Part I

Principles and general methods

1

Introduction: Animal modeling – a precious tool for developing remedies to neurological diseases

TURGUT TATLISUMAK AND MARC FISHER

Human beings owe a great deal to animals. From the earliest periods of history of mankind, animals have been used by humans for food, clothing, tool making, and for several other purposes. Primitive artists painted animal figures onto stone surfaces; animal figures became parts of religions and tribal identities. Over time, some animals were domesticated, serving as regular sources of meat and milk; additionally, animals were used in farmwork and for transport. Dogs were used to defend property and were trained for rescue missions. Cats were used as pets as early as the ancient Egyptian Kingdom. Interesting additional missions have been given to animals such as searching for illicit drugs, explosives, and mushrooms. Some areas where we are still strictly dependent on animals include the drug industry (e.g., insulin isolated from swine pancreas), but there are also areas subject to intense debate (e.g., fur farming, fox hunting, and the cosmetics industry).

We are very much dependent on animals in medical research and in clinical surgery training. Neurological diseases comprise a major health problem all over the world and their importance continues to grow as the population ages and as neurology moves from being largely a diagnostic field to one with more therapeutic approaches. Neurological diseases already absorb approximately one-fourth of health budgets in industrialized countries. It is urgent to develop novel effective therapies for neurological diseases: the aging of the population will increase the number of neurological patients whereas the labor force available in the health sector appears to be decreasing. Additionally, the burden of the neurological disease to the individual patient and their relatives is more dramatic than diseases of other organs. Many critically important discoveries regarding disease mechanisms and most therapies that are currently being used for neurological diseases have been developed in animal models, and the need for animal models is expanding.

Use of animals for scientific purposes has a long history. One animal dies for scientific reasons every second in the USA and every three seconds in the

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European Union. Whereas total medical research has expanded several-fold over recent decades, the number of animals used for scientific purposes has remained the same or even slightly decreased in absolute terms, and substantially decreased in relative terms. A Medline search with the word "rat" gave 1 100 000 hits and "mouse" gave 770 000 hits (March 2005). It is easy to understand why the rat and the mouse are so popular in medical research. They breed easily, profusely, and continuously, have a short gestational period, are relatively inexpensive, and can be housed in large numbers in relatively small spaces; and their anatomy, physiology, biochemistry, genetical properties, and behavior are well described. Rats and mice are easy to handle and the size of their organs is suitable for staining, antibody generation, and many other research activities. Specimens from rats and mice can easily be stored for later studies. Furthermore, the rat and mouse are not generally considered as pets and their experimental use is more acceptable than other animals.

The "3 R" rule (Reduce, Refine, Replace) refers to the efforts to reduce the number of animals used in scientific experiments, to plan and perform the experiments in a way that decreases the suffering of animals, and to develop alternative methods to replace the use of live animals. Even though the aim is ethically well established, it is difficult to develop alternative approaches and some are unreliable. Although the ethics of using animals in research is not a new issue, standards remain to be established. It must be remembered that the very early ethical principles regarding the use of animals in scientific research were written with efforts initiated by scientists themselves in the 1830s, not by animal activists. Furthermore, animal experimentation is rather expensive. If alternative approaches were available, most researchers would readily abandon the use of animals. Interestingly, possessing a pet animal does not require any training, while fishing or shooting animals does not require more than a permit. Scientific use is strictly controlled, suitable facilities are required, extensive training is a must, and permission must be obtained. Anyone can take a pet animal to a veterinary physician for castration without permission of regulatory authorities, despite the fact that this is not necessary for the animal's health, alters its natural life course, and causes pain. To carry out a similar procedure for scientific reasons would require regulatory approval. The final target of animal experimenting is to develop remedies to cure human diseases. Therefore, it is never unnecessary to mention the importance of treating the experimental animals humanely as the source of scientific research is humane and it must be accomplished in a humane way.

We felt frustrated with the difficulty of finding even the most fundamental information in a centralized source for animal experimentation and to collect information from a large number of articles some of which were published

in journals that were difficult to access and was time-consuming. Therefore, we decided to centralize the joint effort of over 60 universally well-recognized scientists and cover most major issues about neurological animal methodology under one cover. This is a first-of-its-kind book in its comprehensiveness. The methods included in this book may improve animal welfare, decrease the number of animals required, and may increase the quality of experiments. Success in animal experiments and the reliability of the results largely depend on the proper use of techniques including proper handling of the animals, suitable anesthesia and analgesia, clean and least-damaging surgery, and use of most appropriate models and techniques. Failure in following the crucial steps in animal experimenting will lead to unreliable results and unnecessary suffering of animals and sometimes of the researcher when he/she is bitten.

This book comprises 30 chapters and is divided into two parts. The first part of this book deals with general principles and methodology, whereas the second part delivers comprehensive data on animal models of individual neurological disorders. In each disease chapter, the authors first discuss the magnitude of the problem in epidemiologic and economic dimensions followed by detailed and critical information on present models. References are generally limited and readers who are interested in more in-depth knowledge are encouraged to further explore the references listed in each chapter or directly contact experts in that field. This book is planned to deliver a broad view to young neuroscientists, but may even be useful to more experienced scientists. Animal modeling deals mostly with rodents and with other animal species where appropriate.

We are grateful to all the authors for their contributions and the time spent in preparing them. Even though we engendered our best to cover all major neurological experimental issues in this book, space limitations led to compromises. We hope that scientists will benefit from this book. Feedback from readers is most welcome. It is of our mutual interest that, in the long run, these animal models can be replaced with novel technologies that are at the same time ethically more acceptable and scientifically more reliable, making new editions of this book unnecessary.

2

Ethical issues, welfare laws, and regulations

DAVID WHITTAKER

2.1 Introduction

The ethics, morals, and laws of any culture or nation are intimately interwoven and dependent upon each other for their continuation within that society. At the same time most cultures are under continual evolution, change, and development due to many factors, but usually due to ingress, influences, and pressures from other external factors and cultures. This is best illustrated by the notion that "developed" nations frequently bring about cultural changes in very old "traditional societies" through their presence and financial impact. Once there is cultural change, then almost certainly it will be followed by changes in the ethical and moral stances taken. Ultimately the laws and regulations will no longer reflect or uphold the current "values" of that society and will need modification.

This point is made to emphasize the fact that ethics and morals are not only diverse in a global sense but also dynamic. What was once ethically acceptable in history (e.g., slavery) may now be locally or globally seen as morally wrong and laws enacted to reflect those views.

With regard to laws it must also be acknowledged that as the "global village" becomes an increasing reality so does a meeting of minds on points of ethics and morals. As the meeting of minds becomes a reality, then it is possible to develop and implement international laws and regulations. This is particularly pertinent when considering the European position with regard to the Council of Europe (CoE) and the European Union (EU).

Finally in this introduction it must be emphasized that the roots of ethics and moral principles lie in the considerations and writings of the great philosophers. It is not the intention of this paper to concentrate on the history and theories which consider the rights and wrongs of animal experimentation. However, consideration will be given to the more practical and pragmatic ethical considerations surrounding animal use.

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2.1.1 Ethical issues and considerations

There are probably three simple categories for those practically considering the ethical rights and wrongs of animal experimentation. They are:

- *All* animal experimentation (indeed man's use and killing of all animals) is wrong and therefore should be stopped, absolutely. This is the position of the abolitionists.
- It is ethically wrong to use animals for some *types* of experimentation. Easy examples here are the use of animals for testing cosmetics, alcohol, tobacco, and for offensive weapons research.
- It is ethically wrong to use certain *species* of animals for research, e.g., the great apes, lower-order primates, and perhaps dogs and cats.
- There is a fourth perspective, which is that of complete dominion over animals where any treatment of animals is acceptable without moral guilt. I believe that such a category of ethical consideration for all uses of animals is largely redundant.

In considering these views we can postulate that those falling within the first category do believe that all animals have equal rights to humans and should not be "exploited," simply because we have the physical and mental capacity to do so. People holding this view frequently draw analogies of animal exploitation with slavery or the possibility of using babies or mentally handicapped people for research.

People holding the second view probably do so more on their moral values and ethical view of the research topic in question, e.g., it is wrong to drink alcohol or smoke in the first place, and therefore animals should not die to help save people from their self-inflicted disease, or to prosecute war.

Those people holding the third ("speciesist") view may be more concerned about the cognitive ability or neurophysiological sensitivity of the animals being used, i.e., their "closeness" to humans, and perhaps more commonly are more concerned with "animal welfare" than they are with "animal rights."

Should the "burden of guilt" (if any exists) be spread amongst those who benefit from animal experimentation and not laid fully on those who conduct it? In short, currently societies around the world welcome the benefits of animal research whilst largely remaining ambivalent to the work itself and will probably choose to remain ignorant of the issues for as long as possible. Is the guilt of benefiting from a medicine any the less because it was developed using animals (perhaps chimpanzees) in another country?

What about animal welfare? So much is said about animal welfare, so little is understood! Many aspects of animal welfare are tangible, indeed often quantitative. Body weights can be measured and disease is detectable by the veterinary

clinician, as is malnutrition. Extremes of behavioral abnormalities can be observed (e.g., stereotypic behavior). Fear is readily detected through the "fight or flight" responses. All of these characteristics contribute to measuring animal welfare. Perhaps the area of greatest interest and of most conflict is currently that of measuring behavioral "needs/drivers" and assessing whether containment in laboratory conditions allows animals to sufficiently satisfy these needs.

The UK Farm Animal Welfare Council (1993) defined the "five freedoms" that should be given to every farm animal. They are:

Freedom from malnutrition
Freedom from injury and disease
Freedom from thermal and physical discomfort
Freedom from fear and stress
Freedom to express most normal patterns of behaviour.

These five freedoms (of welfare) are equally applicable to laboratory animals and serve as an excellent measuring template. It is clear however, that thankfully many of these markers of freedoms are now taken for granted in our animals, specifically freedom from hunger, thirst, malnutrition, (incidental) pain, injury, and disease. Therefore if we were to simply draw up a welfare-scoring template and compare our laboratory animals' lives to those of the average farm animal, they would probably compare very favorably.

However, we are, when using these freedoms to measure welfare, left with some challenges for some laboratory animals. As already considered, the freedom to express normal behavior perhaps remains the greatest concern of the challenging but responsible animal welfare groups. Within the research community we refer to this aspect of welfare as social and environmental enrichment. Whilst there still remain elements of the unknown and doubt regarding suitable and appropriate enrichments, a large degree of the concern surrounds sufficient funding for their implementation and overcoming concerns that they do not prejudice the scientific integrity and regulatory validity of experiments.

The scientific community as a whole frequently broadcasts that the "inflicted" discomfort, pain, suffering, distress, and lasting harm to animals in experiments is mostly minimal. However, there are still opportunities using refinements to reduce these further, thereby better fulfilling this freedom.

Finally a challenge for every laboratory animal-user and handler is to provide totally the freedom from fear and distress. So much of providing this freedom centers on the staff and on policies and procedures regarding both staff and animal training.

To summarize and conclude this review of animal rights and welfare it is probably best to give a quotation. It is taken from *Animal Welfare: A Cool Eye towards Eden*: "It is not what you think of the animal that matters. It is what you do to it that counts!"[1]

As an example, the rabbit kept for meat production, for experimental purposes, or as a pet has still the same basic welfare needs and knows not (nor cares) why it is there, or what its fate is to be, nor what its carer thinks of it. But the consequences of negligence and neglect on each of the three rabbits will be the same!

2.2 Laboratory animal welfare laws and regulations

National laws and regulations tend to reflect the ethics and moral attitudes of the prevailing culture. I believe it therefore inappropriate to take any piece of national legislation in isolation of a nation's cultural position or in context of its total legislative framework and decide on its "value" or "worth" in achieving what that law sets out to do. Often it is as important to reflect on the cultural attitude to and interpretation of law enforcement within a country as it is to simply evaluate the words.

It must also be remembered that in most countries statute law is frequently supplemented with further "regulations" in the form of guidelines and codes of practice.

I believe in and work to three fundamental concepts, or objectives, of the laws governing animal experimentation. Those concepts or objectives are:

- To bestow a privilege on individuals to conduct research on live animals, which may cause pain, suffering, distress, or lasting harm.
- To provide the opportunity and ability to advance science for the overall benefit of mankind.
- To secure as far as possible the highest welfare standards compatible with the research objectives.

2.2.1 Council of Europe (CoE) and Convention ETS/123

The Council of Europe (CoE) is the continent's oldest political organization, founded in 1949, and currently has 46 Member States.

The CoE was set up with primary aims:

- To defend human rights, parliamentary democracy, and the rule of law.
- To develop continent-wide agreements to standardize Member States' social and legal practices.
- To promote awareness of a European identity based on shared values and cutting across different cultures.

In summary the CoE was established to harmoniously bring Europe back together after the war, and as such it works through mutual understanding, handshakes, and gentlemen's agreements, in contrast to the European Union which keeps order through legal enforcement.

The CoE uses Conventions as instruments of harmonization, and Member States of the CoE can choose to respond to a Convention in one of three ways. They can refuse to recognize a Convention and in doing so make no obligation to comply in whole or part. They can "sign" a Convention and in doing so acknowledge the existence of the Convention but again make no binding obligations in respect of compliance. Finally a Member State can "ratify" a Convention and on ratification acknowledge a "moral" obligation to comply. In reality should a Member State which has ratified the Convention fail to comply, then there is little the CoE can do to enforce compliance short of dismissing the state from the CoE.

Clearly such a method of implementation combined with a historical picture of enrolment to the CoE (many Eastern states joining in very recent times) leads to significant variations in the degree of compliance between Member States.

The relevant Convention regarding animal experimentation in the CoE is titled the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes 1986 and is commonly referred to as Convention ETS/123 (1986).[2] This Convention is one of five relating to animal welfare.

Of most relevance today is Article 30 of the Convention which requires multilateral consultations by the parties within 5 years of its enforcement and every 5 years thereafter. The first multilateral consultation took place in 1993 and since then they have been held regularly, involving not only the competent authorities responsible for its implementation at national level but also all interested European and global stakeholder organizations including user groups, animal welfare organizations, and animal rights representatives. In such a way the CoE attempts to progress the Convention in terms of meeting current needs of all stakeholder organizations through consensus.

The multilateral consultations have addressed many issues over the last 14 years or so including the introduction of new technologies such as transgenic manipulation, as well as topics such as education, training, and the collection of statistics.

Most recently and for the last 5 years or so the multilateral consultation has concentrated on reviewing and revising Appendix A to the Convention wherever possible basing any modifications on scientific evidence. Appendix A of the Convention provides guidance on the care, housing, and husbandry of the species commonly used in experimentation.

Ratification by the Council of Europe of the revised version of Appendix A can be expected in 2005. It will then be for Member States to decide on individual implementation plans. Of most concern will be the changes of recommended minimum cage sizes and stocking densities, which almost certainly will result in most users across the CoE having to commit to extensive and expensive upgrading programs.[3]

In concluding a short consideration of the Convention it must be emphasized that whilst enforcement is difficult within the CoE, we will go on to see how it is closely mirrored within the European Union and where enforcement at national level is much more closely monitored and applied.

2.2.2 European Union (EU) and Directive 86/609

The European Union is a much smaller (25 Member States in 2004) but is also a growing community. The EU Directive 86/609[4] is a Council Directive on "The approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes."

The CoE Convention and the EU Directive were implemented in the same year (1986) and given the two groups had Member States in common it is not surprising their contents mirror each other closely in format and content. However, they differ in two significant ways:

(1) Their aims are different. The primary aims of the Convention (for the author at least) appear to be directed to the protection of the animals through the "3 Rs" (Refine, Reduce, Replace) with a desire to make this protection common across Member States. The Directive on the other hand places much more emphasis on the aim for the adoption of approximated laws, regulations, and provisions so as to avoid affecting the establishment and functioning of the common market, in particular by distortions of competition or barriers to trade. It seems to the author that the Directive is more concerned with having a level playing field of conditions than it is with the welfare of the animals it covers.
(2) The second major difference, as alluded to earlier, is the "enforceability" of the Directive through national legislation compared with the Directive. It is a mandatory requirement of the EU that national legislation exists in each Member State to cover all Directives. Compare this to the "gentleman's handshake" of the Convention. Sticks and carrots come to mind!

A project carried out for the Eurogroup for Animal Welfare in 1992[5] reported how slowly some Member States were implementing the Directive either through ongoing lack of national legislation or poor administrative support.