The University of Pennsylvania 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 neuromuscular blocking drugs (NMBs) and (2) to ensure that anesthetized, paralyzed animal patients do not experience pain or distress.

It is recognized that the administration of NMBs (which paralyze all voluntary muscles, including extraocular muscles, with no provision of analgesia) may be necessary to minimize self-generated animal movements for a particular protocol. Unfortunately, the use of these agents also eliminates typical indicators of anesthetic depth (such as responses to stimuli and respiratory rate changes), and confounds assessments of whether the animal is experiencing pain or distress. In animals, acute stress is believed to be a consequence of paralysis in a conscious state (Guide, p. 123). Further guidance is based on the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (pp. 88-91; hereafter referred to as the Red Book).

Without exception, the scientific need to use NMBs must be explained in the animal use protocol and approved by the IACUC. While the use of paralytics may be commonplace in human anesthesia, paralytics are not considered standard practice in veterinary medicine. The justification should include the specific physiologic response that NMBs will be controlling for.

This guideline offers direction on the following topics:
- Anesthesia
- Pilot Study
- Computerized monitoring
- IACUC Review/Literature Search
- Euthanasia
- Exemptions

ANESTHESIA
NMBs may be used only in a fully anesthetized patient. 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 involved with procedures using NMBs, the Principal Investigator (PI) must ensure adequate training and qualifications of those individuals who will participate in anesthetizing and monitoring the paralyzed patient. Therefore, the NMB protocol describing the maintenance of anesthesia and monitoring of the anesthetized patient will undergo strict review by the IACUC and ULAR veterinarians (see IACUC Review below).

Induction of anesthesia
After consultation with a ULAR veterinarian regarding agents, doses, and administration of the anesthetic and analgesics most appropriate for the proposed model, anesthesia may be induced as with any patient. The IACUC expects that, as is typical for any anesthetized animal, relevant notations should include the time, date (if appropriate), drugs or solutions administered, and the name or initials of the person making the entry.
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 these surgical procedures will be the (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, p. 88-89). 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 is under the effect of paralytics and unable to respond.

Monitoring the anesthetized patient
Prior to administering NMBs, depth of anesthesia must be established and verified and the physiologic stability of the anesthetized patient must be confirmed at a constant dose of anesthesia (Red Book).

In a paralyzed patient, standard 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). A number of physiologic measures are helpful in monitoring paralyzed animals. Description of 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 with guidance and recommendations from a ULAR veterinarian. Such signs are generally not adequate alone, may not be exhibited by all species, 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

**preferable for rodent assessments

At the very least, adequate ventilation must be provided, heart rate constantly monitored, and body temperature maintained and documented at least every 5-10 minutes during the procedure, as consistent with other Penn IACUC Guidelines.

Animals under anesthesia should NEVER be left alone. In an anesthetized, paralyzed patient, the anesthetist must be in proximity to the patient at all times, in other words, within direct visual contact and able to immediately respond physiologic changes in the patient.

When and if one is recovering the animal 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, p. 43). The IACUC expects that, typical for recovering any anesthetized animal, monitoring should continue and extra attention be provided until the animal is alert and mobile.
IACUC Guideline
USE OF NEUROMUSCULAR BLOCKING AGENTS

PILOT STUDY
"If paralyzing agents are to be used, the appropriate amount of anesthetic should first be defined on the basis of results of a similar procedure using the anesthetic without a blocking agent (Guide, p. 123).

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 and a ULAR or OLAW veterinarian must be present for the pilot experiments. 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 to those proposed for paralyzed animals. Pilot studies must be included in your animal use protocol and approved by the IACUC before they can be performed.

The minimum requirements for designing and performing a NMB pilot study are as follows:

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

2. 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.

3. 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 frequently than 5-15-minute intervals, consistent with the species relevant IACUC surgery guidelines. Additional monitoring modalities may be considered, based on specific animal models.

4. 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.

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

Following protocol approval, the complete anesthesia records, including the name of the ULAR or OLAW veterinarian, will be submitted to iacuc@pobox.upenn.edu and a designated IACUC member and ULAR veterinarian will review the results. The IACUC Chair or Vice Chair will, in writing, notify the PI of the authorization to continue using NMBs with the prescribed anesthesia regimen. Further use of NMBs may not continue until this authorization is granted. 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 and 3-year resubmissions.
IACUC Guideline
USE OF NEUROMUSCULAR BLOCKING AGENTS

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). Like the animal’s clinical record (maintained by ULAR), and animal use 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 at the end of the project (Red Book).

IACUC REVIEW
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 sufficient as a justification.

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 #12).

EUTHANASIA
NMBs may NEVER be used as sole agents for euthanasia (AVMA). Euthanasia methods that are deemed “acceptable” or “acceptable with conditions” must be undertaken (e.g. CO2, barbiturates, etc.) if animals are to be euthanized while under the influence of NMBs. The IACUC expects that the American Veterinary Medical Association Guidelines for Euthanasia of Animals (2013) (AVMA) will be followed.

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” request in ARIES and will be reviewed by the IACUC in consultation with ULAR veterinarians.

REFERENCES
American Veterinary Medical Association Guidelines for Euthanasia of Animals (2013) (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)
Public Health Service Policy (PHS Policy)