The Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (hereafter referred to as the Red Book) states,

“Systemic paralysis is commonly used in neuroscience experiments. These experiments require that the animal be paralyzed with a neuromuscular blocking agent to prevent movement, such as movement of the ocular muscles during visual experiments. Neuromuscular blocking agents are used only in fully anesthetized animals. They do not interact substantially with anesthetics and analgesics, but they leave an animal unable to respond behaviorally to pain or distress.”

While it is recognized that the systemic administration of skeletal muscle relaxants may be a necessary component of a particular protocol, the use of these agents renders assessment of the level of general anesthesia much more difficult than is usually the case. With the advent of new anesthetic agents, innovative monitoring techniques, and creative anesthetic regimes, many cases where neuromuscular blocking agents (NMBs) have been used in the past, may not require the use of NMBs in the present and future.

Penn’s Institutional Animal Care and Use Committee (IACUC) has adopted the following guidelines (1) to assist the research community by clarifying the federally-mandated requirements regarding the use of NMBs and (2) to ensure that anesthetized, paralyzed animal patients do not experience pain or stress.

Guidance is based on the Red Book and concentrates on the following topics regarding the use of NMBs:

- Anesthesia
- Surgery
- Pilot Study
- Computerized Monitoring
- IACUC Review
- Euthanasia
- Exemptions

ANESTHESIA

Without exception, NMBs may be used only in a fully anesthetized patient. This mandate is repeated in the Animal Welfare Regulations and PHS Policy. Paralyzed animals cannot physically respond to surgical pain or may experience distress if not fully anesthetized. Due to the technical complexity and potential for animal pain involving procedures using NMBs, the PI must ensure adequate training and qualification of those individuals who will participate in anesthetizing and monitoring the paralyzed patient. Therefore, the NMB protocol describing the maintenance of anesthesia and monitoring the anesthetized patient must undergo strict review by the IACUC and ULAR veterinarians (see IACUC Review below).

Maintenance of anesthesia
After consultation with a ULAR veterinarian regarding agents, doses, and administration of the anesthetic and analgesics most appropriate for the neuroscience model, anesthesia may be induced as

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with any patient. As with the induction of any anesthetized animal, “notations should include the time, date (if appropriate), drugs or solutions administered, and the name or initials of the person making the entry” (Red Book).

Prior to administering NMBs, depth of anesthesia must be assured and the physiologic stability of the anesthetized patient must be confirmed at a constant dose of anesthesia (inhalation or constant rate infusion) (Red Book). Physiologic parameters and anesthetic depth must be measured and remain constant for at least 15 minutes before NMBs may be administered. Following at least 15 minutes of physiologic stability, the NMBs may be administered and additional attention to monitoring the anesthetized and paralyzed patient is required.

Monitoring the anesthetized patient
In a paralyzed patient, the most useful methods of determining depth of anesthesia in most species are abolished (Red Book, Guide), i.e. jaw tone and response to noxious stimuli (e.g. toe pinch). Therefore, more technically advanced methods of monitoring physiologic responses to pain or distress are required when using NMBs.

“[NMBs]...leave an animal unable to respond behaviorally to pain or distress. That can make it difficult to evaluate the depth of anesthesia and the adequacy of analgesia, so other signs of pain or distress must be used...” Red Book

A number of physiologic measures are helpful in monitoring paralyzed animals. Describing the signs of pain or distress and monitoring anesthesia with NMBs must be included in the IACUC protocol. A combination of the following clinical signs should be included, as appropriate to the model, expertise of the anesthetist, and recommendations from a ULAR veterinarian. Such signs are generally not adequate alone, but in combination they can provide valuable information about an animal’s physiologic status (Red Book).

- Lacrimation
- Salivation
- Pupil size
- Heart rate
- Arterial blood pressure
- Electroencephalographic recordings
- Blood oxygen saturation
- End-tidal CO2
- Rectal temperature
- Urine production

With guidance from a ULAR veterinarian, selecting a subset of these parameters may be appropriate for the model. At the very least, adequate ventilation must be provided, heart rate constantly monitored, and body temperature maintained and documented during the procedure.

The frequency of such measurements should also be based on the procedure, be documented in the animal’s record, and approved by the IACUC (Red Book). If the animal is surgicated immediately before or during the administration of NMBs, then the anesthetist must document physiologic parameters at least every 5-10 minutes during surgery—as consistent with the IACUC Guideline for USDA-Species Surgery. Physiological monitoring must occur at least every 15 minutes for procedures that do not involve surgery.

As with any anesthetic procedure, anesthetized animals should NEVER be left alone. In an anesthetized, paralyzed patient, the anesthetist must be in proximity to the patient at all
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times—within direct visual contact and able to immediately respond physiologic changes in the patient.

As when recovering any anesthetized animal, monitoring should continue and extra attention provided until the animal is alert and mobile. If recovering the patient from anesthesia and neuromuscular blockade, “care should be taken to ensure that the animal has recovered control of respiration and locomotion before it is returned to the home cage” (Red Book).

SURGERY

Aseptic technique
Regardless of species, rodent or non-rodent, survival surgery must be performed using aseptic technique, i.e. the surgeon must use sterile apparel and instruments and the animal must be steriley prepared for surgery. For rodent species, further guidance is provided in the IACUC Guideline for Rodent Surgery and Post-Anesthetic Monitoring. For USDA-species, further guidance on surgical facilities and aseptic surgery is provided in the IACUC Guideline for USDA-Species Surgery.

Surgery prior to NMB administration
It may be necessary to perform specific surgical procedures in preparation for the procedure, e.g. cutdown for vascular access, catheter placement, tracheostomy, or the implantation of devices in order to assist in data collection of the neurologic or behavioral data. It is highly likely that the “surgery” will be the most [potentially] painful portion of a complex procedure using NMBs. Therefore, unless scientifically and clinically justified, all surgical procedures performed in preparation for the experiment must be completed prior to the administration of the neuromuscular blocking agent (Red Book). With proper anesthesia, this requirement will assure that the patient will not experience pain as part of the surgical procedure—an assurance one could not make if the animal would be unable to respond if under the effect of paralytics.

PILOT STUDY

“The IACUC may request direct observation of a procedure (particularly a new or unusual procedure), pilot studies, and particular experimental measurements or monitoring procedure to evaluate and ensure animal well-being.” Red Book

“If paralyzing agents are to be used, it is recommended that the appropriate amount of anesthetic be first defined on the basis of results of a similar procedure that used the anesthetic without a blocking agent.” Guide

In order to assure that the planned anesthetic regimen will be effective during the entire period of time that subsequent animals will be paralyzed, a pilot study must be completed. The goal is to demonstrate that the administration of the proposed anesthetic regimen to un-paralyzed animals prevents them from experiencing pain or distress when they are exposed to procedures identical in duration and invasiveness of those proposed for paralyzed animals.
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1. The pilot study must include at least two (2) animals. These animals must be requested in the protocol submission and the numbers description should specify the numbers to be used in the pilot study.

2. Determination of the adequacy of anesthesia throughout the period of analysis will be assessed by continually monitoring the animal’s heart rate, respiratory rate, and body temperature. Reaction to a stimulus (e.g. firm toe pinch, palpebral or corneal reflex, etc.) must be monitored and recorded at no less than 15-minute intervals.

3. The analysis of physiologic parameter during the pilot study must continue for at least 30 minutes longer than the expected duration of the period of paralysis in the actual procedure.

4. Prior to protocol submission, it is strongly recommended to discuss the specific anesthetic regimen(s) and plan the proposed pilot study with a ULAR veterinarian.

5. The results of these observations must be provided to the IACUC, in writing, prior to any additional work involving the use of NMBs may proceed.

Any significant change in the anesthetic regimen (e.g. drugs, reduction of doses, administration) or any change in the procedure (e.g. invasiveness, lengthening of duration) will require another confirmatory pilot study. Data from an individual pilot study may be used on different protocols and may be “shared” among collaborators—as long as all parameters (e.g. species, drugs, procedures, duration, etc.) are identical. **The pilot data must be submitted with each protocol submission or resubmission.**

COMPUTERIZED MONITORING

The IACUC supports the appropriate use of technologic advances for physiologic monitoring during procedures and to create a historic record when such technology is used to improve animal welfare and investigator compliance (Red Book).

As described above in “Monitoring the anesthetized patient”, the anesthetist must be in proximity to the patient at all times—within direct visual contact and able to immediately respond to alarms or changes in computerized physiologic monitoring devices (Red Book). Even with continuous computerized monitoring, the anesthetist must document individual recordings of at least ventilation, heart rate, and body temperature at least every 15 minutes.

Like the animal’s clinical record (maintained by ULAR), protocols and other records (maintained by the IACUC), the computerized physiological monitoring data should be archived and maintained for a minimum of 3 years after the animal is euthanized or the end of the project (Red Book).

IACUC REVIEW

“It is imperative that any such proposed use [of NMBs] be carefully evaluated by the IACUC to ensure the well-being of the animal because acute stress is believed to be a consequence of paralysis in a conscious state and it is known that humans, if conscious, can experience distress when paralyzed with these drugs.” Guide
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In describing the animal use in an IACUC protocol, the PI must **describe how NMBs will be used** (including appropriate anesthesia and suitable monitoring techniques) as well as **specifically justify why NMBs must be used**. With modern anesthetic drugs and advanced training available to anesthetists, the desire to artificially ventilate the patient is not an adequate justification standing alone.

The paralyzed patient should not be subjected to any pain or distress if NMBs are used properly (including appropriate anesthesia and suitable monitoring techniques). In the case of anesthetic failure, the awake, but paralyzed patient surely will experience pain or distress. Thus, the IACUC requires a literature search for alternatives to the use of NMBs on each protocol describing their use (**USDA Policy #11**).

> “Animals that are paralyzed must be ventilated artificially, and standard veterinary practices should be followed when selecting the gas mixture and anesthetic or analgesic used.” Red Book

To further ensure appropriate use of NMBs, protocols will be specifically reviewed by a ULAR veterinarian prior to approval by the IACUC. The IACUC Office will assign a Vet Review; however the PI is strongly encouraged to consult with a ULAR veterinarian during the planning stage of the protocol.

**EUTHANASIA**

NMBs may NEVER be used as sole agents for euthanasia (**AVMA, OLAW**). **“Acceptable” or “conditionally acceptable” methods of euthanasia must be used** (e.g. CO₂, barbiturates, etc.) if animals are to be euthanized while under the influence of NMBs.

**EXEMPTIONS**

Any departures from these guidelines will require the PI to provide strong scientific and clinical justifications for the exemption. These justifications must be submitted via an **“Exemption Form”** and will be reviewed by the IACUC in consultation with ULAR veterinarians.

**REFERENCES**

American Veterinary Medical Association Guidelines for Euthanasia (**AVMA**)
Animal Welfare Act and Regulations “Institutional Animal Care and Use Committee” (**AWR**)
Guide for the Care and Use of Laboratory Animals (**Guide**)
Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (**Red Book**)
Office of Laboratory Animal Welfare IACUC Guidebook (**OLAW**)
Public Health Service Policy (**PHS Policy**)
USDA Animal Care Policy “Painful Procedures” (**USDA Policy #11**)