17. Experimental Protocol

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

Pilot Study – Primate Model of Menopausal Hot Flashes

General: Study will use 2 groups of two animals each (adult premenopausal female rhesus macaques with documented menstrual cycles), each group of two recorded at the same time, 3x/week (or 2x/week if running both groups of two animals at the same time), during normal lights-on portion of lighting cycle. Specifically, in this pilot study, we will record data for a maximum of 30 sessions during each phase of the experiment (baseline, post ovariectomy, estrogen replacement, washout period, estrogen replacement and washout period). Each session will consist of <8hours of total chairing time: 0.5 hours for electrode placement and removal and a maximum of 7.0 hours of data recording. The room temperature will be varied at different recording times. The neutral temperature 22 °C (71.6 °F) will be the one at which they're normally housed; the warmer temperature will be to a maximum of 32^{0}C (89.6^{0}F); and the cooler temperature will be to a minimum of 20 °C (68^{0}F). Serum levels of estradiol (E2), progesterone (P), LH, and FSH will be measured at weekly, bi-weekly or monthly intervals (dependant on data required to accurately monitor levels) during each phase. Estrogen replacement will be accomplished through constant estrodiol release from a surgically implanted capsule (See surgery description for details). The second set of

animals will follow the same experimental outline as the first set. The additional set of animals is required to obtain a larger N value for demonstrating the validity of the data.

Specifically: Study will be divided into six phases of data collection:

1. **Maximum of 30 sessions** of baseline data collection
   (Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).

2. Ovariectomy, followed by a **maximum of 30 sessions** of data collection.
   (Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).

3. Estrogen replacement followed by **Maximum of 30 sessions** of data collection. **((Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).**

4. Washout period followed by **Maximum of 30 sessions** of data collection.
   (Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).

5. Estrogen replacement followed by **Maximum of 30 sessions** of data collection.
   (Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).

6. Washout period followed by **Maximum of 30 sessions** of data collection.
   (Max 7 hrs data collection/day, maximum 3 sessions/week on non-consecutive days).

The animals will receive water *ad libitum* while chaired, and treats will be used as a method of positive reinforcement. Chaired monkeys will be in visual and auditory contact with another monkey at all times during the experiment.

General Experimental Outline:

	Subgroups	**Sessions**
Baseline	1	Neutral (~22 °C)
	2	Neutral (~22 °C) Warm (<33 °C) Cool (>19 °C)
One week of rest after ovariectomy-no chairing		
Post Ovariectomy	3	Neutral (~22 °C)
	4	Neutral (~22 °C) Warm (<33 °C) Cool (>19 °C)
One week of rest after capsule implanting-no chairing		
E_2 Replacement (2 capsules)	5	Neutral (~22 °C)
	6	Neutral (~22 °C) Warm (<33 °C) Cool (>19 °C)
Washout period	7	Neutral (~22 °C)
	8	Neutral (~22 °C) Warm (<33 °C) Cool (>19 °C)
One week of rest after capsule implanting-no chairing		
E_2 Replacement (2 capsules)	9	Neutral (~22 °C)
	10	Neutral (~22 °C) Warm (<33 °C)

Cool (**>19** °C)

Washout period

11 Neutral (**~22** °C)

12 Neutral (**~22** °C)
Warm (**<33** °C)
Cool (**>19** °C)

Body temperature and skin conductance will be recorded for a maximum of 7.0 hours while the animals are chair restrained. In addition, it will take approximately 0.5 hour to place and remove electrodes. Animals will have a minimum of 40 hours of unrestrained activity between chairing episodes, and will have a maximum of three recording sessions per week. At approximately one month prior to the experiment, monkeys will be adapted to chairs, starting with a short period and gradually increasing to the full 7 hour period. The normal schedule for feeding will be followed (chow offered in the morning and fruit in the afternoon*) and water will be available *ad libitum*. Each chair-restrained monkey will be in visual contact with another monkey at all times during the experiment. The animals will be monitored **visually at least once every 5 minutes** by research or animal care staff during the entire chairing period. Chow will be specialized-Harlan-texlab purified diet. Animals will be given time to adjust to this chow.

1. Body temperature measurement: YSI#729 thermistors will be placed on the inner thigh, forehead, and ear pinna.). Electrodes will be securely fastened with Dermicel hypo-allergenic tape, which may be wrapped around the leg or torso several times. These electrodes will be connected to a physiological data logger and body temperature will be logged continuously over the 7 hour period.

2. Skin conductance: skin conductance is an electrical measure of apocrine sweat output, measuring a tiny current across the dermal surface and converting that to sweat gland activity. Self-adhesive ECG electrodes will be placed on the sternum and/or lateral thigh,. If further adhesion is required, electrodes will be securely fastened with Dermicel hypo-allergenic tape, which may be wrapped around the leg or torso several times. These electrodes will be connected to a physiologic data logger and skin conductance will be logged continuously over the 7 hour period.

3. Blood samples: Blood samples will be collected for analysis by venipuncture from either the saphenous or femoral veins. The blood volumes will vary, depending on research needs and limited by animal size/frequency of bleeding. Each animal will be immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at up to 15 mg/kg, and then any additional anesthesia will be administered only in consultation with a lab animal veterinarian. The animal will be observed throughout this period and until it has completely recovered from the anesthesia. Alternately, an animal will be transferred to a carrier cage, placed in an animal restraining apparatus, temporarily restrained from moving and then blood samples taken by an experienced person.

   Animals will be sampled at the beginning of the study and then routinely at weekly, bi-weekly or monthly intervals (dependant on data required to accurately monitor hormone levels) throughout the study. Samples will be assayed for estrogen, progesterone, LH and FSH levels. The amount of blood required at each time point would be a maximum of 15 mls.

   Total blood volumes per blood draw and per month over time will be monitored so as to remain within limits outlined in the SOP 4.01 (Blood volumes guidelines - rhesus) of the Primate Center. If clinical observations or blood work indicate problems with the animal's health and well being (as determined by a veterinarian), the total blood amounts drawn will be adjusted downward or temporarily discontinued.

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

The only method available for obtaining continuous date recordings requires restraining the animal in a chair for that period of time. (<8hrs) Without proper adaptation, chairing can be stressful for the animal. Therefore, all animals will be adapted to the chair prior to the experiment. This adaptation will begin by lightly sedating the animal with ketamine (<5mg/kg-if required) and placing it in the chair for a very short period (1/2 to 1 hour) with continual monitoring. Visual observation by an experienced animal handler will insure that each animal is not overly stressed while accomplishing chair training. If a problem develops at any time, the animals will be removed from the chair immediately. As the animal adapts to the chair, the time period will be gradually increase to a maximum of **<8 hours**. The animal will receive water *ad libitum* and treats will be used as a method of

positive reinforcement. Chaired monkeys will be in visual and auditory contact with another monkey at all times during the experiment.

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

Food will be withheld up to 20 hours and water withheld up to 3 hours prior to induction. Animals will be allowed water *ad libitum* during chairing sessions, but will not have access to food during the <8 hours of chairing. However, treats will periodically be offered during the chairing sessions.

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

During a portion of each experimental phase recordings will be performed at varying temperatures from >19 °C (68^0F), to <33 °C (89.6^0F), per <8 hrs. chairing per day. Previous work in our laboratory and others has shown that HF frequency is increased by heating (3,4) and reduced by cooling.(7) Therefore, we will need to record the same parameters in ovariectomized rhesus macaques at warm, cool and ambient temperatures during each phase of the experiment.