Development of a Standard Operating Procedure for Warming Hypothermic Mice Due to Flooded Caging
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ABSTRACT
Leakage from the lixit on rodent automatic water systems can cause flooding and animals to become hypothermic. Heat lamps and heating pads have been utilized to dry and warm wet mice but these methods have not been critically evaluated for effectiveness. The purpose of this study was to determine the optimal timing and distance when using a heat lamp to warm mouse cages. Cages were heated to ≤ 100°F and then checked to determine how long the cage could retain warmth. An empty microisolator cage containing one cup of Teklad bed-o-cob with the lid on and food in the hopper was placed 6, 12 or 18 inches from a standard heat lamp with a 250 watt bulb. The temperature inside the cage was measured with a data logger every minute up to 4 hours. Temperatures were measured at 6 inches from the heat source to determine maximal temperatures attained. Measurements were performed in triplicate under a class II Biosafety cabinet with the blower turned on. Humidity within the cage was 29.7 ±0.2% for all experiments. At 6 inches from the heat lamp, it took 35 ± 3 min, at 12 inches 74 ± 6 min and at 18 inches 234 ± 22 min to reach 99.8 ±0.1°F. Cages heated at 6 inches to target temperature and returned to the ventilated rack maintained elevated temperatures >60 min. In conclusion, cages can be safely warmed by placement 6 inches from a heat lamp for 35 min and then returning to the ventilated rack. Cages under a heat lamp should not be left unattended as overheating can rapidly occur. This provides a reproducible method for maintaining mouse health and welfare in the face of unexpected cage flooding.

MATERIALS AND METHODS
Determining easily measured distances from the heat lamp
A standard microisolator rodent cage is 6 inches in width and was used to measure the distance from the heat lamp so that in a real time situation additional measuring devices would not be needed. Distances of 6, 12 and 18 inches were tested (FIG.1).

Recording temperature at measured distances
Within a class II Biosafety cabinet with the blower on, cages were set under a heat lamp containing a standard 250 watt bulb. A data logger was placed in a dry empty cage filled with one cup of Teklad bed-o-cob with food in the hopper and the lid on. The temperature and humidity were recorded every minute for 90 to 280 minutes.

Maintaining a warm cage on the rack
After heating to the target range of ≤ 100°F the rodent cage was placed back on the ventilated rack and the temperature was measured every minute for a maximum of 90 minutes.

Safety
The cage was placed at the desired distance from the heat lamp for 6 hours to determine the maximal temperature within the cage if left unattended.

Heat lamp alternative
An alternative to a heat lamp was the Deltaphase heat pack. The pack was heated for 5 minutes in the microwave and placed beneath the microisolator cage and measurements taken as described for the heat lamp.

BACKGROUND
Cage flooding is a significant health concern for rodents housed on automatic watering systems and can cause hypothermia if not found early. Heat lamps and microwavable heating pads have been utilized to warm cages, but none of these methods have been critically evaluated for effectiveness. The purpose of this study was to determine the optimal timing and distance for placement of a heat lamp to warm mouse cages after flooding.

OBJECTIVES
- Warm a new clean cage quickly to ≤ 100°F
- Determine the duration of heat lamp exposure
- Determine the appropriate distance between heat lamp and cage that is effective and easily measured
- Maintain a warm cage temperature on the ventilated rack

RESULTS
A target temperature of 100°F was selected based on previous experience. At 6 inches from the heat lamp, it took 35 ± 3 min to warm to ≤ 100°F, at 12 inches 74 ± 6 min and at 18 inches 234 ± 22 min to reach 99.8 ± 0.1°F (FIG. 2). Humidity did not change throughout the experiment. Cages heated for 6 hours reached a maximal temperature of 122°F by 180 min. Cages heated at a distance of 6 inches to target temperature (35 min) and returned to the ventilated rack maintained elevated temperatures >60 min.

FIGURE 1 Cage and heat lamp set up within the Biosafety cabinet

FIGURE 2 Temperature elevation over time at different distances for the heatlamp compared to the Deltaphase heat pad.

CONCLUSIONS
Mice from flooded cages can be easily and safely warmed by placement in a dry cage 6 inches from a heat lamp for 35 min and then returning to the ventilated rack. Cages should never be left under a heat lamp unattended! This provides a rapid, reliable and inexpensive method for warming mice in the event of unexpected cage flooding.
Sanitation of Rodent Port Charger Stations: Implications for Bacterial Contamination of Drinking Water
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ABSTRACT
Water from port chargers necessary for ventilated rack systems is not routinely collected and tested for quality assurance purposes. This study was designed to develop guidelines on how frequently port chargers should be sanitized to prevent bacterial colonization of this equipment. Rodent racks are flushed daily. Microbiological testing of water samples is performed quarterly at the source and the level of the line or rack as indicated by room status. Charging stations were tested to determine the cleanliness of port chargers over a 7-d period. Data was collected in a single rodent facility utilizing reverse osmosis, filtered city water. Water samples were collected sterilely from the port chargers in 7 animal rooms containing ventilated rodent racks. Samples were tested before and after lines were changed at the start of each week and then 3 to 4 times a week afterwards for 6 wk. Water was cultured in BHI broth for 48 h and positive samples indicated by flocculent appearance. Positive samples were characterized by gram stain, API 20E, and API 20NE biochemical identification strips. An additional week of testing from 4 rooms was done for bacterial identification. All chargers testing positive were immediately removed along with the connecting hood water lines and sanitized by high pressure chlorine flush, and/or standard cycles in rack washer followed by autoclave. All testing of water at the source and from the racks were negative. From 37 individual room tests, water samples taken at the level of the charger were positive 39% of the time by 48 h and 50% of the time by 7 d. All bacterial species cultured were common environmental, water organisms. Port chargers could offer a potential source of contamination, especially for immunocompromised rodents, and weekly sanitation is recommended for this small equipment.

INTRODUCTION
The Guide for the Care and Use of Laboratory Animals identifies the need for periodic monitoring of water quality; however, there are currently no established national organizational standards for the drinking water of laboratory animals. Microbiological testing is performed on all equipment and water systems quarterly, but port chargers are not routinely tested. Although no disease outbreaks have been recorded, the potential for the spread of bacterial contamination from the port charger to immunocompromised rodents is a concern. The purpose of this investigation was to closely examine our current strategies for monitoring the quality of rodent drinking water. In this regard we attempt to develop performance standards for use in cleaning and maintaining port charger units and connecting water lines free from bacterial contamination.

MATERIALS AND METHODS

A. The Biosafety cabinet, port charging station, and water lines
B. PPE, charging station, Sporklenz, sterile lixit, sterile gauze, sterile collection tube, watch, and towels
C. Clean the connection port
D. Place the sterile lixit in the connection port and flush the charging station for 60 seconds
E. Collect a sample from the charging station
F. Culture 1 ml of water in lactose broth for 48 hours

RESULTS
Positive samples were identified by a cloudy appearance after 48 hours in lactose broth and were further identified. Initial microbiological testing of the existing lines with no flush yielded only one room (out of the four tested) with a positive result of Stenotrophomonas maltophilia. Positive results of Acinetobacter lwoffi, Pseudomonas alcaligenes, Stenotrophomonas maltophilia, and Chryseobacterium indologenes were found in 3 out of the 4 rooms during the course of testing of the “uncleaned” lines with a 1 min flush. Testing of the “cleaned” lines/charger yielded no growth for 3 consecutive tests. The last testing of the new lines/chargers was positive for Ochrobactrum anthropi in all four rooms. All of these species are common environmental, specifically water, organisms.

CONCLUSIONS
The need for consistency in animal experimentation requires adequate environmental monitoring and quality assurance. Providing a clearing flush prior to using the port charger proved to be highly effective in removing microbial contamination from water lines during daily use. High pressure flushing with hyperchlorinated water was used to clear lines that tested positive and at the end of each test period. The clearing flush is recommended prior to every use and the high pressure wash should be performed no less than every 2 weeks.

ACKNOWLEDGEMENTS
Special thanks to Dr. Laura Gallaugher, Justin Lahmers and Sallion Wolfe for editorial and technical support.
The effects of caging systems and cage locations on ventilated rack on mouse oocyte yield and breeding production

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ABSTRACT

While static and ventilated caging systems are both widely utilized in transgenic mouse production facilities, the effects of caging on mouse oocyte and progeny production have not been sufficiently analyzed. In this study, we first compared the oocyte yield of FVB and B6 mice on individual ventilated and static isolated cages. Our data indicated that there were no differences. The average oocyte per female in the static vs. ventilated caging system was 18 vs. 21 in FVB and 21 vs. 31 in B6, respectively. We subsequently tested the females' oocyte yield when they were housed in different areas of the ventilated rack (VR). It appears that animals in cages located at the bottom or far right side of the VR produced more oocytes than those animals placed at the middle or top of the rack. To further verify whether the "location effect" has any influence on mouse progeny yield, FVB and ICR dams - six groups per strain at two mice per cage, were paired with one male of their own strain per cage. The breeder trios were housed at the middle and bottom of VR, and their litters and litter size were tracked for one year. Coincidentally, dams housed at the bottom of the VR produced more litters than those housed at the middle of the rack. At each cage location temperature (T), relative humidity (RH), light (L), and airflow (AF) were recorded. Temperatures were ranging from 72 to 76°F (Average 74°F), RH was in 54-68% (Average 56%), light levels were between 8 to 12 Foot-Candles (Average 9), and airflow ranges from 29 to 35 CFM (average 34). This information should prove useful for colony management for both transgenic mouse production and general breeder colony maintenance.

MATERIALS AND METHODS

Test of mouse oocyte yield: Juvenile female mice at 3–5 weeks old were used as oocyte donors. Animals were purchased from Tecniplast or Harlan. They were housed on a ventilated rack (VR) (Lab Products Inc.) or a conventional static rack, three mice per cage (Table 1). Females were superovulated by subcutaneous injection of 5 IU of PMSG, followed 48-48 h later with 5 IU of HCG (Sigma). Females were subsequently mated with stud male mice. Oocytes were harvested from oviduct ca. 24 h post-HCG injection (8.5 dpc). Total number of oocyte was determined, and fertilized embryos (zygotes) were used for microscopy were maintained in CZB culture (Nagy, et al., 2003) at 37°C, 5% CO2 incubator.

Test of mouse breeding production: FVB and ICR dams were housed in six groups per strain, two mice per cage. Each group was paired with one male of their own strain per cage. The breeder trios were housed at the middle and bottom of VR (Table 1), and their litters and litter size were recorded. All animal experiments were performed in a Specific Pathogen Free (SPF) barrier animal vivarium according to the appropriate guidelines for animal use approved by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University (OSU). The animal facility is fully accredited by the Association for Assessment and Accreditation for Laboratory Animal Care International.

Table 1. Experiment design – cage location on ventilated rack

<table>
<thead>
<tr>
<th>Strain</th>
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<th>Total</th>
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<td>29.0</td>
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</tbody>
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BACKGROUND AND PURPOSE

A ventilated caging system offers a number of advantages over conventional static caging. It improves the health of both animal care workers and mice, and reduces the space requirement and animal husbandry cost. However, when such a system was introduced onto the OSU animal vivarium, the transgenic mouse facility experienced a major reduction in all aspects of animal production. Breeders exhibited low birth rate, produced smaller litters than usual or no progeny. Consequently, the animals for transgenic production were either poorly conceived or died during the gestation period. The gross increase in neonatal mortality and unexpected deaths of the animals prior to reaching the end of the 12-month trial period. The exact cause of this increased mortality was not determined although the deaths occurred predominantly at the middle of the VR. At each cage location temperature (T), relative humidity (RH), light (L), and airflow (AF) were tested. The relevance of these environmental parameters to the increased mortality has not been fully analyzed, and needs further investigation.

RESULTS

1. There were no apparent differences in oocyte yield between the two caging systems. FVB mice housed on ventilated rack produced a slightly more eggs than that of on static rack (average 18 vs. 21 ova per animal). For B6 mice, the average egg yield was similar on both racks (Fig 1). 2. Animals in cages located at the top left of the VR produced fewer oocytes than animals housed at the middle or bottom and the right side of the rack (Fig 2). 3. Animals in cages placed at the right side of the VR produced more oocytes than those animals at the left and middle of the rack (Fig 3). 4. For both FVB and ICR breeders, dams housed at the bottom of the VR produced more litters than the dams housed at the middle of the rack. The total progeny produced by the group at the bottom was 90 for FVB and 97 for ICR more than the animal groups housed at the middle of the VR (Table 2).

CONCLUSIONS

Comparison of a static vs a ventilated caging system revealed no difference in mouse oocyte and progeny production. However, our data also show that there was a location effect on the ventilated rack. Animals in cages located at the bottom or far right side of the VR produced more oocytes than those housed at the middle or top of the rack. Similarly, breeders also appeared to produce better mice when housed at the bottom of the VR. In a 12-month trial, the group of FVB moms housed at the bottom of the VR produced nearly double litters with total 9 more progeny than FVB housed at the middle of the VR. Similarly, ICR moms produced 10 more litters with total 97 more progeny than dams housed at the middle of the rack. The lower productivity of these mouse groups was mostly caused by unexpected deaths of the animals prior to reaching the end of the 12-month trial period. The exact cause of this increased mortality was not determined although the deaths occurred predominantly at the middle of the VR. At each cage location temperature (T), relative humidity (RH), light (L), and airflow (AF) were tested. The relevance of these environmental parameters to the increased mortality has not been fully analyzed, and needs further investigation.

BIBLIOGRAPHY

Ming Lu is a Laboratory Animal Technician at Laboratory Animal Resource (LAR), The Ohio State University (OSU). This position requires transgenic and colony maintenance, and is required for training and maintenance of the Animal Resource (LAR). Dr. Judy M. Hickman-Davis is a Clinical Veterinarian and a faculty member in Veterinary Preventive Medicine, The Ohio State University. Dr. Dr. H. Michael Kubisch is the Head of the Unit of Reproductive Biology at Tulane National Primate Research Center in Covington, Louisiana and an adjunct faculty member in the Department of Physiology in the School of Veterinary Medicine at Tulane University (New Orleans). Dr. Anthony P. Young is a Professor and the Director of The Center for Molecular and Neurobiology, The Ohio State University.
Comparison of Ketamine/Xylazine and Ketamine/Dexmedetomidine Anesthesia and Their Effects on Electrocardiograms in Rice Rat (Oryzomys palustris)

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MATERIALS AND METHODS

Prior to the final research endpoints, the animals were divided in three groups to evaluate three parenteral anesthetic combinations without P3 extraction. Each animal was sedated using 5% isoflurane via induction chamber. Following sedation, an anesthetic combination was administered intraperitoneally (IP) and the animal was returned to its original cage. Group A received ketamine-xylazine (75 and 10 mg/kg, IP). Group B received ketamine-dexmedetomidine (41 and 0.136 mg/kg, IP), and Group C received ketamine-dexmedetomidine (71 and 0.235 mg/kg, IP). Following the loss of the righting reflex, each animal was placed on a heating pad and electrocardiogram (ECG) leads were attached. Pedal withdrawal reflex was evaluated to determine onset of deep anesthesia. Twenty minutes following the loss of pedal withdrawal a reversal agent was provided. Animals in group A were given yohimbine (4 mg/kg) IP, whereas animals in group B and C received atipamezole HCl (1.3 and 2.5 mg/kg, respectively) IP. After the reversal agent was given, initial recovery was noted with the return of the pedal withdrawal reflex, while complete recovery was defined by return of righting reflex and full mobility. Additionally, respiratory rate (RR), heart rate (HR), and ECG biomarkers were obtained at three specific time points relative to the administration of the reversal agent.

RESULTS

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The rice rat (Oryzomys palustris) is a unique lab animal species frequently used for their high degree of susceptibility to destructive periodontal disease. They are native to several countries in South America. This animal appears mouse-like with a coarse pelage and a long tail displaying annulations through the sparse hairs.2 The research project required animals to be anesthetized for a 30 minute duration to allow extraction of M2 (2nd molar in the maxilla and mandible) bilaterally and placement of a mini-implant. Isoflurane was initially proposed due to the wide safety margin, efficacy, and fast recovery. Unfortunately, inhalant anesthesia could not be used successfully due to the required access to the oral cavity. The classic parenteral general anesthesia combination using a dissociative anesthetic (ketamine) paired with an alpha-2 agonist (xylazine) were recommended. Following extraction of the tooth, yohimbine, an alpha-2 antagonist, was given via intraperitoneally (IP) at a dose of 2 mg/kg yet prolonged recovery was still observed. Following the reversal agent, pain was managed by giving a mu opioid agonist (buprenorphine 0.1mg/kg subcutaneously) prior to full recovery. The rats were offered Motrin (100mg/5ml) at a dose of 30mg/kg continuously in the drinking water (500ml water bottle) for 2 weeks. Following the initial findings, we hoped to evaluate the use of an alternative alpha-2 agonist, dexmedetomidine and its reversal agent, atipamezole HCl in the rice rat.

The goal was to maintain the animals at a deep anesthetic plane for a 20 minute duration of time, this was 100% successful only when the higher ketamine/dexmedetomidine dose was used. The pedal reflex returned prior to the 20 min time point in 40% of the animals in group A (2/5) and 25% of animals in group B (1/4). Although anesthesia appeared to be at the low end of published values for a standard lab rat. Evaluation of the ECG noted that atrial premature depolarization was observed in animals in group A (4/5). No abnormal cardiac rhythm was observed in animals in group B and C.

CONCLUSIONS

The results of this study, based on changes in recovery time, suggest that IP ketamine-dexmedetomidine is a preferable parenteral anesthesia combination for the rice rat when compared to a ketamine-xylazine combination. Furthermore, a combination of ketamine-dexmedetomidine at a dose of 710/235 mg/kg successfully provided 20 minutes of deep anesthesia which could be consistently reversed using atipamezole at a dose of 2.5 mg/kg.

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Use of Laminated Cards to Validate Weaning Technique: A Direct Application of the “3Rs”

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**ABSTRACT**

Weaning 21-day-old mice can be challenging, especially in genetically-engineered mice. After animal care personnel have received training on mouse weaning procedures, they are required to demonstrate their proficiency as part of the validation process. Live animals are preferred for training, but they are not always available thus limiting the flexibility in scheduling training. Development of alternatives to the use of live animals in training fulfills the goal of the “3Rs” of animal research: replacement, reduction, and refinement. The purpose of this project was to develop an alternative that would increase the flexibility in scheduling weaning validation and decrease the number of live animals required for training. Adult and weanling-age mice with various coat colors were photographed. The photographs were laminated and placed in empty cages for separation according to age and gender. Cage cards, breeding cards and census sheets were also laminated to avoid repeated use. Marks made with a dry-erase marker or china pencil could be easily removed and the laminated materials disinfected between uses. A laminating check sheet was developed to allow the trainer to ensure that no important steps were skipped during the validation session. Some difficulty was encountered in determining the age and gender of the mice in the photographs. However, because this can occur when working with live mice, it provided an opportunity to determine how the trainee might handle the situation. Although this method has only recently been implemented, it has proven to be a realistic alternative to using live animals when assessing the weaning proficiency of animal care personnel.

**MATERIALS AND METHODS**

- ** Biosafety cabinet (hood), empty microisolator rodent cages, cage card holders
- ** Weaning validation checklist, laminated photos of adult and weanling mice, laminated cage cards (parent/weanling), breeding cards, laminated Census Activity Sheets, dry-erase marker or china pencil, paper towels

To set up for the training session, a ventilated mouse rack with several empty cages was moved into an empty housing room with a functional hood. Laminated photographs of adult and weanling mice were placed into each of three parent cages. A cage card holder containing a parent cage card and a breeding card were added to each parent cage and these cages were then returned to the rack. The rest of the cages remained empty to house the weanlings. A binder containing laminated sample census sheets and a box containing laminated cage cards were placed on a table in the room. Using an empty room allowed for a mock simulation of the weaning technique as well as eliminating the need for personal protective equipment, thus saving resources. Prior to the start of the session, the trainee was instructed to verbally indicate any simulated procedures, e.g. wiping with disinfectant, placing food and a water bottle, etc., to ensure no steps were overlooked. A brief description of the laminated photos was provided so the trainee could determine which mice were adults and which were weanlings. An initial review of the purpose for and responsibilities of weaning was conducted before the hands-on portion of the training. To start the session, the trainee was instructed to identify the cage(s) to be weaned and to carry out the weaning procedure. Although the trainer was present, the trainee was encouraged to perform the techniques independently, i.e. without asking questions about any uncertainties. Discussion was limited to prompting the trainee to verbalize what they would do in an uncertain scenario. Once the weanlings were separated into the new cages and documentation (breeding card, parent and weanling cage cards, census sheet) was performed, the thoroughness of the procedure was evaluated and the training documents completed accordingly.

**OBSERVATIONS**

Overall, this method of training was well received because it provided an opportunity to build self-confidence outside of the live animal setting. Although half-hour sessions were scheduled, the focus was performing the procedures correctly rather than how fast they were performed. Those employees who were proactive in preparing for the training were more successful at the time of evaluation. Those less confident in some procedures were more likely to ask questions during the training session, but were encouraged to perform independently. The laminated photographs proved helpful to those employees for which English is a second language, especially if they had received written material in advance and translated the information. The photographs could be handled extensively to ensure the age and gender were correctly identified, which live mice do not always tolerate.

Although this method eliminated the need for the availability of live animals, the following limitations were identified. The individual photographs were taken with different scales of size so they did not show a true contrast between adults and weanlings. However, age was not the focus of this training because it was assumed that litters will be identified prior to reaching 21 days of age. Even so, our solution was to instruct the trainees prior to starting the hands-on portion that the weanlings would be identified by the presence of a nestlet in the photograph. Another common problem for the trainees was not transferring all the information from the parent cage card to the weanling cage cards. The information of this imagery to the research personnel, as well as other difficulties observed during the hands-on training, was emphasized to the trainees during a group presentation at a later time.

**CONCLUSION**

This alternative training method for weaning is a valuable addition to our training program. It is effective for evaluating the proficiency of the trainees. It also fulfills the replacement, reduction and refinement goals of the “3Rs.” The laminated photographs replace live mice, thus reducing the need for live mice for training. Extensive handling of the photographs allows the trainees to refine their skills in determining mouse gender before handling live mice. Additionally, this training method can be adapted to fit the needs of various rodent facilities across all disciplines, i.e. academia, industry, or government.

**REFERENCES**


**PROBLEM**

A breeding cage policy was developed by University Laboratory Animal Resources (ULAR) together with IACUC to prevent overcrowded rodent cages, thus meeting the recommendation for cage density as provided in the Guide for the Care and Use of Laboratory Animals. Fluorescent green breeding cards were designed to place behind the parent cage card. These cards indicate the presence of new litters, birth and weaning dates and the rack location of the cage(s) of weanlings. Staff training is vital for the success and support of this policy because ULAR staff takes an active role in weaning within the vivarium. As part of the ULAR training program, a weaning validation checklist was created to ensure that sufficient training documentation was in place for seasoned staff members and new hires. However, litters of weaning age were not always present when the training was scheduled. Therefore, an opportunity was presented to create a training method for weaning that eliminated the need for live mice, which fulfills the goal of the “3Rs” as defined by Russell and Burch.

**APPROACH**

A validation checklist (Figure 1) to be used during training was devised listing the purpose, responsibilities, materials needed, and procedures to follow for weaning. The remaining training materials were designed based on this checklist. Digital photographs were taken of various ages and strains of mice as well as the corresponding anogenital region. The photographs were printed then layered back-to-back to represent live mice (Figure 2). The trainees were then able to determine the age and gender of the laminated “mice” (Figure 3). Trainees were required to record information on census sheets, cage cards and breeding cards as expected when weaning live mice (Figure 4). The photos, sample census sheets, cage cards, and breeding cards can be laminated for reuse between training sessions. For example, information recorded during one training session could be wiped off prior to the next session. After all materials were compiled, personnel were individually scheduled for a one-half hour training session.
Anesthetic Protocol for an Extended Duration Electrophysiology Study in Cynomolgus Macaques

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2 Division of Physical Therapy

BACKGROUND
Electrophysiological mapping of motor outputs evoked by microstimulation in the brain was performed twice each for two cynomolgus macaques (Macaca fascicularis) (Figure 1). The duration of anesthesia ranged from 12 to 20 hours. Correlation of microstimulation with muscle movement was the primary objective set forth by the investigator. Therefore an anesthetic plan to produce unconsciousness without inhibiting muscle movements was required. Volatile anesthetics, such as isoflurane, act primarily in the CNS to limit initiation of action potentials and produce immobility, making correlation of stimulation with muscle movements impossible. Ketamine is a dissociative anesthetic which produces unconsciousness without inhibiting skeletal muscle stimulation; it was the appropriate selection for this protocol.

MATERIALS AND METHODS

**Anesthesia**
- Pre-operative Atropine: 0.03 mg/kg atropine SO to decrease secretions and facilitate intubation.
- Ketamine: Continuous rate infusion (CRI) with boluses as needed: CRI titrated on an individual basis. Diluted in 0.9% NaCl or Lactated Ringer’s Solution (LRS) depending on acid-base status. Administered IV in the saphenous vein.
- Dexmedetomidine: Administered during ketamine CRI period. For procedure not involving microstimulation, 1-2 mg/kg in 100% oxygen.

**Local Anesthetic**
- 2-3 ml bupivicaine around the incision every 3-5 hours.

**Electrolyte Balance**
- Venous blood gases every 2-6 hours.
- 1 mEq/kg sodium bicarbonate IV as needed for metabolic acidosis.
- 0.25 mEq/kg potassium chloride IV for hypokalemia, second dose of 0.25 mEq/kg given if necessary based on subsequent blood gas results.

**Emergency Drugs**
- Doxapram: 2 mg/kg IV to stimulate respiration.
- Atropine: 0.03 mg/kg IV for bradycardia or arrest.
- Epinephrine: 0.3 mg/kg IV for cardiac resuscitation.
- Furosemide: 1-4 mg/kg IV to treat pulmonary edema.
- Lidocaine: 1 mg/kg IV as needed for ventricular arrhythmias (i.e. premature ventricular contractions).

**Fluid Balance**
- Mannitol: 1.0 g/kg IV over 15-20 minutes every 8 hours after craniotomy performed.
- Atropine: 0.03 mg/kg IV for bradycardia or arrest.
- Administered in saphenous vein not being used for ketamine CRI.
- Furosemide: 1-4 mg/kg IV to treat pulmonary edema.

**Electrocardiogram, pulse oximetry, capnography**
- Electrocardiogram, pulse oximetry, capnography.

**Setup for the intracortical microstimulation.** A microelectrode was inserted at a stereotaxically defined location in the motor cortex. A train of stimulus pulses was delivered through the electrode. This activated corticospinal tract neurons that project to the spinal cord. Alpha motorneurons and associated spinal circuits were then activated, producing a visible muscle twitch. Intramuscular fine-wire EMG electrodes recorded the muscle activation. In this example, the upper trapezius muscle responded, as evidenced by the EMG. The stimulus train itself lasted about 100 ms, matching the duration of the EMG response.

RESULTS
All objectives set forth at the outset were accomplished:
- Threshold stimulus intensities required to evoke twitches were comparable to the intensity required in a fully awake subject.
- Heart and respiratory rate remained within normal parameters (Figure 2).
- Average ketamine calculated to be 12 mg/kg/hr (Figure 3).
- Mannitol and dexmedetomidine limited cerebral edema and pulsations within the cortex.
- Urine output approximated total fluids administered, pulmonary edema never developed.
- Electrolyte abnormalities detected by frequent monitoring were easily corrected.

CONCLUSIONS
- For surgical procedures requiring dissociative anesthesia without total immobility in cynomolgus macaques, administer ketamine initially at a dose of 12 mg/kg/hr IV as a continuous rate infusion. Titrate as needed based on plane of anesthesia.
- Administer dexmedetomidine to maintain hemodynamic balance and reduce the amount of ketamine required to reach an appropriate plane of anesthesia.
- Provide mannitol to prevent cerebral edema, and carefully monitor and adjust subsequent fluid and electrolyte imbalances.

REFERENCES