

Animal Care and Use Issues in Movement Disorder Research

Jeanne M. Wallace and Paul Sikoski

Abstract

Animal models of movement disorders can present special challenges for the research institutions that use them. Such models often affect the animals' ability to ambulate and perform normal body functions, and these potential effects on health and well-being mandate additional steps to ensure humane animal care and use. Indeed, the appropriate level of care for these models may call for actions that go beyond what is required or considered standard for other protocols. A proactive team approach to animal use protocol development and animal management is important. Through the commitment and involvement of the entire team—researchers, facility personnel, and institutional animal care and use committee members—research institutions that use these valuable models can ensure both the fulfillment of research objectives and the implementation of the best practices for animal care. Among the most commonly used animal models of movement disorder are models of stroke, brain and spinal cord injury, dystonia, Parkinson's disease, and Huntington's disease. Despite their relatively wide use, there is very little in the literature that describes the specific needs of individual models and the challenges those needs may present in today's regulatory environment. In this article, we discuss animal use considerations and provide the available animal care information on specific models. Interested readers are also referred to the additional information in the accompanying articles in this issue of *ILAR Journal*.

Key Words: animal models of movement disorder; animal welfare; dystonia; Huntington's disease; neurological injury; Parkinson's disease; tremor

Jeanne M. Wallace, D.V.M., DACLAM, is Assistant Vice Chancellor for Research and Director, Division of Animal Care at Vanderbilt University Medical Center, Nashville, TN. Paul Sikoski, D.V.M., is an Instructor in the Department of Pathology, Section on Comparative Medicine, and Assistant Director of the Animal Resources Program, Wake Forest University, Winston-Salem, NC.

Address correspondence and reprint requests to Dr. Jeanne M. Wallace, Assistant Vice Chancellor for Research and Director, Division of Animal Care, AA-6206 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232, or email jeanne.wallace@vanderbilt.edu.

Introduction

Humane animal care and use in biomedical research facilities are of the utmost importance, not only for regulatory and ethical reasons but also to ensure the generation of high-quality data. The performance-based recommendations in the *Guide for the Care and Use of Laboratory Animals (Guide*¹; NRC 1996) and the regulations (AWR 1985) set forth in the Animal Welfare Act (AWA)¹ describe expected levels of care for research animals. However, animal models of movement disorders can present special challenges for the research institutions that use them. Such models often affect the animals' ability to ambulate and perform normal body functions, and these potential effects on health and well-being mandate additional steps to ensure humane animal care and use. Indeed, the appropriate level of care for these animal models may call for actions that go beyond what is required or considered standard for other protocols. In this context, close communication and a team approach become essential for providing an appropriate level of care while meeting research objectives.

In this article we highlight some of the important considerations for researchers, institutional animal care and use committees (IACUCs¹), and animal resource programs that are or will be using animal models of movement disorders. Among the most commonly used animal models of movement disorder are models of stroke, brain and spinal cord injury, dystonia, Parkinson's disease, and Huntington's disease. A review of the literature reveals that despite their relatively wide use, there is a paucity of information on the specialized care that some movement disorder models require. In the text below, we provide the available animal care information on specific models, and we refer interested readers to the additional information in the accompanying articles in this issue of *ILAR Journal*. Excellent information of a general nature is also available in the National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (NRC Neurosci-

¹Abbreviations used in this article: AWA, Animal Welfare Act; *Guide*, *The Guide for the Care and Use of Laboratory Animals*; IACUC, institutional animal care and use committee; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NRC *Neuroscience Guidelines*, National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*; PD, Parkinson's disease; PHS Policy, Public Health Service Policy on the Humane Care and Use of Laboratory Animals; USDA, US Department of Agriculture.

ence Guidelines¹; NRC 2003). These *Guidelines* are designed to assist institutions in interpreting and applying current animal welfare laws, regulations, policies, and guidelines for neuroscience research. The information is directly applicable to movement disorder research, and we encourage readers to consult this text for additional guidance.

Animal Use Considerations

The IACUC is responsible for assuring all governing bodies that research involving animals complies with applicable laws, regulations, and guidelines, and that the care and use of animals is appropriate. The IACUC must evaluate every aspect of the proposed project to ensure that there is adequate forethought, planning, training, and resources to complete the project successfully. The importance of such an evaluation applies to all animal research but is especially true for research that involves the use of animal models of movement disorders. An active and involved IACUC is essential from inception to completion of projects that utilize these models. Available guidelines emphasize the need for a proactive and collaborative approach that involves researchers, veterinarians, and IACUCs (Klein and Bayne 2006; NRC 2003). Such an approach minimizes delays that may otherwise impede research productivity and/or cause unnecessary suffering of the animals involved, and it helps ensure data quality and integrity. The NRC *Neuroscience Guidelines* (NRC 2003) provide a helpful overview of protocol development strategies.

Before Protocol Submission

Before selecting a model, investigators should have a good understanding of the model and its appropriateness for the proposed research. The text *Animal Models of Movement Disorders* (LeDoux 2005) provides a comprehensive discussion of the use, selection, and limitations of animal models of movement disorders, as well as information on selected invertebrate and vertebrate species and their genetic, molecular, and phenotypic characteristics. In this issue of *ILAR Journal*, readers will find excellent reviews that assess the strengths and weaknesses of particular disease models. A number of reviews published elsewhere provide additional information on the established models used in this important area of research (Evinger 2005; Levine et al. 2004; Raike et al. 2005; Smeyne and Jackson-Lewis 2005; Traystman 2003).

Before submitting a protocol, researchers should consult with the institution's veterinarian and operations manager to ensure that the physical facilities and practices required to support the model are in place before procuring the animal(s). This consultation is an important opportunity for research and facility personnel to identify potential areas of concern and to reach consensus on how issues will be ad-

dressed. It is essential to clearly define roles and responsibilities at this time and to develop standard operating procedures if warranted. In writing the IACUC protocol, investigators should include a complete description of experimental procedures, any special care considerations, and deviations from animal care regulations and guidelines. It is important to provide scientific justification for any proposed deviations in animal care standards.

In cases that call for the creation of new animal models of movement disorders (e.g., genetically engineered mouse models), there may be little or no knowledge of the specific needs of a new model before procurement. Researchers must then make a reasonable effort to anticipate the special needs by having a good understanding of the human condition and associated complications. The protocol must include a complete description of expected outcomes and how they will be addressed. Given that some unintended consequences cannot be anticipated, it is important for researchers, animal facility staff, and IACUCs to be flexible and responsive when they encounter these situations.

Protocol Review

As for all proposed use of animals, IACUCs must ensure the fulfillment of federal, state, and local regulations and guidelines during protocol review and approval. The specific requirements for protocol review and approval are outlined in the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS Policy¹; NIH 1986), the AWA (PL 89-544 1966), and associated guidelines and regulations (AWR 1985; NRC 1996).

As for all experiments, IACUCs should ensure that a protocol provides appropriate justification for the number of animals to be used. This is especially true if a breeding colony will be maintained because some genetic models of movement disorders have disturbances or consequences that are potentially painful and distressful. IACUCs have an obligation to ensure the use not only of an appropriate number of animals to answer the experimental question but also of the minimum number of breeding animals to generate the experimental population. Appendices B and C of the NRC *Neuroscience Guidelines* offer helpful discussion and examples of methods for determining appropriate animal numbers for both experimental and breeding purposes (NRC 2003).

When studies involve the use of species that the United States Department of Agriculture (USDA¹) regulates, IACUCs must also ensure the placement (and ultimately reporting) of the animals in the appropriate pain/distress category. The USDA's Animal Care Policy Manual defines a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied" (APHIS 1997, p. 11.1). Historically, IACUCs have tended to focus on the experimental manipulation when considering which category is appropri-

ate, but for naturally occurring and genetically engineered models of movement disorders, the condition itself may dictate a particular category.

One of the most important considerations for IACUCs when reviewing protocols that call for the use of animal models of movement disorders involves the criteria for the removal or euthanasia of animals. These models have historically presented special challenges for both research and veterinary personnel in part because there is often only a slight difference between unrelieved pain/distress and the condition that is being studied. Approaches for establishing humane experimental endpoints are available in the literature (Morton 2000). The challenge for researchers, veterinarians, and IACUCs is to balance the humane treatment of the animals with the scientific goals of the study (NRC 2003). Researchers, veterinary personnel, and IACUCs must partner proactively to reach consensus on how to manage health issues. Consensus is essential for ensuring that animals receive the best possible care if/when a particular complication develops. In addition to the proposed endpoints, the IACUC protocol should include a complete description of the roles and responsibilities of both research and animal facility personnel.

As described in more detail below, animal models of movement disorders often require special husbandry and veterinary care procedures. IACUCs should consider these special requirements at the time of protocol review. Protocols that include deviations from mandated or generally accepted standards should provide a full description of the deviation(s) and scientific justification, which the IACUC must then approve. IACUCs must consider these models' unique requirements for both housing (e.g., alternative caging, exemptions from floor space requirements, exercise) and veterinary care (e.g., treatments, maintenance of bodily functions). A complete description of maintenance and monitoring methods for affected animals is essential. Depending on the frequency with which a particular model is used, IACUCs may need to develop written policies and procedures that outline expectations of care rather than address these issues on a protocol-by-protocol basis.

IACUCs must give special attention to the training and qualifications of research and animal care personnel who work with movement disorder models. All personnel involved must clearly understand the model and its special needs. Personnel should be trained to differentiate between "normal," "expected presentation," and "unexpected presentation," and to consult with others who have appropriate expertise when they observe deviations. Training must be comprehensive and also cover areas related to human health. For example, the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP¹) used in many Parkinson's disease (PD¹) models is toxic and has been linked to acute parkinsonism in exposed humans (Jakowec and Petzinger 2004). Although the effects of human exposure to MPTP are still not completely understood, this report illustrates the need for IACUCs to consider all potential risks and to ensure that personnel at all levels are trained to

manage the risks appropriately. IACUC members themselves must have a good understanding of the model and the research in order to make fully informed decisions if/when situations occur that require IACUC action.

Postapproval Monitoring

The IACUC's responsibility to ensure appropriate care and use does not end with approval of the protocol. IACUCs may conduct semiannual program reviews and facility inspections to ensure that what is happening at the cage level is consistent with what was approved. The special challenges that animal models of movement disorders present may require increased monitoring by the IACUC or more intense review during scheduled semiannual inspections. The IACUC must take whatever steps are necessary to ensure that care and use provisions are adequate and suitable for the animals. It is also essential for the IACUC to pay special attention to ensure that the pain/distress category (for USDA-regulated species) is suitable, and that the humane endpoints described in the original protocol are appropriate and consistently used. If there are deviations, the IACUC must take corrective action or confirm that the protocol is revised to reflect current practices.

Reporting Requirements

When the model involves a USDA-regulated species, the IACUC must ensure that animals are tracked and reported in the appropriate pain/distress category. In some instances, there may be different opinions among committee members about how to classify certain models, and observation of the animals may be necessary to reach consensus. It is important to document and report minority views. For models/experiments classified as Category E, additional information must accompany the USDA annual report. Specifically, "animals counted and listed in Column E must have a detailed statement explaining the procedure(s) and the basis for withholding pain-relieving medications" (APHIS 2006, p. 17.3).

The annual report to the USDA (APHIS 2006) must include exceptions to USDA regulations or standards. Examples of such exceptions include but are not limited to exemptions from exercise requirements for dogs, exemptions from the nonhuman primate plan for environmental enrichment, and food presented on the floor of a regulated rodent cage. The IACUC must consider any exceptions at the time of protocol review. Thereafter, the annual report must, at a minimum, include a written summary that identifies and describes the IACUC-approved exception(s) and lists the species and number of animals affected (APHIS 2006).

Depending on the model and the specific circumstances, additional reporting requirements may apply. It is important for IACUCs to have accurate database management systems

in place in order to reliably track and retrieve information on these protocols. IACUCs must also have effective monitoring programs to ensure the reporting of noncompliance consistent with regulatory requirements.

Animal Care Considerations

The challenge of the *Guide*, as well as the AWA and associated regulations, is that these resources provide only the minimum acceptable standards, requiring professional judgment when the minimum is not sufficient. For many research protocols, the minimum standards are more than adequate for proper animal care; however, some protocols require care above and beyond the minimum standards. Establishing which protocols require a different level of care and what level of care is appropriate is often difficult, and one must refer to the scientific literature and to accepted standards of veterinary medical care to help define appropriate care. Animal models of movement disorders often require a higher level of animal care than the minimum standards state. This is not true for all aspects of animal care, or every model, but most movement disorder-related protocols require specialized care both to ensure animal welfare and to provide quality data.

There are many factors to consider when caring for animals, including the animals' environment, access to food and water, facilitation of normal biological processes, environmental enrichment, social housing, and appropriate veterinary care and monitoring. It is also necessary to consider the species and experimental procedures because interspecies differences and variations in procedure vastly affect the level of care that may be required. We summarize below some of the animal welfare considerations associated with animal models of movement disorders. We also refer readers to the discussion of general animal care considerations associated with a variety of neurological disease models in the NRC *Neuroscience Guidelines* (NRC 2003).

Environment

Countless parameters of an animal's environment require monitoring and maintenance at a standard that accommodates normal physical, biological, and social processes. These parameters can be as simple as maintaining the correct temperature and humidity for the species housed, or as complicated as providing an adequately enriched social environment. Temperature, humidity, noise levels, and illumination are relatively static parameters for which straightforward guidelines are available. There is usually no need to adjust these parameters, but there are some instances in which body temperature fluctuations require environmental temperature interventions. For example, a mouse model of Parkinson's disease in which investigators inject mice with the neurotoxin MPTP (Przedborski et al. 2001) causes a drop in body temperature that can lead to acute death unless

the environmental temperature is elevated using an external heating device such as a water blanket. Other models require surgical procedures after which thermal support of some kind can often aid surgical recovery.

Caging

The size of an animal's caging is normally straightforward and established by regulations or guidelines. However, specific research protocols may require deviations from these standards. Animals that undergo spinal cord injuries have paralysis or paresis of multiple limbs, making movement in the cage difficult. It might be important to increase the cage size to better facilitate the animal's movement, or decrease it if the movements are at an uncontrolled level that could lead to injury. When research with monkeys involves the use of MPTP to create a model of PD, it can often leave the animals disoriented (NRC 2003). In such cases it is prudent to house the animal in a cage versus a large indoor/outdoor pen that requires navigation. It is also appropriate to consider experimental measurements in models of PD. Activity monitoring of animals is used to measure the level of hypokinesia, which is a common feature of PD (Emborg 2004). Altering the cage size of an animal could easily influence the animal activity level and have a subsequent effect on research data. Researchers, IACUCs, and laboratory veterinarians should work together to establish the best environment for the animal while accomplishing the research goals and maintaining regulatory compliance.

Cage complexity and environmental enrichment are usually important for the psychological well-being of all species housed in the laboratory. Depending on the research, though, it may be necessary to alter the normal practices used to enrich an animal's environment. Monkey cages should have elevated perches or some means to utilize vertical space, but perches may be contraindicated in PD models, spinal cord injury models, and other models that result in motor impairments. Reduction of vertical space and removal of perches could prevent a fall and subsequent injury. Withholding other forms of environmental enrichment (e.g., nesting boxes or tubes) from rodents with movement disorders may be necessary so that the animal does not become trapped in such devices. There are reports of environmental enrichment that attenuates the effects of MPTP on mice (Faherty et al. 2005; MacNeil 2004), thus establishing experimental justification to withhold enrichment. Conversely, for many behavioral tests and manipulations used in rodent PD models, there are no reported effects of environmental enrichment on behavioral outcomes (Würbel et al. 2005). Clearly, it is important to consider the potential for enrichment to produce unintended consequences for laboratory animals and research results (Bayne 2005). The best advice when it comes to enrichment is to be consistent and objective in decision making to balance the research outcomes with the psychological health of the animal.

All cages must be free of sharp edges, keep the animal

clean and dry, and be secure and safe. The primary enclosures used to house animal models of movement disorders may have additional requisites. For example, it may be necessary to pad cage edges for monkeys used in PD studies because these animals' lack of coordination may make them more prone to injury on edges that would normally be considered benign. Padding may also be necessary for monkey models of spinal cord injury, which results in severe impairment of locomotion and causes animals to remain sedentary for long periods of time. To some degree, padding can also decrease the incidence of decubitus ulcers, which are common sequelae (Santos-Benito et al. 2006). Padding is not as practical for rodent models of spinal cord injury, but the use of softer, more absorbent bedding substrate, as well as more of the substrate, may help decrease the sequelae of spinal cord injury.

One of the greatest challenges in accommodating animal models of movement disorder is fulfilling sanitization requirements while providing an appropriate environment that meets the specialized needs of the model. There are guidelines that govern the frequency of sanitation, but there is also the expectation that cleaning will be done as frequently as is necessary. For example, for paraplegic monkeys that may soil themselves without being able to move to a clean part of the cage, it may be necessary to sanitize the cage multiple times a day (Santos-Benito et al. 2006). For paraplegic animals that require padding, it may be necessary to wrap the padding in a smooth, impermeable liner so that it can be appropriately sanitized (Santos-Benito et al. 2006). In contrast, some experiments using rodent models to study PD involve extensive behavioral testing. The cages of these animals might require less frequent sanitization to avoid the potential of confounding behavioral outcomes.

In each instance, one must balance the experimental needs with the guidelines and welfare of the animal. It is essential to establish policies and procedures that minimize cage disturbances while effectively keeping the animal's environment clean. For rodents, it is possible to accomplish this objective by adjusting the bedding substrate so that it is very absorbent and by increasing the air changes, if it is a ventilated rack cage, to remove excess ammonia and moisture from the cage.

Access to Food and Water

All animals should have daily access to water and food that is palatable, free of contaminants, and nutritionally adequate (NRC 1996). If an animal has trouble accessing food and water due to the experimental procedures it has undergone, it may be necessary to provide access in multiple ways. Monkeys receiving MPTP usually require supplemental feeding and watering immediately after the drug is administered (personal communication, M. Emborg, University of Wisconsin, Madison, November 2006). Rodents that have normally been fed and watered from an elevated position may need after spinal cord injury to be fed and

watered on the floor of the cage. If animals are group housed and have varying stages of the disease or differing severities of lesions, it is necessary to make provisions to allow all of the animals to have adequate access to food and water. Such cases may require temporarily separating the animals, hand feeding animals in advanced stages of the disease, or placing food in multiple locations in the cage.

Experiments that utilize animal models of movement disorders often include behavioral conditioning and/or tests of motor function to assess baseline deficits and response to therapy. It may be necessary to use food or water regulation to sufficiently motivate an animal. In studies involving food regulation, it is important to monitor body weight at regular intervals and it may be appropriate to supplement vitamins in an animal's diet to ensure adequate nutrition for growth or maintenance. Another instance in which food restriction may be used is when a specific body weight range is required for experimental purposes. In rat spinal cord injury studies, keeping animals at a defined body weight is sometimes necessary to compare the motor improvement achieved by different animals (Santos-Benito et al. 2006). There are also some instances of required water regulation. Importantly, limited access to water usually indicates more frequent measurement of body weight than the regulation of only food. Blood chemistries and urine-specific gravity may also be indicated to monitor animals for excessive dehydration and kidney damage. In some instances, it may be important to restrict access to water for reasons related to animal health. For example, in the monkey spinal cord injury model, early stages after injury often leave the animal unable to micturate properly. During the day it may be possible to express the bladder multiple times; however, at night the frequency with which the bladder is expressed is typically reduced. Limited access to water during evening hours is an effective method to prevent bladder distension until the animal regains some urinary function (Santos-Benito et al. 2006).

Veterinary Medical Care

Veterinary medical care is a legal requirement for all animal research, and different experimental protocols require varying levels of attention from the veterinary staff. Spinal cord injuries require intensive veterinary care, including bladder expression multiple times a day for several weeks (Golding et al. 2006; Santos-Benito et al. 2006). In addition, it may be necessary to evaluate the urine daily with test strips for early indications of inadequate hydration or voiding (Santos-Benito et al. 2006). Other examples of medical care that may be required for study animals include abdominal massage to facilitate intestinal motility, anticoagulant therapies to avoid thrombosis and pulmonary embolism, and daily massage of bony prominences to reduce ulcer formation (Santos-Benito et al. 2006). Peripheral nerve regeneration studies in rats and rabbits may result in self-induced trauma, loss of joint mobility, decubitus ulcer formation, and de-

creased grooming habits (P.S., personal experience). Thick bandages made of a durable material can cushion and protect the area to prevent ulcer formation and reduce self-induced trauma. Commercially available Elizabethan collars are effective for protecting rabbits from self-directed trauma. General supportive nursing care is also frequently a necessity after experimental manipulations in PD and stroke models (NRC 2003).

Summary

Animal models of movement disorders often present unique challenges for research institutions. When these models are used, IACUCs, researchers, and veterinarians must establish a culture of respect for the animals and maintain a commitment to follow established guidelines (e.g., the Guide [NRC 2003], PHS Policy, and the AWA). Although helpful information of a general nature is available (LeDoux 2005; NRC 2003), there is very little in the literature that describes the specific needs of individual models and the challenges those needs may present in today's regulatory environment. We advise consultation with research and veterinary personnel already familiar with a particular model. It would also be helpful for researchers and veterinarians to partner with each other to publish the special care considerations associated with the more commonly used models of movement disorders. The recent article by Santos-Benito and colleagues (2006) serves as an excellent example of the type of publication that is of benefit to the research community.

A proactive team approach to animal use protocol development and animal management is important for all types of research, but is essential when investigators use animals in neuroscience and behavioral research (Klein and Bayne 2006; NRC 2003). It is through commitment and involvement of the entire team—researchers, facility personnel, and IACUC members—that institutions using these valuable models will ensure both the fulfillment of research objectives and the implementation of the best practices for animal care.

References

- APHIS [Animal and Plant Health Inspection Service]. 1997. Animal and Plant Health Inspection Service Animal Care Policy Manual. Policy No. 11. Washington DC: US Department of Agriculture.
- APHIS [Animal and Plant Health Inspection Service]. 2006. Animal and Plant Health Inspection Service Animal Care Policy Manual. Policy No. 17. Washington DC: US Department of Agriculture.
- AWR [Animal Welfare Regulations]. 1985. Code of Federal Regulations (CFR), Title 9, Volume 1, 9 CFR 3.6. Animals and Animal Products: Chapter 1. Animal and Plant Health Inspection Service, Department of Agriculture. Washington DC: USDA.
- Bayne KA. 2005. Potential for unintended consequences of environmental enrichment for laboratory animals and research results. *ILAR J* 46:129-139.
- Emborg ME. 2004. Evaluation of animal models of Parkinson's disease for neuroprotective strategies. *J Neurosci Methods* 139:121-143.
- Evinger C. 2005. Animal models of focal dystonia. *NeuroRx* 2:513-524.
- Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ. 2005. Environmental enrichment in adulthood eliminates neuronal death in experimental parkinsonism. *Brain Res Mol Brain Res* 134:170-179.
- Golding JD, Rigley MacDonald ST, Juurlink BH, Rosser BW. 2006. The effect of glutamine on locomotor performance and skeletal muscle myosins following spinal cord injury in rats. *J Appl Physiol* 101:1045-1052.
- Jakowec MW, Petzinger GM. 2004. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned model of Parkinson's disease, with emphasis on mice and nonhuman primates. *Comp Med* 54:497-513.
- Klein HJ, Bayne KA. 2006. Establishing a culture of care, conscience, and responsibility: Addressing the improvement of scientific discovery and animal welfare through science-based performance standards. *ILAR J* 48:3-11.
- LeDoux M. 2005. Animal models and the science of movement disorders. In: LeDoux M, ed. *Animal Models of Movement Disorders*. New York: Elsevier Academic Press. p 13-31.
- Levine MS, Cepeda C, Hickey MA, Fleming SM, Chesselet MF. 2004. Genetic mouse models of Huntington's and Parkinson's diseases: Illuminating but imperfect. *Trends Neurosci* 27:691-697.
- MacNeil JS. 2004. Improving the lives of laboratory animals: No easy task for mice or men. *The Scientist* 18:43.
- Morton DB. 2000. A systematic approach for establishing humane endpoints. *ILAR J* 41:80-86.
- NIH [National Institutes of Health]. 1986. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Washington DC: US Department of Health and Human Services.
- NRC [National Research Council]. 1996. *Guide for the Care and Use of Laboratory Animals*. Washington DC: National Academy Press.
- NRC [National Research Council]. 2003. *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. Washington DC: National Academies Press.
- PL [Public Law] 89-544. 1966. *Animal Welfare Act of 1966*. Washington DC: USDA.
- Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M. 2001. The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): A technical review of its utility and safety. *J Neurochem* 76:1265-1274.
- Raike RS, Jinnah HA, Hess EJ. 2005. Animal models of generalized dystonia. *NeuroRx* 2:504-512.
- Santos-Benito FF, Muñoz-Quiles C, Ramón-Cueto A. 2006. Long-term care of paraplegic laboratory mammals. *J Neurotrauma* 23:521-536.
- Smeyne RJ, Jackson-Lewis V. 2005. The MPTP model of Parkinson's disease. *Brain Res Mol Brain Res* 134:57-66.
- Traystman RJ. 2003. Animal models of focal and global cerebral ischemia. *ILAR J* 44:85-95.
- Würbel H, Lipp HP, Nitsch RM, Wolfer DP, Morf S, Litvin O. 2005. Environmental enrichment does not disrupt standardization. 3R Research Foundation. Available online <http://www.forschung3r.ch/fr/publications/bu30.html>, accessed January 3, 2007.