



Behavioral Standard Operating Procedures

Non-Human Primate SOPs

Standard Operating Procedures in non-human primate components of INIAstress

Endocrine Profiling with Pharmacological Challenges:

Adrenocorticotropin (ACTH) Challenge. This test assesses adrenocortical secretion of cortisol to exogenous ACTH after endogenous HPA activity has been suppressed by overwhelming negative feedback achieved with a large dose of dexamethasone. Following an overnight fast the animals will be administered dexamethasone (0.5 mg/kg im). Four to six hours later, during the maximum dexamethasone suppression of adrenal activity, a blood sample will be taken for baseline measures of cortisol and the animals will be administered the ACTH challenge (Cortrosyn, 10 ng/kg iv). Blood samples will then be taken at 15 and 30 min following ACTH infusion. Peak concentration and area under the curve measure of cortisol indicate adrenal responsiveness to ACTH stimulation (Kaplan et al., 1986)

Dexamethasone Suppression. The purpose of this test is to assess the sensitivity of the hypothalamus and pituitary to negative feedback from circulating levels of cortisol (Davidson et al., 1989; Mossman and Somoz, 1989). Since dexamethasone binds with great affinity to the cortisol receptor it is used in relatively small amounts to test the sensitivity to negative feedback. A morning (8:00 am) blood sample is taken for a baseline measure of cortisol. That evening (10:00 pm) a low dose (130 ug/kg im) of dexamethasone is administered. The next morning (8:00 am) another blood sample is taken for cortisol assay. The difference between the first and second morning cortisol concentrations is used as an indicator of sensitivity to negative feedback (Kalin and Takasheki, 1988).

CRF Challenge: Pituitary response to CRF will be assessed using CRF challenge test (Gold et al., 1984; Sapolsky 1989, Waltman et al., 1994). Monkeys will be fasted overnight. The next morning a blood sample will be taken followed by administration of 1 µg/kg ovine CRH into the saphenous vein. Blood samples (2 ml each) will be obtained at 15, 30, 45 and 60 min following CRH challenge.

Naloxone challenge: Naloxone results in elevated cortisol by blocking inhibitory opiod input to CRF neurons in the hypothalamus (Wand et al., 1998). We will first test 0, 125 and 375 µg/kg naloxone in tests separated by at least 72 hrs. Blood samples (2 ml) will be drawn at 15, 30, 45, 60, 90 and 120 min. Peak values and area under the curve values of cortisol, ACTH and neurosteroids assess the negative inhibitory tone of opiod input on hypothalamic CRF neurons.

Ethanol challenge: Ethanol can changes in circulating cortisol (Schuckit et al., 1987; see Background section). We will test 1.0 g/kg ethanol and five days later 1.5 g/kg ethanol. A baseline blood sample (3 mls) will be taken and 30 minutes later the monkeys received the challenge dose of ethanol (30% w/v in tap water) intragastrically. Blood samples will be drawn at 15, 60, 90 and 120 min following administration of ethanol. Peak values and area under the curve values for cortisol and ACTH will be used to assess the stimulatory effect of ethanol on HPA axis function. In addition, saphenous blood samples will be drawn for BEC at 60, 90 and 120 min.

Time and number of blood samples for each pharmacological challenge

Pre drug sample Post drug sample
ACTH - 15 min 15,30 min
Dexamethasone - 24 hr 10 hr
Naloxone none 15,30,60,90,120 min
Saline * none 15,30,60,90,120 min
Ethanol - 30 min 15,60,90,120 min
CRF - 15 min 15,30,45,60 min

* timepoints to serve both ethanol and naloxone ANOVAs

Training for the Challenge Tests: Following quarantine monkeys are trained to comply with awake veinipunctures to collect blood for the dexamethasone assay and for BEC analysis. Each step in the behavioral training is considered complete when the animals performed the behavior readily and with minimal observable distress. Briefly, twice a day each monkey is trained with positive reinforcement to move to the front of the cage and present its leg through an opening in the cage (10 X 10 cm). As the animal becomes comfortable with this behavior, the animal's upper leg at the femoral triangle was lightly pricked with a dental pick to simulate a needle stick before advancing to the actual blood draw. Training is approximately 2-3 weeks. Once the animal is trained, a 3 cc blood sample is drawn for dexamethasone challenge and 20 ul is taken for BEC analysis.

To administer the ethanol (i.g.) and ACTH (i.v.) and CRF (i.v.) challenges, as well as take repeated blood samples as close as 15 minute intervals, monkeys will be trained to sit in a primate restraining chair. Each monkey is first trained to move to the front of the cage and allow a pole to be fastened to their collar by a technician. A second technician attached another pole and the animal was then guided to the primate chair. The monkey is secured to the chair and trained to sit quietly for up to 3 hours by giving fruit and treats. As the animals becomes comfortable sitting in the chairs, blood draws are obtained via the femoral vein to simulate the blood sampling during the pharmacological challenges. The next phase in training is to train the animals to accept a nasal-gastric feeding tube for ethanol administration as part of the pharmacological challenge. During this training, only tap water is administered in appropriate volumes. Finally, we train the animals to sit quietly while an intravenous line is inserted in the saphenous vein for the eventual administration of cortrosyn and CRF. A final blood sample is obtained the week before pharmacological challenges for a baseline hematocrit which was used to assess the packed red cell volume over the course of the multiple blood draws. Each week during endocrine profiling, a blood sample is obtained for a hematocrit value to assess for anemia. For each challenge test, the order in which the animals are handled is randomly assigned although each animal is assigned a specific chair for the entire endocrine profile to ensure proper fit.

Blood Sampling

Femoral blood samples are obtained with a 22G x 1 ½ inch Vacutainer needle and a 3 ml Vacutainer hematology tube (Becton Dickinson). All blood samples are stored on ice until centrifuged (approximately 5 minutes). Samples are spun at 3000 rpm for 15 minutes at 4°C in a Beckman Coulter refrigerated centrifuge (Model Allegra 21R). The plasma is pipetted into 2 ml microtubes in 100 µl aliquots (n=5/draw). Plasma samples for cortisol analysis are frozen at -20°C and samples for ACTH and neurosteroid analysis are frozen at -80°C and stored until processing. Blood samples (20 µl) for blood ethanol concentrations (BEC) were obtained from the saphenous vein. Blood samples were sealed in air-tight vials containing 500 µl of distilled water and 20 µl of isopropanol (10% internal standard) and stored at -4°C until assay using gas chromatography (Hewlett Packard 5890 Series II, Avondale, PA) supplied with headspace autosampler, flame ionization detector, and a Hewlett Packard 3392A integrator.

Ethanol Self-Administration

Apparatus: Attached to one wall of the home cages will be an operant panel that allow access to all fluid and food requirements. These panels are constructed with two drinking spouts, two sets of three discriminative stimulus lights (red, amber and green) positioned above the spouts, a device that registers a "finger poke" by the monkey lever positioned below one of the spouts, and a centrally positioned opening. Within this opening is a dowel, which must be pulled to close a microswitch to provide access to fluids and food. An active panel is signaled through illumination of the amber stimulus lights, correct placement of the hand and a dowel-pull will be signaled through illumination of the green stimulus lights. Illumination of green stimulus lights further indicates that fluid and food are available. We have developed many changes to the drinking apparatus and

the controlling software over the past funding period and these are available upon request (grantka@ohsu.edu)

Training and Water Induction: Monkeys will be trained to operate the drinking panel in their home cage in daily 60-min sessions in which illumination of amber stimulus lights above both drinking spouts signals the onset of the session. Initially, a green stimulus light will be illuminated to coincide with the availability of fluid through the right-side drinking spout. The next training sequence involves reinforcing successive approximations in placing a finger inside the “finger poke” apparatus in order to activate the feeder and deliver a food pellet. At this point in training, one finger poke (fixed ratio schedule one; FR 1) will result in presentation of a banana pellet. Training will be complete (approximately 2-3 wk) once the monkey reliably operates the panel, drinks from the spout (90% confidence interval of the mean fluid intake of the last three sessions) and responds under the FR 1 schedule for banana pellets. Subsequently, monkeys will be induced to consume water (in the volume required for a 1.5 g/kg dose of 4% (w/v) ethanol) under schedule-induced polydipsia) in daily 22-hr sessions. During this portion of training, 1-g banana-flavored food pellets will be delivered at fixed time (FT) interval of 300 sec.

Ethanol Induction: After this one-month water induction period, ethanol (4% w/v) will be the only fluid present for the initial component of each daily 22-hr session. The amount of ethanol consumed in the first component of the session will be increased in a stepwise fashion over 30-day epochs. Specifically, monkeys will be induced to drink 0.5 g/kg ethanol/day (2-3 drinks) for 30 consecutive days, then 1 g/kg/day (4-5 drinks) for 30 consecutive days, and finally 1.5 g/kg/day (6-7 drinks) for 30 consecutive days. After drinking this allotment of ethanol, the ethanol spout will be no longer operative and only water can be obtained from the panel for the next 3-hr. Following these 3-hr, water and the remaining food (the daily ration minus the pellets delivered to induce alcohol drinking) will be available for the time remaining in the session. The delivery of each food pellet will require the monkey to make a single finger poke.

Ethanol Maintenance and Self-Administration: Following induction of ethanol consumption, there will no longer be a schedule induction and both 4% (w/v) ethanol and water will be available at all times during the daily 22-hr sessions. In addition, a “meal structure” will be imposed where the monkeys are required to eat their daily allotment of food in no less than three “meals,” with at least 2-hr between each meal. A meal will be defined by the proportion of daily food allotted to each monkey and the pace of the animal to obtain the food. The meal will end if one-third of the daily food allotment is obtained at a time, or if the monkey takes longer than 2-min to obtain pellets (inter-response interval 2-min). Following the end of a meal, food will not be available for 2-hr, followed by another meal, until the daily food ration is eaten.

Blood Ethanol Concentrations: Blood samples (20 ul) will be taken from the saphenous vein every fifth day from every monkey at approximately 7-hr following the onset of the session and assayed for blood ethanol concentration. Blood samples will be sealed in air-tight vials containing 500 ml of distilled water and 20 ml of isopropanol (10%; internal standard), and stored at -4 °C until assay using gas chromatography (Hewlett Packard 5890 Series II, Avondale, PA) supplied with a headspace autosampler, flame ionization detector, and a Hewlett Packard 3392A integrator.