INFANT PRIMATE RESEARCH LABORATORY

STANDARD OPERATING PROCEDURE

FOR THE EVALUATION OF

TEMPERAMENT AND DAILY WEIGHTS

Document Name: Temp/Weight.SOP. Rev5.doc

Begin Date: November 1, 2007

End Date:

This Standard Operating Procedure (SOP) was prepared by Brenda Crouthamel, Noelle Liberato, Kimberly Grant and Jim Ha.
**Introduction:** Temperament can be defined in many ways. In general, prominent theorists believe that it is a set of heritable personality traits that emerge during infancy and are distinct from other dimensions of development such as cognition, arousal or motivation (Goldsmith, H., et al., *Child Development, 58*:505-529, 1987). Temperament is best expressed when the subject is faced with an external demand or stimulus. For example, when approaching a task, infants and children may have the same motivation and ability level but can vary in their adaptability, mood, persistence and degree of distractibility. These latter characteristics are believed to be part of temperament and form the basis of personality development. There have been three traits of temperament that have been identified in animals other than humans. These are:

1) Emotionality (equivalent to distress)- Responses may range from general indifference to intense emotional outbursts (crying, tantrums, inability to be soothed)
2) Activity- The two primary components of this dimension are cadence (tempo, rhythm) and vigor. This can be described as behavior that varies in energy from lethargic to hyperactive or manic.
3) Sociability- This is best represented by the preference of the subject to be with others or to be alone. Individuals may range from reclusive (strong inclination for being alone or isolated) to a preference for social interaction and the presence of others.

It is now known that the roots of temperament develop during the prenatal period (DiPietro, JA, et al., *Child Development, 67*(5):2568-2583, 1996). This suggests that prenatal treatments or variables such as stress or teratogen exposure may affect this important and defining characteristic. Data have also shown that temperament is associated with later psychopathology such as mental illness and conduct disorders. Data are collected at the IPRL to document the normal expression of temperament as well as to understand the effects of pre, peri, and postnatal treatments on this dimension of behavioral development. The test paradigm developed at the IPRL is based on one created by Schneider and colleagues for use with nonhuman primates (*American Journal of Primatology, 47*(1):137-155, 1991). To maximize efficiency, temperament data are recorded as a routine part of the daily weight procedure.

**Essential Readings**


**Supplies and Equipment:**

- Weight scale, weighing pan, Igloo carrier, the Daily Schedule, the Daily Weights Sheet, rag diapers, pen, disinfectant spray bottle for cleaning, water spray bottle for cleaning cage fronts, box of gloves, and ice cube tray for storing fruit reward (at least one for each animal).
Schedule:
Temperament and weight data are collected 5 days a week from Day 1 until Day 300. After 300 days of age, temperament data are no longer collected and animals are weighed on a weekly basis. In the case that an animal is not weaned by 300 days, both temperament and weights will continue to be collected daily. When an animal is moved to a run through cage it is weighed one a week. When an animal is moved to a gang cage it is weighed one a month. Temperament and weight data are collected on a laptop. Weights are recorded on a weight sheet in addition to being entered into the Access program on the laptop. The weight sheets are used by the clinical staff to track the general health of the animals. Temperament and weight data should be collected between 9AM and Noon.

Procedure for Weights and Temperament:

Step 1. Go to the treatment room and check the treatment sheet to determine if any of the animals you plan to test today are on treatment. Record the eartags of the monkeys on treatment on the laminated treatment square that is kept on the Weights clipboard.

Step 2. Go to the Weekly feeding and housing schedule and check to see if any animals are scheduled for diaper or surrogate removal. The tester should plan on making the housing changes after assessing the animal. The tester should also review the schedule to see if any animals are in the process of weaning. Animals on the weaning schedule should have their weight closely monitored to make sure they are adequately drinking and maintaining their weight.

Step 3. Retrieve some reward fruit, which typically consists of banana slices or small cut of pieces of apple. The fruit needs to be thoroughly washed, cut up if necessary, and put in ice cube trays (ice cube trays allow the pieces of fruit to be separated out so that the tester has less of a chance of contaminating the fruit with gloves that might have feces on them).

Step 4. Determine the order in which testing will take place. Typically, the animals in RR040 (nursery) should be tested first. After that, the animals in RR035 should be tested according to social group with the youngest groups tested first and the older groups tested last.

Step 5. Bring the weights clipboard, ice cube trays filled with fruit, and rag diapers to the weight cart. The Weights/Temperament laptop used to collect the data is attached to the weight cart. The cart needs to be wheeled into the housing room and the scale and laptop need to be connected to their power sources (this is only done in RR035). The laptop has a wireless connection so the Ethernet connection cord does not need to be plugged in. Once the Windows login screen appears, the tester needs to enter in the login name and password. Once the laptop has finished the login process, click the CompTemp.mdb icon on the desktop. This will bring up the CompTemp Access database. Click on the “Forms” tab on the left side of the window and then click on the “Temp” icon within the “Forms” window of the database.

Step 6. The Temperament data entry form will appear at this time and the curser will be in the “Tester” cell. Enter the following pre-testing info;

-Tester number
-Probe
-Record a “0” if not wearing a costume, or 1-5 depending on which costume is being worn on that day. See the Weekly Novel Probe description under the Special Procedures section on page 8 of this document.
-Eartag
If the database recognizes the eartag, then the monkey’s corresponding animal number will automatically enter in the “Animal” cell. If the animal number doesn’t appear, check the eartag and make sure it’s a legitimate eartag. If it’s the correct eartag, then the monkey might be new to the lab and the monkey’s ID data hasn’t been added to the database.

-Treatment (1 = not on treatment, 2 = on treatment)
Refer to the laminated treatment card where you recorded the treatment info prior to testing.

-Sedation (1 = not sedated, 2 = sedated)
If the monkey was sedated earlier in the day, then do not perform the Temperament assessment. The monkey needs to have the housing info recorded and its weight taken. The remaining cells on the form should be left as “0”s and then type in the comments section why the animal was sedated.

Step 7. Then enter the correct codes for the following housing variables:

Room
1- nursery
2- RR035
3- other

Cage Type
1- isolette
1- six pack
2- small four pack
3- large four pack
4- other

Cage Position
1- top
2- middle
3- bottom
4- not applicable

Diaper
1- no
2- yes

Surrogate Cover
1- no
2- yes
3- not applicable (typically used if the monkey is in an isolate or doesn’t have a swinging surrogate setup in the cage)

Step 8. Turn on the scale and press the “kg” button. The scale will give a read out of .00kg. When the weight is recorded on the hard copy form and electronic form, the tester will need to add an additional “0” to the value. Place a diaper in the pan and press the button labeled “Tare”. This will zero the diaper’s weight and when the diaper is removed the value on the read out display will show a negative number.
Step 9. Remove the diaper from the pan and place it over your left forearm and hand. Approach the animal’s homecage from a distance of 5 feet. Observe the animal’s state as you are approaching the cage and code the state according to the descriptions below before your hand reaches toward the cage.

**Animal’s Response to Tester’s Approach (cage door shut)**

For multiple behaviors, score the behavior observed just prior to lifting hand to open door.

1. Asleep/Drowsy/Unaware (not aware of tester’s approach)
2. Full or extreme withdrawal – Withdrawal to back of cage or top of cage or animal remains at back or top back of cage displaying extreme fear.
3. Partial withdrawal—Withdrawal to surrogate or a few steps back, up or away from the direction of the door
4. No response – animal continues behavior, no apparent response to tester but aware of approach, can also includes circling behavior, stepping forward then backwards (or vice versa), or moving away from a spot in the cage and then returning to the same location (this behavior typically occurs in relation to the swinging surrogate).
5. Partial approach – Approach from back of cage to surrogate, or a few steps down or towards the direction of the door, but not right in front of door
6. Full approach – From anywhere in the cage to right in front of the door, or already at door intensely watching tester

Step 10. With the diaper still draped over your left forearm and hand, open the cage door and extend your left arm into the front section of the cage. Allow the animal to climb onto your hand/forearm. The tester can encourage the animal to move forward by gently patting the diaper. Do not verbally encourage the animal. The tester should observe the animal until he/she crawls on the diaper or until 5 sec. have elapsed. Select from the following codes:

**Animal’s Response to Presentation of Diaper (cage door now open)**

For multiple behaviors that are not 6-8, score the most reactive behavior observed within the 5 sec window

1. Remains asleep (or not aware of tester’s presence)
2. Withdrawal to back wall – *Same as code 2 above*
3. Partial withdrawal – *Same as code 3 above*
4. No approach and no withdrawal in 5 sec. – *Same as code 4 above*
5. Approaches tester but does not touch or climb on diaper (code should be used for older animals that attempt to leave the cage by either touching tester or climbing on tester)
6. Looks at diaper and intentionally touches diaper but does not climb onto diaper within 5 sec.
7. Slowly, delayed and/or hesitantly climbs onto diaper (after approx 3 seconds)
8. Quickly climbs onto diaper

Note: codes 7 & 8 require one of the following;
- 4 limbs (torso doesn’t need to touch diaper)
- two limbs and torso touching diaper
- fully sitting on the diaper, with both butt pads, doesn’t have to include arms, legs, feet or hands.

If the animal remains asleep/unaware after the tester opens the cage door, gently rub the head. If the animal continues to fall back asleep and cannot be fully awakened, let the 5 sec. window
elapse and record code 1. If the animal wakes up, score the behavior observed in the 5 sec window.

Step 11. Once the presentation to diaper has been scored, the animal needs to be removed from the homecage. For animals already on the diaper, remove your arm from the homecage while securing the animal with your other hand. For animals not on the diaper, reach in and physically remove the animal from their cage by placing the left hand under the belly and the right hand on the infant’s back. From Day 1 to Day 30, an infant may tightly grasp the cage wall but not be actively resisting capture. If the infant is grasping the side of the cage without other signs of active resistance, score this behavior with a code 1 (physically passive). Place the animal on the diaper draped over your left forearm and hand, securing the animal with your right hand. Score the animal’s general response to being removed from their cage according to one of the following codes (see below).

**Response to Capture/Removal from Cage**

1- Physically Passive
2- Physically Resists
3- Will not stay on arm or animal exits or attempts to exit cage while being captured.
4- Escapes from cage, never able to put on arm.

Step 12. With the animal properly secured on the diaper draped over the underside of your left forearm and hand, turn your back to the animal’s cage. This minimizes the probability that the animal will attempt to jump back into its homecage. With your back to the animal’s homecage, code how the animal is clinging to the underside of your forearm (see codes below). Gently shake your arm to more accurately determine the clinging behavior of the infant. Do not turn the infant over. If the infant is self-clasping with at least one hand or foot, this behavior (code 3) takes precedence over codes 1 and 2.

If the animal is struggling and will not stay on your forearm/diaper (code 4), firmly hold the monkey to your left arm with your right hand and quickly walk over to the weighing pan. If this behavior occurs for more than two consecutive days then refer to the Special Procedures section on page 8 of this document.

**Animal’s Cling On Tester’s Arm**

1- No cling (would fall off if not held)
2- Cling (holds onto arm with at least one limb and does not fall off)
3- Self-clasp with an one limb
4- Does not cling, struggles against tester’s arm, failure to cling not due to weakness or lethargy

Step 13. Carefully carry the animal on your forearm, with your right hand on the animal’s back, to the weight pan. Place the animal and the diaper in the weight pan. Observe the animal’s reaction to the pan environment during the first 5 sec. of being placed in the weight pan. If the infant displays more than one behavior when in the pan, code the predominant behavior. If the behaviors are evenly split across the 5 sec. observation period and you can not determine the most predominant behavior, score the most reactive of the different observed behaviors before the 5 sec. window elapses. Score according to one of the following codes (see below).
If the animal is unable to be confined to the weight pan, it will need to be weighed using an Igloo carrier with the lid shut. If this behavior occurs for more than two consecutive days then refer to the Special Procedures section on page 8 of this document.

**Reaction to Pan Environment**
1. Drowsy, asleep, not attentive, rooting*
2. Passive tactual or visual exploration (laying down or staying low in the pan with or without movement)*
3. Active tactual or visual exploration (sitting or standing upright in the pan, with or without movement)*
4. Cowers, cringes, may hide under diaper (not exploring and typically accompanied by some degree of irritability)
5. Rapid or disordered movement that can be accompanied by flailing, jerking, distress vocalizations, exaggerated emotionality or fear grimacing
6. Cannot assess (in carrier with lid shut)*

*-No irritability displayed or can display mild irritability

Step 14. After the 5 sec observation period has elapsed, give the animal a small fruit reward and record the animal’s weight from the scale (in grams) on daily weight sheet. Next, score the last 5 observed behaviors by typing in their scores in the appropriate cells on the Access form.

Step 15. Next, using the overall behavior of the animal during the weighing process, score the following behavioral categories in the appropriate cells on the Access form:

**Defecate, Urinate, and/or Vomits**
1. No defecation, urination, and/or vomit
2. Defecates, urinates, and/or vomits

**Irritability (e.g. fussy, agitated, struggling, convulsive jerking, grimacing, distress vocalizations)**
1. None
2. Some irritability
3. Significant, sustained irritability
4. Cannot assess (in carrier with lid shut)

**Consolability**
1. Not necessary to console (calm throughout procedure, relaxed)
2. Able to console with little effort (can calm animal down with minimal soothing)
3. Consoles with difficulty (can console with extended soothing but tends to quickly become agitated again)
4. Cannot console (unable to calm animal down)

*Note*: Consolability is scored when the tester is interacting directly with the monkey and can have some influence of his/her behavior. These instances occur during capture and removal from cage and when the monkey is on the tester’s arm.

**Vocalizations**—Auditory sounds clearly coming from the test subject and need to be heard by the tester and not just felt when the tester is holding the infant.
1. None
2. Positive vocalizations (e.g. cooing)
3. Negative vocalizations (e.g. hooting, screeching, gecking)
4. Both positive and negative vocalizations
Now code whether the weight pan or Igloo carrier was used, and if there were any outside disturbances that could have affected the animal’s behavior (see below)

**Pan vs. Igloo**
- 1- Weight Pan
- 2- Igloo Carrier

**Outside Disturbances**
- 1- No
- 2- Yes (disturbance are coded if the behavior of the infant clearly changes in response to conditions such as loud noises or the presence of strangers---changes in gaze do not represent a clear behavioral change)

Step 16. Lastly, record and relevant comments in the comments section on the Access form, and then type in the animal’s weight in the designated cell on the Access form. Next, push return to advance the form to the next blank form, allowing you to enter the data for the next animal to be tested. Note: if any of the cells were left blank the form will not advance because it will not accept blank cells. Review the cells and fill in the appropriate score in the blank cell(s).

Step 17. Remove the animal and diaper from the pan. Place the animal on the diaper on the underside of your left forearm and hand and return the animal to its cage. If the monkey is allowed a diaper and/or surrogate cover then replace them with clean ones. If you are unsure if a monkey should have a diaper and/or surrogate cover, check the animal’s ID tag because it is frequently updated with the diaper/surrogate info. Place dirty diapers and surrogate covers in one of the dirty laundry barrels.

Step 18. After all members of a social group have been weighed and assessed for temperament then clean the insides of each animal’s cage front using water from the spray bottle and rag diapers. Any monkey on “No Soc” or treatment should have its cage front cleaned last.

Repeat steps 6 through 18 until all animals have been tested.

Step 19. After all the monkeys have been evaluated for the day the data then needs to be queried for possible errors. Click on the Queries tab on the left hand of the Access database window. A new window will pop up and a list of queries used for checking the data collected that day will be displayed. Click each query and check the results. If there are errors, go back to the database table and make the corrections. Re-run the queries that produced the data errors to make sure the correction were properly made.

Step 20. Once all the queries have been run, click on the Reports tab on the left hand side of the Access database window. This will bring up all the rows of data that were collected that day. Review the date to make sure the report is actually accessing the current data and then click on the Print icon on the tool bar. Lastly, drag the CompTemp.mdb icon over to the ‘Shortcut to Access’ folder to backup the data. A window will pop up asking you if you want to replace the previous backed-up version with this current version, so click ‘yes’ on the prompt window.

Step 21. Clean the weight pan with disinfectant and return it to the bottom shelf of the weight cart. Next, shut down the laptop and clean the keypad, keyboard, mouse, scale buttons, the scale platform and the top of the weight cart with disinfectant and a rag diaper. Unplug the power cables for the laptop and scale and wheel it out of RR035 and into the hallway. Return the
Weights clipboard back to the kitchen, and clean the ice cube tray and return it to the kitchen shelf.

**Special Procedures**

**Igloo Carrier Procedure**
If there are two consecutive days of an animal refusing stay on your forearm/diaper once you remove him/her from the home cage or if he/she will not stay in the weight pan, he/she will need to be weighed in an Igloo carrier. Record the animal’s eartag on the laminated square on the laptop to remind you and other testers that this monkey has a tendency to either struggle when removed from its homecage or escape from the weighing pan. Make sure that you have zeroed the Igloo carrier and diaper prior to starting the assessment of this monkey. If the monkey has a tendency to escape from the carrier then the lid will need to be closed during the assessment (Code 7).

**Novel Probe:** The true temperament of an individual may be best expressed under stressful or challenging conditions. To capture these changes, the tester wears a designated costume once a week, typically on Thursdays, and performs the assessment as outlined above with the appearance change being the only variable that has changed. Infants are randomly assigned ages at which they start the novel probe testing (postnatal ages 91, 121 & 151 for *macaca nemestrina* and postnatal age 60 for *macaca fascicularis*). Animals starting probe testing for the first time will be listed on the Daily Schedule. Animals that are tested on the probe condition on a weekly basis have a (P) designator by their animal number on the Weights Sheet. Prior to starting the weekly novel probe, the animals will see the costumes but will not be handled by the tester while wearing the costumes. See below for a description of the probe costumes and their corresponding numbers.

1 = black vinyl top, shower cap, blue bandana around neck, blue mask, face shield, and grey or blue shoe covers
2 = blue lab coat, blue scrubs, red poncho with hood up, shamrock head piece, blue mask, face shield, and grey or blue shoe covers
3 = white tyvek suit, red bandana around neck with large yellow bow attached to bandana, tinsel wig on head, face shield, blue mask, and grey or blue shoe covers
4 = green rain slicker with hood up, white bone head piece on head, face shield, blue mask, and grey or blue shoe covers
5 = long yellow slicker, shower cap, blue halo head piece, blue bandana around neck, face shield, blue mask, and grey or blue shoe covers
**General Notes:**
The tester must change gloves and clean the weighing pan with disinfectant before handling members of different social groups. All animals have their social group number recorded on their ID tags and members of the same social group tend to be housed in the same four-packs. If a monkey is on treatment or has a “No Soc” tag on its cage, then it should be assessed after all the members of its social group have been assessed. After weighing these designated monkeys, the tester needs to change gloves and then clean the weighing pan with disinfectant and a clean rag diaper.
The tester also should change his/her lab coat if it became soiled during the assessment process.

Ideally, it is best to maintain the testing order and assess the monkeys from youngest to oldest, but occasionally the lab’s testing and social schedule conflicts with the Weights/Temperament testing order. Under these circumstances it is acceptable to test out of order as long as the tester thoroughly cleans the weight pan and has new gloves and an unsoiled labcoat after working with each social group.

If the monkey is struggling to such a degree so that the tester may be at risk for a bite or scratch at any point during the assessment, the monkey has to be placed in an igloo carrier.

**Procedure for Removing Diapers and Surrogate Covers:**
If not already done by the Nursery Tech earlier that morning, the tester is then responsible for removing diapers and surrogate covers from animals’ cages on the appropriate postnatal day. Diapers should be removed on day 30 and surrogate covers should be removed on the Friday closest to postnatal day 140. This information is scheduled on the Weekly Feeding and Housing Sheet. It is also the tester’s responsibility to correct instances where a monkey has a diaper or surrogate cover but is not scheduled to have one. When the diaper or surrogate cover is removed, the tester should update the Diaper and Surrogate info on the infant’s ID tag.

**Monitoring Weights**
It is the tester’s responsibility to track the weights of each monkey. If there has been a significant weight loss (greater than 10% of the monkeys total weight), report it to the clinical staff and/or the person responsible for scheduling the feedings as soon as possible.
Training and Reliability:

New Tester Training and Reliability
Prior to beginning training, both testers should closely review the SOP. The SOP should be available during all training sessions so differences in the codes can be discussed and reconciled. For training, the new tester observes the testing of the testing supervisor. The testing supervisor calls out the codes while testing and offers coding explanations to the new tester. After a few days, the new tester then calls out the codes to the testing supervisor. The testing supervisor should give feedback to the new tester on his/her coding accuracy. Once the new tester is reliably calling out accurate codes (typically a week or more), then reliability testing can begin.

For reliability sessions, testers no longer review the SOP or discuss scores. All the animals in the lab less than 10 months should be evaluated and should be tested over 8 consecutive days. On the 1st day, the testing supervisor performs the assessment for all the animals. The new tester observes the test procedures at a distance and simultaneously scores the animals. On Day 2, the new tester conducts the assessment on all the animals and the testing supervisor scores from an observational vantage point. The testing supervisor conducts the procedure on Day 3, the new tester again on Day 4, and so on until the testing supervisor has conducted 4 sessions and the new tester has conducted 4 sessions. Testers record data independently and do not discuss responses or repeat trials.

To pass reliability testing, there must be an 80% agreement between the scores of the new tester and the scores of the testing supervisor. If behavior categories are less than 80% then the data needs to be reviewed for scoring differences. Retraining and retesting takes place until an 80% or greater agreement is reached for each category.

Nine Month Reliability Sessions.

Testers who are routinely taking data partake in reliability sessions every 9 months. Testers should not review the SOP or discuss their coding with other testers prior to reliability sessions. This ensures that the testers are coding in the same manner since the last reliability session and are not changing their coding behaviors based on the SOP document. The 9 month reliability sessions are conducted as outlined above for the initial reliability sessions.

If testers become concerned about how they (or others) are coding a particular behavior after they have passed reliability, they should discuss their concerns with Kimberly Grant or Noelle Liberato prior to attempting to reconcile the situation. Testers should not discuss or implement changes in scoring without a thorough discussion with one of the above. This is being done to keep testers from changing the procedures, which negates reliability. Any changes in testing that come out of these discussions have to be listed in the log of changes section of this document, even if they pertain to only one tester.