

2. In order to determine the distribution of various enzymes and peptides in the brain, immunocytochemistry (ICC) and *in situ* hybridization (ISH) will be performed (Aims 1-3, 2-2a/2b, 3-1, 3-2). To obtain the tissues needed, these animals will be terminally perfused with 4 % paraformaldehyde (or 0.125% glutaraldehyde plus 4% paraformaldehyde) with the protocol described in question 21. If appropriate brain tissues (age, sex, and condition) are available from colony necropsies, we will use them and any left over tissues from our animals will be distributed through the pathology service unit.

3. In order to determine the distribution of various proteins and mRNAs in the brain, RNase protection assay (RPA) and western blots will be performed (Aims 1-1, 2-1, 4-3). To obtain the tissues needed these animals will be euthanized (no fixative perfusion) with the protocol described in question 21. Some animals (Aims 1-1b/1d, 2-1b, 4-3c/3d) will receive ovariectomy before euthanization to see the role of the ovary. Animals will be ovariectomized at least one month before euthanasia. If appropriate brain tissues (age, sex, and condition) are available from colony necropsies, we will use them and any left over tissues from our animals will be distributed through the pathology service unit.

Project 2 (see Table 2 in question 12):

1. Embryos (E30-42 and E45-160) will be delivered by Caesarean section from pregnant female monkeys for culturing of the olfactory placode and the hypothalamus, and for histological investigation. Cultured LHRH neurons from olfactory placode will be used for *in vitro* experiments.

To obtain time-mated embryos we will use the following procedures:

Experimental Breeding Procedure – All females will be bred to provide time-mated embryos. Female sex skin coloration is monitored daily. Starting approximately 8 days after the onset of menstruation, up to 4 ml blood samples may be taken daily to determine LH values and supplement color data. Females are drawn until their LH levels rise or until their sex skin begins to “break down” (decline in color and tumescence) indicating changes in sex steroid levels during ovulation. Blood samples will be taken as stated above (in general procedures) and will adhere to the same parameters. Shortly before ovulation, a female is transferred from her home cage to live with a behaviorally compatible male. The male-female couple is observed for compatibility. The female may live with the male for approximately one week or until she ovulates. The pair is checked daily for ongoing compatibility.

Pregnancy Determination - In some cases, we monitor serum hormone levels during early pregnancy. In this situation we will implement a sampling regimen. We will collect daily blood samples starting as early as the day of mating until the day of Caesarean section, no later than day 42 of pregnancy. Again, blood samples will be taken as stated above (in general procedures) and will adhere to the same parameters. Throughout pregnancy the status of the fetus will be periodically monitored by transabdominal palpation of the uterus, or ultrasound under anesthesia when needed, as described above in general procedures section.

Semen collection methods –

Electro-ejaculator method: Male macaques will be fitted with a standard primate collar prior to initiation of chair restraint training. During a pre-study physical exam, the animal will be sedated, as described above and the collar will be placed. These collars are designed to fit comfortably and rotate freely around an animal's neck. The collars come in many sizes and are changed as the monkey grows. Once adapted to the collar, the monkeys are trained to accept the attachment of two long guide poles to special hooks on the collar. This “pole and collar training” will be done up to 3 times a day, up to 7 days a week. All training involves positive reinforcement for the performance of correct behaviors. Once the animal accepts attachment of the poles readily, the poles are used to guide the animal into a standard nonhuman primate chair restraint device. The poles are detached once the animal is secured in the restraint chair. Animals are highly variable in their ability to learn and therefore may need different amounts of time to accept the restraint chair. Typically, pole and collar training and restraint chair training can be accomplished in 30 days. Once the animal is comfortable with chair restraint, electro-ejaculation will be attempted. For electro-ejaculation, the penis of the chair-restrained macaque will be extended and applied with EKG cream on the entire shaft except the glans. The base of the penis will be wrapped with a pre-sized defibrillator gel strip (electrode) connecting to the negative lead. Another gel strip will be positioned immediately behind the glans and connected to the positive lead. (The skin should not be pinched.) The penis will be electro-stimulated at up to 25-Volt level until ejaculation, or for up to 25 seconds. If no ejaculate is obtained, the electro-stimulation process may be repeated again in 1 to 3 minutes. No more than three consecutive stimulus attempts will be made per macaque in a single day. There will be at least 48 hours between collection attempts, and no more than 3 samples will be collected in a one-week period. Once the electro-ejaculation procedure is completed, the chair restraint

