral lesion of a structure in a first operation (e.g. amygdala, hippocampus, orbital prefrontal cortex, nucleus accumbens, rhinal cortex), be retested on the behavioral task, receive a second operation involving unilateral removal of a different structure in the opposite hemisphere (e.g. amygdala, hippocampus, orbital prefrontal cortex, nucleus accumbens, rhinal cortex), be retested on the behavioral task, and, finally, receive a section of the forebrain commissures (corpus callosum and anterior commissure). The order of the operations would typically be counterbalanced across animals within a group, such that some animals, for example, might receive section of the forebrain commissures as the first operation. After the third operation, monkeys would again be tested on the behavioral task, and perhaps some additional tasks, and then euthanized. In all cases, there will be a minimum of 2 weeks intervening between successive surgeries, and a maximum of 3 planned major survival surgeries per monkey. Upon postoperative assessment of the lesions using MR imaging, if the lesion appears incomplete, an additional major survival surgery may be conducted to complete the lesion. See Section G for details.

Neurotransmitter-specific permanent lesions: Some experiments will employ injection of neurotransmitter-specific toxins intended to remove monoamine neurons or their terminals. Two classes of agents have been employed for this purpose: a) 6-hydroxydopamine and b) cell toxins (e.g., saporin) fused to specific antibodies to the dopamine transporter (DAT). Although the lesion may require a 2-stage operation, animals receiving this treatment will not undergo any other surgical procedure. As is the case for Surgical lesions, above, and as detailed in Section G., all neurotransmitter-specific lesions will be carried out in anesthetized animals using aseptic procedures. Injection of the toxins will be performed via gas-sterilized Hamilton syringes using standard neurosurgical approaches. In addition, animals will receive one treatment or the other (a or b), not both. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a sterile vial after they have been dissolved in the appropriate carrier.

a) 6-hydroxy dopamine (6-OHDA): 6-hydroxy dopamine (Sigma) has been used to ablate dopaminergic neurons extensively for nearly two decades. 6-OHDA is injected intra-cortically. Doses used in the literature range from 2 – 12 µg/µl per injection site. Typically, around 100 µg would be required to ablate dopaminergic fibers in specific cortical areas. Injection volume will be limited to 1.0 µl per site, although the total volume would depend on the size of the structure under investigation. For example, depletions within perirhinal cortex would require injection of a total of approximately 20 µl (distributed over 20 sites), and total volume for any given experiment would typically range from 20 - 50 µl. Intra-peritoneal co-administration of agents that block uptake at norepinephrine and serotonergic terminals (desipramine, 20 - 40 mg/kg IP; pargyline, 5 - 20 mg/kg IP; citalopram, 5 - 10 mg/kg IP; talsupram, 15 - 25 mg/kg IP) are also administered to enhance selectivity for dopaminergic terminals.

Desipramine is counterindicated with ketamine for pre-isofluorane anesthetic preparation. An alternative anesthetic regimen will be employed for pre-surgical preparation. Use of dexmedetomidine (0.005-0.05 mg/kg IM) plus butorphanol (0.3 mg/kg IM) has been successfully used in other settings and would be employed here.

b) Anti-Dopamine Transporter-Saporin (anti-DAT-SAP): Saporin conjugated to antibodies targeting specific cell types has recently been developed for specific ablations of cell types in the nervous system. Saporin is a cytotoxin that targets the ribosome and blocks protein synthesis. Unconjugated saporin cannot penetrate the cell membrane and, therefore, cannot enter cells. When conjugated to a specific antibody targeting a cell surface protein, like the dopamine transporter, it is taken up endosomally and retrogradely transported to the cell body. Here, saporin is cleaved from the construct due to the pH of the endosomal compartment, and targets the ribosomal apparatus. Because the saporin is specifically targeted to neurons that express the dopamine transporter, it poses relatively minimal risk in terms of general pathology. Injection of these constructs into specific cortical areas (0.02 – 0.6 µg/ul anti-DAT-SAP in sterile saline) would lead to ablation of dopamine fibers selectively in that area. As in the case for 6-OHDA (above) we would inject 1.0 µl per site, but the total volume (distributed over multiple sites) would depend on the target structure; the total volume injected would typically range from 20 to 50 µl. From previous experience in LN working with these agents we do not expect any adverse effects on the animals related to the lesions.

Systemic drug injections: Some experiments will employ systemic injections to help elucidate the complex neurochemical circuits that underlie learning and motivated performance. Compounds generally categorized as neurotransmitter receptor agonists and antagonists, and reuptake inhibitors, will be administered either 10 - 60 minutes before task onset or during task performance. In most cases a dose-response function will be determined for all compounds, starting with a very low, ineffective dose. Increments in dosing will cease if any animal exhibits distress (e.g., impaired performance due to dyskinesia, sedation, agitation).

All systemic injections will be administered intramuscularly in a volume of 0.10 ml/kg. The drugs to be evaluated will be chosen from available compounds for which dosing and toxicity measures have been established. Systemic injections will occur with a frequency not to exceed 2 per week using alternating injection sites. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, whether pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a sterile vial after they have been dissolved in the appropriate carrier. For systemic (intramuscular) injections, solution acidity may range from pH 3.0-7.5.

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The following types of receptor agonists and antagonists may be administered: dopaminergic (D2) receptor agonists (e.g., quinpirole, 0.01µg/kg - 0.50 mg/kg) and antagonists (e.g. haloperidol, 5.6µg/kg - 17.8µg/kg); dopaminergic (D1) receptor agonists (e.g., SKF 38393, 0.3 – 15.0 mg/kg) and antagonists (e.g., SCH 23390, 0.50-32.0 µg/kg); cholinergic mimetics or receptor agonists (e.g., physostigmine, 1.0 - 100.0 µg/kg) and antagonists (e.g., scopolamine, 1.0 - 32.0 µg/kg); NMDA receptor glycine site (NMDAR/GS) partial agonists (e.g., d-cycloserine, 32.0 µg/kg - 1.0 mg/kg) and NMDAR antagonists (e.g., MK-801, 3.2 - 56.0 µg/kg); GABAa receptor agonists (e.g., muscimol, 0.01 µg/kg – 1.0 mg/kg) and antagonists (e.g., bicuculline, 0.01 µg/kg - 1.0 mg/kg).

Dopamine, serotonin, and norepinephrine reuptake inhibitors may be administered. Examples of reuptake inhibitors that may be used are: for dopamine, GBR 12909 and nomifensine; for norepinephrine, yohimbine; for serotonin, citalopram and fluoxetine. Doses will be determined using careful escalating dose procedures. Generally, estimates can be obtained from the rodent literature. Doses reported in the rodent literature, for example, include GBR 12909 (15 mg/kg), yohimbine (5 mg/kg) and citalopram (10 mg/kg). These doses have been reported to not cause any adverse effects.

Yohimbine, citalopram and fluoxetine are available pharmaceutical grade (Janssen Pharmaceuticals). GBR 12909 and nomifensine are not. In general, the drugs to be injected will be dissolved in sterile saline. In the event that a compound to be tested is not readily soluble in sterile saline the lowest possible concentration [ranges] of an effective vehicle {e.g., (2-hydroxypropyl)-b-cyclodextrin [1-25%], Tween-80 [0.1-0.2%], Emulphor [1-3%], or DMSO [0.1-0.2%]} will be used to suspend, or dissolve, the compound, and this stock solution will be further diluted in sterile water or sterile saline to achieve the desired molarity.

From the relevant scientific literature, and from experienced investigators in the Laboratory of Neuropsychology, the doses provided above have been shown to be non-toxic. However, if at any time during the experimental process the animal appears unwell we will immediately stop the experiment and consult with the NIMH veterinarian and provide treatment. If the veterinarian, in consultation with the investigator, determines that an animal is experiencing distress that cannot be relieved by applying acceptable medical treatments and procedures, the animal will be euthanized.

Implantation of headposts and chambers: Using aseptic surgical techniques a headpost and chamber, or in some cases a headpost only, will be attached to the animal's head. Both the headpost and chamber are fabricated from MR-compatible materials. Because the headpost takes roughly 4 weeks to heal (i.e., become bonded to the bone), the headpost and chamber are typically added in separate operations. This is for at least two reasons. First, the monkey needs a period of accommodation to headpost restraint, so having this procedure carried out well in advance of the chamber implant allows the restraint training to take place prior to the chamber implant. Second, some of the chambers we use are quite large. In these cases the best surgical approach is to use a skin incision at right angles to the incision used for the headpost. In the experience of LN investigators, using a two-stage surgery gives the best overall outcome for the headpost and chamber implants. A third operation is required to remove the bone inside the chamber to allow access to the brain. Although this bone removal could be carried out at the time of the chamber implant, there is often a months-long delay between the chamber implant and the initiation of the experimental manipulations carried out within the chamber. Accordingly, to avoid risk of infection, we feel it is safer to remove the bone in a separate operation. Other LN investigators have had good outcomes after covering the bone inside the chamber with a thin layer of dental acrylic at the time of the chamber implant. This procedure maintains the integrity of the bone within the chamber indefinitely. Typically, the headpost and chamber are implanted on the skull based on localization of brain structures obtained from MR scans.

An animal will undergo a maximum of three planned major survival surgeries. On occasion, monkeys with chamber implants will require additional major survival surgeries for the purpose of removing bone that has regrown inside the chamber. Monkeys may also require anesthesia to undergo additional, relatively minor procedures to remove excess granulation tissue from the dura mater. Other relatively minor procedures might be carried out in circumstances requiring repair or replacement of a headpost or chamber (see Section G).

Intracerebral injections: After placement of the headpost and chamber, as described above, animals may receive intracerebral drug administration to produce one of two types of treatment: 1) reversible inactivation of the targeted brain structure, which acts as a reversible "lesion"; and 2) pharmacological manipulation of specific neurotransmitter systems. MR scans are used to determine the stereotaxic coordinates for accurate placement of injection needles over brain regions of interest such as the orbital and medial frontal cortex, hippocampus, amygdala, entorhinal and perirhinal cortex, striatum, and thalamus. All intracerebral injections will be made in awake animals. Before the experimental phase requiring intracerebral injections, animals will be acclimated to head restraint using the headpost affixed to the skull. This will be achieved by successive approximation, i.e. starting with short periods of restraint and moving to progressively longer periods of restraint until the restraint period matches the time needed for the injections, roughly 30 minutes. Once this acclimatization is completed, intracerebral injections may be carried out. First the animal will have its head restrained. Then the cover of the plastic chamber will be removed. Using aseptic techniques, a small hole will be made in the *dura mater* with a sterile 24 - 28 gauge needle (the guide cannula) to facilitate the insertion of injection needles. On rare occasions it appears that an animal is distressed by the insertion of the guide cannulae; in such case we will apply a local analgesic (e.g. lidocaine) onto the dura before insertion. Finally, the injection needles will be inserted through the guide cannulae into the selected brain area using the coordinates obtained from the MR images. The materials used for the intracerebral injections (i.e., guide cannulae, injection needles, plastic tubing and Hamilton syringes) will be gas-sterilized prior to each injection. To achieve reversible inactivation, we will use: GABAA receptor agonists (e.g., muscimol, 10 - 150 ng/µl) and possibly GABAA/BZR agonists (e.g., CDP, 10 - 50 µg/µl). Compounds are administered via a programmable pump that holds the syringes while advancing the plunger at a given rate of speed; the Hamilton syringes are attached via plastic tubing to the injection needles, which in turn dispense the compound into the selected brain location. The drugs to be infused will be dissolved in sterile saline (pH 7.0 – 7.5) and injected in volumes ranging from 0.3 - 3.0 µl/site, at rates of 0.15 - 0.25 µl/min. Depending on the size of the target structure, a range of 1 - 5 sites per hemisphere may be required to address the tissue adequately. The entire procedure typically takes 15 - 30 minutes. When the injections are completed, the chamber will be rinsed with sterile saline, the chamber cap replaced, the head restraint removed, and the animal's behavioral task will be initiated. Intracerebral injections will occur with a frequency of 1 to 2 per week (no more than 2 per week), and the total number of injections per site will not exceed 30. Experience in LN has shown no adverse effects of administration of these compounds in the range of doses described above. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, whether pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a

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sterile vial after they have been dissolved in the appropriate carrier.

Pharmacological manipulations made via intracerebral injections may involve the following compounds: D2 antagonists (e.g., sulpiride, 5 - 25 µg / µl); D1 antagonists (e.g., SCH23390, 10 - 80 µg / µl); cholinergic (M1) antagonists (e.g., scopolamine HBr, 1 - 50 mM); cholinergic (M2) antagonists (e.g., SCH 217443, 5 - 75 µg / µl); GLUR antagonists (e.g., NBQX, 1 – 10 µg / µl). In previous experience in LN working with these agents, and from relevant scientific literature, we have found that the doses provided above are nontoxic. Compounds will be administered 10 - 60 min before task onset or during task performance.

We do not expect any problems related to the drug infusions. However, one potential adverse effect of making intracerebral injections, encountered infrequently, is inadvertent puncture of blood vessels leading to cerebral hemorrhage, infarctions, and raised intracranial pressure, in which case an animal might exhibit distress (e.g., dyskinesia, agitation). If at any time during the experimental procedure the animal appears unwell we will immediately stop the procedure and consult the NIMH veterinarian. If the veterinarian so advises, treatment will be provided. If the veterinarian, in consultation with the investigator, determines that an animal is experiencing distress that cannot be relieved by applying acceptable medical treatments and procedures, the animal will be euthanized.

Cleaning and maintenance of cranial implants: Careful attention, including regular cleaning and disinfection, is paid to the cranial implants (i.e., headposts and chambers) to reduce the possibility of infection. We frequently consult with the NIMH veterinarians and quickly move to treat infections if they occur. The frequency of cleaning depends on the nature of the implant and its clinical state. The materials used for cleaning and disinfection and the antibiotics used to treat any infection are continuing to evolve; accordingly, treatment is based on consultations with the NIMH veterinarian. Current typical materials include hydrogen peroxide and Betadine cream or ointment. Occasional vigorous cleaning and shaving of hair is required. If any sign of an infection appears, samples are sent to the laboratory for culture, and appropriate antibiotic therapy is initiated. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain / or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be anesthetized and subsequently euthanized according to Section J.

It may occasionally be necessary to remove a small piece of bone or enlarge the burr hole inside the chamber using either a drill or a small rongeur. If the removal is very minor and will last only a few minutes it is generally carried out using ketamine (5.0 - 20.0 mg/kg IM), but a ketamine / xylazine mixture (ketamine, 8 mg/kg, plus xylazine, 0.4 mg/kg IM) is used if the procedure involves more extensive drilling or if the animal shows any reaction to stimulation. A dose of atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) will also be given as pre-treatment for this procedure.

After a period of weeks or months the dura mater sometimes thickens. When this happens the monkey will either be sedated with ketamine (10 - 20 mg/kg), first pre-treating with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), as appropriate, or taken to the OR to be done under isoflurane (1.0 – 4.0%, to effect) and the fibrous connective tissue will be removed using aseptic technique. These procedures are performed in either the implant treatment room or in the surgical suite.

Long-term maintenance of chambers: We have developed a 2-day procedure to implement placement of an elastomer seal within the chamber. This procedure has been shown by others to result in a hermetically-sealed chamber that provides long-term sterility, in some cases up to 24 months. On day 1, to prepare the dura mater, the animal will undergo a dural scraping to remove excess granulation tissue followed by treatment with 5-fluorouracil (5-FU; 5-10 ml, depending on the size of the chamber) in physiological saline (25.0 mg/ml) for 1 - 5 minutes to slow growth of fibroblastic connective tissue. On day 2, the chamber will be rinsed with sterile saline, cleaned with 0.5% chlorine (Dakin's Solution), and rinsed again with sterile saline. The chamber, bone, and dura mater will be dried with ocular / optic wicks and sterile silicone elastomer (Kwik-Sil, World Precision Instruments, FL) will be poured into the chamber. The elastomer will cover the craniotomy, bone and bottom of the chamber, creating a silicone elastomer seal, or plug. This procedure has been shown to result in a hermetically-sealed chamber that provides a long-term sterile environment within the chamber, thereby obviating the need for twice-weekly (or more) cleaning. An additional benefit of the elastomer seal is that it appears to prevent growth of granulation tissue. However, this procedure will not be used for chambers that communicate with other cylinders.

Because this is a new procedure, our current plan for monitoring the elastomer seal is to check the elastomer seal one day (roughly 24 hrs) and one week after placement to verify the integrity of the seal. If no fluid is found in the chamber, the seal will be left in place. If fluid is found in the chamber, the old elastomer will be removed, the chamber will be washed, cleaned and dried, and a new elastomer seal applied. We will continue visual inspection at weekly intervals until such time as the seal is removed. When using this procedure, we will consult with the NIMH veterinarian regarding the preparation for the long-term seal and the monitoring of the animal.

It is possible that our chamber lids will need to be modified to create a better seal to maintain the sterility of the chamber. We are currently working with engineers to fabricate new chamber lids that differ from the standard lid in two ways: 1) fabricated from clear plastic (Lucite), to allow the experimenter to see inside the chamber without removing the lid; and 2) fabricated with an O-ring fitted inside the lip of the lid to create an air-tight seal.

Chair restraint: Animals to be chaired will wear loose-fitting collars and will be chaired in accordance with NIMH Guidelines for the Use of Restraint Chairs with Nonhuman Primates. They will be transferred to the chairs using the standard pole and collar technique. Animals acclimate quickly to the collars. The collars are placed on the animals under ketamine sedation (Ketamine hydrochloride 10 - 20 mg/kg IM). The animals are allowed to recover for approximately one day, getting used to the collar, before pole training is begun. Pole training typically involves successive approximation. First, the investigator briefly clips the pole onto the monkey's collar, at the same time giving the monkey food rewards. This procedure is carried out repeatedly, across several days, before attempting to transfer the animal to the restraint chair. Once in the restraint chair the animal is taken to the testing room where it sits in the chair and is given food rewards for, perhaps, 15 minutes. The animal is then returned to his home cage. This procedure is repeated for several days until the monkey is acclimated to the chair and test environment. The acclimation of the animal to the collar, chair, and testing procedures will be tailored to accommodate the individual needs of each animal.

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Structural Magnetic Resonance Imaging (MRI) Scans:

The NIMH Guidelines for MRI and MRS will be followed.

location Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with ketamine (10 - 15 mg/kg IM), followed by either 0.005-0.05 mg/kg (IM) dexmedetomidine (Dexdomitor® or 0.5 - 1.5 mg/kg (IM) valium (diazepam), with supplements as needed. The ketamine/ dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. (If dexmedetomidine is not available, medetomidine (Domitor®) may be substituted. In this case, if used in combination with ketamine, 0.02-0.1 mg/kg (IM) medetomidine would be used.) The animal is then transported to location location where it is placed in an appropriate head holder and scanned. This technique is completely noninvasive and lasts approximately 1-2 hours. Following the scan, if either dexmedetomidine or medetomidine is used, we will administer atipamezole (Antisedan®) 0.2-0.4 mg/kg (IM). The animal is then returned to its home cage by the investigators where it is watched and monitored during recover from anesthesia, until it is able to right itself. A single dose of ketoprofen (1 – 2 mg/kg IM) will be administered just prior to or immediately following this procedure.

Diffusion weighted imaging:

Diffusion weighted imaging scans allow for examination of the route of specific fiber pathways in intact animals and the nature and location of changes in white matter pathways that occur as a result of these various lesions.

The DTI and DSI performed under general anesthesia will take place in a horizontal scanner in the NMR center. The anesthesia protocol is similar to that for surgery, comprised of intubation and general isoflurane anesthesia. The animal will be fasted for 12 hours prior to surgery; however, oral fluids will not be restricted. The animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), lightly anesthetized with ketamine (10 - 20 mg/kg IM), and then given isoflurane (1.0 - 2.0%, to effect) as a general anesthetic. The glycopyrrolate (or atropine), ketamine, and isoflurane are administered by the NIF animal technician, and the dosage and route of administration are determined by the veterinarian (the isoflurane is adminstered to effect, to reach a surgical plane of anesthesia). Anesthetized scans lasting four hours are typical for DTI sessions achieving 1 mm isotropic resolution. An interval of at least seven days will occur between consecutive anesthetized scans.

We do not expect any adverse effects from the procedures outlined above. If, however, there is any indication that an animal's condition is changing we seek immediate veterinary medical consultation. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and/or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized as described in Section J.

location In some cases the animals are scanned during stable, balanced anesthesia (1-2% isoflurane, to effect). Induction is generally performed with the animal in a horizontal position, and the chair is then brought to a vertical position for insertion into the vertical magnet. The vertical position, while a great advantage for the awake preparation, poses challenges for anesthesia. Safe and robust procedures for anesthetized monkeys have been developed and are in place, but great effort is being made to constantly improve these paradigms for the continued and increased safety of the animals.

For most structural studies to achieve good high-resolution images full anesthesia is not required but some sedation is necessary, since awake animals tend to make small movements (e.g. ear wiggling, chewing, vocalizing) that disrupt the image quality with motion artifacts. As an alternative to full anesthesia, a combination of three safe drugs will be used:

1) Ketamine (5 - 15 mg/kg IM)
2) Diazepam (0.5 - 1.5 mg/kg IM 30 minutes prior, or 0.1 mg/kg slow IV at the time of anesthesia) – valium, a benzodiazepine sedative.
3) Glycopyrrolate (15 mcg/kg IM) -- anticholinergic, lasts a bit longer than atropine.

Supplemental doses of the above drugs will be given as needed.

While the sedate preparation does not afford as complete monitoring as the full, intubated gas anesthesia, we routinely measure and record the CO_2 level and breathing rate, using a gas sensor positioned just in front of the nose. We also use a camera on the eye and face in order to continually monitor the animal's appearance for movement. Finally, in addition to the CO_2 monitoring, we will attempt to measure the SPO_2 level and the non-invasive blood pressure.

Computed Tomography (CT) Imaging:

All monkeys will be sedated throughout the transport and scanning procedures. We will use the same anesthestic drugs and doses as we normally use for MRI scans. Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with 10 - 15 mg/kg ketamine IM, followed by either dexmedetomidine (0.005-0.05 mg/kg IM) or diazepam (0.5 - 1.5 mg/kg IM) given 30 min prior to the scan. Supplemental doses will be ketamine alone. The ketamine/dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. The animal is then transported in an approved, specifically designed, covered, opaque transport box via an approved route within location During the transport the animals are placed on their side and checked to ensure the airways are open. Once in the scan room they are placed in a stereotaxic head holder which secures their head in a stationary and standardized orientation. This orientation also ensures the airway is open through the scanning session. Total scanning time per monkey will be about 5 minutes. Once the scan is complete the monkeys will be returned to their home cage and monitored until awake and sitting upright. Total time from sedating the animal to return to its home cage for a single animal is about 45 min and no more than 60 minutes for two.

There will be two people with experience in working with monkeys present at all times. During transport of the animals we will not wear PPE but once the animal is in the CT room and before opening the cart we will put on the appropriate PPE (e.g. lab coats, gloves, and face shield). The monkey will be fully wrapped in a disposable absorbent pad with moisture-proof plastic lining. The bed of the scanner will be covered with absorbent pads. The door to the CT scanner will be kept closed. At the end of the scanning session we will remove and discard in a trash bag all

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disposable items. These items will be brought back to [location] where they will be disposed of in MPW waste. The CT bed, inside the scanner, and floor will be cleaned with disinfectant. All [location] NIH Transportation Guidelines will be followed.

Functional MRI Scans (fMRI):

Acclimation to the scanner: To acclimate the animals to the noise of the scanner (same noise levels human subjects are exposed to), monkeys will be exposed to the scanner for brief periods of time at first, followed by increasingly longer periods. Using this successive approximation procedure, the monkey will be exposed to the sounds of the magnet until fully acclimated for the period of testing, which ranges from 2 to 6 hrs. A closed circuit camera is available for monitoring purposes. If for some reason the monkey has trouble acclimating to the magnet environment, a mock scanner will be used. The sounds of the magnet that are present during the scanning session will be present in the mock scanner, but at first they will be at much lower levels and gradually increased over several testing sessions to the same levels in the actual magnet.

Scanning: Monkeys will be scanned during performance of behavioral tasks described under the section on *Behavioral testing*. During a typical scanning session, the head of the subject will be fixed in place using the headpost attached to its skull. Each monkey will be restrained for a period of approximately 2 - 4 hours per daily session. Monkeys may be scanned 1 to 4 times per week with a minimum of 24 hours between successive scans, continuing for 70 to 90 scans per year. The animals will be transported to the [location] See Section C) in the chair and placed in the NIMH 4.7T magnet. The NIMH Guidelines for MRI and MRS are followed. In some scanning sessions we will acquire conventional BOLD (Blood Oxygen Level Dependent) measures of functional 'activity', based on endogenous variations in cerebral blood flow and related variables. In other scanning sessions, we will measure functionally-related variations in cerebral blood volume. The latter measurements require an intravenous injection of monocrystalline iron oxide nanoparticles (MION) in the leg vein of the animal prior to each scanning session (i.e. one injection per day, 3 to 4 times per week maximum, dose: 6 - 20 mg/kg/day).

An intravenous catheter will be placed for IV injections. This catheter will be inserted into the saphenous vein. The monkeys are acclimated to the IV injection while in the chair using a procedure that mimics the injection procedure. First, we will gently take hold of the animal's leg until the animal is acclimated to our touch and restraint; this may take several days. Once the animal remains calm during the leg restraint we pinch the leg with a hemostat or similar instrument to mimic the cannula insertion. The leg is then wrapped as it would be during the procedure once the cannula is removed. The procedure is repeated three or more times a week until the animal has little response to the procedure. It then is done only on a few days prior to the scanning sessions that require the MION infusions. Once acclimated the animals show little aversion to the injection procedure.

The established dose for MION in functional brain imaging in monkeys is 6 - 20 mg/kg. While long-term iron accumulation in the liver may ultimately limit the number of injections, this limit has not yet been identified. An early published study showed that animals can tolerate at least 60 mg/kg and up to 166 mg/kg with no ill effects. More recent work has shown that rhesus monkeys can receive up to 200 doses of 6 - 10 mg/kg (total nearly 2 g/kg iron) without showing any obvious negative health or cognitive effects.

MION is commonly used (D Tsiao et al, PNAS, 2008; S Moeller et al, J Neurosci, 2009). All authors specifically point out that there have been no MION-attributable complications or side effects many using doses of 10 mg/kg (total number of doses is unspecified in publications, but the use of over 200 doses has been reported to us from both Tsiao and Vanduffel's groups). Nonetheless, MION may accumulate in tissues, causing an artifact in MRI images. There are two accepted techniques to slow or prevent the accumulation of MION; the method employed will be the one that best fits the study design. One technique employs single doses of an iron chelator, desferoxamine (500 mg/day IM) given following every session involving MION administration and functional imaging. This has been done at other facilities (Tootell, personal communication) with no known adverse side effects. Given that animals may undergo functional imaging and MION administration two to three times per week, with at least one or two intervening rest days, desferoxamine will be given after MRI procedures involving MION two to three times per week. A second method to reduce iron accumulation is to treat the animal with desferoxamine during periods in which they are receiving no MION. In this method, MION will be used in daily testing for a period lasting 2 - 3 weeks in a row. Thereafter, the animal will be placed on 'rest' for one week while he starts desferal (desferoxamine, 1 g/day over 2 injections) treatment, during the first three days of that rest. This approach has been used routinely with the Massachusetts General Hospital and Leuven imaging groups. While desferoxamine is considered to be an immunosuppressive agent, we expect minimal immunosuppression in the monkeys at the dose and duration of administration that we will be using. Desferoxamine is routinely used in treating humans with thalassemia. The most common side effects in humans are injection site reaction that may include pain, swelling, itching, and redness, blurred vision, and hearing difficulties. These side effects are reversible by discontinuing the administration of desferoxamine.

In addition, for animals that receive MION, we will monitor the iron accumulation by sampling blood transferrin saturation and total blood iron concentration every 2 - 4 months.

Earmold / Ear Protection Efficacy: The sound level inside the magnet itself has been measured to be 103 dB during the functional imaging sequences. Since extended exposure to this sound level can produce hearing impairments in humans, we will provide ear protection in the form of plugs or specialized headphones. The monkeys will be fitted with special ear molds. The silicone molds are the same as those used with young children. Animals are easily acclimated to these earmolds by having them placed within the ear for short durations, 30 to 60 minutes, over the course of 2 to 3 testing sessions. This is a non-invasive procedure that is routinely used on humans of all ages, including infants, in clinical and research settings. See also Section M.

Monkeys have been tested systematically on potential hearing loss before and after multiple scanning sessions using sound protection with silicone molds. Specifically, the monkeys' hearing ability between 892 and 7996 Hz, as well as their middle ear function, was tested on multiple occasions by audiologists using distortion product otoacoustic emission (DPOAE). The audiological results showed normal function before and after full data acquisition (K Tanji et al, Neuroimage, 2010)..

PET Scanning: PET procedures using the newly developed radioligand of synaptic vesicle glycoprotein 2A (SV2A) allow measurement of synaptic density in humans. We would like to employ the same PET radioligand in our monkeys with brain lesions under a collaborative agreement with non-transfer of ownership and two-investigator accountability. The collaboration will serve two

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purposes. First, in accord with the study objectives of our ASP, we expect the PET procedure to aid us in assessing the location and extent of the brain lesions made with excitotoxins. Although we currently assess the extent of lesions using postoperative T2-weighted MRI scans acquired within a week of surgery, having a second method to localize lesions would be extremely beneficial. Second, performing PET studies with the new ligand in our monkeys will provide a negative control for the ligand. If the radioligand is truly selective for synaptic vesicle glycoprotein, which is co-localized with synaptophysin, then there should be a reduction in SV2A binding at the site of brain lesions. Conducting PET in our monkeys with excitotoxic brain lesions allows us to test this prediction, and thereby indirectly benefit the work in humans.

We plan to conduct procedures already carried out by investigators in the Molecular Imaging Brain of NIMH, as described in ASP MIB-02 (PI = Robert Innis), titled Evaluation of PET Radiolabeled Probes in Monkeys. The specific procedures to be conducted are all listed under General PET procedures and include: fasting for approximately 12 h prior to the PET procedure, placement of an endotracheal tube, isoflurane anesthesia, establishment of an IV catheter to inject the radioligand and provide isotonic fluids, injection of the radioligand SV2A intravenously in the scanning room, and blood withdrawal (arterial samples) during the scan. The PET scans would take place location

Staff on MIB-02 would be responsible for arranging pre-scan fasting, all procedures associated with the PET scans, and for post-scan monitoring and recovery of the animals.

After PET scans, the staff of MIB-02 will also take responsibility for signage related to the use of radioactive materials. The location room as well as the location room in location s permanently posted for radioactive materials use and is restricted to the public. A radioactive material use survey will be completed monthly. A copy of each survey, documented on an NIH 88-12 monthly survey form, will be sent to the Division of Radiation Safety by the MIB staff.

Selective satiation: On occasion monkeys will be given a selective satiation procedure, which means they are allowed to eat as much of one food as they will eat (i.e. eat to satiety). This has the effect of altering (reducing) the value of the food reward. We use this experimental manipulation to test the animal's ability to represent food value. The selective satiation procedure always involves one of the foods that the animals receive as food rewards in behavioral testing, and typically lasts no longer than 30 minutes. The procedure is carried out no more than twice per week. The amount and type of food eaten is recorded in either the blue food/water log or the nursing notes of the animal's medical record chart.

Tastants: Tastants, or types of fluid outcomes, are typically used in both human studies (WK Simmons et al, Nat Neurosci, 2013; DM Small et al, Neuron, 2008) and nonhuman primate investigations (C Padoa-Schioppa and Assad, Nature, 2006; Rolls et al, J Neurophysiol, 1990), not only as a reward for performing a task, but also as a means of identifying areas responsive to tastes. These studies have used a variety of tastants ranging from positive (sucrose, sucralose, salt, various fruit juices) to neutral (water, artificial saliva), to mildly negative (quinine, HCl, citric acid). We will be using different tastants to map responses to differing tastes or flavors, and to test monkeys' abilities to form associations with visual cues and different taste outcomes. Taste outcomes will be different types of solutions in three categories: positive, neutral, or mildly negative. A positive reward will be an aqueous solution with sucrose [0.1M], sucralose [1mM] or fruit juice. A neutral reward will consist of a solution containing sodium bicarbonate [0.3mM] and potassium bicarbonate [0.3mM] (personal communication, A. Fontanini, January 29, 2015) to mimic saliva. A more complex saliva substitute contains NaCl (0.002M), KCl (0.005M), sodium bicarbonate (0.003M), potassium bicarbonate (0.003M), magnesium chloride (0.00025M) and potassium phosphate (0.00012M). Saliva substitutes will be used at these concentrations and at 75%, 50% and 25% dilutions. Quinine [1mM], a bitter tastant, or a dilute solution of either citric acid [10mM] or hydrochloric acid [1mM] (VL Smith-Swintosky et al, J Neurophysiol, 1991) are mildly negative. The monkeys will be presented with these fluids in a testing booth as well as the fMRI scanner. Use of these and similar tastants is commonplace in research settings and is expected to have no adverse consequences.

Air puff: In addition to using food or fluid rewards to study the neural substrates of learning using pleasant/agreeable events as reinforcement, we also plan to study the behavioral and neuronal systems underlying learning associated with mild unpleasant/disagreeable events as reinforcers. To study such behavior, it is necessary for the monkey to learn by trial and error which behavior leads to a negative stimulus (reinforcer). We will use a mild stream of compressed air (i.e., air puff, 20-60 PSI) as an unpleasant stimulus to be delivered for a short duration (typically, 100-1000 ms) to the cheek, through a tube positioned at about 5 cm from the side of the monkey's face. The air puff is mildly annoying. Delivery causes an automatic blink response and one is inclined to withdraw. There is, however, no pain associated with the puff. An equivalent human experience would be someone blowing through a straw at your face. This stimulus is neither painful nor does it cause discomfort or distress to humans. The air puff should be only mildly aversive, enough to elicit instrumental behavior from the monkey to avoid its delivery. In some cases the air puff will be presented obligatorily after the presentation of a set of visual stimuli in order to study how monkeys learn to associate the sensory stimuli with the upcoming air puff. To maintain the monkeys' cooperation in the experiment, we will deliver fluid rewards with the air puffs. Continuation of the usage of the air puff will be terminated immediately in case the monkey shows overt reactions suggestive of discomfort, pain or distress including grimacing, squirming, vocalization, urination or defecation.

Genotyping: We specifically need to determine a monkey's status with regard to the serotonin transporter polymorphisms so that their genotype (SS, SL or LL) for the serotonin transporter can be correlated with cognitive measures. Monkeys will be anesthetized (10 mg/kg ketamine IM) and 5 – 10 ml blood withdrawn using a 19 – 23 gauge needle, from the femoral vein, or alternative (e.g. saphenous vein) where necessary, for the purpose of determining the monkeys' genotypes. The blood sample will only be needed once per animal, barring unforeseen and rare problems with the processing of the samples, in which case a second sample would be taken.

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Tracer studies: In order to determine whether certain neuroanatomical projection pathways are intact, we will inject tracer substances into the brains of some intact monkeys and some monkeys that have already received lesions. Aseptic surgical procedures will be employed (see Section G), and intracerebral injections of retrograde tracers (1 - 5 µl 2% Fast Blue and / or 1 - 5 µl 4% Diamidino Yellow), spread over 1 - 20 sites, will be made into either the medial thalamus, amygdala, prefrontal cortex or related regions, via a gas-sterilized Hamilton syringe needle. Although this procedure may entail multiple survival surgeries, it is the only way to determine whether certain fiber bundles are intact after our lesions. No procedure-specific adverse side effects (i.e. those beyond what might be associated with any major survival surgery) are expected. In all cases, there will be a minimum of 2 weeks intervening between successive surgeries. For most studies, there will be a maximum of 3 major survival surgeries per monkey. On occasion, we may need to inject tracers into a monkey that has already undergone multiple major survival surgeries for behavioral experiments. On those rare occasions, the tracer injection would be the fourth and final major survival surgery.

Euthanasia: For histological verification of the lesions, monkeys in experimental groups must be sacrificed at the conclusion of the experiment, which will follow a variable period of behavioral testing ranging from approximately 6 months to 6 years. At this time, monkeys will be sedated with ketamine (10 mg / kg IM or to effect) followed by either Euthanasia-III (1.0 ml/5kg IV), an overdose of barbiturates (sodium pentobarbital, 100 mg/kg IP or 100 - 200 mg/kg IV), or another equivalent euthanasia solution given at the manufacturers recommended dose, and then perfused through the heart with normal saline followed by formaldehyde solution. The brain will be removed for histology and the carcass will be double-bagged in heavy plastic bags and stored in the appropriately marked refrigerator until disposal.

Environmental enrichment: Some monkeys with brain lesions are to be exempt from pair housing. Removal of certain brain regions (e.g. amygdala, orbital frontal cortex, medial frontal cortex) results in alterations in emotional and social behavior. Specifically, after certain brain lesions, the dominance structure within a pair may be altered or the animals may respond inappropriately to social signals. Therefore, pairing could result in severe physical injury or death of the animal secondary to fights. In such cases, animals will no longer be suitable for pairing. In addition, because the controls for these experiments must be treated the same way as subjects in the operated groups, these animals, too, must be single-cage housed. Whenever animals are exempted from pair housing, other means of environmental enrichment will be implemented as per the NIMH Nonhuman Primate Enrichment Policy. See also Section M.

Other: As a general statement it must be emphasized that these animals are extremely important to us. We make every effort to keep them comfortable and healthy, and, if there is any indication that their condition is changing, we seek immediate veterinary medical consultation. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and / or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized according to Section J.

G. SURVIVAL SURGERY - If proposed, complete the following:

None☐ Major☒ Minor☐

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional sheets if necessary):

For all surgeries the animal will be fasted for 12 hours prior to surgery; however, oral fluids will not be restricted. The animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), lightly anesthetized with ketamine (10 - 20 mg/kg IM), and then given isoflurane, (1.0 – 4.0 %, to effect) as a general anesthetic. The atropine or glycopyrrolate, ketamine, and isoflurane are administered by the surgical staff of VMRB (under the supervision of the NIMH veterinarian), and the dosage and route of administration are determined by the veterinarian (the isoflurane is adminstered to effect, to reach a surgical plane of anesthesia). After the animal is anesthetized, it will be placed in a headholder appropriate for the procedure. An intravenous drip of normal saline or Ringer's solution is maintained, body temperature is maintained with a heating pad, and heart rate, temperature, and respiration are monitored with a physiological monitor. All procedures are performed using aseptic technique. At the end of surgery the animal will be monitored until it is awake (trying to sit up) and then be placed back in its home cage or [redacted] location. There will be a 10 - 14 day recovery period before animals are retested. Standard postoperative treatment includes analgesics and antibiotics administered after consultation with the attending NIMH veterinarian or Central Animal Facility veterinarian and may include, for example, Ketoprofen (1 mg/kg bid x 3 days), buprenorphine (0.02 – 0.05 mg/kg IM or IV, q 6 – 12 hr.) acetaminophen (80 mg, oral), ibuprofen (100 mg PO), Banamine (1 mg/kg / day for 2 days IM), as needed. If cerebral edema is considered a problem either mannitol (2 g/kg IV) and/or dexamethasone (1cc/2x/day; 3 mg/cc) for 2 - 5 days, IM) is administered.

Headpost and Chamber Implants: Headpost implants are made of MR-compatible plastic or metal (metals can be either titanium or silver coated with tantalum) and have 3 - 5 adjustable legs, and fastened to the skull with titanium or ceramic screws. Cement and acrylic is avoided when possible, although sometimes it is required to reinforce the primary screws. Chambers are also made of MRI-compatible material. For both headpost and chamber implants the scalp is retracted and the underlying muscles are retracted or removed. Approximately 10 - 12 small (1 - 2 mm diameter) holes are drilled into the skull (not penetrating the dura). The headpost is attached to the skull with either screws only or with screws plus acrylic; to attach the chamber to the skull screws plus acrylic will be used. The skin is then sutured around the implanted headpost or chamber. The monkey is returned to its home cage after it can sit upright without the aid of the caregiver. Normally, these implants will stay in place until the monkey is euthanized. However, if the monkey loses its implant, a new one may be re-implanted using the same procedure.

Bone removal inside the chamber is undertaken under aseptic conditions. The animal is anesthetized and placed in a headholder, the cap of the chamber is removed, and a small opening is made with either a burr or hand drill. The opening is then expanded, as needed, with

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micro-rongeurs.

Surgical Lesions and Neurotransmitter Depletions:
The monkey's vital signs (heart rate, respiration rate and temperature) will be closely monitored. Aseptic procedures will be employed.

All ablations, transections, and injections will be made either: 1) under visual control with the aid of an operating microscope, or 2) via MR-guided stereotaxic approaches. In addition, an animal will undergo some but not all of the surgeries listed in this section. For example, as shown in the sample flow charts depicted in Section F, an animal may undergo a unilateral amygdala lesion in one operation followed by a unilateral lesion of the premotor cortex in another operation.

For aspiration lesions, the dura mater is opened and the desired tissue removed using suction with a small gauge pipette. After completion of the tissue removal, the dura is closed, the bone sewn back in place, or, in those cases where it is not possible, cranioplasty will be done using small pieces of teflon. The incision will be closed in anatomical layers.

Excitotoxic lesions and neurotransmitter depletions will be made using a Hamilton microsyringe using MRI-guided stereotactic procedures or under direct visual guidance with the aid of a operating microscope. The total volume injected per site depends on the size (volume) of the brain target. Once the injections are complete the dura is closed and the bone flap sewn back in place and the skin closed in anatomical layers.

After surgery the monkeys will be given appropriate analgesics and antibiotics as determined by consultation with the NIMH veterinarian. In most cases the animal recovers for ten to fourteen days before postoperative testing begins. The maximum number of planned major survival surgical procedures that an animal will undergo is three. However, as described in Section F, animals may on occasion require additional major survival surgeries to complete a lesion or to remove bone that has regrown inside a chamber.

a. Amygdalar removal: a craniotomy will be performed over the frontal cortex, just behind the orbit. The dura mater will be reflected, the frontal cortex gently lifted, and the amygdala and its subjacent cortex removed by aspiration. In some experiments, selective amygdalar removal will be made with the neurotoxin ibotenic acid (10 – 15 µg/µl solution in phosphate buffered saline) or other neurotoxins such as NMDA. For this procedure, a large bone flap over the frontal cortex will be turned. Then a syringe needle will be lowered, through a small slit in the dura mater, until it reaches the amygdala, where the ibotenate (total of 24 - 30 µl per side) is slowly injected. A series of penetrations and injections, derived in part from measurements obtained from magnetic resonance images, will be used for a complete but selective amygdalar ablation.
b. Hippocampal removal: a triangular-shaped craniotomy will be performed just superior to the external auditory meatus. The dura mater will be reflected, the ventrolateral inferior temporal cortex gently lifted, and the ventromedial temporal cortex (parahippocampal gyrus) removed by aspiration. The white matter in the ablated area will be entered in order to gain access to the temporal horn of the lateral ventricle. The hippocampus, which is lying in the ventricle, will be identified and removed by aspiration. In some experiments, selective hippocampal removal will be achieved through use of the neurotoxin ibotenic acid or other neurotoxins such as NMDA. For this procedure, a large bone flap over the frontal and parietal lobes will be turned. Then, through a small slit in the dura mater, a syringe needle will be lowered into the hippocampal formation and the ibotenate (total volume of 10 - 24 µl per side) injected. A series of penetrations and injections will be required to complete the lesion.
c. The combined amygdalar and hippocampal removal is simply the combination of procedures 1 and 2, above.
d. Rhinal cortex removal: first, the zygoma will be removed to allow access to the portion of the cranium overlying the ventral temporal region. A craniotomy will be performed over the frontal and temporal regions. The dura mater will be reflected, the rhinal sulcus visualized, and the tissue lining the banks of the rhinal sulcus will be removed by aspiration. In some experiments, rhinal cortex lesions will be made with the neurotoxin ibotenic acid (a total of approximately 40 µl per side). Procedures will be similar to those described for ibotenate lesions in 1 and 2, above. In addition, injections may be made directly into the rhinal cortex via a hand-held Hamilton syringe rather than via a stereotaxic approach.
e. Forebrain commissurotomy: a large unilateral bone flap, encompassing the dorsal midline, will be turned and the dura mater reflected to expose the midline. The corpus callosum will be identified and cut with a glass sucker. Then, at approximately the level of the superior precentral sulcus, the anterior commissure, which passes through the third ventricle, will be identified and sectioned as well.
The dura mater will be pulled back to its original position but not sewn. (Since no brain tissue is being removed, the dura mater is specifically not sutured to allow the brain space for some expansion if there is cerebral edema.) The bone flap will be replaced.
f. Inferior temporal cortex ablation: to allow for removal of the most ventral portion of the cranium overlying the inferior temporal cortex, the zygoma is removed. Then a craniotomy is performed over the inferior temporal cortex. Tissue lying between the superior temporal sulcus and the rhinal sulcus, from the temporal pole to just rostral to the inferior occipital sulcus, is removed by aspiration. In some experiments, visual-limbic disconnection will be achieved by section of the optic tract in one hemisphere (see below), rather than an inferior temporal cortex lesion in that hemisphere.
g. Inferior prefrontal cortex ablation: the craniotomy is the same as that used for the amygdalar lesion described in #1a, above. The dura is reflected, and the cortex lying ventral to the principal sulcus, rostral to the fundus of the arcuate sulcus, and dorsal to the lateral orbital sulcus is removed by aspiration.
h. Orbital prefrontal cortex ablation: the craniotomy is the same as that used for the amygdalar lesion described in #1a, above. The dura is reflected, and the cortex lying medial to the lateral orbital sulcus, extending medially to the rostral sulcus, is removed by aspiration. The caudal boundary of the lesion is roughly 5 mm rostral to the junction of the frontal lobe and temporal lobe, and the rostral boundary of the lesion is an imaginary line joining the rostral tips of the lateral and medial orbital sulci.
i. Optic tract section: the craniotomy is the same as that used for the amygdala lesion described in #1, above. The dura is reflected, and the optic tract is located by gently elevating the frontal cortex and following the sphenoid bone to its end. At this point the optic tract may be visualized and sectioned with a glass sucker.
j. Surgical approaches for procedures for injections of retrograde tracers (Fast Blue or Diamidino Yellow) or DNA constructs will resemble

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in many respects the protocol for amygdala lesions or rhinal cortex lesions with ibotenic acid described above. As already indicated in Section F, the number of injections will range from 1 to about 50, and the typical amount of material injected will be 1.0 µl or less per site. All injections will be made directly into cerebral tissue via the needle of a Hamilton syringe.
k. For procedures f-h, the lesion may be achieved by injection of excitotoxins via a Hamilton microsyringe.
l. Frontal cortex: a craniotomy will be performed over the frontal region. The dura mater will be reflected, and portions of the premotor or prefrontal cortex removed by aspiration or injection of ibotenic acid.

From our previous experience working with excitotoxins (e.g. ibotenic acid, NMDA), we do not expect any long-term adverse effects on the health of the animals. However, during surgery respiratory arrest is an occasional side effect of cerebral injection of large volumes of ibotenic acid. Also when NMDA is injected we might observe tachycardia. If this occurs we administer propranolol or other agents, under the guidance of the NIMH veterinarian, and will stop the injections until the heart rate returns to normal. When such procedures are being undertaken, the NIMH and CAF staffs are alerted. The animal is watched closely while being maintained on the respirator and the veterinarian is advised of the situation and consulted as needed. Past instances of respiratory arrest or tachycardia have usually resolved (i.e. the animal resumes breathing on its own) within 30 minutes to 5 hours.

2. Who will perform surgery and what are their qualifications and/or experience?

Dr. Elisabeth Murray, [redacted], or her designee with the appropriate training and experience, will perform the surgery with the assistance of other personnel listed on the protocol. Dr. Murray has over 25 years of experience with aseptic neurosurgical techniques in monkeys, including those described above.

3. Where will surgery be performed, Building and Room? [redacted]
4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:

It will be the responsibility of the principal investigator to ensure that the monkey is adequately cared for until it has completely recovered from surgery. Postoperative care includes a regimen of dexamethasone phosphate and antibiotics, to reduce inflammation and to prevent infection respectively. In addition, analgesics will be administered. The VMRB staff, in consultation with the institute veterinarian, will specify the treatment regimen. Potential postsurgical complications include brain edema, seizures, and respiratory depression. After recovery from surgery the monkeys will be able to feed and care for themselves in the same manner they did before surgery.

Postoperative analgesics may include, for example, Ketoprofen (1 mg/kg bid x 3 days IM), buprenorphine (0.02 – 0.05 mg/kg IM or IV, q 6 – 12 hr.), acetaminophen (80 mg, oral), ibuprofen (100 mg PO), Banamine (1 mg/kg/day for 2 days IM), as needed. If cerebral edema is considered a problem either mannitol (2 g/kg IV) or dexamethazone (1cc/2x/day; 4 mg/cc IM for 2 days) is administered. The NIMH veterinarian is consulted to ensure the proper analgesics and antibiotics and appropriate doses of each are administered. Problems are quickly brought to the attention of the PI and the facility veterinarian and appropriate treatments are given.

After complete recovery from surgery, a minimum of 10 to 14 days based on an uneventful recovery period, the animal will resume postoperative testing. Postoperative recovery normally proceeds without difficulty. However, when the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and/or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized.

5. Has survival surgery been performed on any animal prior to being placed on this study? Y/N Y If yes, please explain:

43 monkeys being transferred to this new ASP have been used in the previous version of this ASP and have had major surgery:
4 monkeys have received bilateral excitotoxic lesions of the entire orbital prefrontal cortex
2 monkeys have received bilateral excitotoxic lesions of lateral orbital prefrontal cortex
2 monkeys have received bilateral excitotoxic lesions of medial orbital prefrontal cortex
3 monkeys have received bilateral excitotoxic lesions of caudal orbital prefrontal cortex
4 monkeys have received crossed lesions of the amygdala in one hemisphere and the entire orbital prefrontal cortex in the other hemisphere
4 monkeys have received crossed lesions of the amygdala in one hemisphere and the prelimbic cortex in the other hemisphere
4 monkeys have received crossed lesions of the amygdala in one hemisphere and the premotor cortex in the other hemisphere
3 monkeys have received crossed lesions of the amygdala in one hemisphere and the premotor cortex in the other hemisphere along with a split brain
1 monkey has received bilateral lesions of the anterior cingulate cortex
3 monkeys have received bilateral lesions of the nucleus accumbens
4 monkeys have received bilateral lesions of the amygdala
4 monkeys have had bilateral excitotoxic lesions of the ventrolateral prefrontal cortex
5 monkeys have received craniotomies with chambers (to allow introduction of cannulae for infusions)

6. Will more than one survival surgery be performed on an animal while on this study?
Y/N Y If yes, please justify:

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Monkeys that are subjects in disconnection experiments will receive multiple survival surgeries (see Section F). The design of "disconnection" experiments is a very powerful one. As already indicated, this is widely accepted as the only way to determine whether a given function, in this case a specific kind of sensory memory, is dependent upon the integrity of the anatomical connections between certain specified brain structures. This experimental design will require 2 or sometimes 3 operations to complete the disconnection (three if section of forebrain commissures is required). Some monkeys that receive ibotenate lesions or injections of DNA constructs will also require multiple survival surgeries. In this case, some of the injection series (e.g. combined amygdala and hippocampal lesions) will take so long to complete that a bilaterally symmetrical lesion must be carried out in two or more stages. Occasionally, the lesion of the intended target appears to be incomplete (as judged from postoperative MR scans) and the injections are repeated to achieve complete removal of a specific brain structure before the postoperative behavioral training is initiated. In other cases, a crossover design requires different animals to receive injections of different DNA constructs in a different order (e.g. treatment A followed by treatment B or vice versa), so this procedure, too, requires multiple survival surgery. Finally, operated monkeys that receive injection of retrograde tracers will in some cases require a minimum of 2 surgeries (see section F). In all cases of multiple survival surgeries, at least two weeks will intervene between successive stages. A maximum of 3 planned major survival surgeries, including operations for lesions and for injections of retrograde tracers, will be performed in one animal. On occasion, however, animals may require additional major survival surgeries to complete a lesion (if postoperative MR imaging shows the original lesion to be incomplete), or to remove bone that has regrown inside a chamber. Finally, on rare occasions we may need to inject a tracer in an animal that has already undergone three major survival surgeries. Approximately 8 monkeys a year will receive multiple-survival surgeries.

H. RECORDING PAIN OR DISTRESS CATEGORY - *The ACUC is responsible for applying U.S. Government Principle IV.: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.* Check the appropriate category or categories and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. FOR USDA REGULATED SPECIES, PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA. FOR ALL OTHER SPECIES, THE JUSTIFICATION FOR SUCH STUDIES MUST BE PROVIDED IN SECTION F. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT

NUMBER OF ANIMALS USED EACH YEAR			Year 1	Year 2	Year 3
	USDA Column C	Minimal, Transient, or No Pain or Distress			
X	**USDA Column D**	Pain or Distress Relieved By Appropriate Measures	109	20	20
	USDA Column E	Unrelieved Pain or Distress			

Describe your consideration of alternatives to procedures listed for Column D and E, and your determination that alternatives were not available. [Note: Principal investigators must certify in paragraph N.5. that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.] Delineate the methods and sources used in the search below. **Database references must include the databases (2 or more) searched, the date of the search, period covered, and keywords used.**

As already indicated in Section E, lesion/inactivation experiments are the only way of determining whether a given brain structure is necessary for a particular function. At present, all brain lesion methods that have the requisite specificity require the aseptic surgical methods described in the ASP, and these methods, in turn, are used only with concurrent administration of appropriate anesthetics. Discussion of alternatives with experts in the field suggested that there was no noninvasive alternative approach. Literature searches covering the years from 1990 to the present using Pubmed, Primate Lit, and Agricola also yielded no alternatives.

On 10/25/2016 PubMed was searched using the following keywords or phrases:
inactivation monkey brain: 2808
lesioning monkey brain: 431
perirhinal lesions monkey: 123
hippocampal lesions monkey: 1326

A total of 4688 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

On 10/25/2016 PrimateLit was searched as follows:
inactivation: 494
lesions: 942
perirhinal lesions: 1

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hippocampal lesions: 38

A total of 1475 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

On 10/25/2016 Agricola was searched:
primate brain lesions: 1
perirhinal lesions: 1
hippocampal lesions: 30
inactivation primate brain: 1

A total of 33 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration. All substances administered to an animal must be pharmaceutical grade unless otherwise justified in accordance with the NIH ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals. None ☐

There will be regular consultations with the NIMH veterinarian in order to ensure the most effective analgesics and anesthetics are used.

Agents routinely used:

For surgery: Pre-treatment with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM).
Ketamine hydrochloride 10 - 20 mg/kg IM; isoflurane gas, 1% to 4%, to effect, inhalation

Desipramine is counterindicated with ketamine for pre-isofluorane anesthetic preparation. An alternative anesthetic regimen will be employed for pre-surgical preparation. Use of dexmedetomidine (0.005-0.05 mg/kg, Dexdormitor®) plus butorphanol (0.3 mg/kg) has been successfully used in other settings and would be employed here.

For MRI scans in the [redacted] *horizontal scanner*: pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) then anesthetized with 10 - 15 mg/kg ketamine IM, followed by either dexmedetomidine (0.005-0.05 mg/kg IM) or valium (0.5 - 1.5 mg/kg IM), with supplements as needed. Supplemental doses will be ketamine alone.

For MRI scans in the [redacted]: In some cases the animals are scanned during stable, balanced anesthesia (1 - 2% isoflurane, to effect). As an alternative to full anesthesia, a combination of three safe drugs will be used:
1) Ketamine (5 - 15 mg/kg IM)
2) Diazepam (0.5 - 1.5 mg/kg IM 30 minutes prior, or 0.1 mg/kg slow IV at the time of anesthesia) – valium, a benzodiazepine sedative.
3) Glycopyrrolate (15 mcg/kg IM) -- anticholinergic, lasts a bit longer than atropine.

For anesthetized CT scans [redacted]: pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) then anesthetized with 10 - 15 mg/kg ketamine IM, followed by either 0.005-0.05 mg/kg dexmedetomidine IM or 0.5 - 1.5 mg/kg valium IM (diazepam), with supplements as needed. Supplemental doses will be ketamine alone.

Postoperative analgesics: will be administered by [redacted] Facility staff and may include, for example, Ketoprofen (1 mg/kg bid x 3 days), buprenorphine (0.02 – 0.05 mg/kg IM, or IV, q 6 – 12 hr.) acetaminophen (80 mg, oral), ibuprofen (100 mg PO), Banamine (1 mg/kg/day for 2 days IM). If cerebral edema is considered a problem either mannitol (2 g/kg IV) or dexamethasone (1cc/2x/day; 3 mg/cc IM) for 2 days is administered.

For nonsurgical procedures such as cleaning headposts and guide holders: Ketamine (5.0 - 20.0 mg/kg IM). To remove a small piece of bone or to enlarge the burr hole inside the chamber, procedures lasting only a few minutes, a ketamine / xylazine mixture (ketamine, 8 mg/kg, plus xylazine, 0.4 mg/kg IM) is used. A dose of atropine at a dose of 0.05 - 0.10 mg/kg, SC or glycopyrrolate 13 - 17 ug/kg IM or SC, will also be given for this procedure.

After a period of weeks or months, the dura mater sometimes starts to thicken. In this case, the monkey will either be sedated with ketamine (10 - 20 mg/kg IM) or taken to the OR to be done under isoflurane (1.0 – 4.0%, to effect) and the fibrous connective tissue will be removed using aseptic technique.

J. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY:

Indicate the proposed method, and if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Guidelines on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

None ☐

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The animal is sedated with Ketamine HCL (10 mg/kg IM or to effect) followed by either Euthanasia-III (1.0 ml/5 kg IV), an overdose of sodium pentobarbital (100 mg/kg IP or 100 - 200 mg/kg IV), or another equivalent euthanasia solution given at the manufacturers recommended dose. After a deep level of anesthesia is attained the animal is perfused through the heart with normal saline followed by aldehyde fixatives. The brain will be removed for histology and the carcass will be double-bagged in heavy plastic bags and stored in the appropriately marked refrigerator until it is transported to DVR for necropsy or disposed of as MPW.

K. HAZARDOUS AGENTS: None ☐

Biological Agents with Pathogenic Potential: NONE X (check if none) For guidance, see ORS/DOHS Biological Safety and Compliance. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.		
Agent:	HPRD #:	ABSL:
Additional occupational health and/or animal facility handling safety considerations.		

Recombinant DNA: NONE X (check if none) For guidance, see NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules FAQs. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.		
Recombinant DNA:	RD #:	ABSL:
Additional occupational health and/or animal facility handling safety considerations.		

Ionizing Radiation: (Radionuclides & radiation producing equipment) NONE ___ (check if none) For guidance, see ORS/DRS/Policies/Radiation Safety Protocols Animal Studies Proposal Requirements
☐ Yes, I will use radionuclides or radiation producing equipment as part of the experimental procedures on the ASP and all operators will be registered with Division of Radiation Safety. If an irradiator is to be used, then all individual users must comply with Division of Radiation Safety requirements for irradiator training, and all individual assessors will comply with applicable security requirements for escorts and proxy card access approval.
List of Radionuclides:
Radiological safety considerations:

Hazardous Chemicals or Drugs: NONE ___ (check if none) For guidance, see NIH Policy Manual 3034 – Working with Hazardous Chemicals Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (Title 29, Part 1910.1200(b)(3)(ii), CFR)
List of Agents: Aldehyde Fixatives, isoflurane, 5-fluorouracil
Additional occupational health and/or animal facility handling safety considerations: All material that has come into contact with monkeys or monkey by products is disposed of in a MPW box, when appropriate. Appropriate protective equipment (i.e. lab coat, chemical resistant gloves, and mucous membrane protection) shall be worn. When working with aldehyde fixatives and anesthetic gasses adequate local exhaust ventilation (e.g. chemical fume hood or downdraft table) shall be used and appropriate protective equipment (i.e. lab coat, chemical resistant gloves, and eye splash protection) shall be worn. All NIMH Guidelines for the Use and Disposal of Aldehyde Fixatives will be followed. Isoflurane gas will be scavenged via an activated charcoal (F-air) canister attached to the anesthesia machine(s) in the [redacted] location. **5-fluorouracil:** The solid form of the chemical is mixed into solution in a chemical fume hood by a worker wearing chemically-resistant gloves and a disposable laboratory coat. The material involved in the preparation of the solution will be disposed of as medical pathological waste (MPW) and the aspirated and waste fluids are disposed of as chemical waste. Drugs will be prepared outside of the animal facility. Appropriate safety materials are available, and procedures are in place for any accidental spillage of 5-fluorouracil (5-FU). The procedure involves absorbing the material if the spill is less than 10 ml, washing the exposed area with sodium hydroxide (10%), then water, and disposing of the collected material as MPW. If the spill is greater than 10 ml, the area is to be evacuated and the [redacted] location Hazard Response Unit called by 911.

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Monocrystalline iron oxide nanoparticles (MION) refer to a suspension of iron oxide particles falling within the nanometer range (generally 10-200 nm diameter). This substance is routinely used in human medical cases of iron deficiency anemia (http://www.feraheme.com) and as an intravenous contrast agent for MRI (Stabi and Bendz, Ann. Pharmacother, 2011, 45:1571-1575). MION has been used routinely and safely as an intravenous contrast agent for non-human primate MRI in this lab and elsewhere for more than twelve years. The MION suspension is stored in small (10mL-20mL) vacuum-sealed vials. In the Neurophysiology Imaging Facility (NIF), the vials are stored in the animal preparation room. Intravenous injection made by an experienced person is undertaken in restrained monkeys, either in the home cage or within a specially designed primate chair. The experimenter wears 2 disposable gloves (e.g. Nitrile) gloves and other appropriate PPE when handling the vials, extracting the suspension into the syringe, and injecting into the animal. Used vials, still sealed but including small amounts of unused MION, are disposed of as chemical waste. Any MION waste, contaminated gloves, bench paper, or spill cleanup materials will be double bagged, labeled as 'nanoparticle waste: 'Monoionic Iron Oxide Nanocolloids' and disposed of as chemical waste". Recommendations of the NIH DOHS Nanotechnology Safety and Health Program' will be followed.

Additional safety considerations: None

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.): None ☐

Section L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS
NONE __X__ (check if none)
List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.

Material:	Source:	Sterile? Y	Sterile? N
If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)			
Have the tested materials been passed through rodents outside of the animal facility in question?			
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?			
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens.			

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs. None ☐

5- fluorouracil

Procedures are in place for any accidental spillage of 5-fluorouracil (5-FU). The procedure involves absorbing the material if the spill is less than 10 ml, washing the exposed area with sodium hydroxide (10%), then water, and disposing of the collected material as MPW. If the spill is greater than 10 ml, the area is to be evacuated and the location [redacted] Hazard Response Unit called by 911.

Intracerebral injection of excitotoxins

From our previous experience working with these lesion-producing agents we do not expect any long-term adverse effects on the animals related to the excitotoxic lesions. However, during surgery respiratory arrest is an occasional side effect of cerebral injection of large volumes of ibotenic acid. Also when NMDA is injected we might observe tachycardia. If this occurs we administer propranolol or other agents, under the guidance of the NIMH veterinarian, and will stop the injections until the heart rate returns to normal. When such procedures are being undertaken, the NIMH and CAF staffs are alerted. The animal is watched closely while being maintained on the respirator and the veterinarian is advised of the situation and consulted as needed. Past instances of respiratory arrest or tachycardia have usually resolved (i.e. the animal resumes breathing on its own) within 30 minutes to 5 hours.

Hearing protection in fMRI experiments

Monkeys have been tested systematically on potential hearing loss before and after multiple scanning sessions using sound protection with silicone molds. Specifically, the monkeys' hearing ability between 892 and 7996 Hz, as well as their middle ear function, was tested on multiple occasions by audiologists using distortion product otoacoustic emission (DPOAE). The audiological results showed normal function before and after full data acquisition (K Tanji et al, NeuroImage,2010).

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MION

The established dose for MION in functional brain imaging in monkeys is 6 - 20 mg / kg. While long-term iron accumulation in the liver may ultimately limit the number of injections, this limit has not yet been identified. An early published study showed that animals can tolerate at least 60 mg / kg and up to 166 mg / kg with no ill effects. More recent work has shown that rhesus monkeys can receive up to 200 doses of 6 - 10 mg / kg (total nearly 2 g / kg iron) without showing any obvious negative health or cognitive effects.

MION has been used frequently as described in Leite et al (2002), and is commonly used now, for example, Tsiao et al, PNAS (2008), and Moeller et al (J Neurosci 2009). All authors specifically point out that there have been no MION-attributable complications or side effects many using doses of 10 mg / kg (total number of doses is unspecified in publications, but the use of over 200 doses has been reported to us from both Tsiao and Vanduffel's groups). Nonetheless, MION may accumulate in tissues, causing an artifact in MRI images. There are two accepted techniques to slow or prevent the accumulation of MION; the method employed will be the one that best fits the study design. One technique employs single doses of an iron chelator, desferoxamine given following every session involving MION administration and functional imaging. This has been done at other facilities (Tootell, personal communication) with no known adverse side effects. Given that animals may undergo functional imaging and MION administration two to three times per week, with at least one or two intervening rest days, desferoxamine will be given after MRI procedures involving MION two to three times per week (500 mg/day), either as a solution of 213 mg/ml for IM administration or as a solution of 95 mg/ml for IV or subcutatneous administration. A second method to reduce iron accumulation is to treat the animal with desferoxamine during periods in which they are receiving no MION. In this method, MION will be used in daily testing for a period lasting 2 - 3 weeks in a row. Thereafter, the animal will be placed on 'rest' for one week while he starts desferal (desferoxamine, 1 g / day over 2 injections) treatment, during the first three days of that rest. This approach has been used routinely with the Massachusetts General Hospital and Leuven imaging groups. While desferoxamine is considered to be an immunosuppressive agent, we expect minimal immunosuppression in the monkeys at the dose and duration of administration that we will be using. Desferoxamine is routinely used in treating humans with thalassemia. The most common side effects in humans are injection site reaction that may include pain, swelling, itching, and redness, blurred vision, and hearing difficulties. These side effects are reversible by discontinuing the administration of desferoxamine.

In addition, for monkeys that receive MION, we will monitor the iron accumulation by sampling blood transferrin saturation and total blood iron concentration every 2 - 4 months.

Social housing

Animals will be housed and enriched in a manner consistent with the NIMH Guidelines for Psychological Well Being and Enrichment of Nonhuman Primates. Some monkeys with brain lesions are to be exempt from pair housing. Removal of certain brain regions (e.g. amygdala, orbital frontal cortex, medial frontal cortex) results in alterations in emotional and social behavior. Specifically, after certain brain lesions, the dominance structure within a pair may be altered or the animals may respond inappropriately to social signals. In such cases, animals will no longer be suitable for pairing. In addition, because the controls for these experiments must be treated the same way as subjects in the operated groups, these animals, too, must be single-cage housed. Whenever animals are exempted from pair housing, other means of environmental enrichment will be implemented as per the NIMH Nonhuman Primate Enrichment Policy.

Scientific Justification for Exemption to NIMH Pair-housing Policy

Cognitive behavioral studies (e.g. studies designed to study the brain mechanisms by which information is acquired and utilized), unlike some other behavioral, physiological, or toxicological studies, are more susceptible to change in the conditions under which an animal is housed. Cognitive behavioral studies are designed to monitor small changes in an animal's ability to learn, retain, and use information over time. The majority of the tasks under study measure the rate of change in learning over time or, once the task is mastered; assess performance at different levels of cognitive difficulty on the task (e.g. mnemonic difficulty by increasing delays or number of stimuli to remember). During these testing periods it is mandatory that changes in the animal's environment (e.g. housing, husbandry, etc.), which could adversely impact the animal's performance, are minimized or eliminated. Performance changes secondary to environmental changes can affect comparisons within an animal over time, as well as planned comparisons between groups of animals over time.

Experience in the Laboratory of Neuropsychology over the past 30 years, as well as from laboratories of others, has demonstrated that the outcome of cognitive behavioral experiments can be altered by changes in the animal's environment, most importantly, the animal's housing situation (e.g. single vs. pair vs. group housing) or position within a social group. Although a singly housed animal does not have physical contact with a cohort they are still part of a social group of animals housed within the same room, and all maintain some visual, olfactory, and auditory interaction. It is our experience that these "extended" social groups have a social structure. For example, changes in an animal's cage location in the room or changes in the numbers of animals within the room (i.e. introduction or removal of animals, etc.) can benefit or adversely impact an animal's social status and dramatically change its behavior and performance within our studies. These changes often result in changes in an animal's expression of emotional behavior, which subsequently affects the way it interacts within its environment as well as its performance in behavioral studies. Changes of this nature have been shown to alter an animal's behavioral performance, as demonstrated by either their lack of completion of a task that is normally easily completed or by a large decrease in the animal's overall performance level. Indeed, one of the quickest ways for an investigator to detect that a change in the housing environment has occurred (without prior knowledge) is by a noticeable change in an animal's performance on a task.

Another example of how an animal's environmental conditions can alter its behavioral performance is the change observed when a monkey gets loose in a room. The excitement of a loose monkey, coupled with the actions taken by care staff to recover the animal, routinely have an adverse impact on an animal's behavioral performance. The difference in changes of this nature compared to those outlined above is that these changes tend to be more transient, whereas the housing changes outlined above may lead to more prolonged or permanent changes in an animal's current and future behavioral performance.

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We have also found that pair-housing is a factor that can affect anatomical measures. In a recent preliminary study we found a decrease in neurogenesis in an animal that was the submissive member of a pair. This decrease was largely unexpected, as we had increases prior to this in animals treated in the same manner but individually housed. Initially, we thought that pair-housing would actually benefit the animal, not have a potentially negative effect. It has been shown that stress and the social environment, including newly added animals, are important factors in regulating the amount of neurogenesis in the primate brain (Gould et al., (1998) PNAS, 95:3168-3171). To the extent that neurogenesis is linked to memory and emotion (Gould et al., (1999) Nat. Neurosci. 2:260-265) it may represent an underappreciated factor in our cognitive and emotional measures as well.

All of these situations can result in compromised data from the animal's behavioral testing session(s) which can cause serious difficulty in the interpretation of the data by altering learning or performance curves that, in many cases, are the critical parameter being used for scientific comparison. Studies like ours are designed to measure small changes in cognitive-related behavior over time as a result of a brain manipulation (e.g. lesion, fiber cut, pharmacological infusion). It is imperative that changes in the animal's environment that may confound or mask potential changes in an animal's behavior be kept to a minimum or prevented.

Therefore, to prevent compromises in the scientific integrity of these studies, investigators may request an exemption from the NIMH NHP pair-housing policy for animals under ASPs that fall in the following categories:

1. The introduction or loss of a cage mate can change an animal's behavioral pattern for weeks, or even months, depending on the ease of introduction or the degree of bonding between the two animals. Although these changes are problematic at any time during a study, they are especially problematic if they occur during a critical performance-measuring period (e.g. relearning of a task) or near the end of the study as the animal completes the performance assessments. Therefore, investigators may request an exemption from pair-housing if the animal is in a critical performance period or within six (6) months of the end of the study. If a further exemption is required for the animal(s) under consideration, a written request for the exemption will be forwarded to the NIMH ACUC for their review and approval. These exemptions will be reviewed with the veterinarian on a monthly basis.

2. Much of our research involves the study of brain regions known to contribute to socio-emotional behavior, including dominance and aggression, for example, limbic regions including the amygdala and frontal cortex. Animals that have undergone brain lesions in these and related areas have demonstrated dramatically altered behavior patterns making them unpredictable and sometimes incompatible with social groups (Rosvold, et al., (1954) J. Comp. Physiol. Psychol. 47:173-178; Mirsky, et al., (1957) J. Neurophysiol. 20:588-601). Lesions in these regions have resulted in changes in an animal's behavior patterns which have made it vulnerable to aggression and attacks from other non-human primates. Therefore, in order to ensure the safety of an animal with a brain lesion in one or more of these areas, as well as the safety of other animals within the colony, investigators may request a permanent exemption from pair-housing for animals with lesions in these areas.

3. This type of research involves comparing the effects on behavior subsequent to a brain manipulation (e.g. lesion or drug manipulation) with other groups with different manipulations (eg. drugs or lesions). Thus, to make valid scientific comparisons it is necessary to treat all the animals in the different groups in the same manner. If this is not done, it could be difficult for data interpretation and publication of the data. For example, if one group of animals had to be individually housed and their data compared with animals that were pair-housed, and there appeared to be a difference in the performance of the two groups, it could be questioned whether this was a result of the pair-housing or the brain manipulation. In the past, data has had to be discarded from some animals that were pair-housed when trying to compare it with data from animals that were individually housed. In addition, journals may question behavioral data derived from situations where all animals were not treated in an identical manner. Therefore, investigators may request an exemption from pair-housing when valid comparisons might be compromised

Individually-housed animals can still participate in other aspects of the NIMH NHP environmental enrichment program. Individually-housed animals can be provided with the use of exercise/play cages, manipulanda, foraging activities, and the use of novel food items as appropriate for the species. Also, whenever possible, visual and auditory contact can be maintained with other animals.

In addition to the above, there is extensive positive human interaction via the provision of food treats by both research and animal care staff. In most cases there are daily positive reinforcement training sessions that serve to provide a dynamic and enriched environment. Individually-housed animals will be closely monitored by our technical and veterinary staff to ensure their health and well-being.

While we do not expect any adverse effects from the procedures described above in this proposal the animals will be monitored frequently enough to ensure that their well-being is not compromised. When monitoring for adverse effects the animals will be flagged and the animal holding facility will be alerted. If there is any indication that the animal's condition is changing, we will seek immediate veterinary medical consultation. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and/or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized as described above in Section J.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. I certify that I have attended an approved NIH investigator training course.
   Month/Year of Initial Course Completion: 1985 ; Month/Year(s) of Refresher Training: Aug., 2004, Aug. 10, 2007; Aug 12, 2010; Aug 12, 2013; Oct 5, 2016
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (or equivalent, as applicable, for contract personnel).

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4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal, have completed the course "Using Animals in Intramural Research: Guidelines for Animal Users" will complete refresher training as required, and received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns. I further certify that I am responsible for the professional conduct of all personnel listed in Section A.
5. **FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section H):** I certify that I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) **as noted in section H,** and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the ACUC before initiating any significant changes in this study.

Principal Investigator:

Signature _____ Date 10/31/2016

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O. CONCURRENCES: PROPOSAL NUMBER _____ (LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____ Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name_____ Signature_____ Date_____

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility [location redacted] Name [secondary name redacted] Signature [secondary name redacted] Date 12/15/16
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name [secondary name redacted] Signature [secondary name redacted] Date 12/15/2016
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name_____ Signature_____ Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____ Signature_____ Date_____

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O. CONCURRENCES: PROPOSAL NUMBER _____ (LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name [redacted] Signature [redacted] Date 12/21/16

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:

Name_____ Signature_____ Date_____

DRS Safety Representative

Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)

Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review

Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____

NIMH Institute Veterinarian: Certification of Review

Name_____ Signature_____ Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____ Signature_____ Date_____

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Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____Signature_____Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name [redacted] Signatu [redacted] Date 12/20/2016

DRS Safety Representative
Name_____Signature_____Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature _____Date_____
Name_____ Signature _____Date_____
Name_____ Signature _____Date_____
Name_____ Signature _____Date_____

NIMH Institute Veterinarian: Certification of Review
Name_____Signature_____Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____Signature_____Date_____

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Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____ Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name_____ Signature_____ Date_____

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name James Rabser Signature [signature] Date 12/16/16

P. FINAL APPROVAL:
Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson Richard Saunders Signature [signature] Date 12/16/16

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 07/16/2021

NATIONAL INSTITUTE OF MENTAL HEALTH

ANIMAL STUDY PROPOSAL AMENDMENT FORM

ASP # LN-20

Leave Blank

APPROVAL DATE 12/22/16

A. ADMINISTRATIVE INFORMATION

Principal Investigator: **Elisabeth Murray, PhD**

APPROVED
ACUC

ASP Title: **Neural Substrates of Sensory Memory, Reward and Emotion**

(List name and affiliation of any persons being added) In addition, training and experience forms must be provided for these individuals.

NAME	Position Title, Affiliation
No Change	

B. ANIMALS NEEDED (List species, strain, number, and source of any additional animals)

SPECIES	STRAIN	SOURCE	NUMBER

No Change

C. TRANSPORTATION (Indicate any changes in transportation. This section must be addressed if housing in the ACRF facility is being added):

No Change

D. PURPOSE OF AMENDMENT (Explain any addition or changes to the previous objectives of this study):

Many of our studies involve daily behavioral testing sessions carried out in automated (computer-run) test apparatuses. During performance of behavioral tasks, monkeys typically earn small food rewards (e.g., 1/2 peanut, 1 banana pellet) for successful completion of each trial. In association with this behavioral testing, we plan to implement a new procedure: once per day feeding of NHPs with a "wet mash/chow", as well as fruit and nuts. This procedure has the advantage of reliably linking the completion of a discrete testing session to the delivery of a large food reward via a computer controlled "lunchbox". On 5-6 days/week on which behavioral testing is conducted, this large reward would constitute almost the entire daily diet of the animal and it can be obtained by the monkey within the testing apparatus on completion of a specified number of trials. Monkeys then feed free from interruption from the experimenter, and closely associate the entire sequence of events from leaving the home cage to finishing the testing session with the large daily reward. The chow is provided as wet mash so that the monkeys are able to eat or pouch this large reward without access to water and in a quicker time than is seen on a biscuit diet.

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Under these conditions - because monkeys are required to complete a fixed number of correct trials to finish the test session and earn the lunchbox - they are highly motivated to perform to a high level because they can obtain the lunchbox more quickly. Other labs that use this procedure also find that behavioral training can proceed more quickly with this approach, as opposed to the standard feeding in the home cage.

E. JUSTIFICATION FOR NUMBER OF ANIMALS REQUESTED (You must provide numbers justification for any additional animals):

No Change

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES (Describe all new experiments and/or changes in existing experiments and animal procedures. Be sure to include study endpoints and possible adverse effects. The same level of detail should be provided as would be in a full ASP.)

Behavioral testing:

Currently, accurate performance through the testing session is rewarded by delivery of small food rewards (usually 190mg dry banana pellets). This standard way of reinforcing correct responses with delivery of food rewards will be continued. In addition, however, the final correct trial of each session will result in the opening of the "lunchbox" that contains most of the monkey's daily food ration, including "wet mash/chow", fruit and nuts. Wet mash will be a mixture of ground biscuits (of the same type given to NHPs daily in LN – Purina LabDiet 5038) and water, combined with seeds or monkey forage mix. The monkey is allowed time to consume all of the food in the test cubicle (about 15 minutes) before returning to the home cage. In the initial stages of training monkeys will be allowed longer to finish eating (at least 30 minutes). The lunchboxes will be wiped down after each monkey has finished and thoroughly cleaned with disinfectant at the end of each testing day. Every 2 weeks, the lunchboxes will be removed and cleaned with hot water and soap.

The weight of wet mash given to each animal will vary between 120g and 330g (24 biscuits is approximately 330g wet mash), depending on the monkey's size, motivation and stage of training. In addition, the lunchbox will contain 1-3 pieces of fruit (e.g., from among ¼ apple or orange, ½ banana, handful of grapes) and peanuts. Monkeys will also typically obtain between 50 and 150 small food rewards during the session (approx. 1-3 biscuits caloric equivalent). Based on a typical daily diet of 24 biscuits (approx. 200 g dry weight), this is well within the recommended daily caloric content for a monkey. The weight of wet mash and amount of fruit given to each monkey will be recorded in our data book and in the food log in each monkey's medical record chart. Each monkey's body weight will be recorded a minimum of once a week.

G. SURVIVAL SURGERY (Describe all additional or changes in surgical procedures to be performed. Include information on aseptic technique, identification of individuals conducting procedures, post-operative monitoring, and location. If multiple survival surgeries are proposed, the total number of surgeries must be indicated clearly; this includes cases where animals will have undergone surgery on previous studies.)

No Change

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H. PAIN OR DISTRESS CATEGORY: List the category for new animals to be added under amendment. IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED. PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO USDA.

Number of Animals

Column C - Minimal, Transient, or No Pain or Distress _____**No Change**

Column D - Pain or Distress with relief _____

Column E - Unrelieved Pain or Distress*** _____

H1. LITERATURE SEARCHES (If the amendment warrants, describe additional consideration of alternatives to new procedures listed for Column D and E animals as in the original ASP. If any new animals are classified in Pain Column E, the Column E Justification Form must be completed as well.)

No Change

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION (Describe anesthesia and analgesia methods for all proposed procedures; if identical to methods outlined in original study, indicate so.)

No Change

J. Euthanasia (Describe proposed method of euthanasia; if identical to methods outlined in original study, indicate so.)

No Change

K. HAZARDOUS AGENTS (Identify any additional hazardous agents proposed. Also describe special handling/disposal methods required.)

	YES	NO	LIST AGENTS AND REGISTRATION DOCUMENT NUMBER (IF APPLICABLE)
1. Radioisotopes			
2. Biological Agents			
3. Hazardous Chemicals or Drugs			
4. Recombinant DNA			

No Change

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS (Describe proposed use of any biological materials or animal products in excess of those outlined in the original ASP. Include information regarding Source, intended use, level of sterility, MAP/RAP/Hap Testing Status.)

No Change

M. SPECIAL CONCERNS OR REQUIREMENTS (Describe any special housing, husbandry, or veterinary requirements for the new proposed experiments.)

No Change

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SIGNATURES:

Principal Investigator — Date: 12/22/16

Radiation Safety Officer (if applicable) — Date

Facility Manager** — Date

Facility Veterinarian** — Date

**ACUC Executive Secretary will obtain these signatures for you.

APPROVALS:

Division of Safety Representative — Date

~~NIMH ACUC~~ Attending Veterinarian — Date

NIMH ACUC Chair — Date: 12/22/16

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NATIONAL INSTITUTE OF MENTAL HEALTH

ANIMAL STUDY PROPOSAL AMENDMENT FORM

ASP # LN-20

Leave Blank

APPROVAL DATE 5/17/19

APPROVED
ACUC

A. ADMINISTRATIVE INFORMATION
Principal Investigator: Elisabeth A. Murray

ASP Title: Neural Substrates of Sensory Memory, Reward, and Emotion

(List name and affiliation of any persons being added) In addition, training and experience forms must be provided for these individuals.

NAME	Position Title, Affiliation

B. ANIMALS NEEDED (List species, strain, number, and source of any additional animals)

SPECIES	STRAIN	SOURCE	NUMBER

C. TRANSPORTATION (Indicate any changes in transportation. This section must be addressed if housing in the ACRF facility is being added; **note: all amendments highlighted in red and bold font**):

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported location location, also include the route and elevator(s) to be utilized.

Monkeys will be transported from monkey home cages to the location location and back location following the approved transportation SOP for this facility. Anesthetized monkeys, **scheduled for either MRI or CT scans,** will be transported in covered transportation carts, and awake monkeys will be transported in transportation chairs. Awake monkeys will be covered and restrained with a minimum of two forms of restraint when being transported through public areas. In addition to the restraint provided by the chair itself, a chain attached to the chair will also be hooked to the monkey's collar.

The animals are transported from location to location and back using an approved opaque transport box and following the approved transportation SOP. For CT imaging, there will be two people with the monkey at all times. Anesthetized monkeys will be transported in an approved, specifically designed, covered, opaque transport box. We will enter location location location until the locked door

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location is reached. We will then call for the CT technician to meet us and escort us through the locked corridor that houses the location

All location and NIH Transportation Guidelines will be followed.

D. PURPOSE OF AMENDMENT (Briefly explain the purpose of the amendment and how the proposed changes fit with the objectives of the original ASP):

The purpose of the amendment is to include additional information regarding another site location where Computed Tomography (CT) may take place.

E. JUSTIFICATION FOR NUMBER OF ANIMALS REQUESTED (You must provide numbers justification for any additional animals): No changes.

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES (Describe all new experiments and/or changes in existing experiments and animal procedures. Be sure to include study endpoints and possible adverse effects. The same level of detail should be provided as would be in a full ASP.; **note: all amendments highlighted in red and bold font**)

Computed Tomography (CT) Imaging:
All monkeys will be sedated throughout the transport and scanning procedures **for** location **scans**. We will use the same anesthestic drugs and doses as we normally use for MRI scans. Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with 10 - 15 mg/kg ketamine IM, followed by either dexmedetomidine (0.005-0.05 mg/kg IM) or diazepam (0.5 - 1.5 mg/kg IM) given 30 min prior to the scan. Supplemental doses will be ketamine alone. The ketamine/dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. The animal is then transported in an approved, specifically designed, covered, opaque transport box via an approved route location to location **or** location location. During the transport the animals are placed on their side and checked to ensure the airways are open. Once in the scan room they are placed in a stereotaxic head holder which secures their head in a stationary and standardized orientation. This orientation also ensures the airway is open through the scanning session. Total scanning time per monkey will be about 5 minutes. Once the scan is complete the monkeys will be returned to their home cage and monitored until awake and sitting upright. Total time from sedating the animal to return to its home cage for a single animal is about 45 min and no more than 60 minutes for two.

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There will be two people with experience in working with monkeys present at all times. During transport of the animals we will not wear PPE but once the animal is in the CT room and before opening the cart we will put on the appropriate PPE (e.g. lab coats, gloves, and face shield). The monkey will be fully wrapped in a disposable absorbent pad with moisture-proof plastic lining. The bed of the scanner will be covered with absorbent pads. The door to the CT scanner will be kept closed. At the end of the scanning session we will remove and discard in a trash bag all disposable items. These items will be brought back to [redacted] where they will be disposed of in MPW waste. The CT bed, inside the scanner, and floor will be cleaned with disinfectant. All [redacted] and NIH Transportation Guidelines will be followed.

G. SURVIVAL SURGERY (Describe all additional or changes in surgical procedures to be performed. Include information on aseptic technique, identification of individuals conducting procedures, post-operative monitoring, and location. If multiple survival surgeries are proposed, the total number of surgeries must be indicated clearly; this includes cases where animals will have undergone surgery on previous studies.)
No Changes

H. PAIN OR DISTRESS CATEGORY: List the category for new animals to be added under amendment. IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED. PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO USDA.

	Number of Animals
Column C - Minimal, Transient, or No Pain or Distress	_____
Column D - Pain or Distress with relief	_____
Column E - Unrelieved Pain or Distress***	_____

H1. LITERATURE SEARCHES (If the amendment warrants, describe additional consideration of alternatives to new procedures listed for Column D and E animals as in the original ASP. If any new animals are classified in Pain Column E, the Column E Justification Form must be completed as well.)

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION (Describe anesthesia and analgesia methods for all proposed procedures; if identical to methods outlined in original study, indicate so.) No Changes

J. Euthanasia (Describe proposed method of euthanasia; if identical to methods outlined in original study, indicate so.) No Changes

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K. HAZARDOUS AGENTS (Identify any additional hazardous agents proposed. Also describe special handling/disposal methods required.) No Changes

	YES	NO	LIST AGENTS AND REGISTRATION DOCUMENT NUMBER (IF APPLICABLE)
1. Radioisotopes			
2. Biological Agents			
3. Hazardous Chemicals or Drugs			
4. Recombinant DNA			

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS (Describe proposed use of any biological materials or animal products in excess of those outlined in the original ASP. Include information regarding Source, intended use, level of sterility, MAP/RAP/Hap Testing Status.)

M. SPECIAL CONCERNS OR REQUIREMENTS (Describe any special housing, husbandry, or veterinary requirements for the new proposed experiments.) No Changes

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SIGNATURES:

Principal Investigator — 05/15/19 Date

Radiation Safety Officer (if applicable) — Date

Facility Manager** — Date

Facility Veterinarian** — Date

**ACUC Executive Secretary will obtain these signatures for you.

APPROVALS:

Division of Safety Representative — Date

NIMH ACUC Attending Veterinarian — Date

NIMH ACUC Chair — Date

Theodore B. Usdin -S

Digitally signed by Theodore B. Usdin -S
Date: 2019.05.17 14:21:26 -04'00'

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NATIONAL INSTITUTE OF MENTAL HEALTH

ANIMAL STUDY PROPOSAL AMENDMENT FORM

ASP # LN-20

Leave Blank

APPROVAL DATE 12/4/19

APPROVED
ACUC

A. ADMINISTRATIVE INFORMATION
Principal Investigator: Dr. Elisabeth (Betsy) Murray

ASP Title: Neural Substrates of Sensory Memory, Reward, and Emotion

(List name and affiliation of any persons being added) In addition, training and experience forms must be provided for these individuals.

NAME	Position Title, Affiliation

B. ANIMALS NEEDED (List species, strain, number, and source of any additional animals)

SPECIES	STRAIN	SOURCE	NUMBER

C. TRANSPORTATION (Indicate any changes in transportation. This section must be addressed if housing in the ACRF facility is being added):

D. PURPOSE OF AMENDMENT (Briefly explain the purpose of the amendment and how the proposed changes fit with the objectives of the original ASP):

The purpose of the amendment, specifically for page 12 of the ASP under Section F: "Description of Experimental Design and Animal Procedures," subsection titled "Tastants," is to include additional information regarding the types of tastants that will be used in studies involving the delivery of fluid rewards during testing.

E. JUSTIFICATION FOR NUMBER OF ANIMALS REQUESTED (You must provide numbers justification for any additional animals):

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES (Describe all new experiments and/or changes in existing experiments and animal procedures. Be sure to include study endpoints and possible adverse effects. The same level of detail should be provided as would be in a full ASP.; **note: all amendments to the existing amendment are highlighted in red and bold font**)

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Tastants: Tastants, or types of fluid outcomes, are typically used in both human studies (WK Simmons et al, Nat Neurosci, 2013; DM Small et al, Neuron, 2008) and nonhuman primate investigations (C Padoa-Schioppa and Assad, Nature, 2006; Rolls et al, J Neurophysiol, 1990), not only as a reward for performing a task, but also as a means of identifying areas responsive to tastes. These studies have used a variety of tastants ranging from positive (sucrose, sucralose, **aspartame**, salt, various **flavored** fruit juices or **solutions**) to neutral (water, artificial saliva), to mildly negative (quinine, HCl, citric acid). We will be using different tastants to map responses to differing tastes or flavors, and to test monkeys' abilities to form associations with visual cues and different taste outcomes. Taste outcomes will be different types of solutions in three categories: positive, neutral, or mildly negative. A positive reward will be an aqueous solution with sucrose [0.1 M], sucralose [lmM] or fruit juice. A neutral reward will consist of a solution containing sodium bicarbonate [0.3mM] and potassium bicarbonate [0.3mM] (personal communication, A. Fontanini, January 29, 2015) to mimic saliva. A more complex saliva substitute contains NaCl (0.002M), KCl (0.005M), sodium bicarbonate (0.003M), potassium bicarbonate (0.003M), magnesium chloride (0.00025M) and potassium phosphate (0.00012M). Saliva substitutes will be used at these concentrations and at 75%, 50% and 25% dilutions. Quinine [lmM], a bitter tastant, or a dilute solution of either citric acid [10mM] or hydrochloric acid [lmM] (VL Smith-Swintosky et al, J Neurophysiol, 1991) are mildly negative. The monkeys will be presented with these fluids in a testing booth as well as the fMRI scanner. Use of these and similar tastants is commonplace in research settings and is expected to have no adverse consequences.

We plan to conduct studies that investigate the neural bases for flavor-nutrient learning. This type of learning is thought to rely on signaling via the gut-brain axis. On occasion, monkeys will consume a flavored fluid (tastant) mixed with maltodextrin, a flavorless and odorless food additive, at five different increments (0, 37.5, 75, 112.5, or 150 kilo-calories) at their home cage. This will have the effect of altering (increasing) the value of the flavored fluid (see: Yeomans et al, Physiol & Behav, 2008; de Araujo et al, Curr Biol, 2013; Veldhuizen et al, 2017, Curr Biol). As in the selective satiation procedure described above, we use this experimental manipulation to test the animal's ability to represent food value. Both the flavors and the maltodextrin are commercially available and widely used in studies of human flavor-nutrient learning. We anticipate no adverse consequences following the ingestion of either maltodextrin or the flavored fluids.

G. SURVIVAL SURGERY (Describe all additional or changes in surgical procedures to be performed. Include information on aseptic technique, identification of individuals conducting procedures, post-operative monitoring, and location. If multiple survival surgeries are proposed, the total number of surgeries must be indicated clearly; this includes cases where animals will have undergone surgery on previous studies.)

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H. PAIN OR DISTRESS CATEGORY: List the category for new animals to be added under amendment. IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED. PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO USDA.

Number of Animals

Column C - Minimal, Transient, or No Pain or Distress _____

Column D - Pain or Distress with relief _____

Column E - Unrelieved Pain or Distress*** _____

H1. LITERATURE SEARCHES (If the amendment warrants, describe additional consideration of alternatives to new procedures listed for Column D and E animals as in the original ASP. If any new animals are classified in Pain Column E, the Column E Justification Form must be completed as well.)

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION (Describe anesthesia and analgesia methods for all proposed procedures; if identical to methods outlined in original study, indicate so.)

J. Euthanasia (Describe proposed method of euthanasia; if identical to methods outlined in original study, indicate so.)

K. HAZARDOUS AGENTS (Identify any additional hazardous agents proposed. Also describe special handling/disposal methods required.)

	YES	NO	LIST AGENTS AND REGISTRATION DOCUMENT NUMBER (IF APPLICABLE)
1. Radioisotopes			
2. Biological Agents			
3. Hazardous Chemicals or Drugs			
4. Recombinant DNA			

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS (Describe proposed use of any biological materials or animal products in excess of those outlined in the original ASP. Include information regarding Source, intended use, level of sterility, MAP/RAP/Hap Testing Status.)

M. SPECIAL CONCERNS OR REQUIREMENTS (Describe any special housing, husbandry, or veterinary requirements for the new proposed experiments.)

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SIGNATURES:

Principal Investigator — 10/08/19 Date

Radiation Safety Officer (if applicable) — Date

[redacted] secondary name — 12-02-19 Date

Facility Manager** — Date

Facility Veterinarian** — 12/2/2019 Date

**ACUC Executive Secretary will obtain these signatures for you.

APPROVALS:

Division of Safety Representative — Date

NIMH ACUC Attending Veterinarian — Date

NIMH ACUC Chair — Date

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SIGNATURES:

Principal Investigator — 10/08/19 Date

Radiation Safety Officer (if applicable) — Date

Facility Manager** — Date

Facility Veterinarian** — Date

**ACUC Executive Secretary will obtain these signatures for you.

APPROVALS: secondary name [redacted]

Division of Safety Representative — Date

NIMH ACUC Attending Veterinarian — Date

NIMH ACUC Chair — Date

Obtained by Rise for Animals.
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SIGNATURES:

Principal Investigator — 10/08/19 Date

Radiation Safety Officer (if applicable) — Date

Facility Manager** — Date

Facility Veterinarian** — Date

**ACUC Executive Secretary will obtain these signatures for you.

APPROVALS:

Division of Safety Representative — Date

NIMH ACUC Attending Veterinarian — 12/4/19 Date

NIMH ACUC Chair — Date

Theodore B. Usdin -S

Digitally signed by Theodore B. Usdin -S
Date: 2019.12.02 12:27:26 -05'00'

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NATIONAL INSTITUTES OF MENTAL HEALTH
ANIMAL STUDY PROPOSAL
(Revised NIH 2014, NIMH 2014)
(See NIH PM 3040-2, 2014)

Leave Blank

PROPOSAL # LN 20

APPROVAL DATE 12/16/19

EXPIRATION DATE 12/16/2022

APPROVED
ACUC

A. ADMINISTRATIVE DATA:

Institute or Center: NIMH

Principal Investigator Elisabeth A. Murray

Building/Room location Email murrayc@mail.nih.gov Telephone 301-443-7401 FAX 301-402-0046
Emergency Treatment and Animal Care instructions shall be provided on the attached form at the end of this document.

Division, Laboratory, or Branch Laboratory of Neuropsychology

Project Title Neural Substrates of Sensory Memory, Reward, and Emotion

Initial Submission [ ] Renewal [X] or Modification [ ] of Proposal Number LN 20 (16)

List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., Co-investigator(s)): A brief summary of the training and/or experience for procedures each individual will be expected to perform in this ASP must be documented and available to the ACUC. The name(s) of the supervisor, mentor, or trainer who will provide assurance each individual is/has achieved proficiency in those procedures shall be included in that documentation.

Name: Elisabeth A. Murray Degree: Ph.D. Position title, Affiliation: Chief, Section on Neurobiol. Learn & Mem, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: 301-443-7401 Email address: murrayc@mail.nih.gov

Name: secondary name Degree: Ph.D. Position title, Affiliation: Senior Scientist, Contractor,LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary name/ Email address: secondary name

Name: secondary name Degree: Ph.D. Position title, Affiliation: PI, SLDM, LN/NIMH
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: secondary name/ Email address: secondary name

Name: secondary name Degree: Ph.D. Position title, Affiliation: Staff Scientist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary Email address: secondary name

Name: secondary name Degree: Ph.D. Position title, Affiliation: Research Fellow, LBC
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary name/ Email address: secondary name

Name: secondary name Degree: B.S. Position title, Affiliation: Biologist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary Email address: secondary name

Name: secondary name Degree: B.S. Position title, Affiliation: Postbacc IRTA, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary Email address: secondary name

Name: secondary name Degree: B.S. Position title, Affiliation: Biologist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: secondary name/ Email address: secondary name

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Uploaded to Animal Research Laboratory Overview (ARLO) on 07/16/2021

Name: [redacted] Degree: Ph.D. Position title, Affiliation: Staff Scientist, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone [redacted] Email address: [redacted]

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X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name [redacted] Degree: B.S. Position title, Affiliation: Post-Bacc, LN
X Fully qualified in all relevant animal procedures or o Will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name: [redacted] Degree: BA/MBA Position title, Affiliation: Biologist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name: [redacted] Degree: Ph.D. Position title, Affiliation: Postdoc IRTA Fellow, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name: [redacted] Degree: Ph.D. Position title, Affiliation: Postdoc IRTA Fellow, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name: [redacted] Degree: Ph.D. Position title, Affiliation: Staff Scientist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

Name: [redacted] Degree: B.S. Position title, Affiliation: Biologist, Contractor, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone [redacted] Email address: [redacted]

Name: [redacted] Degree: B.S. Position title, Affiliation: Biologist, LN
X fully qualified in all relevant animal procedures or o will be trained and supervised by:
Office phone: [redacted] Email address: [redacted]

NOTE: Approval of an animal study proposal by an ACUC does not ensure it acceptance by an animal holding facility. Acceptance by a facility is dependent on available space, personnel and resources to support the ASP. Allocation of animal holding space is the role and responsibility of the IC Scientific Director.

B. ANIMAL REQUIREMENTS:

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Species: *Macaca mulatta* Age/Weight/Size: 3.0 kg - adult Sex: M / F

Stock or Strain: N / A Source(s): NIH Approved Sources and/or the NIH NHP Recycling Program

Holding Location(s): location Animal Procedure Location(s): location
location

Estimated Number of New and Existing Animals Required to Support this Proposal:

		Year 1	Year 2	Year 3	Total
1	**Estimated number of <u>new</u> animals required** (Include all future purchases, births, and transfers)	20	20	20	60
2	**Number of <u>existing</u> naïve animals to be transferred into this ASP (any age)**	15	N/A		15
3	**Number of <u>existing</u> animals previously used in any experiment to be transferred into this ASP***	88	N/A		88
	YEARLY TOTALS	123	**20**	**20**	163

*If animals have been in a previous study, briefly describe how animals were used, e.g. any surgeries, procedures that caused pain and or distress (relieved or unrelieved):

There are a total of 88 animals being transferred onto this protocol from the preceding approved version of this protocol. Of these, 15 are naïve and 73 have been studied in the preceding version of this ASP. For the animals engaged in studies, the experimental design, methods, and survival surgeries were the same as described below in Sections F and G.

The 15 naïve monkeys will begin behavioral testing and take part in lesion studies on visual learning and memory.

The 73 monkeys that have been used in the previous version of this ASP are as follows:

9 monkeys are being tested on a social cognition task. 7 currently have headposts. A subset of this cohort (3) received bilateral excitotoxic lesions of the medial prefrontal cortex (anterior cingulate cortex).

12 monkeys are being trained on a spatial memory task. Half will undergo excitotoxic lesions of the medial frontal cortex(anterior cingulate cortex) when they reach criterion; 3 monkeys have received this surgery.

8 monkeys are being trained on an appetitive Pavlovian task to assess autonomic responses. Of these, 4 have received bilateral excitotoxic lesions of the amygdala and 4 are unoperated controls.

7 monkeys are being trained for a pilot study for optogenetic manipulation of perirhinal cortex. Two monkeys have received bilateral excitotoxic lesions of the perirhinal cortex. The behavior of these monkeys will be compared with those who later received optogenetic manipulation of perirhinal cortex.

16 monkeys are performing tests of stimulus-reward association. The monkeys are learning a new version of our stimulus-reward association task with fluids (instead of foods) in order for the behavior to be compatible with fMRI. 4 of these monkeys have crossed lesions of the amygdala and orbital frontal cortex; 5 are unoperated controls. A subset of 7 monkeys are participating in fMRI studies of stimulus-reward association. All 16 monkeys have headposts.

2 monkeys with crossed lesions of the premotor cortex and amygdala are being assessed for stimulus-reward association judgements.

3 monkeys with bilateral excitotoxic lesions of the ventral striatum (nucleus accumbens) are being trained on automated tasks to assess stimulus-reward and action-reward reversal learning.

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7 monkeys have recently been placed on rest and are waiting for their next task. Three of these monkeys have bilateral excitotoxic lesions of the orbitofrontal cortex and four are unoperated controls.

9 monkeys are on rest while waiting for assignment to a new project. 2 have bilateral excitotoxic lesions of the orbitofrontal cortex, 3 have crossed lesions of the prelimbic cortex and amygdala, and 4 are unoperated controls.

At the end of the study animals with lesions will be euthanized and the brains removed for histological processing and analysis.

C. TRANSPORTATION:

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported [redacted] also include the route and elevator(s) to be utilized.

[redacted] monkeys will be transported between [redacted] Awake monkeys will be covered and restrained with a minimum of two forms of restraint when being transported through public areas. In addition to the restraint provided by the chair itself, the monkey's collar will be held in place by either a slide-in neck plate that secures the collar from the back as well as a pin that inserts from the chair into a notch in the side of the collar in the vertical MR chairs or a collar slotting mechanism with a locking pin to secure it in the closed position in the horizontal MR chairs. Anesthetized monkeys will be transported to [redacted] in an approved, specially designed, covered transportation cart. In all cases, animals will be transported according to the NIH Transportation Guidelines.' Animals transported through public corridors will be shrouded during transport.

If [redacted] scanner is unavailable, or if certain procedures are only possible on other scanners, animals will be scanned [redacted] Animals undergoing MRI scans [redacted] will be anesthetized as described in Section F, Standard Procedures. They will be transported between [redacted] in covered, opaque transport boxes approved by the transportation SOP. In all cases, both the NIH Transportation Guidelines and the [redacted] SOP will be followed.

D. STUDY OBJECTIVES:

Provide no more than a 300 word summary of the objectives of this work. Why is this work important/interesting? How might this work benefit humans and/or animals? This should be written so that a non-scientist can easily understand it. Please eliminate or minimize abbreviations, technical terms, and jargon. Where they are necessary, they should be defined.

Earlier work from this laboratory has indicated that certain parts of the brain--the medial temporal lobe limbic structures, the amygdala, hippocampus and rhinal cortex--are critical for information storage in the brain. Specifically, these structures are known to underlie the ability of organisms to recognize previously experienced sensory events, to associate these events with one another, and to associate these events with reward and with motor acts. In addition, we have found that the orbitofrontal cortex is essential for storing and updating information about specific food and fluid rewards, including its current value or desirability. The proposed study will build on these earlier findings in two ways: 1) by contrasting the specific contributions to memory made by the limbic structures with those of other brain structures implicated in memory processes, and 2) by examining the interaction of medial temporal lobe structures with neocortical or thalamic processing areas in the storage and recall of sensory memory, stimulus-reward associations, and stimulus-action associations. These goals will be achieved by examining the effects of selective brain lesions, reversible brain lesions, and pharmacological manipulations on the acquisition and flexible use of different kinds of memory and by use of functional magnetic resonance imaging (fMRI) to identify the larger brain networks involved in reward and memory processes. By determining the parts of the brain that underlie memory in monkeys, we hope to uncover information relevant to the treatment of human disorders involving memory loss. Identifying these neural substrates of memory in monkeys will indirectly benefit the treatment of human amnesic disorders by promoting the use of other investigative tools or approaches (e.g. electrophysiological, neurochemical) in specific areas processing memories. Findings from such studies may lead to greater understanding of the cellular events underlying information storage that will be important in treating patients with Alzheimer's disease, semantic dementia, Parkinson's disease, or Huntington's disease, brain disorders which are characterized, in part, by progressive memory impairment. Moreover, the neural substrates of stimulus-reward association and emotional expression and regulation are relevant to understanding the origins and functional changes of mood and anxiety disorders.

E. RATIONALE FOR ANIMAL USE:

1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used. (Use additional sheets if necessary)

1) Animals must be used to investigate the neural substrates of memory because the same kind of information cannot be gleaned from human studies or from use of alternative techniques. Brain damage in humans is rarely either selective or complete for a given structure. Consequently it is impossible in most instances to draw conclusions regarding the neural basis for an identified memory deficit. Animals, however, can be used for the behavioral evaluation of the effects of selective brain lesions.

2) Nonhuman primates are required for these studies because they are the only readily available animals that can sufficiently master the kinds of cognitive problem-solving tasks being employed. *M. mulatta* is the preferred species. There is a long history of anatomical and physiological study of both limbic and neocortical structures in rhesus monkeys, thus providing a basis for comparison across studies. In addition, rhesus monkeys adapt readily to the laboratory environment. Furthermore, the organization of the brains of these animals is similar to that of humans,

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so any findings concerning the neural bases of behavior may apply to humans as well.

3) In most cases the control and experimental groups will be comprised of 4 animals each; this is the minimum number of subjects that will yield statistically meaningful results. Each year, I have the resources to conduct one new main study using 12 monkeys and another smaller one using 8 monkeys. The larger study would be comprised of two experimental groups and one control group (N = 12). The smaller one would consist of one experimental group and one control group (N=8). Therefore, I will need 3 X 20 monkeys, or a total of 60 monkeys, over the three-year period. In all cases, I will use the procedures as described in Section F, and would have the general experimental goals outlined in Section D.

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following: (Use additional sheets if necessary.)

Injections, Inoculations or Instillations (substances, e.g., infectious agents, adjuvants, medications, drugs, etc.; dose, sites, volume, route, diluent, and schedules). All substances administered to an animal must be pharmaceutical grade unless otherwise justified in accordance with the NIIH ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals

Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)

Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)

Radiation (dosage and schedule)

Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)

Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)

Other Procedures (e.g., survival studies, tail biopsies, etc.)

Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain or distress, ascites production, etc.) For Column E studies provide: 1) a description of the procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress cannot be relieved.

Resultant Effects, if any, that an animal(s) are expected to experience or exhibit (e.g., circling, abdominal distension, weight loss, etc.)

Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.

Breeding Paradigms (Include the maximum age of breeders, use of permanent breeding pairs or trios, weaning age, maximum number of animals in a cage, fate of pups not carrying the required gene, collection of tissues and/or body fluids for genetic testing, etc.)

Past Experience with Identical or Similar Experimental Treatments, Procedures, etc. (e.g. direct experience or the published experience of others related to the well-being of animals undergoing identical or similar experimental procedures, etc.).

Most experiments involve: 1) creating a permanent brain lesion using either subpial aspiration or injection of excitotoxins, OR manipulating neuronal activity using drugs; 2) measuring the effects of these manipulations on behavior; and 3) euthanizing the animal to examine the brain. Descriptions of the different procedures involved in these experiments are provided below.

Identification of animals: Animals will be identified by chest tattoos, subcutaneous transponder, and by the tags attached to their home cage.

Behavioral testing: Monkeys will be trained daily on behavioral tasks administered either in a modified Wisconsin General Testing Apparatus (WGTA) or in an automated (computer-run) test apparatus. In general, the monkeys learn to displace objects or make responses to pictures presented on a color monitor fitted with a touch-sensitive screen to earn food or fluid (juice or water) rewards. Monkeys trained in the WGTA will be held in a transport/testing cage for approximately 20 - 120 minutes per day, whereas monkeys trained in automated apparatuses will be restrained in either nonhuman primate chairs or a testing cage from approximately 30 minutes to 3 hours. In addition, monkeys will have their diet or their fluid intake manipulated in order to achieve prompt responding in the test situation. During training all monkeys will receive food or liquid rewards (e.g. banana pellets, peanuts, fruit juice, water).

In some experiments, monkeys are given their entire daily ration of chow in the test apparatus following completion of the daily test session. After the last trial of the session, an automated latch is released, thereby opening the "lunchbox". The lunchbox contains all required nutrients, including a "wet mash/chow", as well as fruit and nuts. This procedure has the advantage of reliably linking the completion of a discrete testing session to the delivery of a large food reward via a computer controlled lunchbox. Wet mash will be a mixture of ground biscuits (of the same type given to NHPs daily in LN - Purina LabDiet 5038) and water, combined with seeds or monkey forage mix. The monkey is allowed time to consume all of the food in the test cubicle (about 15 minutes) before returning to the home cage. In the initial stages of training monkeys will be allowed longer to finish eating (at least 30 minutes). The lunchboxes will be wiped down after each monkey has finished and thoroughly cleaned with disinfectant at the end of each testing day. Every 2 weeks, the lunchboxes will be removed and cleaned with hot water and soap.

The weight of wet mash given to each animal will vary between 120 g and 330 g (24 biscuits is approximately 330 g wet mash), depending on the monkey's size, motivation and stage of training. In addition, the lunchbox will contain 1-3 pieces of fruit (e.g., from among ¼ apple or orange, ½ banana, handful of grapes) and peanuts. Monkeys will also typically obtain between 50 and 150 small food rewards during the session (approx. 1-3 biscuits caloric equivalent). Based on a typical daily diet of 24 biscuits (approx. 200 g dry weight), this is well within the recommended daily caloric content for a monkey. The weight of wet mash and amount of fruit given to each monkey will be recorded in our

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data book and in the food log in each monkey's medical record chart. Each monkey's body weight will be recorded a minimum of once a week.

On 5-6 days/week on which behavioral testing is conducted, this large reward would constitute almost the entire daily diet of the animal and it can be obtained by the monkey within the testing apparatus on completion of a specified number of trials. Monkeys then feed free from interruption from the experimenter, and closely associate the entire sequence of events from leaving the home cage to finishing the testing session with the large daily reward. The chow is provided as wet mash so that the monkeys are able to eat or pouch this large reward without access to water and in a quicker time than is seen on a biscuit diet.

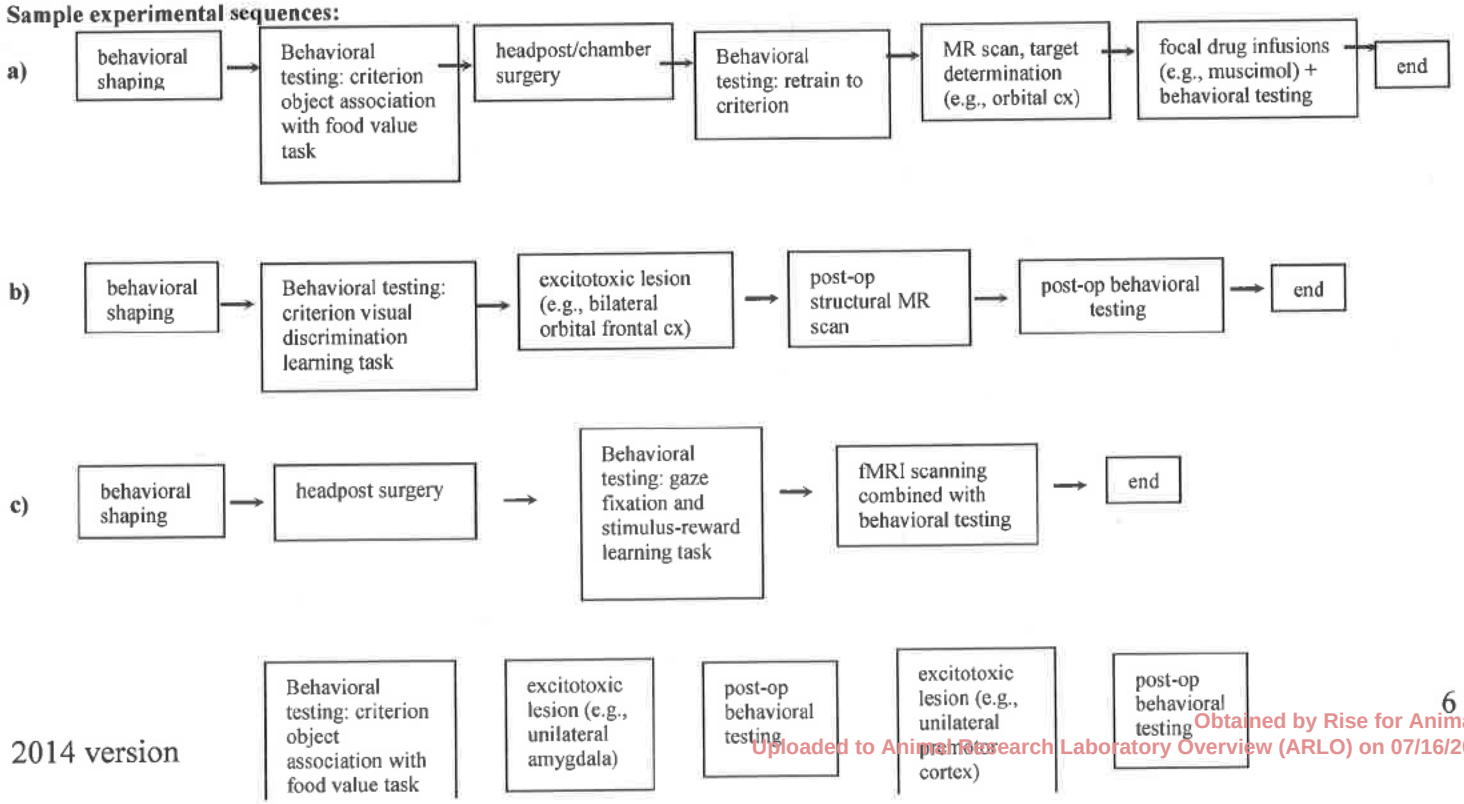
During training the animals' food / fluid may be controlled. All food / fluid control will be in strict adherence to the NIH Guidelines for Diet Control in Behavioral Studies. Daily records of food / fluid intake will be kept and all monkeys on food / fluid control will be weighed weekly. The NIMH veterinarian will be consulted if any problems develop. In the case of water control, the reduction in water provision will be gradually implemented, and continued only until it has the desired effect of inducing monkeys to reliably engage in their behavioral task. The attempt is to tailor the daily water provision for each animal so that it is relatively stable across days. Once a baseline fluid intake has been established on a given task, we typically allow each monkey to earn its full daily fluid allotment in the test apparatus. If that is not possible, a fluid supplement will be provided in the home cage, after behavioral testing has been completed.

Monkeys will be evaluated for their visual learning and memory abilities. This is carried out using a variety of specific tasks, each involving the animal's selection of objects (manual test apparatus), images (automated test apparatus) or locations (manual and automated apparatuses) that leads to delivery of food or fluid rewards. In addition, monkeys will be evaluated for their emotional responses to objects and to unfamiliar humans. We use two different procedures, each of which involves exposing the monkey to a potentially emotionally-charged stimulus and measuring the animal's behavioral responses. One procedure involves exposing the monkeys to several neutral objects and to a fake (rubber) snake and rubber spider. The latter two objects evoke an innate fear response. The emotional response is mild, in that it typically involves freezing behavior, eye and head aversion, and withdrawal (i.e., moving away from the object), and dissipates over time. The exposures will be limited to 30 sec and will occur only a handful of times in the course of the experiment. A second procedure involves exposure to an unfamiliar "human intruder" for roughly 10 minutes. Responses range from submission (e.g. lip smacking, moving away) to aggression (e.g. cage shaking) to defensive behaviors (e.g. freezing). Although these procedures may induce mild fear and anxiety for brief periods of time which are momentary and transient, they are necessary for evaluating the effects of brain lesions on monkeys' innate behavioral repertoire. These are widely used experimental procedures that have no identified long-lasting effects on behavior or on the health of the animal.

In some experiments we will measure monkeys' autonomic responses, including pupil diameter, skin conductance and heart rate, during performance of a behavioral task. The measures can all be obtained using noninvasive procedures. Monkeys first receive extensive training to acclimate them to accepting placement of electrodes on the palm of the hand (for skin conductance responses or SCR) and back (for heart rate). Alternatively, heart rate may also be monitored using a pulse-oximeter clipped onto the earlobe or toe. Both SCR electrodes and heart rate electrodes have conductive gel between them and the skin. To obtain reliable SCR, we apply vetwrap around hand to hold the electrode in place. In addition, it is necessary to restrict the movement of one or both arms. An arm is restrained with either a soft Velcro strap or a ring of aluminum, steel or plastic, sometimes in combination with a loosely fitting tube surrounding the lower arm and hand. Pupil diameter is measured with a camera. To obtain reliable measures of pupil diameter the monkey's head must be immobilized. The pupil diameter is measured using an infrared sensitive camera placed a fixed distance from the monkey's head.

All monkeys assigned to this protocol will not necessarily undergo the same procedures, nor will any monkey undergo all of the procedures described below. The following sample flow charts demonstrate several examples of how individual animals might be used.

Sample experimental sequences:



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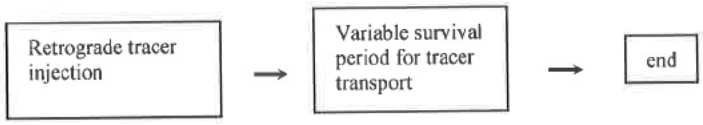
d)

e)



Surgical lesions: Most experiments will examine the effects of bilaterally symmetrical lesions intended to achieve a permanent loss of neurons in a specific brain region. For example, a monkey might be trained on a behavioral task, receive a lesion of either the amygdaloid complex, the hippocampal formation, the rhinal cortex, caudate nucleus, medial thalamus, prefrontal or premotor cortex, or other related structures, and then be retested on the behavioral task. After training on some additional behavioral tasks, the operated monkeys would be euthanized so that we can perform histological examination of the brains to document the extent of the lesions.

Some experiments will employ a "disconnection" technique to test the hypothesis that interaction of two different, but anatomically connected, structures (e.g. a limbic structure and a neocortical area) is critical for the storage/retrieval of the same kind of memories examined after the symmetrical lesions. The disconnection will be achieved by removing one structure in one hemisphere in one stage, and the other structure in the other hemisphere in a second stage, and transecting the forebrain commissures (anterior commissure and corpus callosum) in a third stage, not necessarily in that order. To assess the effects of the individual lesions, training will intervene between each surgical stage. (A positive result would yield a behavioral effect only following the third and final surgical stage, the one that completes the disconnection of the two structures being examined.) The disconnection procedure can only be achieved using multiple survival surgery. In this type of experiment, for example, a monkey would receive a unilateral lesion of a structure in a first operation (e.g. amygdala, hippocampus, orbital prefrontal cortex, nucleus accumbens, rhinal cortex), be retested on the behavioral task, receive a second operation involving unilateral removal of a different structure in the opposite hemisphere (e.g. amygdala, hippocampus, orbital prefrontal cortex, nucleus accumbens, rhinal cortex), be retested on the behavioral task, and, finally, receive a section of the forebrain commissures (corpus callosum and anterior commissure). The order of the operations would typically be counterbalanced across animals within a group, such that some animals, for example, might receive section of the forebrain commissures as the first operation. After the third operation, monkeys would again be tested on the behavioral task, and perhaps some additional tasks, and then euthanized. In all cases, there will be a minimum of 2 weeks intervening between successive surgeries, and a maximum of 3 planned major survival surgeries (i.e., planned as part of the experimental design) per monkey. In addition to the planned major survival surgeries, we may occasionally carry out additional operations that fall in the category of 'repair'. For example, upon postoperative assessment of the lesions using MR imaging, if the lesion appears incomplete, an additional major survival surgery may be conducted to complete the lesion. See Section G for details.

Neurotransmitter-specific permanent lesions: Some experiments will employ injection of neurotransmitter-specific toxins intended to remove monoamine neurons or their terminals. Two classes of agents have been employed for this purpose: a) 6-hydroxydopamine and b) cell toxins (e.g., saporin) fused to specific antibodies to the dopamine transporter (DAT). Although the lesion may require a 2-stage operation, animals receiving this treatment will not undergo any other surgical procedure. As is the case for Surgical lesions, above, and as detailed in Section G., all neurotransmitter-specific lesions will be carried out in anesthetized animals using aseptic procedures. Injection of the toxins will be performed via gas-sterilized Hamilton syringes using standard neurosurgical approaches. In addition, animals will receive one treatment or the other (a or b), not both. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a sterile vial after they have been dissolved in the appropriate carrier.

a) 6-hydroxy dopamine (6-OHDA): 6-hydroxy dopamine (Sigma) has been used to ablate dopaminergic neurons extensively for nearly two decades. 6-OHDA is injected intra-cortically. Doses used in the literature range from 2 – 12 µg/µl per injection site. Typically, around 100 µg would be required to ablate dopaminergic fibers in specific cortical areas. Injection volume will be limited to 1.0 µl per site, although the total volume would depend on the size of the structure under investigation. For example, depletions within perirhinal cortex would require injection of a total of approximately 20 µl (distributed over 20 sites), and total volume for any given experiment would typically range from 20 - 50 µl. Intra-peritoneal (IP) co-administration of agents that block uptake at norepinephrine and serotonergic terminals (desipramine, 20 - 40 mg/kg IP; pargyline, 5 - 20 mg/kg IP; citalopram, 5 - 10 mg/kg IP; talsupram, 15 - 25 mg/kg IP) are also administered to enhance selectivity for dopaminergic terminals. Any of the foregoing drugs administered IP are considered safe at the doses specified.

Desipramine is contraindicated with ketamine for pre-isofluorane anesthetic preparation. An alternative anesthetic regimen will be employed for pre-surgical preparation. Use of dexmedetomidine (0.005-0.05 mg/kg IM) plus butorphanol (0.3 mg/kg IM) has been successfully used in other settings and would be employed here.

b) Anti-Dopamine Transporter-Saporin (anti-DAT-SAP): Saporin conjugated to antibodies targeting specific cell types has recently been developed for specific ablations of cell types in the nervous system. Saporin is a cytotoxin that targets the ribosome and blocks protein synthesis. Unconjugated saporin cannot penetrate the cell membrane and, therefore, cannot enter cells. When conjugated to a specific antibody targeting a cell surface protein, like the dopamine transporter, it is taken up endosomally and retrogradely transported to the cell body. Here, saporin is cleaved from the construct due to the pH of the endosomal compartment, and targets the ribosomal apparatus.

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Because the saporin is specifically targeted to neurons that express the dopamine transporter, it poses relatively minimal risk in terms of general pathology. Injection of these constructs into specific cortical areas (0.02 – 0.6 µg/ul anti-DAT-SAP in sterile saline) would lead to ablation of dopamine fibers selectively in that area. As in the case for 6-OHDA (above) we would inject 1.0 µl per site, but the total volume (distributed over multiple sites) would depend on the target structure; the total volume injected would typically range from 20 to 50 µl. From previous experience in LN working with these agents we do not expect any adverse effects on the animals related to the lesions.

Systemic drug injections: Some experiments will employ systemic injections to help elucidate the complex neurochemical circuits that underlie learning and motivated performance. Compounds generally categorized as neurotransmitter receptor agonists and antagonists, and reuptake inhibitors, will be administered either 10 - 60 minutes before task onset or during task performance. In most cases a dose-response function will be determined for all compounds, starting with a very low, ineffective dose. Increments in dosing will cease if any animal exhibits distress (e.g., impaired performance due to dyskinesia, sedation, agitation).

All systemic injections will be administered intramuscularly in a volume of 0.10 ml/kg. The drugs to be evaluated will be chosen from available compounds for which dosing and toxicity measures have been established. Systemic injections will occur with a frequency not to exceed 2 per week using alternating injection sites. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, whether pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a sterile vial after they have been dissolved in the appropriate carrier. For systemic (intramuscular) injections, solution acidity may range from pH 3.0-7.5.

The following types of receptor agonists and antagonists may be administered: dopaminergic (D2) receptor agonists (e.g., quinpirole, 0.01µg/kg - 0.50 mg/kg) and antagonists (e.g. haloperidol, 5.6µg/kg - 17.8µg/kg); dopaminergic (D1) receptor agonists (e.g., SKF 38393, 0.3 – 15.0 mg/kg) and antagonists (e.g., SCH 23390, 0.50-32.0 µg/kg); cholinergic mimetics or receptor agonists (e.g., physostigmine, 1.0 - 100.0 µg/kg) and antagonists (e.g., scopolamine, 1.0 - 32.0 µg/kg); NMDA receptor glycine site (NMDAR/GS) partial agonists (e.g., d-cycloserine, 32.0 µg/kg - 1.0 mg/kg) and NMDAR antagonists (e.g., MK-801, 3.2 - 56.0 µg/kg); GABAa receptor agonists (e.g., muscimol, 0.01 µg/kg – 1.0 mg/kg) and antagonists (e.g., bicuculline, 0.01 µg/kg - 1.0 mg/kg).

Dopamine, serotonin, and norepinephrine reuptake inhibitors may be administered. Examples of reuptake inhibitors that may be used are: for dopamine, GBR 12909 and nomifensine; for norepinephrine, yohimbine; for serotonin, citalopram and fluoxetine. Doses will be determined using careful escalating dose procedures. Generally, estimates can be obtained from the rodent literature. Doses reported in the rodent literature, for example, include GBR 12909 (15 mg/kg), yohimbine (5 mg/kg) and citalopram (10 mg/kg). These doses have been reported to not cause any adverse effects.

Yohimbine, citalopram and fluoxetine are available pharmaceutical grade (Janssen Pharmaceuticals). GBR 12909 and nomifensine are not. In general, the drugs to be injected will be dissolved in sterile saline. In the event that a compound to be tested is not readily soluble in sterile saline the lowest possible concentration [ranges] of an effective vehicle {e.g., (2-hydroxypropyl)-b-cyclodextrin [1-25%], Tween-80 [0.1-0.2%], Emulphor [1-3%], or DMSO [0.1-0.2%]} will be used to suspend, or dissolve, the compound, and this stock solution will be further diluted in sterile water or sterile saline to achieve the desired molarity.

From the relevant scientific literature, and from experienced investigators in the Laboratory of Neuropsychology, the doses provided above have been shown to be non-toxic. However, if at any time during the experimental process the animal appears unwell we will immediately stop the experiment and consult with the NIMH veterinarian and provide treatment. If the veterinarian, in consultation with the investigator, determines that an animal is experiencing distress that cannot be relieved by applying acceptable medical treatments and procedures, the animal will be euthanized.

Implantation of headposts and chambers: Using aseptic surgical techniques a headpost and chamber, or in some cases a headpost only, will be attached to the animal's head. Both the headpost and chamber are fabricated from MR-compatible materials. Because the headpost takes roughly 4 weeks to heal (i.e., become bonded to the bone), the headpost and chamber are typically added in separate operations. This is for at least two reasons. First, the monkey needs a period of accommodation to headpost restraint, so having this procedure carried out well in advance of the chamber implant allows the restraint training to take place prior to the chamber implant. Second, some of the chambers we use are quite large. In these cases the best surgical approach is to use a skin incision at right angles to the incision used for the headpost. In the experience of LN investigators, using a two-stage surgery gives the best overall outcome for the headpost and chamber implants. A third operation is required to remove the bone inside the chamber to allow access to the brain. Although this bone removal could be carried out at the time of the chamber implant, there is often a months-long delay between the chamber implant and the initiation of the experimental manipulations carried out within the chamber. Accordingly, to avoid risk of infection, we feel it is safer to remove the bone in a separate operation. Other LN investigators have had good outcomes after covering the bone inside the chamber with a thin layer of dental acrylic at the time of the chamber implant. This procedure maintains the integrity of the bone within the chamber indefinitely. Typically, the headpost and chamber are implanted on the skull based on localization of brain structures obtained from MR scans.

An animal will undergo a maximum of three planned major survival surgeries (i.e., planned as part of the experimental design). This number (3) would include operations to induce a lesion as well as surgery for a craniotomy. On occasion, monkeys with chamber implants will require additional 'repair' surgeries for the purpose of removing bone that has regrown inside the chamber or removing excess granulation tissue from the dura mater. Because bone removal and granulation tissue removal from the dura inside chambers are classified as major survival surgeries, these procedures, if needed, may exceed the 'planned' number of major survival surgeries mentioned above. In all cases, however, we will use the minimum number of major survival surgeries necessary to carry out the work. Other relatively minor procedures (not major survival surgeries) might be carried out in circumstances requiring repair or replacement of a headpost or chamber (see Section G).

Intracerebral injections: After placement of the headpost and chamber, as described above, animals may receive intracerebral drug

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administration to produce one of two types of treatment: 1) reversible inactivation of the targeted brain structure, which acts as a reversible "lesion"; and 2) pharmacological manipulation of specific neurotransmitter systems. MR scans are used to determine the stereotaxic coordinates for accurate placement of injection needles over brain regions of interest such as the orbital and medial frontal cortex, hippocampus, amygdala, entorhinal and perirhinal cortex, striatum, and thalamus. All intracerebral injections will be made in awake animals. Before the experimental phase requiring intracerebral injections, animals will be acclimated to head restraint using the headpost affixed to the skull. This will be achieved by successive approximation, i.e. starting with short periods of restraint and moving to progressively longer periods of restraint until the restraint period matches the time needed for the injections, roughly 30 minutes. Once this acclimatization is completed, intracerebral injections may be carried out. First the animal will have its head restrained. Then the cover of the plastic chamber will be removed. Using aseptic techniques, a small hole will be made in the *dura mater* with a sterile 24 - 28 gauge needle (the guide cannula) to facilitate the insertion of injection needles. On rare occasions it appears that an animal is distressed by the insertion of the guide cannulae; in such case we will apply a local analgesic (e.g. lidocaine) onto the dura before insertion. Finally, the injection needles will be inserted through the guide cannulae into the selected brain area using the coordinates obtained from the MR images. The materials used for the intracerebral injections (i.e., guide cannulae, injection needles, plastic tubing and Hamilton syringes) will be gas-sterilized prior to each injection. To achieve reversible inactivation, we will use: GABAA receptor agonists (e.g., muscimol, 10 - 150 ng/µl) and possibly GABAA/BZR agonists (e.g., CDP, 10 - 50 µg/µl). Compounds are administered via a programmable pump that holds the syringes while advancing the plunger at a given rate of speed; the Hamilton syringes are attached via plastic tubing to the injection needles, which in turn dispense the compound into the selected brain location. The drugs to be infused will be dissolved in sterile saline (pH 7.0 – 7.5) and injected in volumes ranging from 0.3 - 3.0 µl/site, at rates of 0.15 - 0.25 µl/min. Depending on the size of the target structure, a range of 1 - 5 sites per hemisphere may be required to address the tissue adequately. The entire procedure typically takes 15 - 30 minutes. When the injections are completed, the chamber will be rinsed with sterile saline, the chamber cap replaced, the head restraint removed, and the animal's behavioral task will be initiated. Intracerebral injections will occur with a frequency of 1 to 2 per week (no more than 2 per week), and the total number of injections per site will not exceed 30. Experience in LN has shown no adverse effects of administration of these compounds in the range of doses described above. When pharmaceutical grade compounds are available (e.g. Sigma, Tocris), they will be used. All drugs, whether pharmaceutical grade or not, will be sterile filtered (0.20 µm syringe-mounted filter, Corning) into a sterile vial after they have been dissolved in the appropriate carrier.

Pharmacological manipulations made via intracerebral injections may involve the following compounds: D2 antagonists (e.g., sulpiride, 5 - 25 µg / µl); D1 antagonists (e.g., SCH23390, 10 - 80 µg / µl); cholinergic (M1) antagonists (e.g., scopolamine HBr, 1 - 50 mM); cholinergic (M2) antagonists (e.g., SCH 217443, 5 - 75 µg / µl); GLUR antagonists (e.g., NBQX, 1 – 10 µg / µl). In previous experience in LN working with these agents, and from relevant scientific literature, we have found that the doses provided above are nontoxic. Compounds will be administered 10 - 60 min before task onset or during task performance.

We do not expect any problems related to the drug infusions. However, one potential adverse effect of making intracerebral injections, encountered infrequently, is inadvertent puncture of blood vessels leading to cerebral hemorrhage, infarctions, and raised intracranial pressure, in which case an animal might exhibit distress (e.g., dyskinesia, agitation). If at any time during the experimental procedure the animal appears unwell we will immediately stop the procedure and consult the NIMH veterinarian. If the veterinarian so advises, treatment will be provided. If the veterinarian, in consultation with the investigator, determines that an animal is experiencing distress that cannot be relieved by applying acceptable medical treatments and procedures, the animal will be euthanized.

Cleaning and maintenance of cranial implants: Careful attention, including regular cleaning and disinfection, is paid to the cranial implants (i.e., headposts and chambers) to reduce the possibility of infection. We frequently consult with the NIMH veterinarians and quickly move to treat infections if they occur. The frequency of cleaning depends on the nature of the implant and its clinical state. For headposts or similar implants without a recording cylinder, the skin-implant interface will be cleaned at least once per month. Recording cylinders or similar implants with an open craniotomy will be cleaned as often as necessary to maintain them free from infection or bacterial growth, but no less frequently than twice per week. The materials used for cleaning and disinfection and the antibiotics used to treat any infection are continuing to evolve; accordingly, treatment is based on consultations with the NIMH veterinarian. Current typical materials include hydrogen peroxide and Betadine cream or ointment. Occasional vigorous cleaning and shaving of hair is required. If any sign of an infection appears, samples are sent to the laboratory for culture, and appropriate antibiotic therapy is initiated. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain / or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be anesthetized and subsequently euthanized according to
Section J.

It may occasionally be necessary to remove a small piece of bone or enlarge the burr hole inside the chamber using either a drill or a small rongeur. If the removal is very minor and will last only a few minutes it is generally carried out using ketamine (5.0 - 20.0 mg/kg IM), but a ketamine / xylazine mixture (ketamine, 8 mg/kg, plus xylazine, 0.4 mg/kg IM) is used if the procedure involves more extensive drilling or if the animal shows any reaction to stimulation. A dose of atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) will also be given as pre-treatment for this procedure.

After a period of weeks or months the dura mater sometimes thickens. When this happens the monkey will either be sedated with ketamine (10 - 20 mg/kg), first pre-treating with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), as appropriate, or taken to the OR to be done under isoflurane (1.0 – 4.0%, to effect) and the fibrous connective tissue will be removed using aseptic technique. These procedures are performed in either the implant treatment room or in the surgical suite.

Chair restraint: Animals to be chaired will wear loose-fitting collars and will be chaired in accordance with NIMH Guidelines for the Use of Restraint Chairs with Nonhuman Primates. They will be transferred to the chairs using the standard pole and collar technique. Animals acclimate quickly to the collars. The collars are placed on the animals under ketamine sedation (Ketamine hydrochloride 10 - 20 mg/kg IM). The animals are allowed to recover for approximately one day, getting used to the collar, before pole training is begun. Pole training typically involves

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successive approximation. First, the investigator briefly clips the pole onto the monkey's collar, at the same time giving the monkey food rewards. This procedure is carried out repeatedly, across several days, before attempting to transfer the animal to the restraint chair. Once in the restraint chair the animal is taken to the testing room where it sits in the chair and is given food rewards for, perhaps, 15 minutes. The animal is then returned to his home cage. This procedure is repeated for several days until the monkey is acclimated to the chair and test environment. The acclimation of the animal to the collar, chair, and testing procedures will be tailored to accommodate the individual needs of each animal.

Structural Magnetic Resonance Imaging (MRI) Scans:

The NIMH Guidelines for MRI and MRS will be followed. Ear protection (silicone ear plugs/molds) is used during fMRI studies or anesthetized scans in either the 4.7T or 3T scanners in the NIF. For the anesthetized scans which require the monkey to be placed into a headholder/stereotax, the ear bars serve this purpose.

[location redacted] Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with ketamine (10 - 15 mg/kg IM), followed by either 0.005-0.05 mg/kg (IM) dexmedetomidine (Dexdomitor® or 0.5 - 1.5 mg/kg (IM) valium (diazepam), with supplements as needed. The ketamine/ dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. (If dexmedetomidine is not available, medetomidine (Domitor®) may be substituted. In this case, if used in combination with ketamine, 0.02-0.1 mg/kg (IM) medetomidine would be used.) The animal is then transported to [location redacted] [location redacted] where it is placed in an appropriate head holder and scanned. This technique is completely noninvasive and lasts approximately 1-2 hours. Following the scan, if either dexmedetomidine or medetomidine is used, we will administer atipamezole (Antisedan®) 0.2-0.4 mg/kg (IM). The animal is then returned to its home cage by the investigators where it is watched and monitored during recover from anesthesia, until it is able to right itself. A single dose of ketoprofen (1 – 2 mg/kg IM) will be administered just prior to or immediately following this procedure.

Diffusion weighted imaging:

Diffusion weighted imaging scans allow for examination of the route of specific fiber pathways in intact animals and the nature and location of changes in white matter pathways that occur as a result of these various lesions.

The DTI and DSI performed under general anesthesia will take place in a horizontal scanner in the [location redacted] The anesthesia protocol is similar to that for surgery, comprised of intubation and general isoflurane anesthesia. The animal will be fasted for 12 hours prior to surgery; however, oral fluids will not be restricted. The animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), lightly anesthetized with ketamine (10 - 20 mg/kg IM), and then given isoflurane (1.0 - 2.0%, to effect) as a general anesthetic. The glycopyrrolate (or atropine), ketamine, and isoflurane are administered by [location redacted] animal technician, and the dosage and route of administration are determined by the veterinarian (the isoflurane is adminstered to effect, to reach a surgical plane of anesthesia). Anesthetized scans lasting four hours are typical for DTI sessions achieving 1 mm isotropic resolution. An interval of at least seven days will occur between consecutive anesthetized scans.

We do not expect any adverse effects from the procedures outlined above. If, however, there is any indication that an animal's condition is changing we seek immediate veterinary medical consultation. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and/or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized as described in Section J.

[location redacted] In some cases, the animals are scanned during stable, balanced anesthesia (1-2% isoflurane, to effect). Induction is generally performed with the animal in a horizontal position, and the chair is then brought to a vertical position for insertion into the vertical magnet. The vertical position, while a great advantage for the awake preparation, poses challenges for anesthesia. Safe and robust procedures for anesthetized monkeys have been developed and are in place, but great effort is being made to constantly improve these paradigms for the continued and increased safety of the animals.

For most structural studies to achieve good high-resolution images full anesthesia is not required but some sedation is necessary, since awake animals tend to make small movements (e.g. ear wiggling, chewing, vocalizing) that disrupt the image quality with motion artifacts. As an alternative to full anesthesia, a combination of three safe drugs will be used:

1) Ketamine (5 - 15 mg/kg IM)
2) Diazepam (0.5 - 1.5 mg/kg IM 30 minutes prior, or 0.1 mg/kg slow IV at the time of anesthesia) – valium, a benzodiazepine sedative.
3) Glycopyrrolate (15 mcg/kg IM) -- anticholinergic, lasts a bit longer than atropine.

Supplemental doses of the above drugs will be given as needed.

While the sedate preparation does not afford as complete monitoring as the full, intubated gas anesthesia, we routinely measure and record the CO_2 level and breathing rate, using a gas sensor positioned just in front of the nose. We also use a camera on the eye and face in order to continually monitor the animal's appearance for movement. Finally, in addition to the CO_2 monitoring, we will attempt to measure the SPO_2 level and the non-invasive blood pressure.

Computed Tomography (CT) Imaging:

[location redacted] ***Scans Using Ketamine/Domitor/Valium:*** All monkeys will be sedated throughout the scanning procedures. We will use the same anesthetic drugs and doses which are normally used for anesthetized MRI scans. Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with ketamine (10 - 20 mg/kg IM), followed by either domitor/dexdomitor (medetomidine/dexmedetomidine; 0.01 - 0.1 mg / kg IM) or valium (diazepam; 0.5 - 1.5 mg / kg IM). As appropriate, supplements of ketamine (5 mg /kg) are administered every ~30 minutes and domitor/dexdomitor (10 ug /kg) every ~60 minutes. The ketamine/dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. Once in the scan room they are placed in an appropriate

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head holder, which secures their head in a stationary and standardized orientation. This orientation also ensures the airway is open through the scanning session. Once the scan is complete, the monkeys will be returned to their home cage and monitored until awake and sitting upright.

[location] ***Scans Using Ketamine/Domitor/Valium:*** All monkeys will be sedated throughout the transport and scanning procedures. We will use the same anesthetic drugs and doses which are normally used for MRI scans. Typically, for structural scans the animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM) and then anesthetized with 10 - 20 mg/kg ketamine IM, followed by either medetomidine/dexmedetomidine (0.01 – 0.1 mg/kg IM) or diazepam (0.5 - 1.5 mg/kg IM). As appropriate, supplements of ketamine (5 mg /kg) are administered every ~30 minutes and domitor/dexdomitor (10 ug /kg) every ~60 minutes. The ketamine/dexmedetomidine and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. The animal is then transported in an approved, specifically designed, covered, opaque transport box via an approved route [location] During the transport, the animals are placed on their side and checked to ensure the airways are open. Once in the scan room, they are placed in a stereotaxic head holder which secures their head in a stationary and standardized orientation. This orientation also ensures the airway is open through the scanning session. Once the scan is complete, the monkeys will be returned to their home cage and monitored until awake and sitting upright.

There will be two people with experience in working with monkeys always present. During transport of the animals we will not wear PPE but once the animal is in the CT room and before opening the cart we will put on the appropriate PPE (e.g. lab coats, gloves, and face shield). The monkey will be fully wrapped in a disposable absorbent pad with moisture-proof plastic lining. The bed of the scanner will be covered with absorbent pads. The door to the CT scanner will be kept closed. At the end of the scanning session we will remove and discard in a trash bag all disposable items. These items will be brought back to [location] where they will be disposed of in MPW waste. The CT bed, inside the scanner, and floor will be cleaned with disinfectant. Al[location] and NIH Transportation Guidelines will be followed.

Functional MRI Scans (fMRI):

fMRI in awake animals. During functional MRI, monkeys perform similar behavioral tasks as the in the neurophysiology sessions but are seated inside either the vertical 4.7T Bruker MRI scanner or the horizontal 3.0T Siemens MRI scanner of the [location] In this environment, any small piece of ferrous metal can become a dangerous projectile, so careful measures are taken to ensure that no parts of the chair, implants, or preparation equipment are ferrous in nature. Through initial training sessions, each monkey is gradually acclimated to the enclosed space and scanning sounds. This initial training involves (1) the animal learning to wear wax plugs inserted into their ear canals, (2) raising/inserting the monkey slowly into the scanner bore while delivering juice reward, (3) leaving the animal inside for increasing periods of time, (4) requiring the animal to perform the behavioral (e.g. eye fixation) task inside the scanner bore, and (5) applying real scanner sounds of increasing intensity. While it can be helpful in some monkeys to use a mock environment with artificial scanning sounds, we have found that this step is in general this is unnecessary, since nearly all animals adapt well to the sequence outlined above.

During echoplanar imaging (EPI) scanning, which is the main functional imaging sequence used for fMRI, the volume inside the magnet bore has been measured to reach 103 dB. In humans, extended exposure to this sound level can produce hearing impairments. For this reason, each animal is required to wear ear protection in the form of wax plugs, silicone molds, or specialized headphones/earbuds, which can attenuate the sound level by 15-25 dB. Most animals easily acclimated to these earplugs by having them placed within the ear for short durations, 30 to 60 minutes, over the course of 2 to 3 testing sessions. This is a non-invasive procedure that is routinely used on humans of all ages, including infants, in clinical and research settings. Monkeys have been tested systematically on potential hearing loss before and after multiple scanning sessions using sound protection with silicone molds. Specifically, the monkeys' hearing ability between 892 and 7996 Hz, as well as their middle ear function, was tested on multiple occasions by audiologists using distortion product otoacoustic emission (DPOAE). The audiological results showed normal function before and after full data acquisition [Tanji, K. et al., (2010). Effects of sound intensity on tonotopic fMRI maps in the unanesthetized monkey. Neuroimage 49(1): 150-7].

During a typical imaging session, the monkey is moved into position inside the scanner bore. In the vertical scanner, the monkey is raised into the magnet by an elevator and, once in position, able to see stimuli on a screen above it through the mirror. In the horizontal scanner, the monkey is slid into the magnet on a railing and, once in position, able to see stimuli on a screen in front of it. The monkey will normally remain in place up to 3.5 hours in total, as this is the length of a scanning slot in the NIF facility. For occasional experiments, this duration can be up to 5 hours. The initial part of the session involves acquiring an anatomical profile of the monkey's brain for that day, a scan that lasts 5 - 10 minutes. This will serve to register experiments from session to session where the monkey's position might have changed slightly. Following this, the animal will begin to perform the behavioral task as the scanner acquires images of the brain continuously. The T2*-sensitive sequences will permit the visualization of regional activity in the brain resulting from blood-oxygenation level-dependent (BOLD) changes. The functional sequences we use typically require that the monkey is exposed to minimal radio frequency (RF) energy, but as an additional safety precaution an RF energy monitor is built into the scanner acquisition software. These sequences are similar in nature to those used in the majority of functional imaging studies in humans.

Monocrystalline Iron Oxide Nanoparticle (MION). MION is a systemic contrast agent for functional magnetic resonance imaging (fMRI) that has been used extensively for functional imaging in rhesus monkeys and has been shown to be safe for repeated injections over weeks and months. In the [location] the MION solution is administered intravenously (IV), usually once per week but on occasion twice in a single week, prior to the beginning of a functional imaging session. The iron nanoparticles in the blood serve to distort the local magnetic field in the microvasculature, with the level of distortion proportional to the total blood volume (i.e. total MION content). This can be detected using a standard functional imaging signal such as echo planar imaging (EPI). It distinguishes itself from the intrinsic blood oxygenation level-dependent (BOLD) response in that, unlike BOLD, it isolates the portion of the functional signal associated with blood volume. In addition, and perhaps more importantly, MION has been identified as showing less session-to-session variability than the BOLD signal.

In some sessions, we will inject MION into the saphenous vein of the animal during or prior to scanning. A catheter and an i.v. lock is generally used to administer the MION. A small volume (1-3 cc) of sterile saline is injected before and after the MION to test the i.v. line and to flush any remaining MION, respectively. The monkeys are successively acclimated to the injection procedure by having their leg held and groomed while in their primate chair, prepping the leg for injection (e.g. shaving the area above the heel and swabbing with an alcohol wipe), and with injections of just sterile saline. Food or liquid reinforcement is usually provided during the acclimation process. Once acclimated, the animals show little aversion to the injection procedure.

The quantity of MION required varies between different vendors, batches, magnets, and monkeys. The efficacy of MION (at distorting local

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magnetic fields) is thus determined empirically using data from different researchers. The optimal dose will reduce the echo planar image (EPI) signal by 50-60%, relative to the pre-injection BOLD signal (Leite et al., 2002). In our experience, this typically requires a MION dose of ~12 mg / kg / day at 3T and ~15 mg / kg / day at 4.7T. In some animals, prolonged MION use has resulted in a chronic drop in EPI signal from certain brain areas, presumably because of accumulation of MION or a MION byproduct in these areas. To prevent or minimize this EPI signal loss we generally avoid commercial MION (Feraheme), use the minimal efficacious dose (~50% signal drop), and wait several days to a week between successive MION doses so the EPI signal can return to its pre-MION levels.

The preference for smaller and less frequent doses of MION is motivated by scientific concerns, not health concerns. MION is commonly used in fMRI studies of NHP (e.g., Tsao et al., PNAS (2008) and Moeller et al., J Neurosci (2009)) and these authors specifically point out that MION use did not lead to any health or medical complications. Furthermore, MION is approved for use in humans to treat anemia (Feraheme, ferumoxytol injection). In our experience in the location where we have used MION in more than 20 monkeys over a period of approximately six years, no monkeys have ever suffered adverse health effects due to MION use. In order to slow or prevent the accumulation of MION, particularly when multiple doses are administered per week, we may prophylactically administer single doses of the iron chelator desferoxamine (Derferal; pharmaceutical grade, 50 mg/kg i.m.) and/or deferiprone (Ferriprox; FDA approved, 25 mg/kg orally, up to 3x per day for a total of 75 mg/kg/day) at the conclusion of imaging sessions involving MION. Desferoxamine and deferiprone has previously been used at the NIH and at other institutions (e.g., MGH, MIT, personal communication) to evacuate MION from the body following monkey imaging experiments with no observed adverse side effects. Deferiprone treatment is often done in combination with deferoxamine (Desferal) as the combination has been shown to have greater efficacy (Pepe et al., 2010; Pantalone et al., 2011).

Anesthetized MRI. In addition to the awake, functional scans, we also carry out anesthetized scanning for several reasons. These include (1) the acquisition of high-resolution anatomical scans that are disrupted by any subject motion, (2) the high-resolution mapping of manganese transport, (3) the acquisition of specialized scan types, such as that for diffusion weighted imaging, (4) medical diagnosis, (5) the testing of new radiofrequency coils or other methods optimization, or (6) the scanning of an animal that does not have a head post or is not yet acclimated to the scanner. While most of our scanning is done in location facility, it is sometimes desirable to scan monkeys in the location In all cases, the NIMH Guidelines for MRI and MRS will be followed.

location *Scans* location *vertical 4.7T) using Ketamine/Diazepam.* In the location facility's vertical 4.7T scanner, nearly all anesthesia procedures are carried out using a combination of ketamine/diazepam. This combination was established previously at the NIH to minimize the loss of blood pressure during vertical posture scanning, which is undesirable and potentially dangerous over extended periods. Over the past several years, we have used this regimen routinely and successfully without incident. The specific anesthesia regimen consists of ketamine (5 - 20 mg / kg IM), diazepam (0.5 - 1.5 mg / kg i.m. 30 minutes prior, or 0.1 mg / kg slow iv at the time of anesthesia), glycopyrrolate (15 mcg / kg i.m.), none of which lowers blood pressure. Supplements of the above drugs are given as needed. While the sedation preparation does not afford complete physiological monitoring, we routinely measure and record the CO_2 level and breathing rate using a gas sensor positioned just in front of the nose. We also use a camera on the eye and face in order to continually monitor the animal's appearance for movement.

location *Scans* location *vertical 4.7T) using Isoflurane.* In some cases the animals are scanned in location during stable, balanced anesthesia (pretreatment with atropine (0.05mg / kg, i.m.) or glycopyrrolate (15 mcg / kg i.m.), lightly anesthetized with ketamine (10 - 20 mg / kg i.m.), and then given isoflurane (0.7 – 2.5%, to effect) for maintenance). Induction is generally performed with the animal in a horizontal position, and the chair is then brought to a vertical position for insertion into the vertical magnet. The animal's vital signs, including heart rate, respiration, blood pressure, expired CO_2 concentration, and SpO_2 are monitored and recorded throughout the procedure. Any sign of instability in anesthesia leads to termination of the experiment.

location *Scans* location *horizontal 3.0T) using Ketamine/Domitor.* Typically, for structural scans the animal is pretreated with atropine (0.05 mg / kg, i.m.) or glycopyrrolate (15 mcg / kg, im.) and then anesthetized (i.m.) with 10 – 20 mg / kg ketamine, followed by either 0.01 - 0.1 mg / kg domitor/dexdomitor (medetomidine/dexmedetomidine) or 0.5 - 1.5 mg / kg valium (diazepam). The ketamine/(domitor/dexdomitor) and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. This technique is completely non-invasive and lasts approximately 2 - 3 hours. The animal's level of sedation will be assessed periodically between scans by physical inspection (e.g, by assessing movement, muscle tone, and pain reflex). As appropriate, supplements of ketamine (5 mg /kg) are administered every ~30 minutes and domitor (10 ug /kg) every ~60 minutes. If monitoring equipment for use with monkeys is available, temperature, heart rate, and pulse oximetry are monitored during the scan. If the heart rate drops too much, we reduce the domitor, and if it drops below 70, we administer another 15 ug/kg Glycopyrrolate. Upon completion of the scan and return to home cage, we may administer the reversal agent Antisedan in an amount equal to that of the total amount of domitor given throughout the experiment. Once the animal is returned to its home cage, it is watched and monitored by the investigators during recovery from anesthesia, until it can right itself. A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

location *Scans* location *horizontal 3.0T) using Isoflurane.* Animals are pretreated with glycopyrrolate (15 ug/kg, i.m.) or atropine (0.05 mg / kg, i.m.) and then sedated with ketamine (10-20 mg/kg) and/or domitor (0.01 - 0.1 mg/kg) prior to intubation. Once in the NIF facility, the animal will be anesthetized using gas, isoflurane gas (1 to 4%, to effect), during the scanning session. At the end of the scan session, the animal will be given a dose of Ketamine (5-10mg/kg) before returning to the animal facility. Once the animal is returned to its home cage, it is watched and monitored by the investigators during recovery from anesthesia, until it is able to right itself. The monkeys can also be given appropriate analgesics and antibiotics as determined in consultation with the NIMH veterinarian.

location *Scans* location *using Ketamine/Domitor/Valium.* Typically, for structural scans the animal is pretreated with atropine (0.05 mg / kg, i.m.) or glycopyrrolate (15 mcg / kg, im.) and then anesthetized (i.m.) with 10 - 20 mg / kg ketamine, followed by either 0.01 - 0.1 mg / kg domitor/dexdomitor (medetomidine/dexmedetomidine) or 0.5 - 1.5 mg / kg valium (diazepam). The ketamine/(domitor/dexdomitor) and ketamine/valium combinations have proven to be a safe and effective anesthetic for this and similar procedures. The animal then transported to the location where it is placed in an appropriate head holder

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and scanned. This technique is completely non-invasive and lasts approximately 2 - 3 hours. The animal's level of sedation will be assessed periodically between scans by physical inspection (e.g. by assessing movement, muscle tone, and pain reflex). As appropriate, supplements of ketamine (5 mg /kg) are administered every ~30 minutes and domitor (10 ug /kg) every ~60 minutes. If monitoring equipment for use with monkeys is available, temperature, heart rate, and pulse oximetry are monitored during the scan. If heart rate drops too much, we reduce the dormitor and if it drops below 70, we administer another 15 ug/kg Glycopyrrolate. Upon completion of the scan and return to [location] we may administer the reversal agent Antisedan in an amount equal to that of the total amount of domitor given throughout the experiment. The animal is then returned to its home cage by the investigators where it is watched and monitored during recover from anesthesia, until it is able to right itself. A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

[location] *Scans* [location] *using Isoflurane.* Animals are pretreated with glycopyrrolate (15 ug/kg, i.m.) or atropine (0.05 mg / kg, i.m.) and then sedated with ketamine (10-15 mg/kg) and dormitor (0.01 - 0.1 mg/kg) prior to intubation and transport. The monkey will be intubated in [location] and then transferred to [location] using an approved, covered, opaque transport cart. Once in [location] the animal will be anesthetized using gas, isoflurane gas (1 to 4%, to effect), during the scanning session. At the end of the scan session, the animal will be given a dose of Ketamine (5-10mg/kg) before returning to [location] At the conclusion of scanning at [location] the animal is returned to its home cage or the [location] by the investigator. The monkeys can also be given appropriate analgesics and antibiotics as determined in consultation with the NIMH veterinarian.

Blood sampling: Awake monkeys will be accommodated to sitting in a primate chair while their leg is held over the course of 2-3 weeks using positive appetitive reinforcement. Once monkeys are able to sit still during the accommodation procedures, small volumes of blood (1-3 mls) will be drawn from the saphenous vein up to 3 times / week. These measurements will be used to quantify hydration levels prior to behavioral tasks or fMRI sessions. We assess hydration levels because this impacts fMRI findings (e.g., Kaskan et al, 2019) and we therefore need to monitor possible effects of hydration levels in our studies. Withdrawn blood will be put in hematocrit tubes and spun in a centrifuge for PCV and protein measurements.

Selective satiation: On occasion monkeys will be given a selective satiation procedure, which means they are allowed to eat as much of one food as they will eat (i.e. eat to satiety). This has the effect of altering (reducing) the value of the food reward. We use this experimental manipulation to test the animal's ability to represent food value. The selective satiation procedure always involves one of the foods that the animals receive as food rewards in behavioral testing, and typically lasts no longer than 30 minutes. The procedure is carried out no more than twice per week. The amount and type of food eaten is recorded in either the blue food/water log or the nursing notes of the animal's medical record chart.

Tastants: Tastants, or types of fluid outcomes, are typically used in both human studies (WK Simmons et al, Nat Neurosci, 2013; DM Small et al, Neuron, 2008) and nonhuman primate investigations (C Padoa-Schioppa and Assad, Nature, 2006; Rolls et al, J Neurophysiol, 1990), not only as a reward for performing a task, but also as a means of identifying areas responsive to tastes. These studies have used a variety of tastants ranging from positive (sucrose, sucralose, aspartame, salt, various flavored fruit juices or solutions) to neutral (water, artificial saliva), to mildly negative (quinine, HCl, citric acid). We will be using different tastants to map responses to differing tastes or flavors, and to test monkeys' abilities to form associations with visual cues and different taste outcomes. Taste outcomes will be different types of solutions in three categories: positive, neutral, or mildly negative. A positive reward will be an aqueous solution with sucrose [0.1M], sucralose [1mM] or fruit juice. A neutral reward will consist of a solution containing sodium bicarbonate [0.3mM] and potassium bicarbonate [0.3mM] (personal communication, A. Fontanini, January 29, 2015) to mimic saliva. A more complex saliva substitute contains NaCl (0.002M), KCl (0.005M), sodium bicarbonate (0.003M), potassium bicarbonate (0.003M), magnesium chloride (0.00025M) and potassium phosphate (0.00012M). Saliva substitutes will be used at these concentrations and at 75%, 50% and 25% dilutions. Quinine [1mM], a bitter tastant, or a dilute solution of either citric acid [10mM] or hydrochloric acid [1mM] (VL Smith-Swintosky et al, J Neurophysiol, 1991) are mildly negative. The monkeys will be presented with these fluids in a testing booth as well as the fMRI scanner. Use of these and similar tastants is commonplace in research settings and is expected to have no adverse consequences.

We conduct studies that investigate the neural bases for flavor-nutrient learning. This type of learning is thought to rely on signaling via the gut-brain axis. On occasion, monkeys will consume a flavored fluid (tastant) mixed with maltodextrin, a flavorless and odorless food additive, at five different increments (0, 37.5, 75, 112.5, or 150 kilo-calories) at their home cage. This will have the effect of altering (increasing) the value of the flavored fluid (see: Yeomans et al, Physiol & Behav, 2008; de Araujo et al, Curr Biol, 2013; Veldhuizen et al, 2017, Curr Biol). As in the selective satiation procedure described above, we use this experimental manipulation to test the animal's ability to represent food value. Both the flavors and the maltodextrin are commercially available and widely used in studies of human flavor-nutrient learning. We anticipate no adverse consequences following the ingestion of either maltodextrin or the flavored fluids.

Air puff: In addition to using food or fluid rewards to study the neural substrates of learning using pleasant/agreeable events as reinforcement, we also plan to study the behavioral and neuronal systems underlying learning associated with mild unpleasant/disagreeable events as reinforcers. To study such behavior, it is necessary for the monkey to learn by trial and error which behavior leads to a negative stimulus (reinforcer). We will use a mild stream of compressed air (i.e., air puff, 20-60 PSI) as an unpleasant stimulus to be delivered for a short duration (typically, 100-1000 ms) to the cheek, through a tube positioned at about 5 cm from the side of the monkey's face. The air puff is mildly annoying. Delivery causes an automatic blink response and one is inclined to withdraw. There is, however, no pain associated with the puff. An equivalent human experience would be someone blowing through a straw at your face. This stimulus is neither painful nor does it cause discomfort or distress to humans. The air puff should be only mildly aversive, enough to elicit instrumental behavior from the monkey to avoid its delivery. In some cases the air puff will be presented obligatorily after the presentation of a set of visual stimuli in order to study how monkeys learn to associate the sensory stimuli with the upcoming air puff. To maintain the monkeys' cooperation in the experiment, we will deliver fluid rewards with the air puffs. Continuation of the usage of the air

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puff will be terminated immediately in case the monkey shows overt reactions suggestive of discomfort, pain or distress including grimacing, squirming, vocalization, urination or defecation.

Genotyping: We specifically need to determine a monkey's status with regard to the serotonin transporter polymorphisms so that their genotype (SS, SL or LL) for the serotonin transporter can be correlated with cognitive measures. Monkeys will be anesthetized (10 mg/kg ketamine IM) and 5 – 10 ml blood withdrawn using a 19 – 23 gauge needle, from the femoral vein, or alternative (e.g. saphenous vein) where necessary, for the purpose of determining the monkeys' genotypes. The blood sample will only be needed once per animal, barring unforeseen and rare problems with the processing of the samples, in which case a second sample would be taken.

Tracer studies: In order to determine whether certain neuroanatomical projection pathways are intact, we will inject tracer substances into the brains of some intact monkeys and some monkeys that have already received lesions. Aseptic surgical procedures will be employed (see Section G), and intracerebral injections of retrograde tracers (1 - 5 µl 2% Fast Blue and / or 1 - 5 µl 4% Diamidino Yellow), spread over 1 - 20 sites, will be made into either the medial thalamus, amygdala, prefrontal cortex or related regions, via a gas-sterilized Hamilton syringe needle. Although this procedure may entail multiple survival surgeries, it is the only way to determine whether certain fiber bundles are intact after our lesions. No procedure-specific adverse side effects (i.e. those beyond what might be associated with any major survival surgery) are expected. In all cases, there will be a minimum of 2 weeks intervening between successive surgeries. For most studies, there will be a maximum of 3 major survival surgeries per monkey. On occasion, we may need to inject tracers into a monkey that has already undergone multiple major survival surgeries for behavioral experiments. On those rare occasions, the tracer injection would be the fourth and final major survival surgery.

Euthanasia: For histological verification of the lesions, monkeys in experimental groups must be euthanized at the conclusion of the experiment, which will follow a variable period of behavioral testing ranging from approximately 6 months to 6 years. For the majority of monkeys on this ASP, the experimental endpoint will be the completion of behavioral testing. Monkeys receiving tracer injections will be euthanized approximately 1-2 weeks after the injection, whichever is required for transport of the tracer being used. At this time, monkeys will be sedated with ketamine (10 mg / kg IM or to effect) followed by either Euthanasia-III (1.0 ml/5kg IV), an overdose of barbiturates (sodium pentobarbital, 100 mg/kg IP or 100 - 200 mg/kg IV), or another equivalent euthanasia solution given at the manufacturers recommended dose, and then perfused through the heart with normal saline followed by formaldehyde solution. The brain will be removed for histology and the carcass will be double-bagged in heavy plastic bags and stored in the appropriately marked refrigerator until disposal.

Environmental enrichment: Some monkeys with brain lesions are to be exempt from pair housing. Removal of certain brain regions (e.g. amygdala, orbital frontal cortex, medial frontal cortex) results in alterations in emotional and social behavior. Specifically, after certain brain lesions, the dominance structure within a pair may be altered or the animals may respond inappropriately to social signals. Therefore, pairing could result in severe physical injury or death of the animal secondary to fights. In such cases, animals will no longer be suitable for pairing. In addition, because the controls for these experiments must be treated the same way as subjects in the operated groups, these animals, too, must be single-cage housed. Whenever animals are exempted from pair housing, other means of environmental enrichment will be implemented as per the NIMH Nonhuman Primate Enrichment Policy. See also Section M.

Other: As a general statement it must be emphasized that these animals are extremely important to us. We make every effort to keep them comfortable and healthy, and, if there is any indication that their condition is changing, we seek immediate veterinary medical consultation. When the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and / or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized according to Section J.

G. SURVIVAL SURGERY - If proposed, complete the following:

None☐ Major☒ Minor☐

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional sheets if necessary):

For all surgeries the animal will be fasted for 12 hours prior to surgery; however, oral fluids will not be restricted. The animal is pretreated with atropine (0.05 mg/kg IM) or glycopyrrolate (15 mcg/kg IM), lightly anesthetized with ketamine (10 - 20 mg/kg IM), and then given isoflurane, (1.0 – 4.0 % to effect) as a general anesthetic. The atropine or glycopyrrolate, ketamine, and isoflurane are administered by the surgical staff of [location] (under the supervision of the [location] veterinarian), and the dosage and route of administration are determined by the veterinarian (the isoflurane is adminstered to effect, to reach a surgical plane of anesthesia). After the animal is anesthetized, it will be placed in a headholder appropriate for the procedure. An intravenous drip of normal saline or Ringer's solution is maintained, body temperature is maintained with a heating pad, and heart rate, temperature, and respiration are monitored with a physiological monitor. All procedures are performed using aseptic technique. At the end of surgery the animal will be monitored until it is awake (trying to sit up) and then be placed back in its home cage or the [location] There will be a 10 - 14 day recovery period before animals begin postoperative testing. Standard postoperative treatment includes analgesics and antibiotics administered after consultation with the attending [location] veterinarian or [location] veterinarian and may include, for example, Ketoprofen (1 mg/kg bid x 3 days), buprenorphine (0.02 – 0.05 mg/kg IM or IV, q 6 – 12 hr.) acetaminophen (80 mg, oral), ibuprofen (100 mg PO), Banamine (1 mg/kg / day for 2 days IM), as needed. If cerebral edema is considered a problem either mannitol (2 g/kg IV) and/or dexamethasone (1cc/2x/day; 3

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mg/cc) for 2 - 5 days, IM) is administered.

Headpost and Chamber Implants: Headpost implants are made of MR-compatible plastic or titanium. The titanium headposts have 3 - 5 adjustable legs, and are fastened to the skull with titanium or ceramic screws. Cement and acrylic is avoided when possible, although sometimes it is required to reinforce the primary screws. Chambers are also made of MRI-compatible material. For both headpost and chamber implants the scalp is retracted and the underlying muscles are retracted or removed. Approximately 10 - 12 small (1 - 2 mm diameter) holes are drilled into the skull (not penetrating the dura). The headpost is attached to the skull with either screws only or with screws plus acrylic; to attach the chamber to the skull screws plus acrylic will be used. The skin is then sutured around the implanted headpost or chamber. The monkey is returned to its home cage after it can sit upright without the aid of the caregiver. Normally, these implants will stay in place until the monkey is euthanized. However, if the monkey loses its implant, a new one may be re-implanted using the same procedure.

Bone removal inside the chamber is undertaken under aseptic conditions. The animal is anesthetized and placed in a headholder, the cap of the chamber is removed, and a small opening is made with either a burr or hand drill. The opening is then expanded, as needed, with micro-rongeurs.

Surgical Lesions and Neurotransmitter Depletions:
The monkey's vital signs (heart rate, respiration rate and temperature) will be closely monitored. Aseptic procedures will be employed.

All ablations, transections, and injections will be made either: 1) under visual control with the aid of an operating microscope, or 2) via MR-guided stereotaxic approaches. In addition, an animal will undergo some but not all of the surgeries listed in this section. For example, as shown in the sample flow charts depicted in Section F, an animal may undergo a unilateral amygdala lesion in one operation followed by a unilateral lesion of the premotor cortex in another operation.

For aspiration lesions, the dura mater is opened and the desired tissue removed using suction with a small gauge pipette. After completion of the tissue removal, the dura is closed, the bone sewn back in place, or, in those cases where it is not possible, cranioplasty will be done using small pieces of teflon. The incision will be closed in anatomical layers.

Excitotoxic lesions and neurotransmitter depletions will be made using a Hamilton microsyringe using MRI-guided stereotactic procedures or under direct visual guidance with the aid of a operating microscope. The total volume injected per site depends on the size (volume) of the brain target. Once the injections are complete the dura is closed and the bone flap sewn back in place and the skin closed in anatomical layers.

After surgery the monkeys will be given appropriate analgesics and antibiotics as determined by consultation with the [redacted] veterinarian. In most cases the animal recovers for ten to fourteen days before postoperative testing begins. The maximum number of planned major survival surgical procedures that an animal will undergo is three. However, as described in Section F, animals may on occasion require additional major survival surgeries to complete a lesion or to remove bone that has regrown inside a chamber.

a. Amygdalar removal: a craniotomy will be performed over the frontal cortex, just behind the orbit. The dura mater will be reflected, the frontal cortex gently lifted, and the amygdala and its subjacent cortex removed by aspiration. In some experiments, selective amygdalar removal will be made with the neurotoxin ibotenic acid (10 – 15 µg/µl solution in phosphate buffered saline) or other neurotoxins such as NMDA. For this procedure, a large bone flap over the frontal cortex will be turned. Then a syringe needle will be lowered, through a small slit in the dura mater, until it reaches the amygdala, where the ibotenate (total of 24 - 30 µl per side) is slowly injected. A series of penetrations and injections, derived in part from measurements obtained from magnetic resonance images, will be used for a complete but selective amygdalar ablation.
b. Hippocampal removal: a triangular-shaped craniotomy will be performed just superior to the external auditory meatus. The dura mater will be reflected, the ventrolateral inferior temporal cortex gently lifted, and the ventromedial temporal cortex (parahippocampal gyrus) removed by aspiration. The white matter in the ablated area will be entered in order to gain access to the temporal horn of the lateral ventricle. The hippocampus, which is lying in the ventricle, will be identified and removed by aspiration. In some experiments, selective hippocampal removal will be achieved through use of the neurotoxin ibotenic acid or other neurotoxins such as NMDA. For this procedure, a large bone flap over the frontal and parietal lobes will be turned. Then, through a small slit in the dura mater, a syringe needle will be lowered into the hippocampal formation and the ibotenate (total volume of 10 - 24 µl per side) injected. A series of penetrations and injections will be required to complete the lesion.
c. The combined amygdalar and hippocampal removal is simply the combination of procedures 1 and 2, above.
d. Rhinal cortex removal: first, the zygoma will be removed to allow access to the portion of the cranium overlying the ventral temporal region. A craniotomy will be performed over the frontal and temporal regions. The dura mater will be reflected, the rhinal sulcus visualized, and the tissue lining the banks of the rhinal sulcus will be removed by aspiration. In some experiments, rhinal cortex lesions will be made with the neurotoxin ibotenic acid (a total of approximately 40 µl per side). Procedures will be similar to those described for ibotenate lesions in 1 and 2, above. In addition, injections may be made directly into the rhinal cortex via a hand-held Hamilton syringe rather than via a stereotaxic approach.
e. Forebrain commissurotomy: a large unilateral bone flap, encompassing the dorsal midline, will be turned and the dura mater reflected to expose the midline. The corpus callosum will be identified and cut with a glass sucker. Then, at approximately the level of the superior precentral sulcus, the anterior commissure, which passes through the third ventricle, will be identified and sectioned as well.
The dura mater will be pulled back to its original position but not sewn. (Since no brain tissue is being removed, the dura mater is specifically not sutured to allow the brain space for some expansion if there is cerebral edema.) The bone flap will be replaced.
f. Inferior temporal cortex ablation: to allow for removal of the most ventral portion of the cranium overlying the inferior temporal cortex, the zygoma is removed. Then a craniotomy is performed over the inferior temporal cortex. Tissue lying between the superior temporal

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sulcus and the rhinal sulcus, from the temporal pole to just rostral to the inferior occipital sulcus, is removed by aspiration. In some experiments, visual-limbic disconnection will be achieved by section of the optic tract in one hemisphere (see below), rather than an inferior temporal cortex lesion in that hemisphere.
g. Inferior prefrontal cortex ablation: the craniotomy is the same as that used for the amygdalar lesion described in #1a, above. The dura is reflected, and the cortex lying ventral to the principal sulcus, rostral to the fundus of the arcuate sulcus, and dorsal to the lateral orbital sulcus is removed by aspiration.
h. Orbital prefrontal cortex ablation: the craniotomy is the same as that used for the amygdalar lesion described in #1a, above. The dura is reflected, and the cortex lying medial to the lateral orbital sulcus, extending medially to the rostral sulcus, is removed by aspiration. The caudal boundary of the lesion is roughly 5 mm rostral to the junction of the frontal lobe and temporal lobe, and the rostral boundary of the lesion is an imaginary line joining the rostral tips of the lateral and medial orbital sulci.
i. Optic tract section: the craniotomy is the same as that used for the amygdala lesion described in #1, above. The dura is reflected, and the optic tract is located by gently elevating the frontal cortex and following the sphenoid bone to its end. At this point the optic tract may be visualized and sectioned with a glass sucker.
j. Surgical approaches for procedures for injections of retrograde tracers (Fast Blue or Diamidino Yellow) will resemble in many respects the protocol for amygdala lesions or rhinal cortex lesions with ibotenic acid described above. As already indicated in Section F, the number of injections will range from 1 to about 50, and the typical amount of material injected will be 1.0 µl or less per site. All injections will be made directly into cerebral tissue via the needle of a Hamilton syringe.
k. For procedures f-h, the lesion may be achieved by injection of excitotoxins via a Hamilton microsyringe.
l. Frontal cortex: a craniotomy will be performed over the frontal region. The dura mater will be reflected, and portions of the premotor or prefrontal cortex removed by aspiration or injection of ibotenic acid.

From our previous experience working with excitotoxins (e.g. ibotenic acid, NMDA), we do not expect any long-term adverse effects on the health of the animals However, respiratory arrest or tachycardia are occasional side effects of cerebral injections of ibotenic acid or NMDA. Animals will be carefully monitored for these side effects If this occurs we administer propranolol or other agents, under the guidance of the NIMH veterinarian, and will stop the injections until the heart rate returns to normal. When such procedures are being undertaken, the [location redacted] staffs are alerted. The animal is watched closely while being maintained on the respirator and the veterinarian is advised of the situation and consulted as needed. Past instances of respiratory arrest or tachycardia have usually resolved (i.e. the animal resumes breathing on its own) within 30 minutes to 5 hours.

2. Who will perform surgery and what are their qualifications and/or experience?

Dr. Elisabeth Murray, [secondary names redacted] will perform the surgery with the assistance of other personnel listed on the protocol. Dr. Murray has over 30 years of experience with aseptic neurosurgical techniques in monkeys, including those described above. [secondary names redacted] each have over 10 years experience with aseptic neurosurgery in nonhuman primates, including implant surgeries, stereotaxic approaches and injections of excitotoxins or tracers with the aid of an operating microscope. [secondary name redacted] has 3 years experience with aseptic neurosurgical techniques, mainly with cranial implants.

3. Where will surgery be performed, Building and Room? [location redacted]
4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:

It will be the responsibility of the principal investigator to ensure that the monkey is adequately cared for until it has completely recovered from surgery. Postoperative care includes a regimen of dexamethasone phosphate and antibiotics, to reduce inflammation and to prevent infection respectively. In addition, analgesics will be administered. [location redacted] staff, in consultation with the institute veterinarian, will specify the treatment regimen. Potential postsurgical complications include brain edema, seizures, and respiratory depression. After recovery from surgery the monkeys will be able to feed and care for themselves in the same manner they did before surgery.

Postoperative analgesics may include, for example, Ketoprofen (1 mg/kg bid x 3 days IM), buprenorphine (0.02 – 0.05 mg/kg IM or IV, q 6 – 12 hr.), acetaminophen (80 mg, oral), ibuprofen (100 mg PO), Banamine (1 mg/kg/day for 2 days IM), as needed. If cerebral edema is considered a problem either mannitol (2 g/kg IV) or dexamethazone (1cc/2x/day; 4 mg/cc IM for 2 days) is administered. The NIMH veterinarian is consulted to ensure the proper analgesics and antibiotics and appropriate doses of each are administered. Problems are quickly brought to the attention of the PI and the facility veterinarian and appropriate treatments are given.

After complete recovery from surgery, a minimum of 10 to 14 days based on an uneventful recovery period, the animal will resume postoperative testing. Postoperative recovery normally proceeds without difficulty. However, when the veterinarian, in consultation with the investigator, determines that an animal is experiencing pain and/or distress that cannot be relieved by applying accepted medical treatments and procedures, the animal will be euthanized.

5. Has survival surgery been performed on any animal prior to being placed on this study? Y/N Y If yes, please explain:

29 monkeys being transferred to this new ASP have been used in the previous version of this ASP and have had major surgery:
5 monkeys have received bilateral excitotoxic lesions of the entire orbital prefrontal cortex
4 monkeys have received crossed lesions of the amygdala in one hemisphere and the entire orbital prefrontal cortex in the other hemisphere
3 monkeys have received crossed lesions of the amygdala in one hemisphere and the prelimbic cortex in the other hemisphere

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2 monkeys have received crossed lesions of the amygdala in one hemisphere and the premotor cortex in the other hemisphere
6 monkeys have received bilateral excitotoxic lesions of the medial prefrontal cortex (anterior cingulate cortex)
3 monkeys have received bilateral excitotoxic lesions of the ventral striatum
4 monkeys have received bilateral excitotoxic lesions of the amygdala
2 monkeys have received bilateral excitotoxic lesions of the perirhinal cortex

6. Will more than one survival surgery be performed on an animal while on this study?
Y/N_**Y**_ If yes, please justify:

Monkeys that are subjects in disconnection experiments will receive multiple survival surgeries (see Section F). The design of "disconnection" experiments is a very powerful one. As already indicated, this is widely accepted as the only way to determine whether a given function, in this case a specific kind of sensory memory, is dependent upon the integrity of the anatomical connections between certain specified brain structures. This experimental design will require 2 or sometimes 3 operations to complete the disconnection (three if section of forebrain commissures is required). Some monkeys that receive ibotenate lesions will also require multiple survival surgeries. In this case, some of the injection series (e.g. combined amygdala and hippocampal lesions) will take so long to complete that a bilaterally symmetrical lesion must be carried out in two or more stages. Finally, operated monkeys that receive injection of retrograde tracers will in some cases require a minimum of 2 surgeries (see section F). In all cases of multiple survival surgeries, at least two weeks will intervene between successive stages. An animal will undergo a maximum of three planned major survival surgeries. This number (3) would include operations to induce a lesion as well as surgery for a craniotomy or tracer injections. On occasion, an excitotoxic lesion of the intended target appears to be incomplete (as judged from postoperative MR scans) and the injections are repeated to achieve complete removal of a specific brain structure before the postoperative behavioral training is initiated. This would be a case where the number of planned surgeries is exceeded. In such cases, we would carry out the minimum number of additional surgeries required to complete the lesion. In addition, monkeys with chamber implants will require additional operations for the purpose of removing bone that has regrown inside the chamber or removing excess granulation tissue from the dura mater. Because bone removal and granulation tissue removal from the dura inside chambers are classified as major survival surgeries, these procedures, if needed, may exceed the 'planned' number of major survival surgeries mentioned above. In all cases, however, we will use the minimum number of major survival surgeries necessary to carry out the work. Other relatively minor procedures (not major survival surgeries) might be carried out in circumstances requiring repair or replacement of a headpost or chamber (see Section G).

Approximately 8 monkeys a year will receive multiple-survival surgeries.

H. RECORDING PAIN OR DISTRESS CATEGORY - *The ACUC is responsible for applying U.S. Government Principle IV.: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.* Check the appropriate category or categories and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. FOR USDA REGULATED SPECIES, PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA. FOR ALL OTHER SPECIES, THE JUSTIFICATION FOR SUCH STUDIES MUST BE PROVIDED IN SECTION F. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT

NUMBER OF ANIMALS USED EACH YEAR			Year 1	Year 2	Year 3
	USDA Column C	Minimal, Transient, or No Pain or Distress			
X	**USDA Column D**	Pain or Distress Relieved By Appropriate Measures	112	20	20
	USDA Column E	Unrelieved Pain or Distress			

Describe your consideration of alternatives to procedures listed for Column D and E, and your determination that alternatives were not available. [Note: Principal investigators must certify in paragraph N.5. that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.] Delineate the methods and sources used in the search below. **Database references must include the databases (2 or more) searched, the date of the search, period covered, and keywords used.**

As already indicated in Section E, lesion/inactivation experiments are the only way of determining whether a given brain structure is necessary for a particular function. At present, all brain lesion methods that have the requisite specificity require the aseptic surgical methods described in the ASP, and these methods, in turn, are used only with concurrent administration of appropriate anesthetics. Discussion of alternatives with

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experts in the field suggested that there was no noninvasive alternative approach. Literature searches covering the years from 1990 to the present using Pubmed, Primate Lit, and Agricola also yielded no alternatives.

On 09/25/19 PubMed was searched using the following keywords or phrases:
inactivation monkey brain: 3282
lesioning monkey brain: 488
perirhinal lesions monkey: 130
hippocampal lesions monkey: 1504

A total of 5404 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

On 10/25/2016 PrimateLit was searched as follows: **(PrimateLit is no longer being updated)**
inactivation: 494
lesions: 942
perirhinal lesions: I
hippocampal lesions: 38

A total of 1475 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

On 09/25/2019 Agricola was searched:
primate brain lesions: 3
perirhinal lesions: 2
hippocampal lesions: 32
inactivation primate brain: 2

A total of 39 references were found. A search of these titles and a review of the pertinent articles did not offer any viable alternatives.

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration. All substances administered to an animal must be pharmaceutical grade unless otherwise justified in accordance with the NIH ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals. None ☐

There will be regular consultations with the NIMH veterinarians in order to ensure the most effective analgesics and anesthetics are used.
Agents routinely used:

For general surgical procedures:
Atropine (0.05 mg / kg, i.m.)
Glycopyrrolate (15 mcg / kg i.m.)
Ketamine (10 - 20 mg / kg i.m.)
Isoflurane (1.0 - 4.0%, to effect)

[redacted] light anesthesia inside the MR scanner and (vertical, [redacted]
Glycopyrrolate (15 mcg / kg i.m.)
Ketamine (5 - 20 mg / kg i.m.; 1-5 mg/kg i.v.) or Telazol (2 - 3 mg / kg i.m.)
Diazepam (0.5 - 1.5 mg / kg i.m. 30 minutes prior, or 0.5 – 1.5 mg / kg slow i.v. at the time of anesthesia)
Supplements of the above drugs will be given as needed.

[redacted] eneral anesthesia inside the MR scanner (vertical, [redacted]
Atropine (0.05 mg / kg, i.m.)
Glycopyrrolate 15 mcg / kg i.m. anticholinergic agent
Isoflurane gas in oxygen for general anesthesia [0.7% - 2.5%, to effect]. Anesthesia is induced by ketamine (10 – 20 mg / kg or to effect, i.m.) followed by isoflurane for maintenance.

[redacted] ight anesthesia inside the MR scanner (horizontal, [redacted]
Atropine (0.05 mg / kg, i.m.) or Glycopyrrolate (15 mcg / kg i.m.)
Ketamine (5 - 20 mg / kg i.m.; 1-5 mg/kg i.v.)
Dexdomitor (dexmedetomidine/medetomidine, 0.01 - 0.1 mg / kg) or 0.5 - 1.5 mg / kg; 0.5 – 1.5 mg / kg slow i.v. valium (diazepam)
Supplements of the above drugs will be given as needed.
A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

[redacted] light anesthesia inside the CT scanner ([redacted]
Atropine (0.05 mg / kg, i.m.) or Glycopyrrolate (15 mcg / kg i.m.)

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Ketamine (5 - 20 mg / kg i.m.; 1-5 mg/kg i.v.)
Dexdomitor (dexmedetomidine/medetomidine, 0.01 - 0.1 mg / kg) or 0.5 - 1.5 mg / kg; 0.5 – 1.5 mg / kg slow i.v. valium (diazepam)
Supplements of the above drugs will be given as needed.
A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

[location] general anesthesia inside the MR scanner (horizontal, [location]
Atropine (0.05 mg / kg, i.m.)
Glycopyrrolate 15 mcg / kg i.m. anticholinergic agent
Isoflurane gas in oxygen for general anesthesia [1.0% - 4.0%, to effect]. Anesthesia is induced by ketamine (10 - 20 mg / kg or to effect, i.m.) followed by isoflurane for maintenance.

[location] light anesthesia inside the MR scanner (horizontal, [location]
Atropine (0.05 mg / kg, i.m.) or Glycopyrrolate (15 mcg / kg i.m.)
Ketamine (5 - 20 mg / kg i.m.; 1-5 mg/kg i.v.)
Dexdomitor (dexmedetomidine/medetomidine, 0.01 - 0.1 mg / kg) or 0.5 - 1.5 mg / kg; 0.5 – 1.5 mg / kg slow i.v. valium (diazepam)
Supplements of the above drugs will be given as needed.
A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

[location] light anesthesia inside the CT scanner (horizontal, [location]
Atropine (0.05 mg / kg, i.m.) or Glycopyrrolate (15 mcg / kg i.m.)
Ketamine (5 - 20 mg / kg i.m.; 1-5 mg/kg i.v.)
Dexdomitor (dexmedetomidine/medetomidine, 0.01 - 0.1 mg / kg) or 0.5 - 1.5 mg / kg; 0.5 – 1.5 mg / kg slow i.v. valium (diazepam)
Supplements of the above drugs will be given as needed.
A single dose of ketoprofen (1 – 2 mg / kg, i.m.) will be administered just prior to or immediately following this procedure.

[location] general anesthesia inside the MR scanner (horizontal, [location]
Atropine (0.05 mg / kg, i.m.)
Glycopyrrolate 15 mcg / kg i.m. anticholinergic agent
Isoflurane gas in oxygen for general anesthesia [1.0% - 4.0%, to effect]. Anesthesia is induced by ketamine (10 - 20 mg / kg or to effect, i.m.) followed by isoflurane for maintenance.

Standard post-operative treatment includes analgesics and antibiotics administered after consultation with the attending NIMH veterinarian or [location] veterinarian and routinely includes ketoprofen (1 - 2 mg / kg BID x 3 days) followed by ibuprofen (100 mg PO BID x 4 days). Other analgesics that could be used are buprenorphine (0.02 - 0.05 mg / kg i.m. q 6 - 12 hr.), acetaminophen (80 mg PO BID), or banamine (1 mg / kg i.m. SID).

After a period of weeks or months, the dura mater sometimes starts to thicken. In this case, the monkey will either be sedated with ketamine (10 - 20 mg/kg IM) or taken to the OR to be done under isoflurane (1.0 – 4.0%, to effect) and the fibrous connective tissue will be removed using aseptic technique.

Desipramine is counterindicated with ketamine for pre-isofluorane anesthetic preparation. An alternative anesthetic regimen will be employed for pre-surgical preparation. Use of dexmedetomidine (0.005-0.05 mg/kg IM) plus butorphanol (0.3 mg/kg IM) has been successfully used in other settings and would be employed here.

J. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY:

Indicate the proposed method, and if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Guidelines on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

None ☐

The animal is sedated with Ketamine HCL (10 mg / kg, i.m., or to effect) and catheterized in the saphenous vein. After a deep level of anesthesia is attained, the animal receives heparin (1mL of 10,000 Units/ mL) via the saphenous vein. This is followed by Euthanasia-III (minimum dose 120 mg/kg pentobarbital), or another equivalent euthanasia solution given at the manufacturers recommended dosage, administered via the IV catheter. Immediately following euthanization, the animal is perfused through the heart with normal saline followed by aldehyde fixatives. The brain will be removed for histology and the carcass will be double-bagged in heavy plastic bags and stored in the appropriately marked refrigerator until disposal.

K. HAZARDOUS AGENTS: None ☐

Biological Agents with Pathogenic Potential:	NONE X (check if none)

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For guidance, see ORS/DOHS Biological Safety and Compliance. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.		
Agent:	~~H~~PRD #:	ABSL:
Additional occupational health and/or animal facility handling safety considerations.		

Recombinant DNA: NONE _X_ (check if none) For guidance, see NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules FAQs. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.		
Recombinant DNA:	RD #:	ABSL:
Additional occupational health and/or animal facility handling safety considerations.		

Ionizing Radiation: (Radionuclides & radiation producing equipment) NONE ___ (check if none) For guidance, see ORS/DRS/Policies/Radiation Safety Protocols Animal Studies Proposal Requirements
_ Yes, I will use radionuclides or radiation producing equipment as part of the experimental procedures on the ASP and all operators will be registered with Division of Radiation Safety. If an irradiator is to be used, then all individual users must comply with Division of Radiation Safety requirements for irradiator training, and all individual assessors will comply with applicable security requirements for escorts and proxy card access approval.
List of Radionuclides:
Radiological safety considerations:

Hazardous Chemicals or Drugs: NONE ___ (check if none) For guidance, see NIH Policy Manual 3034 – Working with Hazardous Chemicals Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (Title 29, Part 1910.1200(b)(3)(ii), CFR)
List of Agents: Aldehyde Fixatives, isoflurane
Additional occupational health and/or animal facility handling safety considerations: All material that has come into contact with monkeys or monkey by products is disposed of in a MPW box, when appropriate. Appropriate protective equipment (i.e. lab coat, chemical resistant gloves, and mucous membrane protection) shall be worn. When working with aldehyde fixatives and anesthetic gasses adequate local exhaust ventilation (e.g. chemical fume hood or downdraft table) shall be used and appropriate protective equipment (i.e. lab coat, chemical resistant gloves, and eye splash protection) shall be worn. All NIMH Guidelines for the Use and Disposal of Aldehyde Fixatives will be followed. Isoflurane gas will be scavenged via an activated charcoal (F-air) canister attached to the anesthesia machine(s) in [location] surgery. Drugs will be prepared outside of the animal facility. Additional safety considerations: None

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.): None ☐

Section L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS **NONE** _X_ (check if none) List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.			
Material:	Source:	Sterile? Y	N

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If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)			
Have the tested materials been passed through rodents outside of the animal facility in question?			
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?			
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens.			

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY:

List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs. None ☐

Intracerebral injection of excitotoxins

From our previous experience working with these lesion-producing agents we do not expect any long-term adverse effects on the animals related to the excitotoxic lesions. However, during surgery respiratory arrest is an occasional side effect of cerebral injection of large volumes of ibotenic acid. Also when NMDA is injected we might observe tachycardia. If this occurs we administer propranolol or other agents, under the guidance of the NIMH veterinarian, and will stop the injections until the heart rate returns to normal. When such procedures are being undertaken, the [location redacted] staffs are alerted. The animal is watched closely while being maintained on the respirator and the veterinarian is advised of the situation and consulted as needed. Past instances of respiratory arrest or tachycardia have usually resolved (i.e. the animal resumes breathing on its own) within 30 minutes to 5 hours.

Hearing protection in fMRI experiments

Monkeys have been tested systematically on potential hearing loss before and after multiple scanning sessions using sound protection with silicone molds. Specifically, the monkeys' hearing ability between 892 and 7996 Hz, as well as their middle ear function, was tested on multiple occasions by audiologists using distortion product otoacoustic emission (DPOAE). The audiological results showed normal function before and after full data acquisition (K Tanji et al, NeuroImage,2010).

Social housing

Animals will be housed and enriched in a manner consistent with the NIMH Guidelines for Psychological Well Being and Enrichment of Nonhuman Primates. Some monkeys with brain lesions are to be exempt from pair housing. Removal of certain brain regions (e.g. amygdala, orbital frontal cortex, medial frontal cortex) results in alterations in emotional and social behavior. Specifically, after certain brain lesions, the dominance structure within a pair may be altered or the animals may respond inappropriately to social signals. In such cases, animals will no longer be suitable for pairing. In addition, because the controls for these experiments must be treated the same way as subjects in the operated groups, these animals, too, must be single-cage housed. Whenever animals are exempted from pair housing, other means of environmental enrichment will be implemented as per the NIMH Nonhuman Primate Enrichment Policy.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. I certify that I have attended an approved NIH investigator training course.
   Month/Year of Initial Course Completion: 1985 ; Month/Year(s) of Refresher Training: Aug., 2004, Aug. 10, 2007; Aug 12, 2010; Aug 12, 2013; Oct 5, 2016; Oct 16, 2019
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (or equivalent, as applicable, for contract personnel).
4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal, have completed the course "Using Animals in Intramural Research: Guidelines for Animal Users" will complete refresher training as required, and received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns. I further certify that I am responsible for the professional conduct of all personnel listed in Section A.
5. **FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section H):** I certify that I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) **as noted in section H,** and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the ACUC before initiating any significant changes in this study.

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 07/16/2021

Principal Investigator:

Signature _____ Date 10/18/19

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 07/16/2021

O. CONCURRENCES: PROPOSAL NUMBER _____ (LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____ Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name_____ Signature_____ Date_____

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility [location] Name [secondary name] Signature [secondary name] Date 12/19/19
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name [secondary name] Signature [secondary name] Date 12/16/2019
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name_____ Signature_____ Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____ Signature_____ Date_____

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Name_____ Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name_____ [redacted]

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name_____ Signature_____ Date_____

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Chairperson_____ Signature_____ Date_____

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Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name secondary name Signature secondary name Date January 6, 2020

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative:
Name_____ Signature_____ Date_____

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____
Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____
Name_____ Signature _____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name_____ Signature_____ Date_____

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Certification of review and approval by the Animal Care and Use Committee Chairperson

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DOHS Safety Representative:
Name_____ Signature_____ Date_____

DRS Safety Representative
Name_____ Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)
Facility_____ Name_____ Signature_____ Date_____
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Facility_____ Name_____ Signature_____ Date_____

COMMENTS:

Facility Veterinarian: Certification of Review
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____
Name_____ Signature_____ Date_____

NIMH Institute Veterinarian: Certification of Review
Name Krystal Allen-Worthington Signature [redacted] Date 12/16/19

P. FINAL APPROVAL:
Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson_____ Signature_____ Date_____

Theodore B. Usdin -S

Digitally signed by Theodore B. Usdin -S
Date: 2019.12.31 14:52:45 -05'00'

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 07/16/2021