

1. PURPOSE

This Standard Operating Procedure (SOP) describes the implementation and withdrawal of water control in nonhuman primates (NHPs).

2. RESPONSIBILITY

Principal investigator (PI, and their research staff, veterinary care staff.

3. INTRODUCTION

- 3.1. Water control is a motivational tool in training NHPs to perform specific tasks required for a research protocol.
- 3.2. Water is used as a reward during operant conditioning.
- 3.3. Three basic issues must be addresses prior to proceeding with water control:
 - 3.3.1. Necessary level of control
 - 3.3.2. Potentially adverse outcomes
 - 3.3.3. Methods used to assess animal health and well-being

4. PROCEDURES

- 4.1. Scientific justification for water control is required by the Facility Animal Care Committee (FACC).
- 4.2. The goal is to establish the minimal amount of water control that will produce the required behavioral performance for the experiment. The figure on page 3 illustrates how baselines are measured.
- 4.3. Establishment of water consumption baseline:
 - 4.3.1. A period of at least one week is required to establish an *ad libitum* baseline for water consumption and the baseline bodyweight.
 - 4.3.2. Limited experimental manipulations may occur during this period. Examples include: chair training, anesthesia for complete physical examination, collar application, blood collection and determination of individual preferences (e.g. fluids other than water).
- 4.4. Implementation of water control:
 - 4.4.1. After a stable *ad libitum* baseline is established, water control may commence.
 - 4.4.2. Body condition, weight and hydration status must be assessed daily by the veterinary care or animal care staff and research staff.
 - 4.4.3. Water may be withheld for a maximum of 24 hours prior to initiation of water control.
 - 4.4.4. Starting with the *ad libitum* baseline, the total volume of water consumed can be reduced by a maximum of 100 mL daily until the total volume of water reaches 350mL. If the animal does not consume this amount during testing then supplemental water must be given to meet the minimal total volume.
 - 4.4.5. If the animal is still not performing the required task, then the total volume of water consumed can be further reduced by 25 mL daily until the animal begins to work for its water. If the animal does not consume this amount during testing then supplemental water must be given to meet the minimal total volume.
 - 4.4.6. The total volume of available water can continue to be decreased as long as the animal does not exhibit weight loss exceeding 15% of its baseline body weight or any clinical signs of dehydration as described in section 4.9.
 - 4.4.7. Once the animal is fully trained to perform the task, it will be allowed to work for as much water as it wants every working day. This is the new *testing* baseline.
 - 4.4.8. On days when animals are not required to work (e.g., weekends), an amount of water equivalent to the *testing* baseline will be provided.

- 4.4.9. Investigators are encouraged to offer alternative rewards (e.g., fruit juices) allowing the animal to increase the *testing* baseline while still maintaining task performance.
- 4.5. Withdrawal of water control:
 - 4.5.1. Withdrawal of water control occurs when testing is no longer required for extended periods of time.
 - 4.5.2. Starting from the *testing* baseline, increase water availability daily at 100 mL increments until the animal is receiving its *ad libitum* baseline.
 - 4.5.3. After reaching the *ad libitum* baseline, the animal can then have free access to unlimited water.
- 4.6. Ongoing health monitoring:
 - 4.6.1. Chronic water control results in the establishment of new physiological set points. Most animals experience an initial weight loss, usually not exceeding 15% of baseline body weight, followed by weight gain and eventual stabilization of body weight.
 - 4.6.2. The following parameters must be monitored as specified below:
 - 4.6.2.1. Body weight:
 - 4.6.2.1.1. Must be obtained in as consistent a manner as possible (e.g. on awake animals, at the same time each day, in a chair with a known weight) each time the animal is removed from its cage.
 - 4.6.2.1.2. Each time an animal is anaesthetized, body weight must be recorded.
 - 4.6.2.2. Body condition:
 - 4.6.2.2.1. Once weekly, research and/or veterinary care staff must perform a cage side assessment (e.g. thin, normal, overweight).
 - 4.6.2.2.2. Body condition scoring may be performed when the animal is anesthetized.
 - 4.6.2.3. Hydration status:
 - 4.6.2.3.1. Once daily, animal care or veterinary care staff must perform cage side assessment through qualitative observation of fresh feces, urine output and mental status.
 - 4.6.2.4. Hematology and chemistry profiles:
 - 4.6.2.4.1. Biannual review with special attention paid to hematocrit, proteins, renal parameters and electrolytes.
- 4.7. Additional considerations:
 - 4.7.1. During the first 4 to 6 years of a monkey's life, growth spurts may lead to weight gain of as much as 2kg in a 12 month period. Water control during this period may result in an inability for an animal to maintain its body condition and therefore careful monitoring is necessary.
 - 4.7.2. The *ad libitum* and *testing* baselines for water control must be adjusted to account for animal growth.
 - 4.7.3. Body weights can vary by as much as 1kg, depending on whether the animals was fed or watered. Daily weighing must take place at approximately the same time each day in order to obtain consistent and accurate results.
 - 4.7.4. Animals that are comfortable in their environment will perform tasks more efficiently than nervous and stressed individuals.
- 4.8. Record keeping:
 - 4.8.1. Individual medical records must be maintained in the animal facility and include the following:
 - 4.8.1.1. Body weight
 - 4.8.1.2. Food and water consumption data and trends
 - 4.8.1.3. Daily observation sheets including body condition, fecal and urine output and mental status.
 - 4.8.1.4. Behavioral assessments, with deviations from normal noted.
- 4.9. Termination of water control:
 - 4.9.1. The following criteria will necessitate evaluation by the veterinarian:

- 4.9.1.5. Significant weight loss (e.g. exceeding 15% of baseline body weight) at anytime throughout the study.
- 4.9.1.6. Thin on cage side assessment.
- 4.9.1.7. Apathy, lack of interest, depression, anorexia or reduced appetite that has not improved despite veterinary intervention.
- 4.9.1.8. Abnormal laboratory data such as hemoconcentration, azotemia and electrolyte imbalances.
- 4.9.2. Upon consultation with the veterinarian, water control may be stopped.
- 4.9.3. Water control can only resume with approval from the veterinarian.

4.10. Reporting:

- 4.10.1. The veterinarian must notify the FACC of any serious or irresolvable issues that arise during water control.

5. REFERENCES

- 5.1. Toth L.A., Gardiner T.W. "Food and water restriction protocols: physiological and behavioral considerations". *Contemp. Top. Lab. Animal Sci.* 39(6), 2000.

Example of initiating NHP water control

