Experimental Surgical Models *in the* Laboratory Rat

Edited by

Alfredo Rigalli
Verónica Elina Di Loreto

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Preface

This book is the direct consequence of many years of work in the surgical theatre where assorted techniques were developed or reproduced in the study of pharmacokinetics and pharmacology of fluorine-containing compounds, diabetes, and pancreatitis. The experience and knowledge accumulated through decades of work and the start of my doctorate career in Biomedical Sciences, where surgical experimentation with rats is one of the disciplines, brought about the writing of this book, which actually began in 1997.

Since then, the compilation of surgical technique descriptions has been an ongoing endeavor and included the help of many associates. Contributors to this book are researchers, professors, and students from the Bone Biology and Mineral Metabolism Laboratory, School of Medicine, Rosario National University; from the Pharmacology Division, Biochemical and Pharmaceutical School, Rosario National University; from the Institute of Cardiovascular Pathophysiology, School of Medicine, Buenos Aires University; and from the Pharmacology Division, School of Veterinary, La Plata National University, all in Argentina. Contributor chapters are based on surgical models developed or reproduced in the course of their research projects.

The main objective of this book, *Experimental Surgical Models in the Laboratory Rat,* is to contribute to the postgraduate studies of researchers in the biomedical area and in the study of the mechanism of action and the efficacy of drugs in different pathologies.

The election of an experimental model is the crucial point in a research project. The knowledge of where this model can be used, how it can be done, and when it can produce valuable results is not always clear for the researcher in the beginning. Once a model has been chosen, the aid of a teacher is important and can help reduce the optimization of the model, a process that usually takes months or years. The development of the methodology includes the correct selection of materials and procedures, the environmental conditions, the sex and strain of animals, the diet, and the personnel to take care of animals. The correct choice of all of these requirements will be crucial for obtaining reliable and reproducible results. This book provides important details that are not always included in the journals where the techniques are published. Sometimes small details are omitted, which can often make the difference between the success or failure of an experiment. It is important to notice that a failed experiment often implies the useless sacrifice of animals. An animal researcher has a moral obligation not to uselessly cause animal deaths without producing results.

Experimental Surgical Models in the Laboratory Rat is organized in sections, each of which contains a definite subject. All chapters are organized with a short introduction and the utility of the technique, the list of materials needed for performing the surgery, a step-by-step description of the surgery, and the precautions and experimental results obtained by the authors. Each chapter also includes detailed figures, which are complemented with sequential photographs of the surgery on a CD that accompanies this book. In the case that contingency plans are available, they are also described as well as the combination with other procedures.

The book contains a vast list of updated references where the theoretical bases of the models are described. Included as well are numerous journal articles where most of the results obtained with the models are included. Normal values of weight, food, and water consumption as well as some common biochemical parameters are included. These values come from an eumetabolic rat, which is no different than the recognized strains, such as Wistar and Sprague Dawley rats. Although there are a wide range of instruments available to assist in the surgery, a chapter explaining simple devices is also included.

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Abbreviations

APSB	alkaline phosphatase substrate buffer
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BSA	bovine serum albumin
CNS	central nervous system
DMF	dimethylformamide
°C	degree Celsius
h	hour
hrGH	human recombining growth hormone
i.g.	intragastric
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IU	international unit
l	liter
KRB	Krebs–Ringer buffer
KRBB	Krebs–Ringer bicarbonate buffer
MFP	sodium monofluorophosphate
ml	milliliter
mM	millimole/liter
NAD^+	nicotinamine adenin dinucleotide
OVX	ovariectomy
PAH	p-aminohippuric acid
p-NPP	p-nitrophenylphosphate
p.o.	oral
PTH	parathyroid hormone, parathormone
RIA	radioimmunoassay or radioimmunoanalysis
s.c.	subcutaneous
SD	standard deviation
SEM	standard error of the mean
STZ	streptozotocin
TPTX	thyroidparathyroidectomy
w/v	weight per volume
wt%	weight percent

Section 1

Introduction

1 Bioethics and Animal Care

Lucas R. M. Brun and Verónica E. Di Loreto

INTRODUCTION

The use of animals with medical and biological objectives has been practiced for centuries. Areas of biomedical research, such as pharmacology, physiology, and toxicology, have based their progress in experiments carried out mainly in animal models or in the accurate observations of spontaneous phenomena in animals. Advances in human health conditions are, in part, the result of the knowledge of biological processes, which were first understood in animal models. Laboratory animals have also contributed in the development of vaccines, methodology for the diagnosis of different illnesses, identification of pharmacological target cells or molecules for new pharmaceutical products, organ transplantation, grafts, and much more.

Science has developed the model alternatives that do not involve animals, such as cell culture, mathematical models, computational simulation models, *in vitro* experiments with cell-free systems, etc. The question is: Is research with animals necessary? And it remains without a proper answer because it is influenced greatly by religion, human behavior, age, feelings and more. Although the question remains unanswered, there is agreement among scientists that even the more sophisticated technologies cannot reproduce the complexity and interactions among cells, molecules, tissues, and organs, which take place in a multicellular organism. However, these isolated systems provide information that cannot be obtained from intact animals because of their high complexity.

The results obtained in animal models provide invaluable information for the design of tests for new pharmacological products in human beings. The test of a new product in human beings must begin by experimenting on intact animals; this is apart from the tests in other models, such as cell culture. As a consequence, research with animals is an obligation in biomedical sciences. According to the Nuremberg Council, the tests carried out in human beings must be designed carefully and must take into consideration the results previously obtained in animals. The Declaration of Helsinki, which was adopted in 1964 by the World Medical Association, also indicates that the research in human beings must be designed based on results obtained with animals. Therefore, biomedical science is impossible without research with laboratory animals. However, the use of animals needs to be justified; it must follow national and international rules and interdisciplinary committees must evaluate the procedure where the experimental design is considered for both scientific and ethical principles.[1]

Along with the scientific knowledge gained from the use of laboratory animals in research, laws have been established that state principles for the use of animals as experimental models. Institutional animal care and use committees were created around the world to control the use of laboratory animals in research and for educational purposes. However, there are countries without this kind of legislation or where it is only now coming into existence.

The International Council for Laboratory Animal Science (ICLAS) is a nongovernmental organization for international cooperation in laboratory animal science.[2] This council's mission's is the advancing of human health by promoting the care and ethical use of laboratory animals. The first ICLAS meeting for harmonization of guidelines was held in 2004. One of the aims of this meeting was to obtain consensus from the most important organizations that deal with the care and ethi-

cal use of laboratory animals to develop guides of animal care. The last harmonization guidelines update was published in 2007.

There are important organizations around the world that regulate and produce guides for the use and management of laboratory animals, such as the Federation of European Laboratory Animal Science Associations (FELASA),[3] the American Association for Laboratory Animal Science (AALAS)[4], and the Canadian Council on Animal Care (CCAC).[5]

THE 3 Rs

The concept of the 3 Rs was established by Russell and Burch[6] in 1959. Since then, a number of changes have taken place in the use and management of laboratory animals in both research and educational objectives. The principle of the 3 Rs proposes the sensible and humanitarian use of animals in scientific work, and the aim is to guarantee the rational and respectful use of experimental animals, **reducing** the number of animals through the correct choice of genetic and environmental conditions, **replacing** the animal by another model when possible, and **refining** procedures in order to minimize stress and pain, but guaranteeing the validity of the results.

The alternatives of **reduction** describe methods to obtain valuable results from experiments carried out with few animals, such as the election of the correct animal model, pilot studies, correct design of the experiment, adequate statistical tests, sanitary quality, genetic and environmental qualities, etc. The correct and efficient bibliographic searching prevents the duplication of information and the realization of unnecessary experiments. The **replacing** alternatives refer to other methods that obtain the same results, but without involving animals, such as *in vitro* systems, cell culture, mathematical models, simulators, human materials, etc. The alternatives of **refining** add methods that alleviate or minimize pain, stress, anxiety and fear, and maintain the welfare of the animal. Refining relies on the knowledge and ability of trained personnel who deal with the animals and who have the capacity to detect pain and discomfort, use the appropriate analgesic and anesthetics, use mini- or noninvasive techniques, and use the correct choice of euthanasia. Refining in techniques produces better results and lower variability. For example, new anesthetics together with training in surgical techniques certainly reduce the number of deaths in the anesthetic procedure. In the same vein, knowledge about statistics and design of experiments contributes to the choosing of the adequate model and test without losing important information.

The researcher must act responsibly in order not to repeat experiments that have already been done. Only those experiments relevant and pertinent for scientific knowledge or the well-being of the community should be accepted.

The researcher must know when the results of the experiment are less important than the suffering and pain of the animals, and euthanasia must be performed even though the results at the end of the experiment are important. So, analgesia and euthanasia play an important role in the end point of an experiment. The welfare of animals must supercede results and conclusions of the experiment. On the other hand, when an animal is not in good health, the intake of food and water is dramatically reduced, resulting in dehydration and multiorganic failure. As a consequence, experiments where samples are obtained only until the animal's death can give distorted results. The end point of an experiment must be defined before the experiment is carried out and should be evaluated through biochemical parameters, behavior of the animal, the model of the illness, and the treatment. An investigator faces the premature end point of an experiment when the animal has alterations in its behavior for reasons not related to the experiment that modify the expected results, when there is unnecessary suffering and data will not benefit the project, and when the decay in health of the animal causes invalid results. The correct decision about the end point of one experiment suggests that there be permanent monitoring of the animal throughout the experiment to establish knowledge of the behavior and suffering of the animal. The sensible choice of the time for the end point of an experiment, instead of reducing the data of the experiment, will produce better results for supporting the hypothesis or enunciate new ones.

ETHICAL PRINCIPLES OF RESEARCH WITH ANIMALS

Basic ethical principles concerning human health state the importance of knowledge in biology and medicine. As a consequence, experiments involving animal models are necessary. However, the use of animals must imply respect and be humane. Therefore, those who carry out experiments with animals must know that animals also have senses, memory, and are susceptible to pain and suffering.

The researcher is responsible for his actions in the context of a research project; therefore, experiments carried out on animals must be done by qualified researchers or controlled by them. The conditions in which the animals are maintained throughout the experiment must be defined and controlled by a veterinarian or by a competent scientist. The experiment must involve the species of animals that can better adapt to the experiment, and the sensorial and psychological properties of the species involved is also an important factor to be considered before choosing the animals.[7] The investigator must care about the experimental condition of the animal and give necessary help in order to avoid physical and psychological suffering. Furthermore, projects that involve animals must contribute to the knowledge of human health or the well-being of animals or human beings.

From the concepts stated above emerge the principle that working with animals is not a right, but a privilege. Researchers who are involved in the experiments, whether they are assistants or are in charge of the project, must incorporate this principle into their thinking. Because animals lack autonomy and have no choice about participation in an experiment, the researcher must not abuse this privilege.

Although it is possible that animals do not suffer pain in the same way as humans do, there is no reason to suppose that animals do not feel pain and suffer as a result of it.[8]

Abnormal behavior, movements, and postures are signals of pain in experimental animals. Other signs, such as aggressiveness, salivation, unusual sounds, facial expression, etc., are also indicators of pain and suffering. Therefore, the person who deals with experimental animals must be aware of the animal's normal behavior, and also have the ability to detect the minor signs of stress, pain, and suffering.

In summary, the researcher must consider that the well-being of animals is as important as the results of the experiment, and he has an obligation to reduce all the possibilities of suffering pain and stress in the experimental animal.

Animal Well-Being

Well-being is a term based on the human perspective, virtues, and ethical values. However, the well-being of animals involves the absence of pain and stress.[9] The animal needs an appropriate environment for its normal behavior, which can be affected by the animal's senses and perception. In summary, animal well-being is an internal state involving quality of life that is affected by the responses to internal and external stimuli, which may or may not be aversive. It is necessary to establish rules for the care and breeding of animals that will cause the least stress on them. These rules must include all aspects of a normal life for the animals, such as nutrition, housing, feeding, treatment and prevention of illnesses, anesthesia, analgesia, and, when necessary, euthanasia. For example, the stated purpose of the *Guide for the Care and Use of Laboratory Animals* "is to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate."

The researcher who deals with experimental animals must be knowledgeable about conditions in the area where the animal is housed and where the experiments are carried out. Experimental conditions must be carefully controlled in order to obtain standardized responses. In this way, a smaller number of animals would be involved in the experiment. In addition, the results would be comparable with those from other laboratories around the world. The environmental conditions that must be controlled include:

1. Climate: temperature, humidity, ventilation
2. Physical–chemical: light, sound, presence of contaminants, composition of air, light–dark cycle
3. Rooms: shape, size, number of animals per cage
4. Nutrition
5. Microorganisms and parasites
6. Transport

In regard to the transport of animals, there must be minimum stress on the animals and the travel must not have an impact on their well-being. In addition, they must be secure and comfortable. The stressors on the animals as they travel can be physical (changes in temperature and humidity, sounds, etc.), physiological (access to water and food), and psychological (exposure to new individuals or environments). The effects of stressors are acute and can remain for several days. Acclimation to the new container where the animal will be housed can contribute to the decrease in stress.[10]

In addition, in order to establish universal principles in the practices of ethical care of experimental animals, categories of discomfort to the animal during experimental conditions have been established:[11]

1. Minor discomfort: collection of blood samples, collection of urine in metabolic cages, treatment with drugs in the drinking water, housing in cages in order to observe normal behavior, administration of substances, experimentation with anesthesia and vaccines.
2. Moderate discomfort: frequent sampling of blood, catheterization and intubation, recuperation from general anesthesia as well as immunization with complete adjuvants, cannulation, and recovery from general anesthesia.
3. Severe discomfort: extraction of ascitic fluids, obtaining large volumes of blood without anesthesia, induction of genetic defects, starvation, periods without *ad libitum* access to water, perturbation during periods of sleep, infections, fractures, diabetes, pancreatitis, and renal failure.

A scale of invasiveness of experimental procedures has also been established. It allows the researcher and the ethical committee to evaluate the necessity of special training before the experiment is carried out, to establish standardized operational procedures, to choose from alternative techniques and procedures, and to accompany and supervise the experiments. All of these topics must be evaluated and approved before the experiments can be carried out.

Category	Procedure
A	Experiments with invertebrates, cells or isolated tissues
B	Experiments that cause no stress or minimal discomfort
C	Experiments that cause minimal stress or short duration pain
D	Experiments causing moderate to severe stress or discomfort
E	Procedures involving severe pain in conscious or nonanesthetized animals

This ranking is not only limited to surgical procedures, but it can also include other situations, such as noxious stimuli or agents whose effects are unknown, exposure to drugs or chemicals, behavior studies, nutritional experiments, etc.[10]

CONCLUSION

Although biomedical science research not involving animals is almost impossible, the researcher has a moral obligation to respect the life of the research animal. Apart from the specific objectives of the project, the researcher must avoid unnecessary pain and create the best conditions for housing, sampling, and euthanasia, if necessary. The international harmonization of biological assays is

a major effort that is necessary in the research world and animal well being has to be a central issue in current times.

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