The use of non-human primates in research

A working group report chaired by
Sir David Weatherall FRS FMedSci

December 2006

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Sponsors' statement

The use of non-human primates continues to be one the most contentious areas of biological and medical research. The publication of this independent report into the scientific basis for the past, current and future role of non-human primates in research is both a necessary and timely contribution to the debate.

We emphasise that members of the working group have worked independently of the four sponsoring organisations. Our organisations did not provide input into the report's content, conclusions or recommendations. The report is aimed at all those involved in non-human primate research, namely government, regulatory agencies, professional bodies, industry, research funders and the scientific community. For our part, we will consider and respond to its recommendations.

We are grateful to the members of the working group for their considerable time and efforts in preparing this report. We especially thank Sir David Weatherall for leading on this important issue and producing a report that will be invaluable in taking forward this debate.

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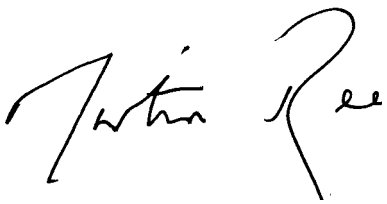
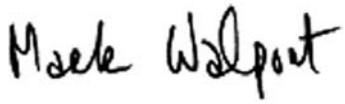


Lord Rees of Ludlow PRS
The Royal Society



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Contents

Abbreviations and acronyms

3Rs	Replacement, Refinement and Reduction
Aβ	Amyloid β protein
ABPI	Association of the British Pharmaceutical Industry
AD	Alzheimer's disease
ADME	Absorption, Distribution, Metabolism and Excretion
AIDS	Acquired Immune Deficiency Syndrome
AMS	Accelerator Mass Spectrometry
APC	Animal Procedures Committee
APP	Amyloid Precursor Protein
A(SP)A	Animals (Scientific Procedures) Act 1986
BBSRC	Biotechnology and Biological Sciences Research Council
BMI	Brain-Machine Interface
BOLD	Blood Oxygen Level Dependent Activity
BSE	Bovine Spongiform Encephalopathy
BUAV	British Union for the Abolition of Vivisection
CAT	Computer Assisted Tomography
CFM	Centre for Macaques
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CNS	Central Nervous System
CSP	Circumsporozoite protein
CTL	Cytotoxic T Lymphocyte
DBS	Deep Brain Stimulation
Defra	Department for Environment Food and Rural Affairs
DNA	Deoxyribonucleic Acid
Dstl	Defence Science and Technology Laboratory
DTI	Diffusion Tensor Imaging
DWA	Dangerous Wild Animals
ECVAM	European Centre for the Validation of Alternative Methods
EEG	Electroencephalogram
EFPIA	European Federation of Pharmaceutical Industries and Associations
EMEA	European Medicines Agency
EPAA	European Partnership on Alternative Approaches to Animal Testing
ERP	Ethical Review Process
ES cells	Embryonic Stem cells
ESG	Expert Scientific Group
EU	European Union
EUMAPP	European Union Microdose AMS Partnership Programme
FDA	US Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
GDNF	Glial Cell Derived Neurotrophic Factor
GM	Genetically Modified
HAART	Highly Active Anti-Retroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HO	Home Office
IAT	Institute of Animal Technology

IPS	International Primate Society
JALAS	Japanese Association for Laboratory Animal Science
LASA	Laboratory Animals Science Association
LGN	Lateral Geniculate Nucleus
MAbs	Monoclonal Antibodies
MCI	Mild Cognitive Impairment
MDR	Multi-Drug Resistant
MEG	Magnetoencephalography
MHC	Major Histocompatibility Complex
MHRA	Medicines and Healthcare products Regulatory Agency
MoD	Ministry of Defence
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MTD	Maximum Tolerated Dose
MVA	Modified vaccinia virus ankara
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NFT	Neurofibrillary Tangle
NHP	Non-human Primate
OECD	Organisation for Economic Co-operation and Development
OHSS	Ovarian Hyperstimulation Syndrome
OPV	Oral live poliovirus vaccine
PCOS	Polycystic ovarian syndrome
PD	Parkinson’s disease
PET	Positron Emission Tomography
PPN	Pedunculopontine Nucleus
R&D	Research and Development
RNA	Ribonucleic acid
SARS	Severe Acute Respiratory Syndrome
SHIV	Simian Human Immunodeficiency Virus
SIV	Simian Immunodeficiency Virus
STAIR	Stroke Therapy Academic Industry Roundtable
STN	Subthalamic Nucleus
TB	Tuberculosis
TMS	Transcranial Magnetic Brain Stimulation
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

1 Summary

The central goal of this study was to examine the scientific case for the use of non-human primates for research into the prevention or treatment of disease, or for fundamental research that has the long-term potential of achieving the same end. The material presented in this report is primarily targeted at policy-makers in government, research funders, universities, scientific societies and relevant professional and regulatory bodies, as well as all other interested parties. It should be emphasised that its conclusions and recommendations reflect the views of the members of the working group; the sponsors played no part in determining its contents or in shaping its conclusions.

There is a particular concern and uncertainty about the acceptability of using non-human primates in medical research, primarily because of their evolutionary proximity to human beings. Debate around this issue has become polarised such that it is pursued by opponents as though no harmful consequences could result from abandoning this work, and by proponents as though its abolition would entail the sacrifice of a large amount of knowledge and the betterment of human health. Although initially sceptical of both of these views, the working group has attempted to address both assertions.

It should be emphasised that, given the breadth and complexity of this topic, it has not been possible to draw firm conclusions on every area of non-human primate research. In several important aspects there was simply insufficient information to achieve this end. However, where possible, the report points out directions for further work and approaches to a continuous and more rigorous process of assessment of the role of non-human primates in the constantly changing scene of the biomedical sciences.

While examining the scientific case for the use of non-human primates in research, ethical issues were considered. The members of the working group accepted a moral case for careful, well monitored and meticulously regulated non-human primate research, provided it is of a high quality and has the potential to benefit mankind, and if it is the only way of solving important scientific or medical questions. This does not preclude the need for consideration of ethics, together with scientific and welfare issues, in the cost-benefit assessment of each research proposal.

The total number of non-human primates used for scientific or medical purposes in the UK has remained fairly constant over the last 10 years (at around 3,300), albeit with fluctuations. Each year approximately 75% of these animals are used for the purpose of toxicity and safety testing of pharmaceuticals, with a small percentage used for procedures in fundamental biological research. The remit of this study, i.e. the use of non-human primates in hypothesis-driven research, dictated a focus on the latter. After an investigation of the current use of non-human primates in research in the UK and internationally, a few research fields were chosen for investigation: principally communicable disease and neuroscience; and, more briefly, reproductive medicine, developmental biology and ageing.

After an assessment of the written and oral evidence submitted to the study, together with the appropriate scientific literature, it was concluded that there is a strong scientific case for maintaining work on non-human primates for carefully selected research problems in many of the areas studied, at least for the foreseeable future. In some cases, however, despite the scientific questions posed being both valid and important, it was concluded that, because of the availability of other approaches, the argument for the use of non-human primates was not as strong. In all instances we emphasise the continued need for each case to be judged individually, according to a rigorous

assessment of the welfare costs to animals involved, the potential scientific or medical benefit of the work and the availability of other approaches.

The working group was aware that many people find research involving animals to be more acceptable if it is clear that it is applied directly towards a medical need, rather than if it is asking a more fundamental biological question. During these inquiries, the distinction and relationship between applied and fundamental research was therefore considered. It was concluded that this distinction is now outdated; modern biomedical research reflects a continuum stretching from basic studies of normal function to its breakdown in disease. Without knowledge of normal function it is often difficult to begin to understand its failure in illness. High quality fundamental biological research, if the questions asked can only be answered by studies with non-human primates, should be judged on a case-by-case basis in the same way as more applied studies directed at the control or cure of disease.

There is an impressive body of work directed at developing alternatives to non-human primates in research. There have been remarkable advances in recent years in molecular and cell biology, non-invasive imaging, computer modelling and systems biology approaches, as well as techniques for human studies. This success is demonstrated by the fact that investment in research and development has increased significantly in the last 10 years, while the amount of animal, including non-human primate, research has remained more or less the same. While some of the research into alternatives has already borne fruit, it is too early to predict the time that will be required for many of these projects to achieve their goals. In the meantime, research funders must take every opportunity to encourage and fund research in this area.

The biological and medical sciences are passing through a period of unprecedented technological development. In most fields of research it is too early to assess the relative roles of animal research, human studies and the approaches mentioned above, in obtaining a fully integrated view of biological function in health and disease. Hence, it is impossible to make any blanket decisions about the future requirements for non-human primates in research; each case will have to be examined individually against this background of rapid change.

With this in mind, we consider that greater effort should be directed at coordinating and constantly reviewing the need for non-human primate research on the part of individual research teams, specialist research societies and granting agencies. Information obtained in this way should be regularly collated, updated and made available to the scientific research community, granting agencies and regulatory bodies. This should be supported by much greater openness about every aspect of non-human primate research on the part of all those involved, including: a review of the outcomes of biomedical research using non-human primates carried out over the last 10 years; steps to make the results of toxicological studies involving non-human primates publicly available; and requirements to improve the publication of experimental details of non-human primate research in scientific journals. Efforts towards greater openness and accessibility of information would provide the much-needed basis for improving and sustaining the scientific and public debate.

Although not a major part of the study remit, areas for potential improvement of the welfare of non-human primates used in biological and medical research have also been considered. These include reporting procedures, housing and transport conditions and training of those who carry out this work. We have also called for an expansion in support for work towards refining research methods involving non-human primates, particularly in the behavioural neurosciences. In all respects, it is crucial that experiences leading to improvements in welfare are shared amongst the non-human primate

community, which can only occur through sustained education and access to information.

Throughout the study, the working group heard claims that the future of UK non-human primate research is threatened by a number of factors, including a climate of intimidation created by some opponents to animal research, a shortage of available animals, administrative difficulties and high costs compared with other countries. Although some genuine difficulties have been identified, the true extent of this problem remains unclear and requires urgent investigation on the part of the government and relevant funding bodies.

We consider that all those involved in non-human primate research should work together in formulating a national strategic plan that should address issues of supply and demand in the short and longer term and include a re-evaluation of the organisation of non-human primate research facilities. In this respect, we urge consideration of the creation of UK specialised centres of excellence in non-human primate research, which could bring significant scientific and welfare benefits. At the very least, consideration should be given to the development of 'virtual' networks between existing facilities, which could improve sharing of knowledge, resources and expertise and ensure that consistently high standards are implemented.

Finally, we urge the bodies that sponsored this study to work to activate the recommendations of this study and to monitor progress in achieving these ends over the next few years.

Recommendations

Recommendation 1	**There is a strong scientific case for the carefully regulated use of non-human primates where there are no other means to address clearly defined questions of particular biological or medical importance.**
Recommendation 2	**In the fields of research considered in this study, namely communicable disease, neuroscience and reproductive biology, there is a strong scientific case for maintaining the use of non-human primates in some aspects of this work, at least for the immediate future.**
Recommendation 3	**The major specialist organisations involved in research fields that utilise non-human primates, particularly neuroscience, communicable disease, and reproductive and developmental biology, should regularly collate information about evolving research technology in their fields, with particular respect to the need for non-human primates. This information should be disseminated to funding bodies, ethics committees and regulatory agencies.**
Recommendation 4	**As part of their ongoing programmes to assess the outcomes of their research, the major funding organisations should undertake a systematic review of the outcome of all their research using non-human primates supported over the last decade.**
Recommendation 5	**UK research funding organisations, both governmental and charitable, should continue to take every opportunity to encourage and fund research into developing alternatives to the use of non-human primates for both research and toxicology. Funders should expand their support for research into refining non-human primate research practices, particularly in the behavioural neurosciences.**
Recommendation 6	**Retrospective reporting on the severity of procedures for non-human primates, as recommended by the LASA/APC pilot study, should be introduced as soon as possible.**
Recommendation 7	**Improvements in the supervised continuous training of research workers in non-human primate research should be instituted.**
Recommendation 8	**Scientific journals should include details of animal welfare and steps taken to ameliorate suffering in all published papers that involve non-human primate research.**
Recommendation 9	**Work should be accelerated towards improving and applying current best-practice regarding housing of non-human primates, including minimum cage size, an emphasis on the avoidance of single housing, how cage fittings and conditions can be accommodated to the purpose of individual experiments, and a better assessment of the advantages of outside access and visual stimulation.**

Recommendation 10	**Further efforts should be made to improve interactions between regulatory bodies at national and international levels and between regulatory bodies and the scientific community. Given the current speed of research in the biological sciences, new approaches to improve these interactions are urgently required.**
Recommendation 11	**Steps should be taken to make the results of toxicological studies involving non-human primates publicly available, in the same way as initiatives to register and publish the results of all human clinical trials.**
Recommendation 12	**It would be premature to make firm recommendations on how a reduction in the number of non-human primates used in regulatory toxicology might be achieved before the completion of the NC3Rs/ABPI study. However, we urge government and other stakeholders to act on the recommendations of this study, and in the light of its findings, to re-examine responses to the 2002 APC report.**
Recommendation 13	**Concerns that costs and harassment by activists are forcing scientists and research companies to pursue non-human primate work overseas require urgent examination by the relevant UK research funding and regulatory bodies.**
Recommendation 14	**The major funding bodies, together with government, other stakeholders, scientists, primatologists, vets and welfare specialists, should give careful consideration to the creation of UK centres of excellence for non-human primate research.**
Recommendation 15	**All bodies involved in engaging the public around issues of science and medicine, including the UK government, should ensure that the whole field of research utilising animals, including non-human primates, has a major place in their future programmes. Given the extremely rapid pace of development in the biological sciences, mechanisms for regular meetings between scientists and the media should be further explored.**
Recommendation 16	**The bodies that sponsored this study should establish a mechanism for monitoring progress in achieving the aims of these recommendations over the next few years.**

2 Introduction and methods of working

2.1 Background, objectives and timeliness

2.1.1 The evolution of concerns about the use of animals in research

Ever since the 17th Century, when, as exemplified by William Harvey's discovery of the circulation of the blood, the biological and medical sciences began to seek quantitative ways of measuring biological functions, there has been widespread uncertainty and controversy about the use of animals to achieve these ends. Early in the 18th Century, Alexander Pope published an essay entitled '*Against Barbarity to Animals'* in an English newspaper and, later, Samuel Johnson denounced doctors who *'extend the arts of torture'*; *'I know not'*, he wrote, *'that by doing any living dissection any discovery has been made by which a single malady is more easily cured'*.[1] In 1875 Charles Dodgson, under his pseudonym Lewis Carroll, wrote a strong attack on vivisection, which he circulated to the governing body of Oxford University in an attempt to persuade them not to establish a physiology department in the university, thus beginning an intermittent series of protests about animal research in UK universities and research institutes that continues today.

A Royal Commission on Vivisection was established in June 1875 and, in 1886, two Private Member Bills led to the enactment of a broad series of regulations directed at the control of research on animals. Between 1907 and 1912 the Royal Commission published six Reports investigating the nature of animal research and its regulation. They included a detailed analysis of the role that animal research had played in medical advances at the time. For example, in the Fourth Report, published in 1908, Sir William Osler described the importance of animal work in seminal discoveries about the mode of transmission of malaria and yellow fever (both of which were decimating huge populations in tropical countries at the time) and in understanding the cause of diseases due to lack of thyroid function, and hence to their control.[2] These Reports, and many that followed, paved the way for the regulation of animal research as it is carried out today.

2.1.2 Current concerns

Opinion on the subject of animal use in research in the UK continues to vary widely. It ranges from the view that any form of animal research is completely unjustifiable, to the belief that it is acceptable, provided it is carefully regulated to cause minimal suffering to the animals concerned, and is directed at alleviating human suffering or for the pursuit of knowledge that might in the long term achieve this end.

Many of these arguments are based on the premise that *Homo sapiens* occupies a special place in the animal kingdom, such that it is appropriate for it to inflict potential suffering on other species to reduce its own suffering (see section 11 for further discussion on the ethics of non-human primate research). Since the arguments for differences in moral status between animal species are so complex, the only pragmatic approach is for individuals to become as well informed as they can about the arguments for and against the necessity of using animals in research and how such research is regulated, examine their consciences, and make up their own minds.

Because this is such an emotive topic, and since no civilised society would wish to cause unnecessary suffering to any living thing, the only way forward is to obtain a consensus opinion of the acceptability, or otherwise, of animal research. This should be based on widespread informed public debate, which must rest on a genuine understanding of the current issues involved; since biological and medical research moves so quickly it is not productive to limit these discussions to past events.

There have been attempts to test public opinion about the use of animals for research

1 See Porter R (1997) *The Greatest Benefit to Mankind: A Medical History of Humanity from Antiquity to the Present*. HarperCollins, London.
2 Osler W (1908) *Fourth Report of the Second Royal Commission on Vivisection.* 1906-1912.

and toxicology testing in recent years. Public opinion polls certainly have their limitations, but the results of those that have been carried out have been useful, in that they reflect some of the central concerns in examining the present state of the experimental use of animals.[3] First, it is clear that there is much more concern about the use of non-human primates than other animals. Second, there seems to be a higher acceptance of animal research when it is clearly shown to be directed towards near-term control of serious disease. Conversely, there appears to be less certainty about the appropriateness of using animals, particularly non-human primates, for more fundamental research that is not perceived to be directly concerned with the control of human disease.

Given their evolutionary proximity to humans, it is not surprising that the most serious public concern about animal experimentation is directed at research using non-human primates. Hence, it is very important to try to determine the extent to which research would be set back by restricting experiments to either non-primates or approaches that require no animals at all. Currently, this debate is extremely complex, based as it is on a large literature that makes strong generalisations for or against the requirement of animals for research. Unfortunately, while much of the material (on both sides of the argument) provides numerous examples of the value, or lack thereof, of animal research, very little of it explores these questions in the depth required to form a reasoned conclusion.

The lack of public enthusiasm for animal research directed at fundamental scientific questions could, in part, reflect these shortcomings in current discussions on animal research. Much fundamental research is directed at understanding the basic processes of living organisms. While some people may feel it is not justifiable to pursue what might be perceived as scientific 'curiosity' by potentially invasive research on animals, the question remains as to whether this stance would endure if the public were convinced, as many researchers are, that numerous medical advances have ultimately stemmed from this type of work and that this will continue to be the case in the future. As the divide between fundamental and applied biological research becomes less distinct, this is another important topic that requires more in-depth consideration.

2.1.3 Healthcare during the 20th Century

Since the aim of utilising animals in research is, either directly or indirectly, to improve human health and/or animal health, this report has to be considered in light of current and future problems of healthcare and the nature of the investigations that will be required to solve them.

During the 20th Century the populations of industrialised nations experienced a steady improvement in their health and longevity. Although many of these advances reflected better sanitation, nutrition, and related social changes, there is no doubt that medical research, at least some of which involved the use of animals, played a major role.[4] The identification of agents responsible for communicable disease, and the later development of powerful vaccines and antibiotics, led to the disappearance of many infectious killers. In the second half of the 20th Century, a better understanding of the mechanisms of disease, together with advances in diagnosis, surgical procedures and pharmacology, resulted in major improvements in the management of many diseases of the developed countries. Epidemiological studies that established links between common disorders such as heart disease and cancer and the action of environmental agents or lifestyle factors, initiated various prevention and public health programmes.

Progress in the provision of healthcare in many poorer countries has been much less satisfactory. Although there has been a modest increase in longevity and a significant reduction in childhood mortality in many parts of Asia

3 MORI (2005) *Use of animals in medical research*. Research conducted for Coalition for Medical Progress.

4 Weatherall DJ, Greenwood BM, Chee H-L & Wasi P (2006) Science and Technology for Disease Control: Past, Present and Future. In: *Disease Control Priorities in Developing Countries* (ed. D Jamison *et al.),* pp. 119-138. Oxford University Press and the World Bank, New York and Washington.

and the Middle East, this has not been the case in sub-Saharan Africa. The pattern of disease in these countries still reflects poverty, poor sanitation, dysfunctional healthcare systems and, in particular, the ravages of communicable disease.

2.1.4 Problems for healthcare during the new millennium

Major advances in preventative medicine and clinical care in the richer countries have had a positive impact on premature deaths due to heart disease, stroke and cancer. However, our lack of knowledge about their basic underlying causes means that they remain a major problem, particularly for older people. The underlying causes of diseases such as Parkinson's disease, Alzheimer's disease and several rheumatic conditions, also remain unknown. Hence, they cannot be prevented or cured and doctors are limited to managing their symptoms, often with only partial success.

For poorer countries, communicable disease is likely to remain a major challenge. In addition to the resurgence of malaria and tuberculosis, and the frightening decimation of large populations by HIV/AIDS, there are many other epidemics of less publicised infections, including dengue, leishmaniasis, schistosomiasis and various forms of encephalitis. Furthermore, as these countries pass through an epidemiological transition following improvements in nutrition and health, they will encounter an increasing frequency of diseases currently typical of the richer countries, such as heart disease, diabetes and stroke. Indeed, it is estimated that by 2020 there will be 300 million cases of insulin-resistant diabetes worldwide, many of which will be associated with obesity.

Clearly, we have not reached the stage at which there will be any decline in the requirement for medical research. This raises the question of whether changes in the approaches and patterns of future medical research will have any effect on the potential requirement for animal studies.

2.1.5 Medical research in the new millennium

Following the remarkable revolution in basic biological science over the second half of the 20th Century, particularly in molecular and cell biology, there has been a change in the focus of medical science away from intact patients and towards cells and molecules.[4,5] There is no doubt that the application of molecular biology to the study of human disease will become increasingly important. It has already led to major advances in the identification of those at risk of inherited monogenic disorders, although, apart from a few exceptions, these conditions are relatively rare. It is also yielding extremely rapid methods for the diagnosis of many communicable diseases and, in the longer term, may identify targets for new forms of treatment and the development of vaccines.

Molecular and cellular techniques are shedding light both on the causes of different cancers and approaches to their early diagnosis and management. It is hoped that the search for genes that increase/decrease susceptibility to the action of diet and lifestyle in causing heart disease and diabetes may provide valuable insights into their underlying mechanisms and hence how they might be better managed. Similarly, for diseases of unknown cause, including the major psychiatric disorders, Parkinson's and Alzheimer's disease, the identification of susceptibility genes may also throw light on their underlying mechanisms.

In the context of this report, a central question is therefore the extent to which the study of disease at the molecular level, backed up by analysis in animal models such as mice and rats (which are increasingly amenable to genetic engineering directed at producing models of human disease), will reduce the necessity for research involving non-human primates.

2.1.6 Genomics and the future of medical research?

When the completion of the human genome project was announced in 2001, expectations

5 Sargent MG (2005) *Biomedicine and the Human Condition: Challenges, Risks and Rewards.* Cambridge University Press, Cambridge, and New York.

were raised that information obtained from this remarkable scientific feat would alter the pattern of medical practice within the next 20 years. However, it is already clear that these hopes were premature, certainly in the case of the complex diseases of the developed countries. Already, new layers of complexity have been discovered with regard to the way genes are regulated and how they interact with each other in health and disease. It has also become apparent that our genetic make-up may be modified during early development, by ageing, and by interactions with our environments.

Hence, understanding the orchestration of our genes and the many proteins they encode will require integration of the fields of molecular biology with whole organ, and ultimately whole organism, physiology. In other words, our current reductionist approach to the study of human biochemistry and physiology, and its breakdown in disease, may not be sufficient. Rather, molecular and cell biology will have to be integrated with studies on human beings and animal models, supported by complex computer modelling and epidemiological analysis over large populations. Current biological and medical research is starting out on a completely new venture that may take the rest of this century to come to fruition; it is far too early to predict the relative roles of genomics and the many other new technologies that are evolving to achieve this end.

2.2 Scope and process of the report

2.2.1 Summary of the rationale for this report

Against this complex background, and in accordance with the terms of reference (see below), this study has focused on the major medical research areas that currently utilise non-human primates and attempted to investigate the extent to which progress in the control of serious disease might, or might not, be set back if non-human primates were not used. It also examines the current role of more fundamental research in forming the groundwork for future medical advances. In particular, we have tried to assess the necessity for research using non-human primates in the context of advances stemming from work in the genomic era, which promise to provide many completely new approaches to the control of human disease (in addition to examples included in sections 5 and 6, general trends are discussed in section 9). This report is intended for policy-makers in government, research funders, universities, scientific societies and relevant professional and regulatory bodies, as well as all other interested parties.

While the focus of the study is primarily scientific, a full analysis cannot be performed in isolation from the associated welfare and ethical issues (discussed in sections 10 and 11). The working group noted that more qualified bodies, such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), are actively developing practical measures to safeguard welfare and promote refinement within non-human primate research. Similarly, detailed considerations of the associated ethical issues have been undertaken previously by the Boyd Group and the Nuffield Council on Bioethics.[6,7] Here, we present only a brief outline of the ethical approach that underpinned the considerations of the working group.

The study remit focused on hypothesis-driven research in the UK, primarily in academia, and the working group were not tasked with investigating the use of non-human primates in the safety testing of new drugs. This issue has been, or is being, tackled by other groups (the Animal Procedures Committee, the NC3Rs and Association of the British Pharmaceutical Industry). However, since drug toxicology studies account for the majority of non-human primate usage in the UK, the use of non-human primates in drug discovery and development is discussed briefly in section 8.

6 The Boyd Group (2002) *The use of non-human primates in research and testing.*
7 Nuffield Council on Bioethics (2005) *The ethics of research involving animals*.

Finally, the report discusses the current shortcomings in the dissemination of information about the scientific arguments for and against research that utilises animals, how such research is carried out and regulated, and briefly examines how this increasingly serious situation might be improved.

2.2.2 Independence

The Chair and members of the working group were drawn from outside the active non-human primate research community; they were appointed as individuals and not as representatives of their affiliated organisations (see appendix I).

While this study was initiated and sponsored by the Academy of Medical Sciences, Royal Society, Medical Research Council and Wellcome Trust, members of the working group have been completely autonomous in their work and in reaching their conclusions.

2.2.3 Evidence gathering

The working group considered the gathering of evidence from a range of perspectives to be an essential component of the study and a public call for written evidence was held from March until June 2005. A total of 62 responses were received from individuals and organisations. The majority of submissions were made by individual UK academic researchers, as well as academic research groups, institutions and funders. Further submissions were received from: commercial organisations; organisations that campaign for a reduction/elimination of animal research; overseas researchers or research institutes; individuals/organisations concerned with animal welfare; patient groups; and government departments/agencies/arms-length bodies

The working group also held eight oral evidence sessions, in which 35 witnesses spoke in either an individual capacity or as representatives of their affiliated organisations. The list of consultees and respondents to the call for evidence is given in appendix II. The use of evidence is discussed in Box 1 (see p16).

2.2.4 Site visits

Given that active non-human primate researchers were not represented on the working group, it was considered important for members to gain direct experience of non-human primate laboratory activities and conditions. Members made site visits to 4 non-human primate facilities, including laboratories at the University of Oxford and an MRC Unit, in addition to the breeding facilities at the Centre for Macaques and the Defence Science & Technology Laboratory (Dstl) at Porton Down.

2.2.5 Review

The draft report was formally reviewed by an external panel (see appendix I) appointed by the four sponsoring organisations and was amended by the Chair in light of the comments received. The four sponsoring organisations did not review the draft report and have not provided input into its content and recommendations.

Box 1. Use of evidence

Submitted evidence, both written and oral, has been integral to the preparation of this report. We have not included a summary of the evidence; rather, references to the submitted evidence are made throughout the report and discussed in the context of the wider literature.

As mentioned previously, the majority of submissions were from members of the scientific community, including researchers who use non-human primates and those who do not. The majority of these respondents argued that non-human primates are the best model to address particular research questions, based on their close phylogenetic relationship with humans. However, other submissions argued that it is precisely this evolutionary link that causes them to object to non-human primate research, since the similarity implies that non-human primates can suffer in the same way as humans. There was a consensus view that non-human primates' highly developed cognitive abilities, and the challenge of meeting their complex social, behavioural and psychological needs in the laboratory environment, increase their potential for suffering relative to other animals.

Respondents exhibited a range of moral and ethical stances in response to this tension. Nearly all respondents acknowledged that non-human primate experiments can deliver relevant knowledge, but some felt that this was at too great a cost to the animals involved. Some respondents felt that physiological and genetic differences between non-human primates and humans undermine the extrapolation of data from one to the other. Other respondents argued that these differences would be even more magnified in non-primate species and non-animal systems. These respondents argued that, whilst differences between non-human primates and humans are inevitable, it is only through more research that the advantages and limitations of non-human primate models can be better understood.

Some respondents drew attention to examples where non-human primate data have confounded human disease research by conflicting with human data, and many respondents expressed disappointment that research, some involving animal models, has not delivered preventative measures or therapies for some of the major common killers, most notably HIV/AIDS, Parkinson's disease and stroke. Researchers stressed that these 'failures' do not invalidate the approach, or the use of non-human primates as an experimental model, but demonstrate that medical research is slow, difficult and sometimes disappointing.

Devising appropriate experimental tools and model systems will depend on the nature of the question under investigation. Regardless of the position taken on the use of non-human primates in research, respondents emphasised the importance of using a scientific approach that is appropriate to the question. Similarly, there was consensus around the complexity of biological systems and processes and the need for a variety of approaches to establish a comprehensive understanding of a particular biological phenomenon. Whilst many respondents emphasised that science is a slow and cumulative process, in which data from different avenues of research are used to build and improve overall understanding, others questioned the necessity for the variety of different animal models developed to address specific questions. Some respondents warned of the danger that individual researchers and research groups might have a mind-set predetermined by their experience of research carried out in specific animal models. However, the view from researchers was that the key to achieving a research goal is to avoid a

narrow focus on a particular experimental model. They expressed a strong belief that non-human primate research should be carefully integrated with research using other animal models, *in vitro* and *in silico* methods and human clinical research.

In focusing our attention on a few areas of research, we have attempted to examine these claims in the context of the importance of the scientific or medical question under investigation, including the range of scientific approaches being pursued, and current and future trends in the development of new techniques.

3 Current position of non-human primate research

3.1 Introduction

This section provides an overview of the use of non-human primates in UK research and considers exactly how many animals are used each year and for what purpose. It includes:

- A brief overview of non-human primate research in the UK and abroad.
- A summary of current UK legal requirements governing the use of non-human primates in research.
- An account of the numbers of non-human primates used annually in the UK, and the numbers and types of procedure carried out for the purposes of both fundamental biological research and toxicology.
- A brief introduction into the fields of non-human primate research and publication trends in the UK.

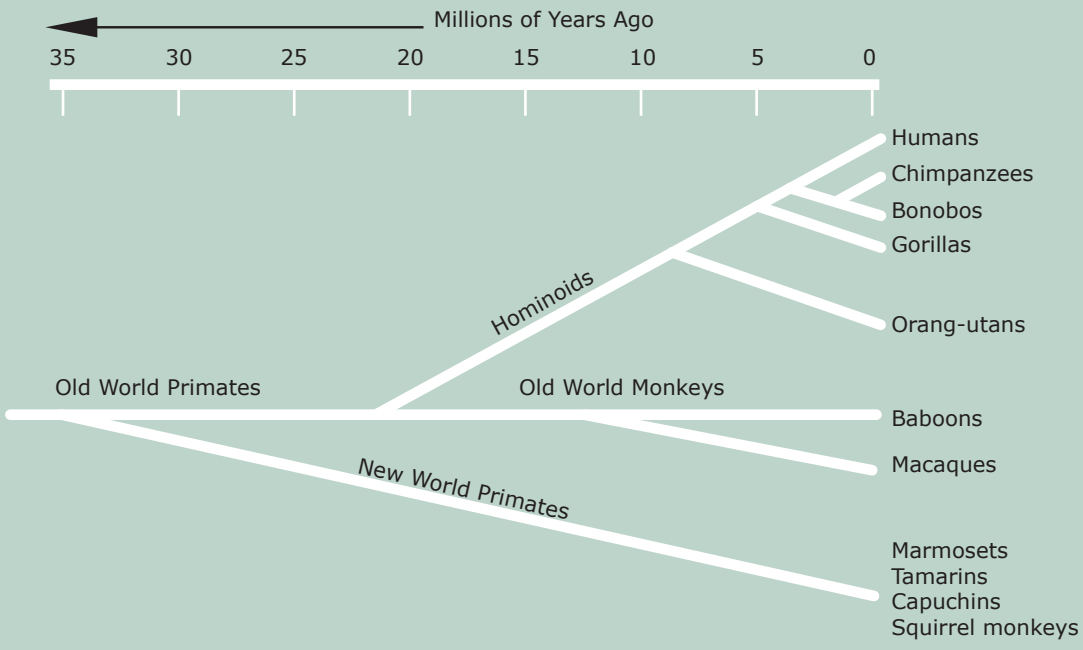
3.1.1 Non-human primate species in UK research

Non-human primates used in UK research include New World and Old World monkeys (see figure 3.1). The genus of Old World monkey most frequently used is the macaque, specifically the rhesus macaque (*Macaca mulatta*), cynomologus or long-tailed macaque (*Macaca fascicularis*) and stump-tailed macaque (*Macaca arctoides*). Of the New World monkeys used, almost all are common marmosets (*Callithrix jacchus*), with a smaller number of tamarins (*Saguinus spp.)* and squirrel monkeys (*Soimiri spp*.). Characteristics of macaques and marmosets are given in Box 2. Baboons are also Old World monkeys, but have not been used in the UK since 1998. Prosimians, which are small primitive primates such as lemurs, have also not been used since 1991. No great apes (common chimpanzee, bonobo, orang-utan, gorilla) have been used in the UK since 1986.

3.2 A global overview

It is relatively difficult to build an overall picture of the global use of non-human primates for research; many countries do not disclose the number of non-human primates used for this purpose, making it impossible to ascertain accurate figures for total global use. However,

Figure 3.1: Evolutionary Tree[8]

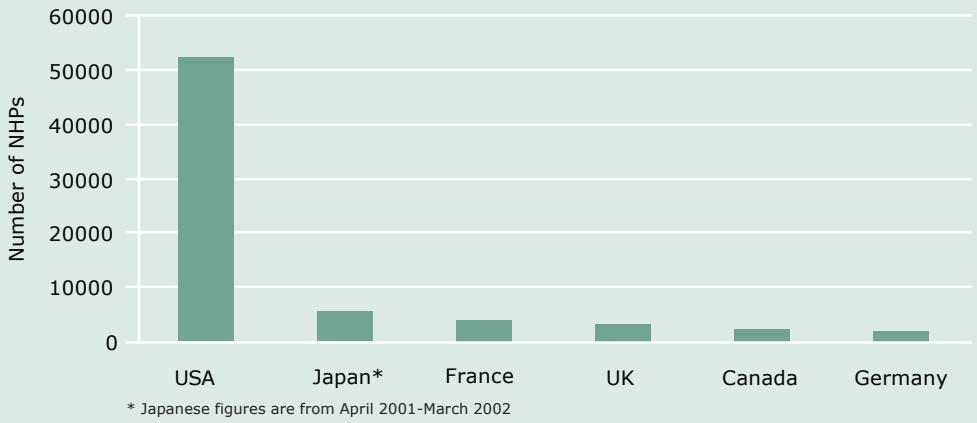


8 Primate evolutionary tree based on DNA comparisons taken from Living Links http://www.emory.edu/LIVING_LINKS/Taxonomy.html

a recent survey estimated that the annual number of non-human primates used in research worldwide is between 100,000 and 200,000,[9] with 64.7% of studies involving Old World monkeys, and 15.5% using New World monkeys.

All European Union (EU) member countries are legally required to provide statistical data on the number of animals used in scientific research each year and the purpose of the procedures involved. According to the most recent European Commission report, 10,362 non-human primates were used for experimental purposes in the EU in 2002.[10] In this year, France and the UK reported using 3,840 and 3,173 non-human primates respectively. Comparative figures from the USA show that 52,279 non-human primates were used in research in 2002,[11] in keeping with an average annual use over the past 10 years of around 54,000.[12] Figures from Canada, which are published annually by the Canadian Council

Figure 3.2 Number of non-human primates used worldwide in 2002



* Japanese figures are from April 2001-March 2002

Box 2. Macaques and marmosets

Macaques are Old World monkeys, with most of those used in research originally native to Asia. They vary in adult body weight from 2.5-10kg and can live for more than 20 years in captivity. In the wild they live in multi male/multi female troops of between 10-100 individuals, with strong coalitions between family members and occasionally between males. They are diurnal and largely arboreal. Females give birth every 1-2 years and siblings and relatives may help care for infants. They are highly visual animals with forward facing eyes and show considerable manual dexterity.

Marmosets are New World monkeys originating from South and Central America. They are genetically more distant from humans than macaques (see figure 3.1). Marmosets are small (typical body weight 250-600g), highly arboreal and diurnal. In the wild they live in family groups of 5-20 individuals. Marmosets are frugivores (fruit eaters), but they also eat insects and specialise in gum feeding. Their lifespan is 10-15 years. In the wild they give birth to twins after a gestation period of 4 to 5 months. They are highly visual and olfactory animals.

9 Carlsson HE, Schapiro SJ, Farah I, Hau J (2004) Use of Primates in Research: A Global Overview. *American Journal of Primatology* **63,** 225-237.

10 Commission of the European Communities (2005) *Fourth report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union*, available at: http://europa.eu.int/comm/environment/chemicals/lab_animals/pdf/com_2005_7_en.pdf

11 USDA (2003) *Annual Report of Enforcement for the Fiscal Year 2002*. Riverdale, MD. Available at: http://www.aphis.usda.gov/ac/awreports/awreport2003.pdf

12 USDA (2004) *Annual Report of Enforcement for the Fiscal Year 2003*. Riverdale, MD. Available at http://www.aphis.usda.gov/ac/awreports/awreport2004.pdf

on Animal Care, show that 2,109 non-human primates were used for research purposes in 2002 (figure 3.2).[13]

A number of countries, including Japan, do not have requirements to report numbers of animals used in scientific experiments. While no official statistics exist, the Japanese Association for Laboratory Animal Science (JALAS) carries out a survey of animal use every three years. The most recent figures available are for 1998 and 2001, when Japanese researchers reported using 9,037 and 5,606 non-human primates respectively.[14,15] Since only 57% of Japanese institutes and organisations responded to this survey, these figures are likely to represent an underestimate of the true numbers used. There is evidence that an increasing amount of research using non-human primates is also taking place in China, and new facilities are being built to attract this type of research to the country.[16] However, the exact figures for the numbers of non-human primates involved in research in China were not available for comparison.

3.3 UK legal requirements

Strict laws and guidelines relate to the use of all animals in research in the UK, with additional restrictions placed on the use of non-human primates. Several respondents to the call for evidence asserted that the UK Animals (Scientific Procedures) Act (A(SP)A) of 1986 is the most comprehensive and rigorous system of regulating animal experimentation in the world. The Act controls any experimental or other scientific procedure applied to a '*protected animal*'[17] that '*may have the effect of causing that animal pain, suffering, distress or lasting harm*.'

The Act enshrines all the 3Rs (i.e. the replacement, reduction and refinement of the use of animals in research) and it must be shown by the applicant for a project licence that the research programme cannot be achieved satisfactorily by any other non-vertebrate animal method.[18] The Act requires that the use of animals in research is based on sound scientific evidence and that every practical step is taken to use the smallest number of animals possible to give significant results, with the minimum amount of suffering inflicted. Differences in interpretation of the Act and real or perceived problems with identifying 3Rs techniques make this an ongoing challenge, hence the important role played by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (see below).

The Act sets out provisions for three types of licence that must be obtained before any animal is used in a scientific procedure. These include:

- A personal licence for the individual researcher.
- A project licence, containing information about the types and numbers of animals involved, severity of the procedures and the objectives of the research.
- A certificate of designation for the establishment in which the research is to be carried out.

The Animals Scientific Procedures Division of the Home Office administers licences and other aspects of the Act. Home Office Inspectors, who are required to have medical or veterinary qualifications, advise the Secretary of State on the granting of licences. Inspectors are tasked with assessing all applications to ensure that only properly justified work is licensed and that full consideration is given to alternatives and the implementation of the 3Rs. This involves weighing the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme of work.[19] Inspectors also make visits to research facilities, often unannounced, to check

13 Canadian Council on Animal Care (2003). Available at: http://www.ccac.ca/en/Publications/New_Facts_Figures/trends/trends_01.htm
14 Matsuda Y (2004) Recent trends in the number of laboratory animals used in Japan. *ATLA* **32**, Supplement 1, 299-301.
15 Committee for Laboratory Animal Care and Use (2003). The number of live animals used in experiments in 2001 -results of a survey. *Exp anim* **52**, 143.
16 News article (2003) China takes steps to secure pole position in primate research. *Nature* **432**, 3.
17 'Protected animals' are defined as all living vertebrate animals, except man.
18 Animals (Scientific procedures) Act 1986. Section 5 (5) a.
19 Animals (Scientific Procedures) Act 1986 Section 5 (4).

compliance with licence conditions and provide advice to certificate holders.

There are a number of guidance documents available to aid researchers in the implementation of the Act, including a detailed document issued by the Home Office in 1990 and updated in 2000.[20] Additional sets of guidelines have been produced by the Home Office and by bodies such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), including codes of practice on housing, care, breeding and transport of animals, some containing provisions particularly relating to non-human primates.[21,22,23] Infringements of licence conditions can result in a number of sanctions including: a requirement for retraining; activation of licence restrictions; withdrawal of personal or project licences; and, in the case of very serious breaches, fines or imprisonment.

3.3.1 Approvals for non-human primate research

The A(SP)A states that a project licence will not be granted for a research proposal involving non-human primates (or dogs, cats and equidae) unless '*no other species are suitable for the purposes of the programme to be specified in the licence or it is not practicable to obtain animals of any other species that are suitable for those purposes'*.[24] Further UK regulations on the use of non-human primates, introduced in 1996, banned the use of wild-caught primates, except where their use can be exceptionally justified, and installed a requirement for special justification for the use of Old World, rather than New World, primates. Since 1997 the Home Office has not issued licences for the use of great apes (including chimpanzees, gorillas and orang-utans). In fact, great apes have not been used in UK research since 1986.

A researcher wishing to use non-human primates must justify their use to the institute in which they work, an ethical review panel (ERP), the Home Office and the source of research funds. Grant applications to funding bodies are subject to independent expert-review of the science, to ensure that the experiments proposed provide sufficient justification for the use of animals and could be carried out in no other way. In evidence to the working group, research funders stressed that 3Rs aspects of project proposals are considered during the expert-review process, which may include reviewers from relevant fields who do not use non-human primates. However, grants committees do not replicate the work of the Home Office or ERP and grants are awarded subject to Home Office and ethics approval.

All applications to use non-human primates in procedures of substantial severity, or to use wild-caught primates, must be reviewed by the Animal Procedures Committee (APC), the Government's statutory, independent, advisory committee on animal experiments. The membership of the APC includes experts in biomedicine, veterinary science, animal welfare and law. The APC also appoints a Primates Subcommittee, which concentrates specifically on issues relating to non-human primate research and welfare.

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established in 2004 following recommendations by a House of Lords Committee report *'Animals in scientific procedures'*.[25] The aim of the NC3Rs is to promote, develop and implement the 3Rs. It is an independent organisation that reports to the UK Science Minister and includes various stakeholders from academia, industry, government and animal welfare organisations. All research proposals involving non-human primates, dogs or cats that are submitted to the MRC, Wellcome Trust or BBSRC are now passed to the office of the NC3Rs for evaluation, advice on welfare and input on implementation of the 3Rs.

20 Home Office (2000) Guidance on the operation of the Animals (Scientific Procedures) Act 1986.
21 Home Office *(1995) Code of practice for the housing of animals in designated breeding and supplying establishments. Part 2: 9. Non-human primates.*
22 Home Office (1989) *Code of practice for the housing and care of animals used in scientific procedures.*
23 NC3Rs (2006) *NC3Rs Guidelines: Primate accommodation, care and use. London, NC3Rs.* (Also see http://www.mrc.ac.uk/pdf_lasa_mrc_primate _breeding.pdf)
24 Section 5(6) of the Animals (Scientific Procedures) Act 1986.
25 House of Lords (2002). *Report of the Select Committee on Animals in Scientific Procedures.* HL Paper 150-1 TSO, London.

3.3.2 Presentation of statistics on animal use in research

Project licence holders must make annual returns to the Home Office, including details of the number of animals used, the species involved and the nature and purpose of the procedures. The Home Office publishes this information in its annual *'Statistics of Scientific Procedures on Living Animals in Great Britain'.*

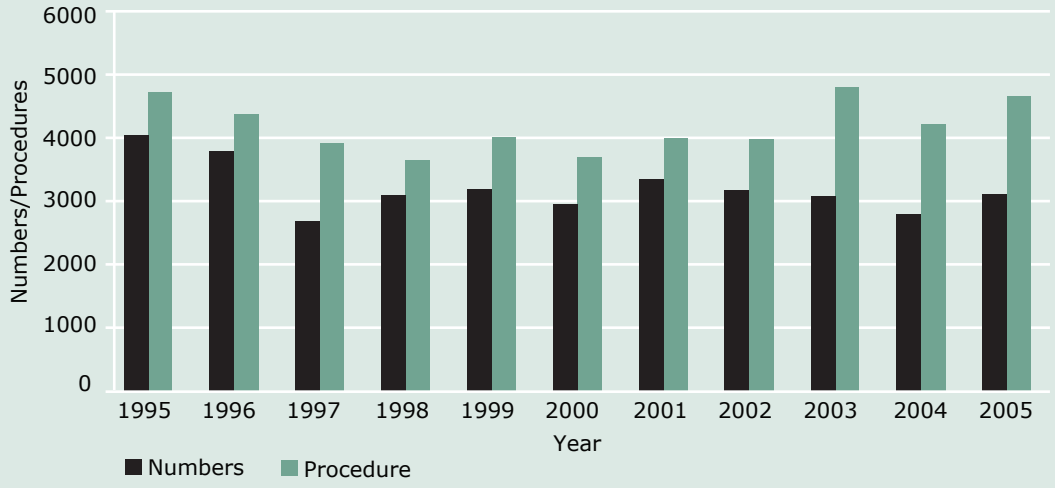
Most of the information in the Home Office's publication relating to the use of non-human primates is given as the number of *procedures* carried out, rather than *numbers* of animals used. In this way:

- The reported number of each species is the number of individual animals on which procedures were carried out for the first time in any one year.
- Each procedure for a given purpose is counted as one *returnable procedure* for the year in which it commenced.

In cases where the same animals are in ongoing use or are used repeatedly, a greater number are often used in procedures during a given year, than are used for the first time. For example, the *number* of 'other New World monkeys' used for the first time in 2005 is given as zero in the statistics, but there were in fact 24 *procedures* carried out on squirrel monkeys in that year.[26] It can therefore be deduced that procedures using the same animals have been ongoing since before 1998, which is the last year that squirrel monkeys were reported as being used for the first time.[27] The statistics do not indicate what is involved in the reported procedures. For instance, a simple procedure, such as an injection, and a complicated procedure, for instance involving anaesthesia and invasive surgery, would each be classified as one procedure.

The statistics do not provide specific information on the re-use of non-human primates, although as discussed above it can be inferred that re-use occurs since the number of procedures exceeds the number of animals used in a given year. Home Office figures indicate an increase in the number of procedures relative to the number of animals used, suggesting that levels of re-use have increased in recent years (see figure 3.3). Section 14 of the A(SP)A contains details on the circumstances where re-use of animals is permitted with prior consent from the Secretary of State. The use of any animal more than once in a procedure entailing severe pain or distress will not be authorised by the Home Office.

Figure 3.3 Total numbers of non-human primates used for the first time and procedures carried out from 1995-2005



26 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain 2005.* HMSO, London.
27 Home Office (1999) *Statistics of Scientific Procedures on Living Animals Great Britain 1998.* HMSO, London.
28 House of Lords (2002). *Report of the Select Committee on Animals in Scientific Procedures.* HL Paper 150-1 TSO, London.

Many organisations have commented previously on the collection and publication of the annual statistics by the Home Office, including the House of Lords Select Committee on Animals in Scientific Procedures (2001), the Nuffield Council on Bioethics (2005) and the APC.[28,29] A recent APC report, published in 2005, comprehensively reviewed the Home Office statistics and made 34 recommendations to improve their presentation.[30] These included calls for:

- Methods of counting animals and procedures to be reviewed.
- More specific categories of the purpose and severity of procedures to be provided in the information tables.
- A database that can be searched easily, including searchable project abstracts, to be made available on the Home Office website.

The government responded to the APC report in January 2006, broadly accepting many of the recommendations.[31] The redesign of the annual statistics to make the data presentation more user-friendly is now being taken forward. The Home Office have modified the presentation of the statistics in their most recent 2006 report, although there is scope for greater efforts to make the information accessible to a wider audience. A large amount of information on different animal species is collated and reported each year; the provision of data in a searchable online format may be the most effective way forward.

Reporting requirements under EU law are also currently under revision, and will probably affect how statistical data are collected and presented in all member states, including the UK.[32,33] Other bodies have already carried out detailed examinations of issues related to the compilation and presentation of annual UK statistics on animal use in research. We will not therefore make any further comments or recommendations on this subject, other than to say that we support the recommendations made by the APC in their 2005 report and welcome progress in this area.

3.4 UK non-human primate use

3.4.1 Species and numbers

In 2005, 4,652 procedures were carried out on non-human primates (0.16% of all scientific procedures involving animals). The actual *number* of non-human primates used in scientific procedures in 2005 was 3,115, an increase of 12% on the 2004 figure of 2792.[34,35] According to the Home Office, the use of macaques for pharmaceutical safety and efficacy testing is the main reason for this increase. Each year, approximately 75% of

Figure 3.4 Numbers of New World and Old World primates used in scientific procedures 1995-2005

29 Nuffield Council on Bioethics (2005) *The ethics of research involving animals*.
30 Home Office: Animal Procedures Committee. (2005) *Report of Statistics Working group.*
31 Home Office (2006) *Report by the Animal Procedures Committee on the Statistics of Scientific Procedures on Living Animals, Government Response.*
32 European Union (1986) Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Paris, France.
33 Full details available on EUROPA website: http://ec.europa.eu/environment/chemicals/lab_animals/revision_en.htm
34 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain 2005.* HMSO, London.
35 Home Office (2005) *Statistics of Scientific Procedures on Living Animals Great Britain 2004* HMSO, London.

the non-human primates used in UK laboratories are involved in toxicological testing of pharmaceuticals and almost all of these studies are carried out to meet legislative requirements.

The annual number of non-human primates used in the UK in the period 1995-2000 has remained fairly constant, at around 3,300 per year (figure 3.3).

In 2005, 643 New World monkeys were used for the first time, all of which were marmosets or tamarins, and 2,472 Old World monkeys were used, all of which were macaques. The number of New World primates used for research purposes has been generally decreasing since 1995, while the numbers of Old World Primates used have fluctuated in recent years (figure 3.4).

Interestingly, while the investment in medical research in the UK has increased significantly over the past 10 years, this has not been matched by a growth in numbers of non-human primates (see figure 3.5).

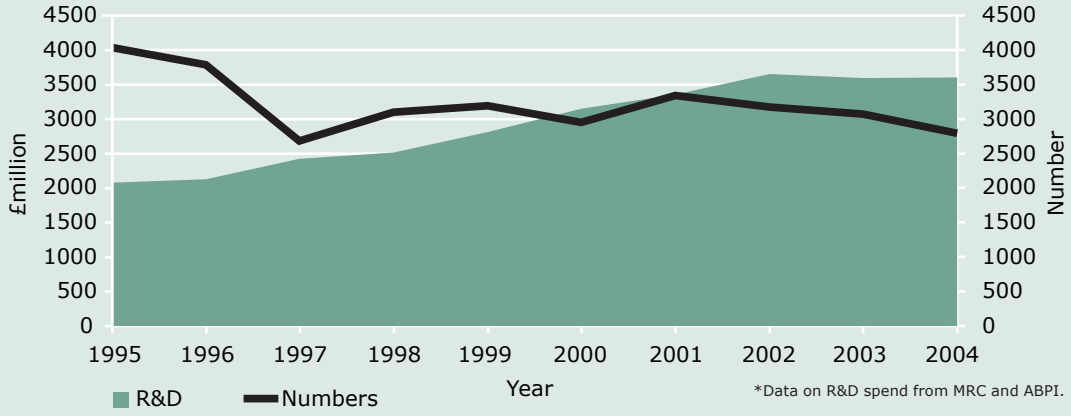
3.4.2 Types of procedure

The annual statistics provided by the Home Office divide types of scientific procedures carried out on non-human primates into the following categories:

- Fundamental biological research.
- Applied studies in human medicine and dentistry, including toxicological tests or other safety or efficacy evaluation.
- Protection of man, animals and environment.
- Direct diagnosis.

In general, many of the procedures carried out on non-human primates are classified

Figure 3.5 R&D investment and non-human primate numbers*



*Data on R&D spend from MRC and ABPI.

Table 3.1 Non-human primate use in 2005 (number of procedures)[37]

	Marmoset and tamarin	Squirrel/Owl/ Spider monkey	Macaques	Total Procedures
Fundamental biological research	**147**	**4**	**96**	**247**
Applied studies-human medicine or dentistry				
Non-toxicology	348	20	9	
Toxicology (incl. safety/efficacy)	378		3217	
	726	**20**	**3226**	**3972**
Protection of man, animals or environment (safety)	**21**		**396**	**417**
Direct diagnosis[38]	**16**			**16**
Total	**910**	**24**	**3718**	**4652**

37 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain* 2005 HMSO, London.

38 According to the Home Office, this category involves the investigation of disease including suspected food poisoning. This caters for procedures carried out for the purpose of diagnosing disease in an individual human or animal patient or a group of such patients. There is no research function; these are essentially applied studies.

as mild to moderate (see 3.4.4) and do not require anaesthesia. In 2005, 3,980 (86%) procedures carried out on non-human primates did not require anaesthesia. 536 (12%) procedures involved animals receiving a general anaesthetic to recovery, and in 67 procedures (1.4% of all procedures) the animals received a terminal general anaesthetic without recovery. There were no procedures using genetically modified non-human primates for breeding or research purposes.[37]

The numbers of non-human primates used for each purpose vary each year, but the most recent statistics from 2005 have been further examined to give an indication of the types of use (see table 3.1).

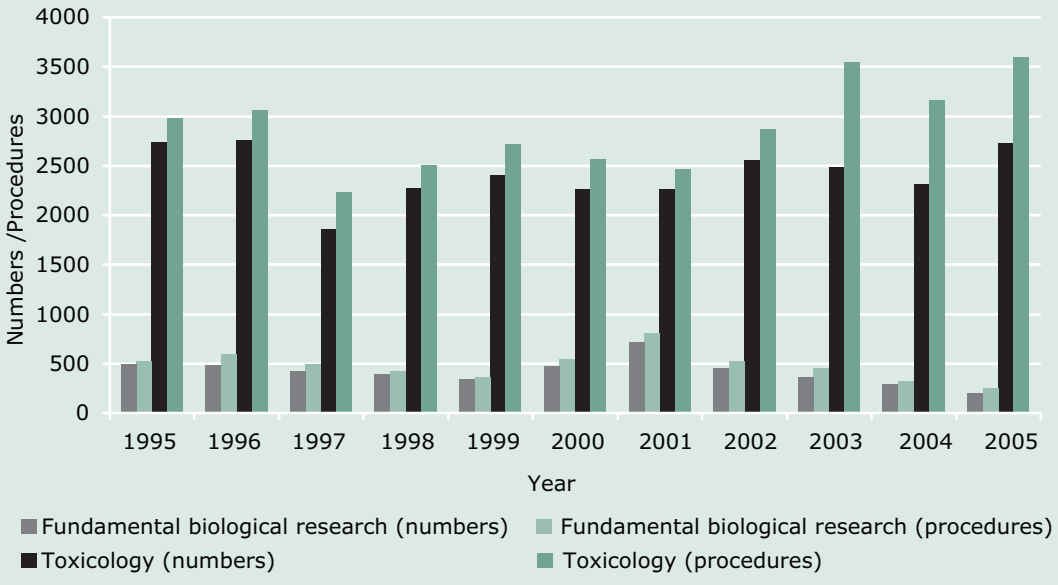
3.4.2.1 Fundamental biological research

Fundamental biological research is carried out with the primary intention of increasing knowledge of the structure, function and malfunction of man and other animals.[39] According to the annual Home Office statistics, this category includes *'physiological, pathological, pharmacological, genetic and biochemical studies, including toxicological evaluation.'*

In 2005, 203 non-human primates were used in 247 procedures for the purpose of fundamental biological research. This is a slight decrease on 2004, when 288 non-human primates were used in 315 procedures.[40] The number of non-human primates used in fundamental biological research has declined since 2001 (figure 3.6). In contrast, the number of non-human primates used in toxicological testing has remained relatively constant during this period, with an increasing trend in the number of procedures carried out for this purpose (figure 3.6).[41]

In 2005, within the category of fundamental biological research, marmosets and tamarins were used mainly for studies in psychology, physiology and pharmacology, with a smaller number being used for immunology and microbiology. Macaques were mainly used for studies in immunology, physiology, microbiology and anatomy. It is difficult to ascertain from the Home Office statistics the exact purpose for which the animals were used, since only broad categories are provided. However, a number of specific (non-toxicology) procedures are listed in the statistics provided by the Home Office.

Figure 3.6 Number of non-human primates and procedures carried out in fundamental biological research and toxicology studies



39 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain 2005.* HMSO, London.

40 Home Office (2005) *Statistics of Scientific Procedures on Living Animals Great Britain 2004.* HMSO, London.

41 The Home Office point out that rodents were used in 80% of all toxicology procedures in 2005, while non-human primates were used in less than 1%.

In 2005:

- 108 non-human primates are listed as having the procedure *'interference with brain'.*
- 8 had *'interference with organs of special sense'.*
- 1 had *'psychological stress'.*
- The majority of procedures carried out are classified as *'other techniques'*.

3.4.2.2 Applied studies in human medicine and dentistry

According to the animal licensing regulations, applied studies in human medicine and dentistry consist of research, development and quality control of products and devices, including toxicological evaluation and safety or efficacy testing. In 2005, a total of 377 procedures involving non-human primates were carried out for the purpose of *'applied studies in human medicine and dentistry',* not including toxicology. Within this classification, the majority were used in pharmaceutical research and development.

3.4.2.3 Toxicology

The highest numbers of non-human primates are used in toxicological testing of pharmaceutical products. Toxicology studies accounted for 77% of the 4,652 scientific procedures carried out in 2005, with 3,217 procedures carried out on macaques and 378 on marmosets or tamarins (table 3.1). The annual Home Office statistics include details of the categories of toxicological tests for which non-human primates are used (table 3.2).

All toxicological procedures were classified as being carried out for the purpose of safety or efficacy testing of pharmaceuticals, 98% of which were performed to satisfy legislative requirements. The percentage of acute or lethal tests carried out is very low in toxicological studies on non-human primates. Whilst most of the procedures were for safety testing purposes, a small percentage was used in method development or for absorption, distribution, metabolism, excretion (ADME) and residue tests. Non-human primates are very rarely used in toxicity testing for substances other than those intended for medical use.

According to the returns from all countries in the EU in 2002, the proportion of non-human primates used specifically for toxicological

Table 3.2 Scientific procedures by species of animal and type of toxicological test in 2005[42]

Type of test	New World (Marmosets)	Old World (Macaques)
Acute lethal toxicity	-	-
Acute lethal concentration	-	-
Acute limit setting	35	-
Acute non-lethal clinical sign	5	8
Subacute limit setting or dose ranging	55	375
Subacute toxicity	52	975
Subchronic and chronic toxicity	155	886
Toxicokinetics	30	316
Other	46	657
Total	**378**	**3217**

The different categories of toxicology procedures involve:

- **Acute** toxicity involves a single exposure to a substance. In the past death was used as a criteria of toxicity with LD50 being the usual test done, but this has now been replaced by alternative methods, which replace death as the endpoint with signs of significant toxicity instead. For acute toxicity testing of pharmaceuticals, maximum tolerated dose (MTD) studies are carried out to aid the later process of dose selection.
- **Subacute** exposure involves repeated exposure of chemicals at subacute doses over a period of one month or less. The "no observable adverse effect level" provides a quantitative measures of toxicity of each chemical in each animal studies. "Safe" levels for humans is then projected at 1/100 of this amount.
- **Subchronic and chronic** refer to exposure between one to three months and of more than three months respectively.
- **Toxicokinetics** includes tests for the absorption, distribution around the body, metabolism and excretion (ADME) of medicines.

42 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain 2005.* HMSO, London.

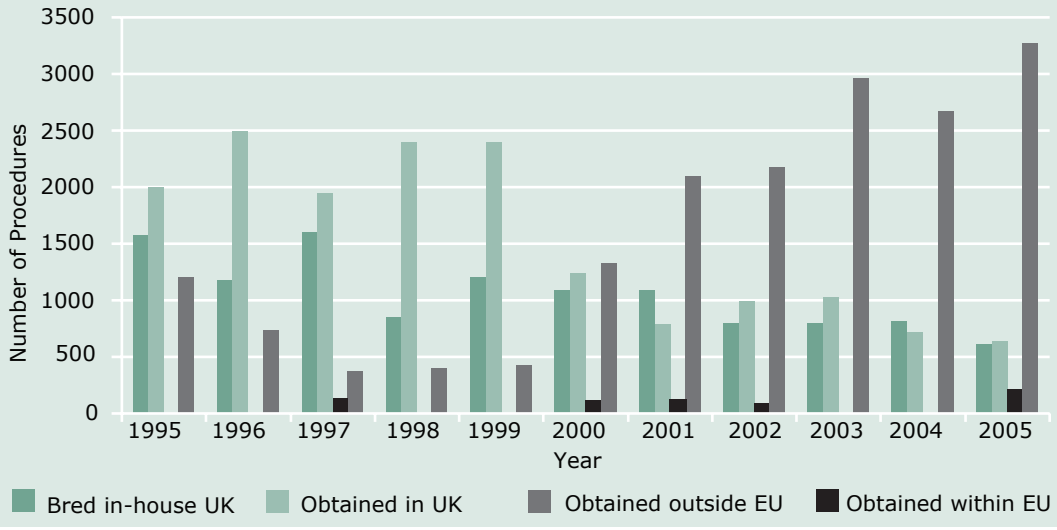
studies was 66%, with 17% of non-human primates used for '*biological studies of a fundamental nature.*'[43] In the UK in 2005, 87% of all non-human primates were used in toxicology-related procedures, with 6.5% used for studies in fundamental biological research.

The APC published a detailed report in 2002 on the use of non-human primates under the A(SP)A, which focused on the analysis of current trends, particularly in regulatory toxicology.[44] In this report, the APC made a number of recommendations aimed at reducing the number of non-human primates used in toxicological testing in the pharmaceutical industry. These included approaches to accelerate the development and implementation of non-animal alternatives by industry and the instigation of a detailed examination of regulatory policies on species selection in toxicity testing. The government held a primate stakeholders meeting in January 2004 to discuss the APC recommendations and the issues and questions contained in the report. In their response, published in April 2006, the government stated that it had already implemented many of the recommendations in the APC report and that the evidence from discussions at the stakeholders meeting suggested that unnecessary research and testing was not being carried out on non-human primates.[45] The use of non-human primates in drug development and testing is further discussed in section 8.

3.4.3 Sources of non-human primates

Researchers are required to provide information on the sources of the non-human primates used in experimental procedures in their annual returns to the Home Office. The Home Office guidance document states that approval for the acquisition of primates from overseas will only be given if the conditions at the breeding or supplying centre are acceptable to the Home Office. Each batch of animals acquired from overseas must be separately authorised and the transport arrangements approved by the

Figure 3.7 Source of non-human primates used in UK research*



* Figure adapted from Rennie & Buchanan-Smith, 2001[52] and HO Annual Statistics 1995-2004.

Table 3.3. The number of non-human primates for which import licenses were issued for biomedical or scientific research according to the UK CITES permit records[46]

Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Number	2161	2224	720	2356	1300	1216	2003	2293	1794	1518	1476

43 Commission of the European Communities (2005) *Fourth report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union*, available at: http://europa.eu.int/comm/environment/chemicals/lab_animals/pdf/com_2005_7_en.pdf

44 Animal Procedures Committee (2002) *The use of primates under the animals (scientific procedures) Act (1986).*

45 Home Office (2006). Government response to the Animal Procedures Committee's report on the use of non-human primates.

46 This data from the CITES database counts gross imports only; re-exports are not included in these figures. The statistics exclude primates traded within EU member states. CITES trade statistics were derived from the *CITES Trade Database*, UNEP World Conservation Monitoring Centre, Cambridge, UK. http://sea-bov.unep-wcmc.org/citestrade/

Home Office. These practices have also recently been reviewed by the APC.[47,48] However, the information on 'source of animals' provided in the annual statistics relates to number of *procedures*, rather than number of *animals*, so it not possible to determine exactly how many animals are sourced from overseas. In addition, details are not provided on the actual origin of the primates, which means that some of the animals from UK suppliers may have been born outside the UK. Both of these points have been highlighted in a recent report on the statistics by the APC and are being considered by the government.[49] The last primate importation and supply establishment closed in 2000 in the UK, which is reflected in the significant increase in animals sourced outside the UK since 2000 (figure 3.7).[50,51]

The actual number of non-human primates imported into the UK is recorded in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) database, which holds records for all trade in wildlife worldwide. Most of the animals imported are long-tailed (cynomolgus) macaques (approximately 85% of imports), with a small number of rhesus macaques and other species. Marmosets are usually bred in-house in the UK. The majority of macaques are imported from Mauritius, in addition to the Phillipines, Israel, China and Vietnam. Information is also given by CITES on the purpose for which the animals are imported (commercial, breeding, scientific or biomedical use) and the source of the animal, i.e. whether they are wild-captured, bred in captivity or born and bred in captivity.

The majority of non-human primates currently imported into the UK for biomedical research are born in captivity (first-generation animals). Table 3.3 provides details of the number of non-human primates imported into the UK annually, according to information on the permits granted.[53] Evidence presented to the working group indicated that the majority of the primates used in academia are sourced from breeding centres within the UK, suggesting that most imported animals are used by industry for toxicological testing purposes.

3.4.4 Levels of severity in procedures

While it is important to know the numbers of non-human primates involved in research, it is also crucial to understand the amount of suffering to which they are exposed. The current UK system requires prospective assessment of the likely costs to an animal from a scientific procedure; procedures are classified into severity levels in the project licence application and agreed with ethical review committees and Home Office inspectors.

There are two types of assessment:

- The overall severity band, which is the anticipated average suffering experienced by all the animals used.
- The severity limit of individual protocols, determined by the maximum level of suffering that may be experienced by an individual animal.[54]

The four levels of severity that can be assigned to a research project are mild, moderate, substantial or unclassified:

- *Mild* includes procedures that give rise to mild or transitory minor adverse effects.
- *Moderate* includes toxicity testing and surgical techniques that do not involve lethal endpoints.
- Procedures in the *substantial* category result in a major departure from the

47 Home Office (1995) *Code of practice for the housing of animals in designated breeding and supplying establishments. Part 2: 9. Non-human primates.*

48 Animal Procedures Committee (2006) *Acceptance of overseas centres supplying non-human primates to UK laboratories: A report by the primates sub-committee of the APC.*

49 Home Office: Animal Procedures Committee. (2005) *Report of Statistics Working group.*

50 Prescott MJ (2002). *Counting the cost- Welfare implications of the acquisition and transport of non-human primates for use in research and testing.* Royal Society for the Prevention of Cruelty to Animals.

51 Home Office (1995-2006) *Statistics of Scientific Procedures on Living Animals Great Britain 1994-2005.* HMSO, London.

52 Rennie AE & Buchanan-Smith (2003) *Report on the extent and character of primate use in scientific procedures across Europe in 2001.*

53 These figures cannot be correlated to the information given in the Home Office Annual Statistics because the imported animal may not be used for scientific procedures in the same year that it was imported, and the information given in the statistics relates to number of procedures carried out rather than number of animals used.

54 Home Office (2005) *Statistics of Scientific Procedures on Living Animals Great Britain 2004*. HMSO, London.

animal's usual state of health and can involve major surgery, toxicity testing leading to death or the use of animals as disease models.

- If all the protocols are under terminal anaesthesia or on decerebrate[55] animals, the overall severity limit of the project is *unclassified*.

Assessments of severity (of individual protocols or the project as a whole) must be reviewed and revised as necessary during the lifetime of a project.

It is difficult to determine the severity of procedures carried out on individual animals from reading published information alone. According to the APC report '*The use of primates under the animals (scientific procedures) Act 1986'*, the only way to gain a comprehensive understanding of the procedures carried out is to read the relevant project licences.[56] Abstracts of project licence applications are now available through the Home Office '*Animals in Scientific Procedures'* website.[57] While the abstracts are not yet in searchable form, the government's response to the 2005 APC report pledged to take this forward. This should make it easier to get a picture of the type of procedures that are carried out on non-human primates in the UK.[58]

The APC Primate Subcommittee receives a breakdown of the categories of projects specifically involving non-human primates each year, but this information is not in the public domain. Statistics on the number of projects on non-human primates granted in the five years from 1997-2001 are published in their 2002 report. This states that an average of 20 projects were approved each year during that period, and that the majority of projects were in the category of mild to moderate severity (27% mild, 67% moderate, 3% substantial and 3% unclassified). The actual number of non-human primate applications assessed by the committee is relatively low, since they generally only review proposals that involve procedures of substantial severity or the use of wild-caught animals. For example, in 2004 no application for a specific project licence involving non-human primates was referred to the committee, but they did report that two licence applications involving the use of non-human primates were received in late 2004, on which they had still to advise.[59] One application involved the investigation of stem cell therapy in Parkinson's disease and the other concerned the efficacy of antibiotics for the treatment of anthrax. In 2003, the APC considered and approved two applications requesting the use of non-human primates, both of which involved marmosets. One application involved the development of an improved vaccine and therapy regime for an infectious disease and the second involved evaluating the efficacy of new drugs to treat Parkinson's disease.[60]

The limitations of the current Home Office classifications of severity have previously been highlighted by a number of organisations; the APC, Boyd Group, RSPCA and Nuffield Council on Bioethics have all made a number of recommendations on this issue in recent reports.[61,62,63] The Nuffield Council report found the terminology used to describe the severity of projects, and information about the suffering of the animals involved, to be unsatisfactory. The report recommended that annual statistics should provide case studies of projects and procedures in each category of severity and that retrospective information about the level of suffering involved should be made available. The report also criticised the system of severity banding, in particular the use of the term 'unclassified', which they believed to be vague and uninformative. We fully support the recommendations made by the Nuffield Council in this area and agree that there

55 Decerebrate animals have had cerebral brain function eliminated by removal of the cerebrum, cutting across the brain stem, or severing certain arteries in the brain stem. As brain destruction is incomplete in decerebrate animals, such animals are considered to be living for the purposes of the Act.
56 Animal Procedures Committee (2002) *The use of primates under the animals (scientific procedures) Act (1986).*
57 http://scienceandresearch.homeoffice.gov.uk/animal-research/publications/001-abstracts/?version=2
58 Home Office (2006) *Report by the Animal Procedures Committee on the Statistics of Scientific Procedures on Living Animals, Government Response.*
59 Animal Procedures Committee (2005) *Report of the Animal Procedures Committee for 2004.* HMSO, London.
60 Animal Procedures Committee (2004) *Report of the Animal Procedures Committee for 2003.* HMSO, London.
61 Animal Procedures Committee (2002) *The use of primates under the animals (scientific procedures) Act (1986).*
62 The Boyd Group and the RSPCA (2004) *Categorising the severity of scientific procedures on animals.* RSPCA Research Animals Department, UK.
63 Nuffield Council on Bioethics (2005) *The ethics of research involving animals*.

is an urgent need to present clearer information about the nature and effects of scientific procedures involving non-human primates.

A report on the retrospective reporting of suffering and severity has recently been published by the Laboratory Animal Science Association (LASA) and the APC.[64] This report looked at the feasibility of a system in which researchers are required to report the level and duration of severity of procedures retrospectively, to give a more accurate indication of the actual level of suffering experienced by animals and report any unexpected adverse effects. The recommendations of this report are discussed further in section 10.

3.5 Main fields of non-human primate research, excluding toxicology

The Home Office statistics give limited information on the purposes for which non-human primates are used in scientific procedures. According to the 2004 statistics, the majority of procedures carried out for the purposes of fundamental biological research were in the nervous system or special senses category, accounting for 159 of the 315 recorded procedures using non-human primates.[65] Within neuroscience, non-human primates are used for fundamental research into the structure and function of the normal and diseased brain, the study of memory, cognition and behaviour and for research into vision. Applied studies in neuroscience are carried out into diseases such as Parkinson's disease, Alzheimer's disease and stroke (see section 6.7). The 2004 Home Office statistics show that 130 non-human primates were used for procedures in fundamental biological research related to reproduction (section 7). Non-human primates are also used in research on infectious diseases, especially HIV/AIDS, with 3% of all procedures in 2004 classified as involving research on the immune system (section 5).

A study by Carlsson *et al* identified the most common research areas for which non-human primates are used worldwide, following a detailed examination of scientific articles published in 2001.[66] These were: microbiology (including HIV/AIDS, 26%);

Table 3.4 Classification of non-human primate publications from UK researchers[67]

	Number of UK research publications						
Field of research	**1995**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
Neuroscience	**31**	**31**	**42**	**38**	**34**	**26**	**41**
Basic neuroscience, brain structure and function	11	18	23	18	18	15	26
Applied Neuroscience:							
Parkinson's Disease	9	6	9	12	7	5	7
Vision	11	2	8	8	6	5	6
Alzheimer's Disease		2	1				1
Stroke		2	1		3	1	
Addiction		1					
Infectious disease	**11**	**2**	**8**	**4**	**4**	**5**	**5**
AIDS	10	2	7	2	2	4	4
Other	1		1	2	2	1	1
Other							
Reproduction	6	7	9	3	2	2	3
Behavioural / Welfare studies	3	3	3	2	7	13	5
Xenotransplantation		2	3	1		1	
Anatomy – basic and applied	4	1	2	4	4	1	
Pharmaceutical R&D	6	1	2		1		
Gene Therapy							1
Total	**61**	**47**	**69**	**52**	**52**	**48**	**56**

64 Laboratory Animals Science Association/Animals Procedures Committee (2005) *Report of a LASA/APC Pilot study to assess the feasibility of collecting and reporting data on the severity of adverse effects caused to animals used in procedures regulated under the A(SP)A 1986.*

65 Home Office (2006) *Statistics of Scientific Procedures on Living Animals Great Britain 2005*. HMSO, London.

66 Carlsson HE, Schapiro SJ, Farah I, Hau J (2004) Use of Primates in Research: A Global Overview. *American Journal of Primatology* **63,** 225-237.

67 Any papers that were reviews, book chapters, abstracts, *in vitro* studies, work in zoos, and field studies that appeared to be carried out overseas but by UK authors were removed from this analysis. The remaining publications were those describing novel experimental research from UK laboratories using non-human primates. The publication was deemed a UK publication if the first author was based in a UK laboratory.

neuroscience (19%); biochemistry (12%); and pharmacology/physiology (11%).

To gain a better picture of UK research involving non-human primates, we used an online resource, '*primatelit*',[68] to investigate the number of papers published by UK researchers using non-human primates as an experimental model (table 3.4). While not exhaustive, this study gives a useful indication of the fields of research where non-human primates are currently being used in the UK.

The results of this analysis do not appear to indicate a reduction in the number of papers published by UK non-human primate researchers over the past 10 years. In accordance with the Home Office statistics, by far the largest number of publications involving non-human primates has been in neuroscience. For example, an analysis of publications from the years 2000-2005 shows that between 54% and 73% of all relevant papers were in a neuroscience-related subject, including both basic and applied research. It should be noted that most non-human primate use in industry and for toxicological testing are not routinely published.

3.6 Other uses of non-human primates

Primates are rarely used for purposes outside of medical or scientific research, but they can be kept as pets (figures for 2000 show that licences were issued to keep 655 primates as pets in the UK[69]) and are also used for defence research. The Ministry of Defence (MoD) maintains facilities for animal breeding, housing and research at Dstl Porton (the defence, science and technology laboratory at Porton Down). In evidence to the working group, MoD representatives stressed that animals are not used to develop weapons or offensive capabilities, but to research protective measures and procedures to safeguard service personnel against battlefield and other chemical and biological hazards. Nearly 90% of research is said to be eventually published in the public domain. Projects include vaccine research for infectious agents and studies on non-human primate behaviour and physiology.[70]

The MoD is required to report the numbers of animals used for research to the Home Office under the 1986 Act. Numbers of procedure reported on primate species in recent years are given in table 3.5. The MoD has established an Animal Welfare Advisory Committee (including external representation), which reviews the care and welfare arrangements of animals in UK defence research and publishes annual reports.

3.7 Discussion

The aim of this section is to provide an overview of the use of non-human primates in UK medical research in recent years, including general trends in numbers, species and the types of procedure carried out. The results of the analysis were used to determine the areas of research, namely neuroscience and infectious disease, on which the working group should focus. Several of the wider themes to emerge from this overview are taken up in later sections of this report, particularly in section 10.

The Home Office is responsible for the regulation of non-human primate use in the UK and for the collection and publication of all information and statistics on their use. A number of expert groups and committees have previously examined the presentation of statistics on animal use and have made recommendations aimed at improving the provision of information and making the

Table 3.5 Number of procedures on non-human primates carried out for defence research purposes from 1995-2004.[71]

Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Number of procedures	17	14	30	128	60	34	68	42	23	30

68 http://primatelit.library.wisc.edu/

69 Greenwood AG, Cusdin PA, Radford M (2001). *Effectiveness study of the Dangerous Wild Animals Act 1976.*

70 Fairhall SJ, Dickson CA, Scott L, Pearce PC (2006) A non-invasive method for studying an index of pupil diameter and visual performance in the rhesus monkey. *J Med Primatol.* **35,** 67-77.

71 Information provided by dstl and the Sixth report of the Animal Welfare Advisory Committee (2002). Available at: http://www.mod.uk/NR/rdonlyres/F4AAE7BE-9D64-4FB5-A41F-481807F4DB21/0/awac_6threport.pdf

statistics easier to interpret. We support the recommendations of the Nuffield Council on Bioethics regarding defining, monitoring and recording severity levels during experimental procedures. In particular, we consider that the data provided in the statistics should be subdivided according to the nature or severity of the procedures and the fate of the animals. This should be part of wider efforts towards increased openness and clarity of the information on levels of suffering that are made publicly available (see also 10.5.2). The recent move to publish licence abstracts on the Home Office website is therefore welcome and further developments and debate in this area are to be encouraged.

The total number of non-human primates used for scientific or medical purposes has remained fairly constant over the last 10 years, albeit with fluctuations. However, closer analysis indicates that there has been a steady decrease in the number of non-human primates used for the purposes of fundamental biological research in the last five years. The reason for this is unclear, although researchers assert that it is due to difficulties in the supply of animals, rather than a reduction in demand.

Despite a reduction in the numbers of non-human primates used for fundamental research, our analysis did not show any corresponding decrease in the number of papers published by UK non-human primate researchers. However, we could not establish if this reflects constancy in the number of UK non-human primate researchers, or whether a decreasing number of research groups are publishing an increasing number of papers.

The number of non-human primates used for toxicology studies has remained relatively constant over the last 10 years, although the number of procedures appears to have increased. This indicates a degree of re-use of individual primates, an issue that has complex scientific and welfare implications (see 8.5.4 and 10.5.6). Figures relating to the source of non-human primates used in UK research suggest the demand for non-human primates in toxicology testing outstrips the supply that can be met within the UK. Again, this has important welfare implications in terms of the long distance transport of animals (see 10.4). The need to import non-human primates for toxicological purposes may also be a contributing factor in the increasing re-use of individual animals, although a conclusive picture would require a more in-depth analysis.

Evidence suggests that it can be easier to meet the needs of New World, compared with Old World, primates in captivity (see 10.5.5). There have been considerable efforts in recent years to promote the use of New World primates over Old World primates, with the latter now requiring special justification by the Home Office. However, this does not appear to have been reflected in usage trends, which show that the proportion of New World primates (relative to Old World primates) has decreased since 1995. This may reflect increases in the use of Old World primates for toxicology testing. Species selection is further discussed in section 10.5.5.

4 Investigation into the scientific basis for current non-human primate research

4.1 Background

There is already a large volume of literature debating the case for research on non-human primates. However, one of the main criticisms of the published material that was presented to the working group (and one with which it had some sympathy) was that many of the cases that either support or dispute non-human primate research deal with a specific set of experiments relating to an individual disease or physiological function. These cases often do not provide a background picture of the importance of the condition or function under investigation, nor the current state of progress in other approaches to its control or better understanding. A clear statement of what measures have been, or could be, taken to avoid the use of non-human primates by alternative experimental systems is also lacking in many instances. In short, these arguments for or against the use of non-human primates are often incomplete and difficult for non-experts, including scientists working in unrelated fields, to assess.

For these reasons, rather than attempt to investigate the entire field of non-human primate research, the working group decided to focus its attention on a small number of fields in which non-human primates are currently being used and to assess the scientific case in greater detail. After considering the pattern of current use of non-human primates in UK and overseas research (section 3) we have focused on neuroscience and infectious disease. We also briefly consider the use of non-human primates in the study of reproductive and developmental biology, and ageing.

In trying to assess the importance of current research involving non-human primates for the study of human disease, particular attention was directed at the overall health burden posed by these conditions. Questions that were addressed included their frequency, whether they are associated with a high mortality or chronic disability, and other approaches that are being explored for their control and management.

As well as the written and verbal evidence received, extensive use has been made of original sources and recent reviews. Due to constraints of space it has not been possible to cite all source articles; readers who wish to consult them will find most of them in the review articles cited. Particular emphasis has been placed on the level of biological or medical importance of each research topic, the complete spectrum of approaches that are available for their investigation, and, in particular, whether the particular scientific questions could be answered without the use of non-human primates, either now or in the future. For cases in which we received conflicting views, we have tried to reach our conclusions through a balanced analysis based on this broader investigation of these complex issues.

4.2 Why does the use of animals in biological and medical research vary so much between different fields?

The pattern of current use of non-human primates in medical research summarised in section 3 raises the question of why it seems to be largely restricted to a few particular fields. Does this reflect the nature of the work in different areas of research or the dogma of research groups using an animal model that is historically associated with a particular field? Does it simply reflect a fashion or does it suggest that researchers only involve non-human primates for particular kinds of research questions? For example, one of the major advances in the biological and medical fields of recent years is a better understanding of the cause and treatment of cancer, yet this field has made very little use of research in non-human primates in recent years. How can this be?

In 1911 Francis Peyton Rous discovered that certain cancers of chickens are caused by a transmissible agent with the properties of a virus. It was later discovered that many tumour viruses exist which carry one or more genes responsible for their ability to produce cancer. Hence these genes were named viral oncogenes. In 1989 Michael Bishop and Harold Varmus were awarded the Nobel Prize in Medicine for their remarkable discovery that all living organisms, including human beings, have genes that are very similar to viral oncogenes. Even more remarkably, it turned out that they are part of our cells' normal genetic machinery, responsible for the control of their proliferation, differentiation and development. More recently, research in the molecular and cellular aspects of cancer have defined many mutations in oncogenes, leading to a better understanding of the basic mechanisms of cancer and how drugs can be designed to interfere with oncogene activity. This work has been augmented by large epidemiological studies which have provided evidence about some of the environmental agents, tobacco smoke for example, that may underlie oncogene mutation. Thus, modern cancer research has moved very much to the cellular and molecular levels, utilising *in vitro* and rodent systems, without a significant requirement for research using non-human primates.

In short, a review of the development of the cancer research field over the last 50 years, and other biological and research fields, indicates that researchers only appear to resort to work requiring non-human primates for questions that cannot be approached in other ways. This principle seemed to be further validated as we explored the areas of major usage chosen for an in-depth review in this inquiry.

4.3 Can research on humans replace work on non-human primates?

As discussed above, some areas of medical research are becoming increasingly tractable using human tissue and *in vitro* approaches. Furthermore, the development of increasingly sophisticated imaging technology, rodent models of disease, new approaches to the analysis of drug metabolites, and other non-invasive technology (see section 9) have the potential to replace and reduce the use of non-human primates in research.

On the other hand, regulatory restrictions on research on human beings have become increasingly stringent in recent years, particularly if children or human tissues are involved. There is no doubt that a great deal of human experimentation (including self-experimentation) that was permissible in the past would be impossible to contemplate at the present time. Against the background of the public's increasing demands for drug safety, regulators are loath to reduce, or be seen to reduce, rigorous processes of drug toxicity testing.

This is not to say, of course, that a great deal of medical research on human beings is not being carried out at the present time. The major communicable diseases, discussed in section 5, provide some particularly good examples of both the advantages and problems that are encountered in research of this kind. Hundreds of healthy volunteers have been infected with potentially lethal malarial parasites in vaccine trials. However, malaria is a disease of rapid onset and, provided that drug-resistant strains are avoided, can be controlled with therapy. On the other hand, research directed at the development of a vaccine for HIV/AIDS or tuberculosis, cannot be carried out in human volunteers; both diseases run a much more chronic course, treatment is difficult, and it would be totally inappropriate to infect patients with these agents for research purposes.

In our investigation of areas of medical research that utilise non-human primates, we have tried to assess whether the questions could be approached by research on human beings, or their organs and tissues. Not surprisingly, in some cases, it was clear that human experimentation would provide more

clear-cut answers, but it was equally clear that such work would not be permissible. Striking a reasonable balance against this complex background has been a key issue in the preparation of this report.

4.4 The distinction between basic and applied research

The working group was aware that many people find animal research acceptable if it is directed towards finding the cause or treatment for serious diseases, but are less certain about its acceptability when it is applied to extending our knowledge of normal biological functions. One of the usual responses to criticisms of the use of animals in basic or fundamental research is that it is impossible to predict what practical issues may follow a better understanding of normal function.

There are certainly some unequivocal examples of invaluable advances in the control of disease that were unexpected and unplanned consequences of good basic science. The discovery of the agent that causes hepatitis B, a disease that affects hundreds of thousands of people worldwide and leads to liver failure and liver cancer, came initially from studies directed at determining genetic variability in the structure of proteins among different ethnic groups. Yet this work led ultimately to the development of the first vaccine for the prevention of hepatitis B. Similarly, studies that led to the first administration of penicillin started out as a fundamental research programme investigating the properties of bacterial cell walls and natural agents that suppress bacterial infection. More recently, research directed towards an understanding of why the growth of viruses is restricted in certain bacterial cells led to the discovery of restriction enzymes, which have since transformed the diagnosis and control of human genetic disease.

How many of the remarkable advances in medicine in the 20th Century arose from research that was not directly aimed at practical outcomes? Surprisingly, this important question has received relatively little attention. One of the only studies to have explored this problem, and one that is often quoted in support of the value of fundamental research, was published by Comroe and Dripps in 1976.[72] They concluded that 40% of the key research papers that had led to the 12 major advances in cardio-respiratory medicine at that time were based on research that had no immediate object other than to advance knowledge. This work has since been criticised, largely for its methodology, but apart from a smaller bibliometric study[73] there have been few serious attempts to analyse the origins of important medical advances with respect to different fields of basic science.[74]

A careful study of the evolution of the medical sciences during the 19th and 20th Centuries suggests that the distinction between fundamental and applied science is becoming increasingly artificial. For example, the historian Roy Porter, in his account of the development of the neurosciences in the second half of the 19th Century describes how research into the function of particular regions of the brain (work that utilised both patients and animals including non-human primates, and which was to form the basis for the later development of clinical neurology) evolved as a partnership between the basic and applied neurological sciences in which the distinction between them became increasingly blurred.[75]

Rapid developments in the biological sciences over the second half of the 20th Century illustrate even more clearly the continuum between basic and applied research. Advances in protein chemistry and molecular biology did not stem from research directed towards a practical or medical goal, yet by the early 1960s, it became apparent to clinical scientists that advances in these new fields would have

72 Comroe JH & Dripps RD (1976) Scientific basis for the support of biomedical science. *Science* **192**, 105-111.
73 Grant J, Green L & Mason B (2003) *From bedside to bench: Comroe and Dripps revisited.* Brunel University, Uxbridge.
74 UK Evaluation Forum (2006) *Medical Research: assessing the benefits to society*. Academy of Medical Sciences.
75 Porter R (1997) *The Greatest Benefit to Mankind: A Medical History of Humanity from Antiquity to the Present*. HarperCollins, London.

important implications for understanding disease mechanisms and the development of diagnostics and new forms of therapy. A recent review of the way in which knowledge was acquired about the inherited disorders of haemoglobin demonstrates the realisation of this ambition. It shows how a largely unplanned partnership between basic science and more clinically directed research can have a remarkable outcome for the control of diseases of this kind, which kill thousands of people globally each year.[76] By amalgamating clinical studies of patients with knowledge gained from basic research into the protein chemistry, molecular biology and genetic control of haemoglobin, it became possible to prevent and improve the management of these diseases, even in many developing countries.

In conclusion, there are many examples of important medical advances that, in the past, stemmed from the unexpected outcomes of fundamental research. Over the years, differences between fundamental and applied research have become much less distinct; biological research now reflects a continuum of work that investigates basic biological processes and their breakdown in disease states. As discussed later in this report, this change in the pattern of biological research is not restricted to the fields of molecular and cell biology, but is also reflected in a wide range of other scientific advances, such as remarkable new imaging processes for studying the function of the brain and developments in the computational technology of systems biology.

Hence in assessing the importance of biological science for our future well-being, the question of whether a piece of research is fundamental or applied science has become outdated. The study of normal function, as well as being central to our understanding of why we are what we are, is often a vital step in the elucidation of the mechanisms that underlie its breakdown in disease. The central issue is whether a programme of research is directed at an important biological or medical question and is designed in a way that has a reasonable chance of answering that question; hence the importance of the case-by-case assessment that forms the basis of UK legislation and practice around animal research.

76 Weatherall DJ (2004) Thalassaemia: the long road from bedside to genome. *Nat Rev Genet* **5,** 625-631.

5 Infectious (communicable) disease

5.1 Introduction

Infectious or communicable diseases are caused by a biological agent such as a virus, bacterium or parasite. Data from the World Health Organization (WHO) show that infectious diseases were responsible for approximately 14.9 million deaths worldwide in 2002 - roughly a quarter of all deaths. The working group elected to focus its attention on the current top three single agent killers: HIV/AIDS, tuberculosis (TB) and malaria. The role of non-human primates in research on other important infectious diseases and in outbreaks of disease due to emerging organisms is also briefly considered. Because of the considerable complexity of this field, and particularly for those who are unfamiliar with the terminology used to describe infectious organisms and different host defence mechanisms, a short summary of the major biological systems involved is provided in Box 3.

While respondents to the call for evidence stressed the need for a range of approaches - both biomedical and non-biomedical - in tackling infectious disease, many argued that education and public health measures alone cannot be relied on to stem an epidemic. They asserted that continued biomedical research, particularly toward developing effective vaccines to the three biggest killers, is essential for making a significant impact on the mortality and morbidity caused by infectious disease. Researchers also pointed out that, for many infectious diseases (including HIV/AIDS, TB and malaria), the major challenge is the lack of definitive information on their pathogenesis and the critical responses that an effective vaccine would need to elicit in order to prevent or control infection. In the absence of clinical data identifying a correlate to protection (or even a mechanism), basic research into the pathogenesis of disease and the impact of distinct vaccine strategies on these processes is essential for vaccine development.

Many respondents emphasised that diseases affecting whole physiological systems, such as the immune system, cannot be adequately modelled *in vitro* using cell-based approaches. They also emphasised that studying interactions between cells, tissues and systems requires a whole animal approach. Similarly, it was asserted that the adverse effects of potential vaccines and therapies can only be elucidated in whole animals. Respondents cited the potential for robust animal 'challenge' models to markedly accelerate the process of vaccine development, arguing that such models offer the opportunity to test potential vaccines in ways that are not possible in humans.

Respondents cited several features of non-human primates that render them superior to other animal models for studying infectious disease. For example, there are major differences between rodent and primate immune systems including natural killer-cell receptors, Toll-like receptors (TLR), carbohydrate–binding lectins such as DC-SIGN, and adhesion molecules such as the ICAMS (see Box 3). Furthermore, pathogens tend to be host-specific, so certain immune-evasion genes or viruses might function in primates but not in rodents. This can lead to differences between rodents and primates in the immunogenicity of virus-based vaccines. For instance, mice may show strong immunogenicity induced by recombinant DNA vaccines, which does not always translate to humans.

However, other respondents drew attention to the number of research programmes involving non-human primates that have not delivered clinical treatments. In light of this, several respondents questioned whether preclinical animal models currently in use are sufficiently valid for, and predictive of, the safety and efficacy of human vaccines and therapies. In this section we have attempted to assess the future role of non-human primates in research into HIV/AIDS, TB and malaria. Although some of the research described in this section

is directed at the discovery of new forms of treatment for infectious diseases, the bulk of it deals with the production of vaccines for their prevention. Since vaccine trials often involve healthy children their development requires particularly high levels of stringency in terms of efficacy, safety and regulation.

5.2 HIV/AIDS

Acquired Immunodeficiency Syndrome (AIDS) was first identified in 1981 and attributed to infection with Human Immunodeficiency Virus (HIV) in 1983. Currently it is estimated that more than 40 million people are infected worldwide, with 4 million new infections each year, of which 10% are children (see figure 5.1). While the number of deaths decreased in almost every infectious disease between 1993 and 2002, it increased four-fold in HIV/AIDS, which now claims the lives of 3 million people per year.[77] There at least 25 million infected people in sub-Saharan Africa and in countries such as Zimbabwe, where about 30% of the population is infected, life expectancy has decreased from 65 years to 37 years in the last 20 years. In the face of this pandemic, enormous efforts have been made to understand the nature, infectious process and pathology of the virus involved and to develop drugs and vaccines that will prevent transmission of the infection or the development of the disease.

HIV is primarily transmitted through sexual intercourse, but can also be contracted by perinatal transmission from mother to child

Box 3. Host/pathogen biology in infectious disease

If an infectious agent is able to penetrate the physical and chemical barriers it encounters on body surfaces, it next meets a barrier imposed by the innate immune system. This consists of a variety of phagocytic cells with the capacity to engulf and destroy bacteria and intracellular parasites. The serum also contains an array of biochemical defences including the complement system which, among its many actions, enhances phagocytosis and facilitates the destruction of bacteria and viruses. Similarly, cytokines are small proteins produced by white blood cells (dendritic cells and macrophages) that initiate inflammation, sensing the danger of an invading micro-organism.

There is a more specific acquired immune system that is mediated by white blood cells called lymphocytes, of which there are two main varieties, B cells and T cells. **B cells** produce antibody that is capable of destroying bacteria and other agents by facilitating phagocytosis, immobilisation, and blocking toxins produced by the organism. Immunity to viruses is largely a function of T-cells. **T cells** are divided into two major subtypes: helper T cells that carry the CD4 glycoprotein and recognise antigens presented by class II human leukocyte antigen (HLA) molecules on antigen presenting cells; and cytotoxic T lymphocytes (CTL) that carry CD8 and respond to peptides presented by HLA class I. Unlike bacteria, viruses live inside cells out of the reach of antibody. T cells are able to kill virus-infected cells and release cytokines such as interferon and tumour necrosis factor (TNF). There are several different classes of helper T cells that release different cytokines on antigenic stimulation.

Micro-organisms also undergo an equally complex series of changes in order to try to evade the immune systems. Some of the proteins and cells of the immune systems and properties of infectious agents that are mentioned in this section are listed opposite.

77 UNAIDS/ WHO (2005) AIDS Epidemic Update.

and by exposure to infected blood, usually through blood transfusion or contaminated syringes used for drug injection. AIDS is characterised by a steady depletion of the body's immune function, reflected by a fall in the level of particular subsets of blood lymphocytes, notably CD4 cells. The complex pathophysiology of HIV's effect on the immune system has been reviewed by several experts in the field in recent publications.[78,79,80,81]

As immune function deteriorates a variety of complications occur, including increased susceptibility to common infections such as

Box 3 (continued)

Host Defence	Components		Function
Skin and epithelial surfaces surfaces	Fatty acids Protective secretions Microflora		
Non-specific (innate) immunity	Complement		Interaction with antibody; lysis; opsonization (ingestion and antibody coating)
	Soluble recognition receptors	C reactive protein; mannose-binding lectin	Complement binding; phagocytosis
	Surface receptors	CR1 CR2 DEC205 TLR, CD14	B-cell activation; antigen presentation; mediation of lipopolysaccharide response
	Cellular components	Macrophages	Ingest and kill pathogens
		Dendritic cells	Antigen presentation; activate CD4 and CD8 cells; surface receptors include DC-SIGN, which binds viruses
		Neutrophils	Ingest and kill pathogens
		Natural killer cells	Cytotoxic to virus-infected cells
		Inflammatory cytokines including: interferons; tumour necrosis factor; interleukins	Anti-viral; augment immune responses; augment inflammatory response
		Cellular adhesion molecules including: ICAM-1; P. Selectin	Cell adhesion in inflammation
Specific Immune Response	Antibody (B lymphocyte) mediated	Specific immune globulins (Ig)	Neutralise virus and toxins; opsonization; phagocytosis; agglutination, complement activation.
	Cellular (T Lymphocyte) mediated	Recognise processed antigen on infected and dendritic cells. Aggregate at sites of infection.	Kill infected cells; release cytokines; develop delayed hypersensitivity response
Genetic Resistance	Genetic variation in many host defence systems modifies susceptibility to infection		

Pathogens	Function
Adhesion and cell entry	Multiple surface proteins bind to specific cell-membrane receptors Toxins disrupt cell-membrane proteins
Spread	Local tissue destruction; blood and lymphatics; shed from blood cells
Evasion of host defence	Bacterial capsules; inactivation of complement, cytokines or Igs Rapid genomic changes leading to change in antigenic structure and acquisition of antibiotic resistance
Virulence factors	Rapid and major changes in genome structure result in activation of virulence genes including toxins, adhesion molecules and secretory proteins.

78 McMichael AJ, Phillips RE (1997) Escape of human immunodeficiency virus from immune control. *Annu Rev Immunol*. **15,** 271-96.
79 Blankson JN, Persaud D, Siliciano RF (2002) The challenge of viral reservoirs in HIV-1 infection. *Annu Rev Med.* **53**, 557-93.
80 Simon V, Ho DD (2003) HIV-1 dynamics in vivo: implications for therapy. *Nat Rev Microbiol.* **1**, 181-90.
81 Kaufmann, S.H. & McMichael, A.J. (2005) Annuling a dangerous liaison: vaccination strategies against AIDS and tuberculosis. *Nat Med* **11,** S33-44.

Figure 5.1. A global view of HIV infection, 38.6 million people (33.4 - 46 million) living with HIV in 2005.[82]

tuberculosis and organisms that do not cause disease in those with intact immune systems, as well as a wide variety of cancers and several neurological disorders. Above and beyond being a lethal disease, AIDS therefore leads to death after variable periods of intense suffering.

5.2.1 Current approaches to the control and management of HIV/AIDS

The major approaches currently used in many countries to curb the AIDS epidemic revolve around public health interventions, the institution of screening programmes and the administration of single or combined antiviral agents.[83] Education programmes are used to try to stem sexual transmission, together with the promotion of condoms, screening and, where available, treatment. A variety of approaches are being attempted to prevent mother-to-child (or 'vertical') transmission, including: restricting the numbers of pregnancies amongst infected mothers; the use of anti-viral therapy for infected mothers, and the application of feeding substitutes to reduce the frequency of breast-feeding.[84]

Several related approaches are being undertaken to reduce the frequency of infection as a result of drug use. For instance, several countries have successfully piloted needle-exchange schemes that give access to safe and clean needles for injecting drug users. Most developed countries now also control blood-borne infection through the appropriate screening of blood and blood products.

Combination therapy has been shown to be associated with prolonged survival. Highly Active Anti-Retroviral Therapy (HAART), involving a combination of several antiretroviral agents, has been shown to reduce the number of viral particles in the bloodstream, leading to improved T-cell counts. Evidence shows that if the levels of HIV particles remain suppressed and the CD4 count remains greater than 200, the quality and length of life can be significantly improved and prolonged.[85] However, these agents have a considerable array of side effects and drug resistance is an ever-increasing problem. Furthermore, there is great uncertainty about the potential long-term toxicity of these agents, which may increase

82 UNAIDS/WHO (2006). *2006 Report on the global AIDS epidemic*.

83 Bertozzi S, Padian NS, Wegbreit J, DeMaria LM, Feldman B, Gayle H, Gold J, Grant R & Isbell MT (2006) HIV/AIDS Prevention and Treatment. In: *Disease Control Priorities in Developing Countries* (ed. by D. Jamison *et al.*), pp.331-369. Oxford University Press and the World Bank, New York, Washington.

84 UNAIDS/ WHO (2005) AIDS Epidemic Update.

85 Bertozzi SM, Bautista-Arredondo S (2006) Modelling the impact of antiretroviral use in developing countries. *PLoS Med.* **3**, e124.

the risk of atherosclerosis, liver failure and cardiac failure.[86]

Another important research field directed at the prevention of HIV/AIDS is the development of microbicides, i.e. anti-microbial medications designed for vaginal administration to prevent the transmission of HIV and, ideally, other sexually transmitted infections. Although no agents of this type have been proven to block the sexual transmission of HIV, at least a dozen are in advanced stages of development.[87] A strategy for the further development of research in this field was released at the XVI International AIDS conference in Toronto in 2006. There is still considerable uncertainty as to whether this approach, together with other public health measures, will control the HIV/AIDS epidemic and hence it seems vitally important to pursue other measures, including research into the development of a vaccine.

The results of prevention and treatment programmes have varied widely between different countries, depending mainly on political issues and the effectiveness of available healthcare programmes. While the burden of disease has fallen in countries such as Uganda and Thailand, the disease remains out of control in many other developing countries. The vital importance of developing a vaccine for the control of HIV/AIDS was emphasised in a recent report assessing future priorities for disease control in the developing countries published jointly by the World Bank, WHO and the Fogarty International Center of the National Institutes of Health.[88]

Developing a HIV/AIDS vaccine has been the subject of intense research in both academia and industry, but unfortunately with only limited success. Researchers emphasise that the field is still hindered by an incomplete understanding of the pathology of the disease. Respondents noted the absence of clinical evidence that individuals infected with HIV can eliminate the virus. This is compounded by the limited evidence that individuals who are repeatedly exposed to the virus, but remain uninfected, maintain this status by immunological mechanisms. There has therefore been limited information upon which to design a rational vaccine strategy and researchers argue that non-human primates provide a vital tool for investigating pathogenesis and developing candidate vaccines and other drug therapies.

5.2.2 The role of non-human primates in HIV/AIDS research: pathogenesis and virology

HIV is caused by two viruses:

- **HIV-1** is the more virulent strain and is globally distributed. It was first transmitted to humans from the chimpanzee subspecies *Pan troglodytes troglodytes*, a conclusion based on the close similarity of the genome sequences of Simian Immunodeficiency Viruses (SIVcpz) obtained from *P.t.troglodytes* to those of human HIV-1.
- **HIV-2** is geographically restricted to West Africa. It is closely related in sequence to SIVmac from macaques and to the SIVsm from sooty mangabeys.

Several respondents noted the limitations of non-human primates to model HIV/AIDs; the only animals known to be susceptible to HIV-1 are chimpanzees and pigtail macaques, but infection does not lead to to the onset of AIDS in either species. Some respondents pointed to the body of HIV research carried out in chimpanzees, claiming that it has produced little data that can be applied to humans. The chimpanzee was a focus for HIV work in the 1980s, given its status as the only known species that could be infected with HIV-1. However, as mentioned above, it was shown that very few chimpanzees infected with HIV-1 progress to a state of

86 Bertozzi S, Padian NS, Wegbreit J, DeMaria LM, Feldman B, Gayle H, Gold J, Grant R & Isbell MT (2006) HIV/AIDS Prevention and Treatment. In: *Disease Control Priorities in Developing Countries* (ed. by D. Jamison *et al.*), pp.331-369. Oxford University Press and the World Bank, New York, Washington.

87 Stone A & Jiang S (2006) Microbicides: stopping HIV at the gate. *Lancet* **368,** 431-433.

88 Jamison DT, Breman JG, Measham AR, Alleyne G, Cleason M, Evans DB, Jha P, Mills A, Musgrove (Eds) *Disease Control Priorities in Developing Countries.* Second Edition (2006). Oxford University Press and The World Bank.

immunodeficiency. Nevertheless researchers argue that the chimpanzee played a critical role in clarifying basic understanding of HIV-1.[89] It is intriguing that most chimpanzees do not appear to get sick when infected with HIV-1; knowing why might shed light on potential human treatments. However, this line of enquiry is now closed and the chimpanzee model has been replaced by work in other non-human primate species, in which HIV-1-like viruses produce infections and clinical signs resembling those in humans suffering from AIDS. Researchers currently use Simian Immunodeficiency Virus (SIV) or SHIV[90] non-human primate models to investigate disease pathogenesis and test novel therapies. SIV is endemic amongst African non-human primates, but only Asian primates, including Indian rhesus macaques, develop an AIDS-like disease upon infection.[91]

Many respondents pointed to the validity of the SIV/SHIV non-human primate model, the successes derived from such research and the difficulty of deriving data using other methods. For instance, the demonstration that cloned SIV causes AIDS in macaques fulfilled Koch's postulates[92] and helped counteract the denial of the HIV-AIDS relationship. Data generated from non-human primates have also given insights into crucial events that occur within days of infection that are responsible for establishing the subsequent course of disease. The equivalent clinical material would be extremely difficult to obtain from humans. Recent studies in macaques have also shown that SIV infection in gut-associated lymphocytes takes place on a huge scale, in contrast to the low level of virus infection in blood lymphocytes. Before this finding indicated that HIV might cause major problems in the gut, it was not ethically acceptable to biopsy the gut of HIV-infected patients. Since the study, it has been possible to confirm that HIV-1 infects large numbers of T cells in the gut of HIV-infected humans, and our understanding of HIV infection has changed markedly.

Some respondents drew attention to differences between SIV/SHIV-infected non-human primates and HIV-infected humans, for example the different rates at which infection with SIV/SHIV or HIV progress. However, researchers argue that this is to be expected when the viruses used for non-human primate experiments have been selected for rapid pathogenicity. There are SIV strains that are slow to progress, taking 5-10 years to cause AIDS, but the obvious time constraints make such strains difficult and impractical to study. It was also asserted that HIV mutates rapidly to escape the strong immune response it generates in humans, but the weaker immune response generated by SIV in non-human primates means it does not mutate so quickly. However, research shows this not to be the case.[93]

Nearly all HIV virus entry into human cells is via CD4 and CCR5 or CD4 and CXCR4 receptors, which is also the case for SIV infection in macaques. Several respondents highlighted that the homology between HIV-1 and SIV is less than 50% (although homology between SIV and HIV-2 is over 90%), which is important in relation to immune responses to viral coat proteins. This is one reason why SHIV hybrids expressing HIV-1 gp120 have been developed and used to test vaccines that stimulate antibodies to the envelope protein.

Researchers have also argued that research involving non-human primates has permitted the use of antibodies to deplete CD8 +ve immune T cells, demonstrating the importance of these cells in controlling acute infection.

89 VandeBerg JL & Zola SM (2005) A unique biomedical resource at risk. *Nature* **437**,30-32.

90 SHIV is a hybrid of HIV and SIV.

91 The reason why African primates do not develop AIDS is unknown, although further investigation could provide valuable insights.

92 Koch's postulates (or Henle-Koch postulates) are four criteria formulated by Robert Koch and Friedrich Loeffler to establish a causal relationship between a microbe and a disease. Koch's postulates are:
1. The organism must be found in all animals suffering from the disease, but not in healthy animals.
2. The organism must be isolated from a diseased animal and grown in pure culture.
3. The cultured organism should cause disease when introduced into a healthy animal.
4. The organism must be reisolated from the experimentally infected animal.

93 See for example, Evans *et al* (1999) Virus-specific cytotoxic T-lymphocyte responses select animo-acid variation in simian innumodeficiency virus Env and Nef. *Nature Medicine* **5**, 1270.

It has also helped to establish the roles of individual virus gene products in infection and pathogenesis.

5.2.3 The role of non-human primates in HIV/AIDS research: drug therapy and vaccine development

Non-human primates have been used relatively little for the development of anti-viral drugs (see Box 4). Their main use in research into the control of HIV/AIDS, as well as that described in the previous section, has been directed at attempts to develop a reliable vaccine.

Box 4. The use of non-human primates in developing HIV/AIDS drug therapy

Except for toxicology studies, anti-retroviral therapy has been investigated to only a limited extent in non-human primates. However, SIV-macaque experiments with the drug tenofovir demonstrated protection of newborn macaques against SIV infection and led to the development of the drug for use in humans.[94] In addition, the SIV-macaque system has been used to show the value of both monoclonal and polyclonal antibody preparations as pre-exposure and post-exposure therapies and to define the parameters of dose and timing required for their transfer to humans.[95]

It has been postulated that one of the major difficulties in developing an effective anti-HIV vaccine is the high rate of variation that occurs during HIV replication, which means that the infecting dose is a mixture of antigenically different viruses. It is difficult to achieve sterilising immunity against such mixtures and, in its absence, the retroviruses that escape partial immune blockade not only replicate, but integrate copies of their genomes into the DNA of host cells. This provides a reservoir of virus 'hidden' from neutralising antibodies and immune cells.

These properties of HIV present formidable challenges for potential vaccines and researchers argue that the SIV-macaque system is an important tool for establishing essential vaccine requirements. Research has focussed on candidate vaccines whose protective effects against virus infection and/or subsequent development of AIDS can only be assessed following deliberate viral challenge. Obviously, it is not possible to challenge human volunteers in this way and researchers argue that differences between rodents and primates (both human and non-human) mean that testing candidate vaccines in macaques is an important pre-selection step between mouse work and costly and lengthy human trials.

Two particular research lines involving non-human primates were drawn to our attention: the demonstration that live attenuated SIV can induce protection against super-infection with homologous virulent virus; and the development of challenge viruses through studies of anti-envelope glycoprotein vaccines of chimeric SHIV viruses containing the HIV-1 envelope glycoprotein. The SIV-macaque model provides a test system for these approaches that is comparatively rapid and permits experimental control of the heterogeneity of the virus challenge and the timing of both challenge and vaccination. Knowledge gained is then applied to the further development of anti-HIV candidate vaccines.

Currently, there are two main avenues being explored in relation to the production of HIV vaccines. First, HIV envelope protein immunogens that might stimulate protective antibodies are being produced. Second, attempts are being made to develop T cell-stimulating vaccines. With regard to the latter, vaccination studies in macaques that are then challenged with SIV have indicated that vaccines that stimulate T cell immunity can work. These findings stimulated a new approach to HIV vaccine development in the mid-1990s, which led to candidate vaccines

94 van Rompay KK, Dailey PJ, Tarara RP, Canfield DR, Aguirre NL, Cherrington JM, Lamy PD, Bischofberger N, Pedersen NC, Marthas ML (1999) Early short-term 9-2-(R)-(phosphonomethoxy) propyladenine treatment favourably alters the subsequent disease course in simian immunodeficiency virus-infected newborn Rhesus macaques. *J Virol.* **73**, 2947-55.

95 Xu W, Hofmann-Lehmann R, McClure HM, Ruprecht RM (2002) Passive immunization with human neutralizing monoclonal antibodies: correlates of protective immunity against HIV. *Vaccine* **20**, 1956-60.

Box 5. Development of a prime-boost strategy for a HIV vaccine; procedures involved and number of non-human primates used

To illustrate the numbers and procedures involved in the development of a vaccine strategy, we have used the example of a potential HIV vaccine (now in human clinical trials) that is based on the discovery that 'priming' with plasmid DNA and then 'boosting' with the same DNA sequence in a virus vector gives a very strong cytotoxic T lymphocyte (CTL) response. After initial testing in mice,[96] the recombinant DNA and modified vaccinia virus Ankara (MVA) prime-boost vaccination regimen has been tested for efficacy and safety in a number of studies in non-human primates.

Studies typically last up to 6 months. The folllowing three studies illustrate the numbers of animals involved:

1. The initial study to test the prime-boost strategy involved 7 rhesus macaques.[97]
2. A second study used 28 macaques.[98]
3. In a subsequent study, to test for safety in phase I clinical trials of HIV-1 infected human subjects, 12 macaques were used.[99]

Procedures for vaccine testing in macaques are generally classified as mild and usually involve 3 stages: inoculation, challenge, and analysis.

Inoculation will usually involve injection by one of a variety routes (e.g. intradermal, subcutaneous, intramuscular, oral, intranasal, intratrachaeal, intravaginal or intrarectal routes), using doses that are likely to be used in humans. A needleless jet injection device to carry out intramuscular injection was used in the initial studies.[99,100] Blood samples are normally taken from the animals at regular intervals after vaccination (e.g. every 4-6 weeks).

Animals may be 'boosted' with vaccine some weeks later, often before challenge with the SIV virus. This involves a further injection, sometimes administered intrarectally.[99] In the second study, 4 naïve control animals, who did not received the inoculation, were also challenged with virus.[100]

On completion of the experiment, animals may be humanely sacrificed so that immune responses can be measured in lymphoid organs at post mortem examination, or they may be kept in the colony and possibly used in subsequent non-immunology experiments. The animal may also develop AIDS, in which case it is sacrificed.

In the second study, the challenge infected all of the vaccinated and control animals, but levels of viral RNA were at least 10-fold lower in the vaccine groups. By 23 weeks after challenge, three of the control animals had succumbed to AIDS and exhibited varying degrees of infections such as enterocolitis, with diarrhoea, cryptosporidiosis (parasitic infection), colicystitis, enteric campylobacter infection, splenomegaly, lymphadenopathy and SIV-associated giant cell pneumonia. All 24 vaccinated macaques maintained their health.[100]

Recent trials in humans have indicated that the vaccine is safe, that it induced HIV-specific immune responses in the majority of volunteers and that the results warrant further testing in larger phase I/II studies.[101,102]

96 Hanke T, Blanchard TJ, Schneider J, Hannan CM, Becker M, Gilbert SC, Hill AV, Smith GL, McMichael A (1998). Enhancement of MHC class I-restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination regime. *Vaccine* **16**, 439-45.

97 Hanke T, Samuel RV, Blanchard TJ, Neumann VC, Allen TM, Boyson JE, Sharpe SA, Cook N, Smith GL, Watkins DI, Cranage MP, McMichael AJ (1999). Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. *J Virol* **73**, 7524-32.

98 Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, Miller J, McClure HM, McNicholl JM, Moss B, Robinson HL (2001) Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* **292**,69-74.

that are now being tested (or about to be tested) in efficacy trials in humans who are at high risk of HIV infection.

In mice, several approaches using plasmid DNA with various recombinant viruses ('prime-boost') and recombinant bacteria have given promising results. 'Prime-boost' regimes do appear to offer enhanced T cell responses in both macaques and humans, though with greater difficulty than in mice. The current position regarding these and other approaches to the development of HIV vaccines are reviewed by Desrosiers and McMichael.[103,104]

Opponents of animal research highlight that none of the 30+ HIV candidate vaccines developed have proved effective in humans, arguing that animal work has produced little data of clinical relevance. While all candidate vaccines have so far proved safe in human phase I trials, only three have got as far as efficacy (phase IIB or phase III) trials. One of these, Vaxgen gp120, has been shown to have no protective effect. Two others (Vaxgen gp120 + Aventis recombinant Canarypox and Merck recombinant Adenovirus-5) are in trial at the moment. These trials take three years to prepare and at least 5 years to conduct and it is therefore unrealistic to expect rapid answers. Researchers stress that some of the other 27 or so may be tested in human efficacy trials, but the careful design and development process discards vaccine candidates where non-human primate experiments of phase I humans trials indicate that that are unlikely to work. The fact that it is taking so long to develop an effective vaccine reflects the extreme difficulty of the problem.

5.3 Malaria

It is estimated that approximately 2 billion of the word's population live in areas where malaria is endemic; there are approximately 160 million cases of malarial infection each year, causing 1-2 million deaths, mainly in childhood.[105,106]

Four species of malaria parasites (*Plasmodium spp.*) have humans as their natural host: *P. falciparum*; *P. vivax*; *P. malariae*; and *P. ovale*. Although the different species produce clinical disorders of differing severity, their overall biology and lifecycles are similar. They are transmitted by a bite from a female *Anopheles* mosquito, after which they invade the liver and are then delivered into the blood stream where they enter the red blood cells. Parasitised red cells, particularly those infected with *P. falciparum*, tend to adhere to the walls of blood vessels, leading to widespread organ damage, and are prematurely destroyed, causing severe anaemia. *P. vivax* and *P. ovale* are different to the other parasites in that they may lie dormant for months or even years in the liver and hence give rise to recurrent attacks. However, *P. falciparum* is by far the most important of the parasites because it causes severe disease characterised by coma, profound anaemia, multi-organ failure and a variety of other complications.

99 Hanke T, McMichael AJ, Dennis MJ, Sharpe SA, Powell LA, McLoughlin L, Crome SJ (2005) Biodistribution and persistence of an MVA-vectored candidate HIV vaccine in SIV-infected rhesus macaques and SCID mice. *Vaccine* **23,** 1507-14.

100 Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, Miller J, McClure HM, McNicholl JM, Moss B, Robinson HL (2001) Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* **292**, 69-74.

101 Mwau M, Cebere I, Sutton J, Chikoti P, Winstone N, Wee EG, Beattie T, Chen YH, Dorrell L, McShane H, Schmidt C, Brooks M, Patel S, Roberts J, Conlon C, Rowland-Jones SL, Bwayo JJ, McMichael AJ, Hanke T (2004). A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. *J Gen Virol.* **85**, 911-9.

102 Cebere I, Dorrell L, McShane H, Simmons A, McCormack S, Schmidt C, Smith C, Brooks M, Roberts JE, Darwin SC, Fast PE, Conlon C, Rowland-Jones S, McMichael AJ, Hanke T (2006). Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers. *Vaccine* **24**, 417-25.

103 Desrosiers RC (2004) Prospects for an AIDS vaccine. *Nat Med.* **10**, 221-3.

104 McMichael, A.J. (2006) HIV vaccines. *Ann Rev Immunol* **24,** 227.

105 World Health Organization (2003) *Shaping the Future.* World Health Organization, Geneva.

106 World Health Organization (2005) *Make every mother and child count.* World Health Organization, Geneva.

5.3.1 Current approaches to the control and management of malaria

Effective control and management of malaria is multi-faceted, involving: attack on the mosquito vector; protection of individuals from bites by the use of bednets; drug prophylaxis; and effective treatment of established cases.[107,108,109,110,111] Establishing such a comprehensive approach can pose considerable problems, particularly in developing countries. Current programmes include attempts to drain or otherwise remove mosquito breeding habitats, the use of chemical larvicides, and the application of residual insecticides to walls and other indoor surfaces. As drug resistance of parasites has increased, there has been a major emphasis on the use of insecticide-treated bednets, combined with personal protection.

Malarial parasites have a remarkable facility for changing their genetic make-up, meaning that drug resistance has proved an increasingly serious problem throughout all malarial regions. For example, chloroquine resistance is now almost universal and the only agents for which resistance had not been encountered (until very recently) are the artemisinin derivatives of the Chinese medicinal herb, *Artemisia annua*. However, as this report was being written, the first descriptions of partial artemisinin-resistance have appeared. Hence, although major progress towards the eradication of malaria was made in many countries in the 1950s and 1960s, problems of vector control and drug resistance, combined with poverty and dysfunctional healthcare systems, have resulted in malaria becoming an even more serious global health problem in 2006, particularly in sub-Saharan Africa. Furthermore, while the work of organisations like Medicines for Malaria Venture is leading to some progress in the development of new anti-malarial agents, some of which may become available by 2008/9, the problem of drug-resistant organisms will always remain a serious hurdle to malaria control.

There is no doubt that a reduction in the mortality and morbidity caused by malaria could be achieved by improving current practices. For instance, better coordination of the international health agencies involved, improvements in public health programmes in the poorer countries, more aggressive vector control, and, in particular, more effective treatment of the disease through drug-combination therapy to reduce the number of drug resistant parasites. However, it is far from clear whether these measures will be sufficiently effective. Furthermore, the problem of vector and parasite drug resistance will always present major difficulties for malaria control. Hence, there has been widespread recognition of the importance of developing an effective malaria vaccine.

5.3.2 Towards a malaria vaccine

The development of an effective malaria vaccine presents formidable problems. Most importantly, a single attack of malaria, unlike many other infections, offers very little protection against future attacks. Those who live in areas where malaria is highly endemic therefore take several years to develop some degree of immunity - at least one factor responsible for the high mortality of malaria in childhood.

The malaria parasite's patterns of proteins (or antigens) that elicit an immune response vary at each stage of its complex life cycle in the human liver and blood, and in the sexual forms that are taken up by the mosquito where they mature for further transmission of the disease. Furthermore, as part of the parasite's adaptation for avoiding immune destruction, it has evolved genetic mechanisms whereby the structure of these proteins is constantly changing.[112]

107 Breman JG *et al.* (2006) Conquering malaria. In: *Disease Control Priorities in Developing Countries* (ed. by D. Jamison *et al.*), pp.413-432. Oxford University Press and the World Bank, New York, Washington.

108 Breman JG (2001) The ears of the hippopotamus: Manifestations, determinants and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene* **64** (suppl. 1-2), 1-11.

109 Breman JG, Alilio, MS, mills A (2004) Conquering the intolerable burden of malaria: whats new, whats needed. A summary. *American Journal of Tropical Medicine and Hygiene.* **71** (suppl. 2), 1-15.

110 Snow RW, Trape JF, Marsh K (2001) The past, present and future of childhood malaria mortality in Africa. *Trends in Parasitology*. **17**, 593-97.

111 Breman JG, O'Meara WP (2005) Intermittent preventive treatment for malaria in infants: moving forward cautiously. *J Infect Dis.* **192**, 1869-71.

112 Walliker D (2005) The hitchhiker's guide to malaria parasite genes. *Trends Parasitol,* **21,** 489-493.

The most important animal model that has been used in malaria vaccine research is infection of New World monkeys with *P. falciparum.* Indeed, the first malaria vaccine to be tried extensively in man, SPf66, was developed largely on the basis of experiments carried out in aotus monkeys and a great deal of background information about malaria vaccine development has come from these studies. A detailed discussion of studies of non-human primates in malarial vaccine development is given by Stowers and Miller.[113]

Currently, research is directed at the development of vaccines that affect every different stage of the malaria parasite's life cycle.[114,115,116] One such strategy is based on early studies demonstrating that irradiation of sporozites (the term for malarial parasites that have entered the body from a mosquito bite and moved to the liver for further development), renders them non-infectious and confers immunity. A great deal of effort has been directed at the development of vaccines based on circumsporozoite protein (CSP; a cell surface protein on the sporozite) of *P. falciparum* and a number of candidates are currently in clinical trials. One of the most promising varieties, called RTS,S, contains the CSP protein fused to the hepatitis B surface antigen, together with several proprietary adjuvants. Studies in Mozambique on over 2000 children have demonstrated a reduction in infection risk by approximately 30% and severity of infection by over 50%.[112] However, it is far from clear how long this protection lasts and larger trials are being planned for the near future.

A great deal of current work is also aimed at developing vaccines that induce cell-mediated immune responses manifested through CD8 and CD4 T cells. In theory, by attacking liver-stage parasites, these vaccines could prevent both blood-stage infection and transmission in malaria-endemic areas. Until recently, researchers found it difficult to develop vaccines that could stimulate T cell responses. However, this is now being tackled using lessons learned from 'prime boost' anti-HIV vaccine research; it has been found that 'priming' with DNA encoding malaria proteins and then 'boosting' with the same DNA in a virus vector, generates a very strong T cell response, both in mice and macaques.[117]

The current approach to identifying anti-malaria T cell responses is to first test the candidate vaccine in a murine model. For example, it was shown that 'prime-boost' strategy can stimulate anti-malaria T cell responses that completely protect mice from infection with two forms of murine malaria, *P. berghei* and *P. yoelli.*[117] The main drawback with this approach is that many vaccines that are safe and highly immunogenic in mice have shown very little immunogenicity in humans and non-human primates. For this reason it has been argued that screening candidate vaccines for immune response in non-human primates can avoid unnecessary clinical trials with non-immunogenic vaccines. In some cases potential vaccines and vaccine adjuvants that should be safe and tolerated in humans are tested directly in human volunteers (see section 4), but particularly with novel DNA constructs or adjuvants, prior safety testing on non-human primates is also required.

Current research is also being directed towards developing protection against the different stages of the parasite's lifecycle within red blood cells, although so far these approaches have been less successful. Similarly, attempts are being made to develop transmission-blocking vaccines that, while they would not prevent individuals developing malaria, they would block transmission of the sexual forms within a community. These might be of greatest use in improving the efficacy of other malaria vaccines by preventing transmission of

113 Stowers AW & Miller LH (2001). Are trials in New World Monkeys on the critical path for blood-stage malaria vaccine development? *Trends in Parasitology.* **17,** 415-419.
114 Targett GA (2005) Malaria vaccines 1985-2005: a full circle? *Trends Parasitol.* **21**, 499-503.
115 Tongren JE, Zavala F, Roos DS, Riley EM (2004) Malaria vaccines: if at first you don't succeed… *Trends Parasitol* **20,** 604-610.
116 Okie S (2005) Betting on a malaria vaccine. *NEJM* **353**, 1877-80.
117 Dunachie SJ & Hill AV (2003) Prime-boost strategies for malaria vaccine development. *J Exp Biol.* **206,** 3771-79.

drug-resistant strains of the parasite. However, while considerable progress has been made in all these approaches to the development of a successful vaccine, it is clear that a great deal more work is required before this important goal is achieved.[119]

5.3.3 Malaria due to Plasmodium vivax infection

This discussion has focussed on malaria due to *P. falciparum* infection because of its greater severity and mortality. However, malaria caused by *P. vivax*, although rather neglected over recent years,[120] is also a major cause of chronic ill health, particularly in children in Central and South America, Asia and the Indian subcontinent. Over one billion people are at risk from infection, with 70-80 million cases reported annually. Furthermore, it has been reported recently that mixed–species malaria infection with both *P. falciparum* and *P. vivax* is posing an increasingly important clinical problem in many tropical countries.[121] The treatment of *P. vivax* malaria poses a particular challenge. Unlike *P. falciparum*, *P. vivax* persists in the liver (in addition to its blood form), giving rise to recurrent attacks unless the liver stage of the parasite is completely eradicated. Although drug resistance has been less of a problem with *P. vivax* than with *P. falciparum*, there is evidence that children require longer courses of treatment than have previously been used to completely eradicate the infection. Also, the main agent used to eradicate the condition, primaquine, causes significant side effects such as haemolytic anaemia in genetically susceptible individuals, which constitute up to 15-20% of some populations.

Research into the pathology and treatment of *P. vivax* malaria has been hindered by difficulties in handling the parasite in the laboratory. Unlike *P. falciparum*, it has so far not been possible to develop a continuous *in vitro* culture system for the growth of the blood stages of the parasite. Rodent models have not been susceptible to infection and much of the current work in this field has relied on the use of different types of primates. For example, rhesus macaques were critical for studies carried out in the 1970s that characterised the invasion pathways whereby the *P. vivax* enters blood cells and the role of the human Duffy blood group system in malarial invasion; the essential electron-microscopic pictures of invasion were obtained from studies of the related parasite, *P. knowlesi*, in non-human primates. More recently the strain of *P. vivax* that was used for the *P. vivax* genome sequencing project was obtained from splenectomized Bolivian squirrel monkeys.[122]

Non-human primates currently play an important role in many different aspects of research directed at the prevention and control of *P. vivax* malaria. Several *P. vivax* antigens offer possible targets for the development of vaccines,[123] including the Duffy-binding protein (DBP), which is essential for invasion of red blood cells. While a great deal of work is being directed at developing *in vitro* assays (such as red blood cell binding) to screen these vaccines, currently they have to be assessed for antibody response and efficacy in non-human primates. Since the establishment of clinical trials for vaccines to protect children against this parasite, particularly those who are already semi-immune, presents formidable problems and will be very costly, there seems to be a strong case for the use of non-human primates in this field, at least for the foreseeable future.

5.4 Tuberculosis

Tuberculosis, or TB, was responsible for millions of deaths between 1700 and 1950 in the developed world, earning the disease the sobriquet 'the captain of the men of death' and 'the White Plague'. However, improvements in public health and sanitation, and the later development of curative chemotherapy, led to a rapid decline in the disease, a phenomenon that,

119 Tongren JE, Zavala F, Roos DS, Riley EM (2004) Malaria vaccines: if at first you don't succeed… *Trends Parasitol*. **20,** 604-610.
120 Mendis K, Sina BJ, Marchesini P, Carter R (2001). The neglected burden of Plasmodium vivax malaria. *Am J Trop Med Hyg.* **64**, 97-106.
121 Mayxay M, Pukrittayakamee S, Newton PN, White NJ (2004) Mixed-species malaria infections in humans. *Trends Parasitol.* **20**, 233-40.
122 Carlton J (2003). The *Plasmodium vivax* genome sequencing project. *Trends Parasitol.* **19**, 227-31.
123 Polley SD, McRobert L, Sutherland CJ. (2004) Vaccination for vivax malaria: targeting the invaders. *Trends Parasitol.* **20**, 99-102.

unfortunately, did not occur in many of the developing countries. The apathy that followed the success in controlling the disease in developed countries, led to a gradual worsening in the situation in many parts of the developing world and, particularly because of its propensity to attack those with HIV/AIDS, TB now presents a major public health challenge.[124,125]

The WHO estimates that more than 2 billion individuals are infected with TB worldwide, of which approximately 10% will develop symptoms of the disease. A combination of poor living standards, the rising incidence of multi-drug resistant (MDR) TB strains, and the increased susceptibility to disease in those who also carry HIV, has resulted in TB becoming a major problem in sub-Saharan Africa, India, China and Eastern Europe. Estimates put the number of annual deaths caused by TB at 2 million. In 2003, 600,000 individuals infected with both HIV and tuberculosis died. In 2004, 40 million were infected with HIV, 2 billion with tuberculosis and 15 million with both.[126]

TB most frequently develops in the lung, although an intense immune response in many cases causes the infecting organism, *M. tuberculosis*, to become enclosed in lesions and thus inactivated. The mechanisms that determine whether it remains in this latent state, which may last for several years, or becomes active, causing a disease characterised by destruction of the lung and eventually other parts of the body, are not fully understood. Associated diseases such as HIV/AIDS, poor living conditions, alcohol abuse, dietary insufficiency, and genetic factors may be involved. Once a destructive process of the lungs is established, patients become highly infective to others.

5.4.1 Current methods of control and treatment

A vaccine against tuberculosis, developed early in the 20th century by the French scientists Albert Calmette and Camille Guerin, consisted of an attenuated strain of *M. bovis*, the agent that causes tuberculosis in cattle. The Bacillus Calmette-Guerin (BCG) vaccine tends to protect against severe forms of childhood tuberculosis but does not lead to the eradication of *M. tuberculosis* and its protective activity weakens during adolescence. Hence it does not protect against the most important form of the disease, which is pulmonary tuberculosis in adults. A review of all published BCG trials performed in the early 1990s found that the protective effect of BCG was about 50% for both the prevention of active tuberculous disease and death.

The current major approaches to the control and treatment of TB include improvements in public health and standards of living, chemotherapy and vaccination.[126] Early trials showed that effective treatment required the administration of two or three anti-TB drugs simultaneously for approximately 6 to 12 months. Unfortunately, this necessitates a complex and rigorous health care regime and hence full courses of treatment are often not sustained, particularly in the developing countries. Partial treatment or the ill-advised use of single chemotherapeutic agents has led to the extensive development of MDR in many populations. The catastrophic increase in TB led the WHO to develop the DOTS strategy (Directly Observed Therapy, Short Course). This involves detection of cases by screening the sputum for *M. tuberculosis*, regular and uninterrupted supply of drugs, 6-8 months of regularly supervised treatment including direct observation of drug taking for the first two months, and reporting systems to monitor treatment progress. Although this approach has been successful in some countries, its expense and heavy requirement for trained personnel have limited its value; the WHO estimated that by 2000 only 25% of patients with TB worldwide were treated within a DOTS programme.[126]

124 World Health Organization (2004) *Changing History.* World Health Organization, Geneva.

125 Kaufmann, S.H. & McMichael, A.J. (2005) Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis. *Nat Med,* **11,** S33-44.

126 Dye C and Floyd K (2006) Tuberculosis. In: *Disease Control Priorities in Developing Countries* (ed. by D. Jamison & e. al), pp.289-309. Oxford University Press and the World Bank, New York, Washington.

The current position regarding MDR is extremely worrying. In several Eastern European countries more than 10% of all cases of TB are caused by MDR strains, with an even worse situation in parts of Africa. In these populations there are no agents available for the treatment of disease, which therefore leads to death and the further spread of infection. It is over 30 years since any new classes of drugs for the treatment of TB were developed, in part due to lack of any financial incentives on the part of the pharmaceutical industry. Currently, the problem with MDR is being approached by clinical trials of existing broad-spectrum agents but, although these may provide a stopgap, a real breakthrough will only come when new agents with genuine sterilising activity are discovered.[128]

Clearly, the current state of the prevention and management of established disease is extremely precarious. Very few new drugs are close to testing in clinical trials, multi-resistance to all the available agents is rife, and an effective vaccine is not available. Better control measures are urgently needed and the essential requirement for an effective vaccine has been emphasised in a recent review of priorities for the control of tuberculosis.[127]

5.4.2 The role of non-human primate research in TB drug and vaccine development

Respondents emphasised that research involving non-human primates plays an important role in developing new drugs to treat TB. *M. tuberculosis* infection of non-human primates generates lesions that closely resemble human pathology. In particular, some mycobacteria are contained in hypoxic lesions in non-human primates in the same way as humans, a situation that is not observed in mice. Such lesions may provide sites for the development of resistance during current drug therapies, leading researchers to argue that non-human primates are therefore important for screening new drugs. Furthermore, unlike mice, non-human primates can develop a latent infection analogous to that in humans when infected with low doses of mycobacteria; no clinical problems are observed, but the disease can be triggered by immune suppression.

As in the case of HIV and malaria, a range of approaches is being explored towards producing an effective TB vaccine.[129] They include: the use of naked DNA with a packaging system; the delivery of particular antigens with adjuvants; viable mutants of *M. tuberculosis*; and 'prime-boost' systems.

The US National Institutes of Health, through its pre-clinical tuberculosis screening programme, and the European Union, through its TBVac integrated project in Framework Programme 6, sponsor experimental vaccine testing in animals. Again, immune system differences mean that immunogenicity results in rodent models cannot unequivocally predict the efficacy of a TB vaccine candidate in humans. Nevertheless, the major thrust of vaccine research using non-human primates is currently focused on trials of candidates that have shown protective effects in mice and guinea pigs. The important objective in the use of non-human primate models is to better inform decisions on taking candidate vaccines into human phase III clinical trials. This is extremely important since human efficacy data require trials lasting several years and involving some 10,000 subjects, at a current cost of about £15 million.

5.5 Other infectious diseases

Vaccines utilising recombinant DNA and related technology are being actively developed in other areas of infectious disease. Since non-human primates are used in some of these programmes, it is important to consider their current status and to highlight some of the problems involved. The following examples reflect two completely different problems in vaccine research, to which researchers argue the solution requires the use of non-human primates; one relates to the size of the parasites involved, the other to the lack of

127 Dye C and Floyd K (2006) Tuberculosis. In: *Disease Control Priorities in Developing Countries* (ed. by D. Jamison & e. al), pp.289-309. Oxford University Press and the World Bank, New York, Washington.

128 Duncan K & Barry CE 3rd (2004) Prospects for new antitubercular drugs. *Curr Opin Microbiol,* **7,** 460-465.

129 Kaufmann SH & McMichael AJ (2005) Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis. *Nat Med.* **11,** S33-44.

any other suitable model systems for studying pathophysiology or testing putative vaccines.

5.5.1 Schistosomiasis

The WHO estimates that schistosomiasis (bilharzia) affects approximately 200 million people in 75 countries, 85% of whom live in Africa. The organisms responsible - *Schistosoma mansoni, S. haematobium, S. intercalatum, S. Japonicum* and *S. mekangi,* - are transmitted by aquatic snails; humans become infected when free-swimming larvae penetrate the skin and undertake a complex migration to sites where they can mature into adult worms (either the blood vessels of the liver or bladder wall, depending on the species). The disease is characterised by anaemia, chronic pain, and severe bladder or liver damage. The number of deaths directly attributable to schistosomiasis in Africa is currently estimated at 280,000 per annum.[130]

There have been major advances in the control of schistosomiasis following extensive health education and the development of therapy such as Praziquantel.[131] For example, the application of these approaches, combined with efforts to reduce the population of snails in

Box 6. The role of non-human primates in schistosomiasis research

A submission to the working group strongly argued that testing of schistosomiasis vaccines required animals for 2 reasons: first, disease processes and immunological responses can only be understood within the context of a whole animal host; and second, a parasite life cycle of 2 hosts, at least 8 different morphological stages and several abrupt transitions of environment has proved impossible to replicate *in vitro*. While the intra-mammalian stages of the parasite are grown *in vitro* to the lung stage, requirement for blood feeding thereafter means that sexually mature adult worms can only be produced in rodents.

There appear to be several limitations of the mouse model of schistosomiasis, the most significant of which is mouse size relative to that of the mature schistosome (approx 1cm long). Even a single worm pair therefore represents an unrealistic parasitic burden in the mouse. Severe disease develops in mice in about 10 weeks (versus many years in humans) and mortality is appreciable by 14 weeks. In the mouse model, protective immunity induced by attenuated or recombinant vaccines must be measured at 5-6 weeks post-challenge. The magnitude of the challenge must also be reduced in mice, making it difficult to achieve statistical significance between test and control groups. Furthermore, infections with human schistosomes carried out in rodents give completely different results depending on the species used. The researchers argue that the mouse is therefore an unsuitable model for long-term vaccine testing.

The submission stated that the 'gold standard' measure for efficacy in human vaccine trials is reduced worm burden. However, it is not feasible to measure this in humans due to location of the parasite in the hepatic portal vasculature. Instead, researchers must rely on indirect estimates of worm burden (e.g. faecal egg output and levels of schistosome circulating antigens). Research in baboons (carried out in the US) has permitted the establishment of a relationship between worm burden and these surrogate markers. This work showed the insensitivity of these indirect measures, due to high thresholds of detection. Tests based on these markers would therefore give a misleadingly positive impression of performance in human trials. The researchers highlight the urgent need to develop more sensitive methods for detecting faecal eggs and circulating antigens in blood and urine. However, they stress that such methods will need to be validated in non-human primates by comparison with actual worm burden.

130 Hotez PJ, Ferris MT (2006) The antipoverty vaccines. *Vaccine* **24,** 5787-99.

131 Fenwick A, Savioli L, Engels D, Robert Bergquist N, Todd MH (2003). Drugs for the control of parasitic diseases: current status and development in schistosomiasis. *Trends Parasitol*. **19**, 509-15.

canals and rivers, has led to a major reduction in the frequency of the disease in Egypt. However, many problems remain, despite efforts to develop public health programmes by the WHO and the donation of drugs by some pharmaceutical companies. High rates of re-infection occur after drug therapy and there is considerable concern about the potential for emergence of drug-resistant strains, particularly as Praziquantel becomes more widely used in sub-Saharan Africa. Praziquantel-resistant schistosomiasis exists already in northern Senegal.[132] For these reasons it is becoming increasingly important to develop effective vaccines for the control of schistosomiasis and other severe helminth infections.[133]

The working group received a detailed submission outlining recent research into schistosomiasis, which argued for the continuing requirements of non-human primates, in concert with alternative techniques (see Box 6).

Research over the last 10 years has shown that non-human primates are the only animals for which it is possible to produce infections that closely mimic those in humans in every respect of the complex life cycles of the parasites.[134,135,136,137] Several different approaches are now being applied for the development of protective vaccines for schistosomiasis[138] and, because of the size of the pathogen, it seems inevitable that non-human primates will be required for their evaluation. For example, a current trial is testing a vaccine based on recombinant schistosome glutathione S-transferase as an antigen that requires non-human primates for its development.[139]

5.5.2 Viral infections: hepatitis B and C

There are several extremely common viral infections for which researchers claim there is no effective rodent (or other small animal) susceptibility model, thus making non-human primates necessary to investigate disease pathology and develop candidate vaccines. We have looked at hepatitis B and C.

Hepatitis B virus (HBV) affects some 3 billion people worldwide, of which about 350 million become chronic carriers and 1-1.5 million die each year of liver failure or cancer of the liver. Although a very effective vaccine was introduced many years ago, approximately 10% of young adults fail to respond to it. Efforts are being made to improve the efficacy of current vaccines using recombinant DNA and adjuvant technology with the objective of producing more effective immune responses.[140,141] Although HBV viruses are found in a wide variety of mammals and birds, chimpanzees and rhesus monkeys are the only animals that can be infected by human HBV.

The more recently identified hepatitis C virus (HCV) also causes chronic liver disease and liver cancer, and is estimated to affect some 170 million people worldwide. Current treatment with interferon, either alone or combined with antiviral drugs, is both expensive and not entirely efficacious. HCV is therefore the subject of intense research towards developing a vaccine, but there are formidable problems, not least that chimpanzees are the only known non-human hosts for HCV.[142] HCV also shows wide genetic heterogeneity, it has a high propensity for promoting chronic persistent infections and can readily re-infect convalescent humans and chimpanzees following further exposure.

132 Doenhoff MJ, Kusel JR, Coles GC, Cioli D (2002) Resistance of Schistosoma mansoni to praziquantel: is there a problem? *Trans R Soc Trop Med Hyg.* **96**, 465-9.

133 Hotez PJ, Ferris MT (2006) The antipoverty vaccines. *Vaccine* **24,** 5787-99.

134 Yole DS, Reid GD & Wilson RA (1996) Protection against Schistosoma mansoni and associated immune responses induced in the vervet monkey Cercopithecus aethiops by the irradiated cercaria vaccine. *Am J Trop Med Hyg.* **54,** 265-270.

135 Wilson RA & Coulson PS (1998) Why don't we have a schistosomiasis vaccine? *Parasitol Today* **14,** 97-99.

136 Wilson RA & Coulson PS (1999) Strategies for a schistosome vaccine: can we manipulate the immune response effectively? *Microbes Infect,* **1,** 535-543.

137 Kariuki TM, Farah IO, Yole DS, Mwenda JM, Van Dam GJ, Deelder AM, Wilson RA & Coulson PS (2004) Parameters of the attenuated schistosome vaccine evaluated in the olive baboon. *Infect Immun.* **72,** 5526-29.

138 Hotez PJ, Ferris MT (2006) The antipoverty vaccines. *Vaccine* **24,** 5787-99.

139 Capron A, Riveau G, Capron M, Trottein F. (2005) Schistosomes: the road from host-parasite interactions to vaccines in clinical trials. *Trends Parasitol.* **21**, 143-9.

140 Pride MW, Bailey CR, Muchmore E & Thanavala Y (1998) Evaluation of B and T-cell responses in chimpanzees immunized with Hepagene, a hepatitis B vaccine containing pre-S1, pre-S2 gene products. *Vaccine* **16,** 543-550.

141 Davis HL, Suparto II, Weeratna RR, Jumintarto Iskandriati DD, Chamzah SS, Ma'ruf AA, Nente CC, Pawitri DD, Krieg AM, Heriyanto, Smits W & Sajuthi DD (2000) CpG DNA overcomes hyporesponsiveness to hepatitis B vaccine in orangutans. *Vaccine* **18,** 1920-1924.

142 Gale M Jr & Beard MR (2001) Molecular clones of hepatitis C virus: applications to animal models. *Ilar J.* **42,** 139-151.

Recently, the discovery in the USA of natural immunity to HCV and vaccine efficacy in the chimpanzee challenge model has led to more optimism about the development of a vaccine against HCV that is effective, albeit partly.[143] Furthermore, the immune systems of some patients can spontaneously clear the virus, whereas others require anti-viral treatment (to stimulate humoral and cellular immune responses). This has led to the investigation of vaccine strategies with the objective of improving treatment outcomes.

In reviewing future directions in the development of an effective HCV vaccine, Haughton and Abrignani[143] emphasise the importance of the chimpanzee model for further definition of correlates of protection, duration of vaccine-mediated protection, the extent of cross-protection against different genotypes and mechanisms of chronicity, and to determine optimal vaccine formulations for both prophylactic and immunotherapeutic approaches to control the disease. In addition, immunogenicity studies are being conducted in macaques using 'prime-boost' regimens with DNA and adenovirus vectors containing HVC sequences.

5.5.3 New or emerging infections

As evidenced by the HIV/AIDS epidemic, the recent emergence of Severe Acute Respiratory Syndrome (SARS), and current fears about the potential for a devastating outbreak of avian influenza, it is clear that humans will always be at risk from potentially overwhelming outbreaks of communicable disease.

The first outbreak of SARS - a respiratory infection characterised by a rapidly progressive atypical pneumonia - occurred in China in 2002. Within a year of the initial outbreak, there were 8,422 cases worldwide, with 916 deaths. The estimated global cost of the outbreak was $60 billion, with $17.9 billion in China alone. Researchers quickly isolated a coronavirus, SARS-CoV, from infected patients. Unfortunately, it was found that SARS-CoV did not produce the typical respiratory disease and associated histological changes in the lung in rodents (or other small mammals). However, it was found to reproduce a very similar disease in cynomolgus macaques, which were used to assess the protective effect of pre- and post-exposure administration of pegylated human interferon-alpha, candidate SARS-CoV vaccines, and convalescent sera from SARS patients.

There may also be circumstances where a non-human primate model would be needed for studies on avian influenza (H5N1), which currently has a greater than 50% mortality in infected humans. There is known to be an overproduction of certain inflammatory cytokines early in infection, which probably contributes to the pathology. Treatments to counter this, such as the use of anti-TNFalpha, could be modelled in macaques ahead of a possible catastrophic pandemic in humans. Against the continued danger of further outbreaks of SARS, or from related viruses, the important role of non-human primate models for the control of SARS has been recently reviewed.[144]

The haemorrhagic fevers causes by filoviruses, such as Ebola and Marburg, result in fatality rates of between 50% and 90% in different outbreaks. There is currently no effective therapy and the development of a vaccine is a priority to restrict the size of outbreaks and to protect healthcare workers. Again, cynomolgus macaques are susceptible to infection and researchers argue that they provide the only suitable challenge model. Experiments using live attenuated, or replication-defective, virus vectors expressing filovirus proteins have been used successfully to protect macaques from infection and to identify virus glycoproteins as the protective immunogens.[145] Clinical trials of these vaccines are now in preparation.

143 Houghton M & Abrignani S (2005) Prospects for a vaccine against the hepatitis C virus. *Nature* **436,** 961-966.

144 Haagmans BL, Osterhaus AD (2006). Nonhuman primate models for SARS. *PLoS Med* **3**, e194.

145 Jones SM, Feldmann H, Stroher U, Geisbert JB, Fernando L, Grolla A, Klenk HD, Sullivan NJ, Volchkov VE, Fritz EA, Daddario KM, Hensley LE, Jahrling PB, Geisbert TW (2005). Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med.* **11**, 786-90.

5.6 Discussion

There is no doubt that communicable disease, particularly as it affects the poorer countries, is still one of the major challenges for medical research. Furthermore, since pathogenic micro-organisms are constantly changing their genetic make-up, the threat of devastating infections will always be with us.

Currently, major efforts are being made by the WHO and international charities to control these diseases by improvements in public health and healthcare delivery and by the provision of drugs for their treatment. However, it is not clear whether these efforts will be successful, particularly given the constant emergence of drug-resistant organisms. The case for continued research directed towards developing new therapeutic agents and vaccines is extremely strong. The major arguments for the continuation of work on non-human primates in this field reflect the necessity for animal models that both closely resemble the human infection and mimic human immune responses. The counter arguments mainly revolve around the imperfections of non-human primate models of human infectious disease, particularly in the case of HIV. Whilst acknowledging the deficiencies and limitations of current non-human primate models, their study has enhanced understanding about the pathology of infectious diseases. In all cases, the welfare impacts of laboratory housing and transport of the animals involved must be considered, but the degree of suffering imposed on animals during the course of these studies ranges relatively widely; in some cases the animals develop a condition identical to the disease in humans while, in others, the animals receive an injection followed by blood sampling to observe an immune response.

Adequate phase III vaccine trials may require as many as 10,000 volunteers and 5 years of study, often in a developing country, making them extremely expensive. Even with the large amounts of money that have been generated for international vaccine research programmes by bodies such as the Bill and Melinda Gates Foundation and the US National Institutes of Health, it has been estimated that funding is available for only about 10 major phase III vaccine trials in the next 10 years. For this reason, the kind of screening studies that have been described in this section, some of them in non-human primates, are likely to be absolutely critical.

To consider the three infectious diseases looked at in detail:

HIV

Comparison of available data from trials of potential vaccines shows that immune responses in the SIV- and SHIV-macaque systems are similar to those reported in humans infected with HIV. It is therefore possible that, in addition to aiding the development of candidate human vaccines, research on macaques may allow prioritisation of vaccine candidates for human trials, as well as providing important safety information. For example, if there were no protection after vaccination and challenge with SIV there would be a strong argument against starting a lengthy and expensive phase III trial. The working group therefore concludes that non-human primates are an essential component of research programmes aimed at preparing the broadly cross-protective vaccines necessary to curb the AIDS pandemic.

Malaria

There is a compelling need for the development of a vaccine against *P. falciparum* malaria. Although some progress is being made in developing new therapies with artemisin derivatives, it may be many years before the new drug targets recently identified by the malaria genome project can be fully exploited and effective drug candidates generated. And drug-resistance will always remain a problem. Although genetic engineering approaches

could conceivably improve rodent models for testing vaccines against *P. falciparum* malaria, differences between the immune responses of rodents and primates (both human and non-human) make it difficult to see how this important field of research can progress without the use of non-human primates for the foreseeable future. Without prior testing in non-human primates it is sometimes impossible to avoid unnecessary clinical trials in humans with non-immunogenic vaccines. Similarly, although whenever possible pilot studies are carried out with human volunteers, until the ideal immunogens, virus vectors and adjuvants are identified, in many cases it will remain important to assess safety in non-human primates before progressing to clinical trials in humans. Non-human primates also have an essential role in research directed at the control of *P. vivax* malaria.

Tuberculosis

The enormous global health problem posed by TB, particularly in relation to its synergism with HIV/AIDS, has led to an urgent need for an effective TB vaccine. The problems of efficacy testing, the limitations of immunogenicity testing in rodents, and the uncertainty about safety when new vectors or adjuvants are used, make it essential for research to continue that includes the use of non-human primates.

In their submissions to this inquiry, several researchers in the vaccine field pointed out the increasing difficulty of pursuing this type of work in the UK. The main reason cited was the expense of UK non-human primate studies compared with other countries; the UK was said to be significantly more costly than the USA and costlier still than Asia. This is a complex issue and respondents noted that potentially higher standards of animal welfare in the UK may contribute to this expense. However, it appears that there is a major drive to carry out vaccine research outside the UK, either as a collaborative programme with workers in the USA or elsewhere, or, in some cases, with the movement of entire programmes overseas. Clearly, the whole question of the future of vaccine research in the UK requires investigation.

From this analysis of current research directed towards the development of vaccines for some of the major infectious killers, we concluded that it would not be possible to continue these programmes at the present time, or for the immediate future, without the use of non-human primates. The pattern of many of these programmes consists of extensive experimental work in rodents, followed by exposure of potential vaccines to a limited number of non-human primates to investigate efficacy and potential toxicity, before moving forward to trials in humans. In short, it involves studies on a small number of non-human primates for the potential benefit to vast numbers of people who are dying of these conditions, particularly in developing countries. In some cases it may be possible to move from studies on rodents directly to humans though, understandably, regulators are particularly sensitive to the dangers of unexpected reactions in vaccine trials, particularly as they involve healthy children or adults.

The ethical argument on whether it is right to use small numbers of non-human primates to benefit large numbers of humans, is for debate; but the scientific argument for the continued use of non-human primates for research in this field is extremely strong.

6 Neuroscience

6.1 Introduction

Neuroscience is the study of what the brain does, how it does it, how it goes wrong and how it might be successfully treated following injury or disease.[146] Neuroscientists are gradually learning more about the normal workings of the central and peripheral nervous system, and this understanding is central to tackling important clinical conditions - neurodegenerative diseases such as Alzheimer's and Parkinson's disease, as well as psychiatric conditions such as schizophrenia and depression. The overall aims of neuroscience research can be summarised as: improved understanding of normal and abnormal brain function; development of novel therapeutics to tackle neurological and psychiatric diseases; and development of artificial devices based on neuroscientific principles.

Neural disease imposes a significant, and increasing, burden on global health services. Two important epidemiological projects, sponsored by the European Brain Council, have established that brain disorders constitute up to 35% of the total burden of disease in Europe, as measured in terms of disability adjusted life years.[147] Estimates of the economic burden, calculated using conservative epidemiological and economic criteria, have been put at 386 billion Euro for 2004.[148]

Fundamental neuroscience draws upon a variety of techniques including molecular biology, brain imaging and computational modelling, and increasingly involves interactions with clinical studies in neurology, neurosurgery and psychiatry. This multidisciplinary approach is a key to the successful translation of fundamental research findings into clinical applications. Evidence from neuroscience researchers asserted that research using non-human primates is one component of this translational interface, alongside studies of other animal models, observational and interventional research involving humans and *in vitro* approaches. Researchers attested that many important discoveries about how the brain works in both health and disease stem from studies using non-human primates. While these findings complement and extend findings derived from other approaches, it was claimed that in many cases they could not have been obtained by other means.

Several respondents highlighted that neuroscience is the field of non-human primate research that raises the most concerns about welfare. Certainly, in the UK many non-human primate experiments categorised as being of 'substantial severity' fall within this discipline (see section 3.4.4). Evidence from those opposed to animal research argued that recent advances in imaging technologies and computational modelling provide viable replacements to non-human primate work. They argued for the replacement of all non-human primate research with non-invasive imaging techniques in human volunteers, the use of human post-mortem tissue, cell culture, computational modelling and other techniques.

In overall terms, work with vertebrate animals constitutes only a proportion of neuroscience research, of which non-human primate research is a very small fraction. Molecular and cellular mechanisms of brain function can be effectively examined using *in vitro* techniques. Insights into simple mechanisms of learning have also been provided by invertebrate animal models, such as the marine snail, where neurotransmitters and signal-transduction mechanisms are evolutionarily conserved with those found in the vertebrate brain,[149] despite radical differences in brain structure and function. Insights into memory and even Parkinson's disease have also been derived from such models, as exemplified by recent work using *Drosophila*.[150]

However, a key claim of several witnesses was that information processing in the brain depends on neural circuits; such circuits

146 Bear MF, Connors B.W & Paradiso M.A (2006) *Neuroscience: Exploring the Brain.* Lippincott, Williams and Wilkins, Philadelphia, USA.
147 Olesen J & Leonardi M (2003) The burden of brain diseases in Europe. *Eur J Neurol.* **10**, 471-477.
148 Andlin-Sobocki P, Jonsson B, Wittchen HU & Olesen J (2005) Cost of disorders of the brain in Europe. *Eur J Neurol.* **12 Suppl 1**, 1-27.
149 Kandel ER, Schwartz JH & Jessell TM (2000) *Principles of Neural Science.* McGraw-Hill, New York.
150 Pallanck L & Greenamyre JT (2006) Pink, parkin and the brain. *Nature* **441**, 1058.

have evolved differently in vertebrates and invertebrates, and non-human primates have a vertebrate brain that is most like that of humans in terms of neural circuitry. With respect to clinical research, it was claimed that similarities with human physiological and behavioural characteristics make non-human primates more accurate models of neurological and psychiatric diseases than other animals. Thus, it was asserted that the efficacy and safety of some prospective therapeutics can be more accurately and predictably assessed in non-human primates. A key aspect of our enquiry was therefore to establish whether non-human primate work remains a necessary component of neuroscience research and the extent to which new technological developments offer alternative ways forward.

6.1.1 Structure of neurosciences section

Whether it is an attempt to define the normal functions of the brain and peripheral nervous system, or to understand the clinical consequences of the disturbance of these functions, neuroscience is undoubtedly one of the most complex fields of all the biological sciences. Indeed, it is just this complexity that has made it so difficult to develop rational debates about the use of non-human primates in research on this subject. The discussion of these complex issues that follows is organised so that readers who are unfamiliar with this topic can most easily follow the arguments that it raises. Hence, it starts with a brief outline of the fundamental activities of the nervous system (section 6.2), continues with a comparison of the structure and function of vertebrate, non-human primate and human brains and the research tools that are required to investigate their structure and function, and, finally, discusses the current and future use of non-human primates in fundamental (6.6) and applied (6.7) medical research. In considering sections 6.6 and 6.7 it is clear that fundamental and applied neurosciences are continually interacting and feeding one off another and that the distinction between them is increasingly artificial.

6.2 Connectivity, neurons and circuits

6.2.1 Connectivity

The basic unit of the nervous system is the neuron, consisting of a nucleated cell body with one or more dendrites and a single axon. The distinctive hallmark of the human nervous system is the manner in which the 10^{12} neurons of the brain, many with up to 10^4 synaptic connections, are organised into circuits and networks. These circuits are surrounded by an elaborate array of glial cells that protect cellular connections, mop up excess transmitters and help to regulate the vasculature. Information processing in the brain involves the 'firing' of electrically active neurons in networks. The patterns of neural activity in such networks are ultimately responsible for the control of behaviour.

Given this complexity, it is perhaps not surprising that we still know relatively little about the detailed wiring of the human brain.[151] The major connections, such as those of the cranial nerves, have long been mapped from studies on human post-mortem tissue. However, understanding connectivity involves injecting chemical 'tracers' into localised areas of living brain and studying the transport of this material from one neuron to another. Tracers are examined under the microscope using thin tissue sections cut from freshly prepared brains. Clearly, it is not ethically acceptable to use tracers in the human brain. Instead, studies of rat, mouse and non-human primate brains have been used to elucidate the cells present in different layers of the brain cortex, showing that the layers receive different connections from, and send different connections to, other parts of the brain.

Very detailed maps of these connections are now available for the macaque brain.[152,153] Anatomical information about the non-human primate brain is available in a web database that presently collates findings from nearly 400 papers (www.cocomac.org).[154] It contains data from over 7000 sites in the brain and has

151 Crick F & Jones E (1993) Backwardness of human neuroanatomy. *Nature* **361**, 109-10.
152 Felleman D J & van Essen, DC (1991) Distributed heirarchical processing in primate cerebral cortex. *Cerebral Cortex* **1**, 1-47.
153 Young MP (1993) The organization of neural systems in the primate cerebral cortex. *Proc Biol Sci* **252**, 13-18.
154 Accessed July 2006.

over 36,000 connection details. This is a huge amount of information, but there are many unresolved questions - such as the chains of connectivity, the layering of local circuits in the cortex and the transmitters that signal within these different connections.

6.2.2 Neurons

Understanding how individual neurons work has also been an important strand of fundamental research over the past century. As far as we know, the mechanisms by which they operate are very old in evolutionary terms; neural transmission along axons operates in essentially the same way in the human brain as it does in a relatively primitive organism. Thus, the neural impulse has been studied very effectively using giant neurons taken from a marine mollusc (the giant squid), while chemical transmission has been studied using a nerve/muscle junction of an amphibian (frog) and a fish (torpedo). This work was carried out with the confidence that the findings were likely to be applicable to all known nervous systems, including those of humans. Several decades of subsequent research have justified that confidence.

6.2.3 Circuits and functions

While research on individual neurons continues, the focus has shifted to understanding the functional interactions between large numbers of nerve cells in ways that are difficult to predict from the behaviour of the individual components. Beyond mere connectivity is the issue of what these circuits actually compute. Animal studies have shown that certain neural circuits in the visual system detect lines and edges, while others determine colour, motion and so on. Similarly, in the auditory system, dedicated circuits allow the brain to locate the source of a sound, while others identify its frequency. Studies using non-human primates have shed light on the categorical perception of sound, in which changes along a continuum are perceived, not as gradual but as instances of discrete categories. This has been particularly relevant to understanding how humans distinguish between phonemes (the smallest unit in a language that is capable of conveying a distinction in meaning, as the m of mat and the b of bat). While the molecular components of these different circuits are likely to be largely the same, adult brains differ in how the circuits are wired up.

6.2.4 Circuits and brain disorders

Key issues for understanding neural circuits include how they develop embryologically and in early postnatal life, and how intracellular molecular cascades enable adult neurons to function. Understanding the interaction of genetic and environmental factors is also proving very important. Developmental abnormalities are now thought to be relevant to a number of psychiatric conditions, while perturbations of reward mechanisms will bias motivational circuits to malfunction in a manner that can contribute to obesity or habitual drug use. The circuits affected may be inter-neuronal (i.e. within brain networks) or intra-neuronal (i.e. in signal transduction cascades that regulate phosphorylation, gene transcription or other biochemical mechanisms). There is also a need to adopt a 'systems approach' for further understanding of neuronal circuits, for instance in terms of localisation of function, how specific anatomical arrangements enable specific algorithms to be computed by the nervous system and the role of electrical rhythms in timing and coordinating activity across the brain.

Some brain disorders do not reflect the dysfunction of neural circuits. For example, multiple sclerosis (MS) involves an immunological breakdown of the insulation that surrounds nerve connections. In this case it is conceivable that a better understanding of the neurodegenerative processes at the molecular and cellular level could be gained from *in vitro* work, perhaps combined with *in vivo* studies using transgenic mice, without having to elucidate the sensori-motor circuits of the affected neurons. However, this is not the case for conditions such as schizophrenia, which affects the way information is represented and processed in the brain, leading to withdrawal, delusions and other symptoms. Schizophrenia is very unlikely to be due to the imbalance of a single neurotransmitter and

we will eventually need to understand how the relevant circuits work, how they represent informatio and how they malfunction. Hence the difficulty of developing new treatments and therapies.

6.3 Comparisons of vertebrate, non-human primate and human brains

6.3.1 Size and structure

The overall organisation of the vertebrate brain is very different from that of invertebrates, even though its cellular components - neurons and glial cells - operate in essentially the same way. Invertebrate brains are organised into ganglia with strands of connective neural tissue. In contrast, all vertebrates have a clearly identifiable central nervous system with a common plan of neural circuit organisation (spinal cord, hindbrain, mid-brain and forebrain).

This common plan means that rats and mice share many similarities in brain architecture with humans, and are therefore useful for a great deal of neuroscience research of direct clinical relevance. For example, an evolutionarily 'old' structure such as the cerebellum is much the same in rodents as in humans. Other structures such as the hypothalamus, hippocampus and amygdala are also present in all mammals. However, certain brain functions mediated by mid-brain circuits in rodents (e.g. aspects of affiliative[155] and sexual behaviour) are subject to neocortical regulation in humans and other primates.[156] Such 'cognitive' modulation of hormones is a distinctive feature of the human and primate brain: one witness advised that neuroendocrine findings derived from rodents can sometimes be misleading for human brain functions involving hormonal influences.

The major difference between the brains of rodents and humans is in size: the human brain (1500g) is approximately 750 times larger than that of a rat (2g). Researchers point out that the human brain is only 15 times larger than that of a rhesus macaque and, because most of the critical cortical cells are in organised layers near the surface, they argue that the relevant ratio from human to macaque is much smaller. In gross appearance, the human and non-human primate brain is packed into the skull with numerous folds of the thin outer layer of its cortical mantle called sulci, whereas those of rodents have a smooth appearance with few sulci. The brain of a rhesus macaque is also more like the human brain in having the major parts of the visual system folded into the medial walls of each hemisphere.

6.3.2 The neocortex, visual and motor systems

Primates, both human and non-human, embody a major evolutionary step-change in vertebrate brain architecture with the massive expansion of the neocortex. The sub-divisions of the neocortex and the sheer number of interconnections in the primate brain are radically different from those of other vertebrates. The primate brain has a greatly enlarged prefrontal cortex that contains the areas responsible for working memory, executive function and aspects of decision-making: some comparative neuroanatomists have even argued that only primates have a true prefrontal lobe.[157] Only the non-human primate brain has a cellular composition of divisions that is in any way directly analogous to that found in humans.[158]

Unlike rodents, non-human primates have forward-facing eyes and complex visual behaviour, making them particularly suitable for vision research of direct human relevance. Specialised areas devoted to different aspects of vision (motion, colour, etc.) are broadly similar in both macaques and humans.[159] The way in which attention can be selectively directed at specific parts of the visual scene is also similar in macaques and humans.[160]

155 Affiliative behaviours are close-proximity behaviours that include touching, grooming and hugging.

156 Keverne EB, Martel FL & Nevison CM (1996) Primate brain evolution: genetic and functional considerations. *Proc Biol Sci.* **263**, 689-96.

157 Preuss TM (2000) Taking the measure of diversity: comparative alternatives to the model-animal paradigm in cortical neuroscience. *Brain Behav Evol.* **55**, 287-99.

158 Kaas JH & Preuss TM (2003) In *Fundamental Neuroscience (Second Edition),* (eds. Squire, L. R. et al.) 1147-1166. Academic Press, San Diego,

159 Zeki S & Shipp S (1988) The functional logic of cortical connections. *Nature* **335**, 311-7.

160 Shipp S (2004) The brain circuitry of attention. *Trends Cogn Sci* **8**, 223-30.

Non-human primates have fine control of their limbs, including independent motor control of the fingers of the hand, in the way that rats and mice do not.

6.3.3 Intelligence

Non-human primates are generally considered to be more intelligent than other mammals, although the criteria for defining intelligence are a constant source of debate. Numerous candidates have been suggested as the major determinants in the evolution of human intelligence, including learning ability, diet, social factors, tool use, language, deception and, most recently, behavioural flexibility.[161] The classical view is that the development of language by humans constituted an evolutionary step-change, and, for this reason, the largest 'cognitive' divide exists between humans and all other animals, including the great apes.[162,163]

Other scientists, including Macphail, have taken the argument further in asserting that language is also central to 'true' conscious awareness.[164] However, opinion on this issue is sharply divided. The psychologist Mark Hauser agrees with Macphail in stating that non-human animals lack self-awareness, but adopts a more 'ecological' stance in arguing that intelligence can only be judged in relation to the context to which an animal is adapted.[165] A recent review of the evolution of intelligence places more emphasis on the continuity of humans with

Box 7. Investigating intelligence in great apes and other non-human primates

A well-known study on self-awareness involves placing different non-human primate species in front of a mirror. In this study, chimpanzees recognise that a mark made on their face belongs to them, as indicated by them rubbing at that point on their face. Other primate species (excluding those of the great ape family) do not do this and always treat the image in the mirror as another individual, irrespective of the extent of previous experience with mirrors.[167] This study has been followed up extensively, including by developmental psychologists using it as a means to appraise self-awareness in young infants, and the sharp distinction between great apes and all other non-human primate species has endured.[168]

Examples of research groups who have been successful in teaching the rudiments of language to individual gorillas and chimpanzees have been widely reported. These have led some researchers to suggest that symbolic reference and the capacity for inferential logic from specific sequences of 'words' are not necessarily unique to humans.[169] However, critics have questioned how much can be concluded from the great ape language experiments, specifically querying why, if great apes have this capability, they have not spontaneously generated at least a gestural 'language'. No one has yet successfully trained other non-human primate species in these language tasks.

The discovery of non-human primate 'mirror neurons' ten years ago (described in section 6.6.3) raised the possibility that the non-human primate brain may possess the neural circuitry necessary for imitation and, if so, confer some sense of the animal's own identity. Subsequent research has suggested that, in non-human primate species excluding the great apes, the mirror system may be involved with understanding the intention of others' actions. However, this is a field of ongoing inquiry.

161 Allman JM (1999) *Evolving Brains.* Scientific American Library, New York.
162 Macphail EM (1982) *Brain and intelligence in vertebrates.* Clarendon Press, Oxford.
163 Pinker S (1994) *The Language Instinct.* Morrow, New York.
164 Macphail EM (1998) *The Evolution of Consciousness.* Oxford University Press, Oxford.
165 Hauser MD (2001) *Wild Minds: what animals really think.* Owl Books, New York.
166 Roth G & Dicke U (2005) Evolution of the brain and intelligence. *Trends in Cognitive Science* **9**, 250-257.
167 Gallup GGJ (1970) Chimpanzees: self-recognition. *Science* **167**, 86-87.
168 Gallup GGJ, Anderson JR & Shillito DJ (2002) In *The Cognitive Animal: Empirical and Theoretical Perspectives on Animal Cognition* (eds. M. Bekoff, Allen, C. & Burghardt, G. M.) 147-165. MIT Press, Cambridge, MA.
169 See http://en.wikipedia.org/wiki/Ape_language for a good discussion of this controversy.

other primates: *'The outstanding intelligence of humans appears to result from a combination and enhancement of properties found in non-human primates, such as theory of mind, imitation and language, rather than from 'unique' properties.'*[166] See also Box 7.

6.4 Neuroscience research tools: direct and indirect techniques

Scientists use both direct and indirect measures of physical and biological entities and processes. For example:

- A *direct* measure of brain activity is the electrical activity of brain cells, recorded by placing an electrode close to or inside them.
- An *indirect* measure of brain activity is the changing blood flow near the neurons of interest, which is known to be closely coupled with their activity, particularly that of their synaptic connections.

A key difference between these two measures is that direct measures require invasive access to the cells in question, while indirect measures may not. Both invasive and non-invasive techniques are widely used in neuroscience research.

6.4.1 Electrophysiology and single cell recording

Direct measures using electrophysiological techniques involve the insertion of a miniature electrode into a living brain. This is rarely appropriate in humans, although electrophysiological experiments have been carried out in the context of the surgical management of epilepsy.[170] Researchers argue that recording brain cell activity in non-human primates and rodents while they perform various tasks provides the most direct method for investigating brain function. New techniques have made it possible to record from multiple single-cells simultaneously.[171,172] Similarly, new algorithms and more powerful computing software are helping neuroscientists who use cell recording techniques to unravel how different parts of the brain communicate with each other.[173]

Evidence presented to the working group suggests that electrophysiological techniques raise some of the most substantial concerns about welfare. The procedure for inserting electrodes into the brain of a primate is invasive. Microelectrodes (the tips of which are about 10 microns, with a shaft of 0.1-0.5mm) are inserted by means of a permanent head cap consisting of a circle of stainless steel fixed during a 4-5 hour surgical procedure carried out under general anaesthetic. The positions of the electrodes are determined from sectional X-rays or from MRI scanning (such procedures having been developed from those used on humans). Recording electrodes are then inserted each day of the experiment and removed at the end of each day's testing. The animals are conscious during testing; their heads are restrained, but they can move their limbs.

Researchers argue that, while these procedures might appear alarming, they are tolerated well by the animals. The lack of sensation in brain tissue means that non-human primates (and humans) can tolerate insertion of microelectrodes. Researchers reported that animals with head-caps could not be distinguished on the basis of their behaviour from those without. However, other respondents raised concerns about the effects of surgery on welfare, including the impacts of postoperative pain, the practice of single housing (to prevent infection or other animals damaging the head caps), the length of time the animals are restrained during recording, and the use of food and fluid control to motivate the animals to carry out the tasks.[174] The evidence suggests that managing infection is one of the most significant problems facing researchers carrying out this type of work, who have requested that the NC3Rs organise a workshop on the subject.

170 Fried I, Cameron KA, Yashar S, Fong R & Morrow JW (2002) Inhibitory and excitatory responses of single neurons in the human medial temporal lobe during recognition of faces and objects. *Cereb Cortex* **12**, 575-84.

171 McNaughton BL, O'Keefe J & Barnes CA (1983) The stereotrode: A new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. *J. Neurosci. Methods* **8**, 391-397.

172 Hoffman KL & McNaughton BL (2002) Coordinated reactivation of distributed memory traces in primate neocortex. *Science* **297**, 2070-3.

173 Rolls ET & Treves A (1998) *Neural Networks and Brain Function.* Oxford University Press, Oxford.

174 Promoting refinements in food and fluid control is the subject of a current NC3Rs initiative, due to report soon.

Further discussion on welfare issues, including single housing, can be found in section 10. Here, we emphasise that procedures involving invasive methods demand excellent laboratories and high standards of anaesthesia, intra-operative and post-operative care for the animals. We also note that evidence presented to the group shows that animals with head caps can be successfully group housed. However, it is clear that further refinements of electrophysiological recording techniques would be beneficial and an expansion of those programmes currently in development (for example see Box 8) would be welcome.

6.4.2 Non-invasive imaging

Certain neural processes happening inside the skull can be studied effectively in humans using indirect non-invasive techniques. The electroencephalograph (EEG) and associated scalp-recorded evoked potentials (ERPs) have long been used in humans, in conjunction with behavioural observations. The most important recent example of an indirect approach to measuring neuronal activity is brain scanning, including techniques such as:

- Computer Assisted Tomography (CAT)
- Positron Emission Tomography (PET)
- Magnetic Resonance Imaging (MRI).

UK scientists have played a major role in the development of these techniques (developments of CAT and MRI scanning were recognised through the award of Nobel Prizes to their developers[175]). Scanning techniques offer unprecedented non-invasive images of the human body and brain that are proving immensely valuable in both the laboratory and the clinic. Functional imaging using PET and MRI has allowed researchers to observe patterns of brain activity during many different kinds of cognition.

The continuing development of new imaging techniques for the human brain promises to yield further insight into normal brain mechanisms and brain disorders. For instance:

- Diffusion Tensor Imaging (DTI) is a mathematical technique that reveals the alignment of water molecules inside nerve fibres. This provides an indirect measure of circuit connectivity in the human brain, although it is still poorly understood in detail.
- Magnetoencephalography (MEG) measures tiny electromagnetic dipoles that occur in the cerebral cortex when rapid information processing is occurring. While it is a more direct measure of brain activity than MRI, it is nevertheless an indirect measure of the firing of brain cells.

Structural MRI pictures of the brain are well known to the public. The artificially coloured outputs of functional magnetic resonance imaging (fMRI) are often described as showing the brain 'lighting up' in response to some stimuli. However, unlike looking down a microscope, fMRI images do not provide

Box 8. Refining electrophysiological recording techniques

A project at University College London, funded by the NC3Rs, aims to refine current methodologies to record electrophysiological signals in non-human primates. This project aims to improve methods for restraining the head of the animal during electrophysiological recording procedures, which is usually done using an inert metal implant. Researchers are using non-invasive Magnetic Resonance Imaging (MRI) to make a 3-dimensional reconstruction of the animal's skull in order to guide the construction of a custom-fitted head-holding device. This new method is tissue-friendly, thus reducing the likelihood of infection and avoiding additional surgery should devices become loose or broken. Researchers also point out this refinement has the potential to improve the yield of data from each animal, so reducing the number of animals required for each project.

175 See Nobel Prize web pages about Sir Godfrey Hounsfield and Sir Peter Mansfield at: http://nobelprize.org/medicine/laureates/1979/hounsfield-autobio.html and http://nobelprize.org/medicine/laureates/2003/mansfield-autobio.html

a direct picture. They in fact depict the changing oxygenation of haemoglobin in blood in capillaries. Research has shown that there are now very good reasons to regard this as a valid, but ultimately indirect, measure of certain aspects of brain activity, e.g. of the field-potentials that reflect synaptic activation (see Box 9).[176]

The use of fMRI is now a major strand of neuroscience research and the working group heard evidence of how human cognitive neuroscientists are using these techniques to make advances in understanding consciousness and perception. However, neuroscience researchers also drew attention to technical limitations on the spatial and temporal resolution of fMRI, MEG and other non-invasive imaging techniques. The current temporal resolution for MRI is 1-3 seconds; MEG promises finer temporal resolution, but at the expense of spatial resolution. fMRI signals have a spatial resolution that is determined, not by the pattern of cells firing in a neural circuit, but by the layout of the capillary vasculature of the brain. In absolute terms, there are thousands of neurons in the region of any single capillary being measured. While single cell electrode recording can distinguish the firing patterns of individual cells separated by as little as 50 microns apart, the spatial resolution of fMRI and MEG reflects the synaptic activation of hundreds or thousands of cells.

Box 9. The neural basis of the BOLD signal measured in non-invasive brain imaging

fMRI outputs are encapsulated in the form of the BOLD signal: a measure of **b**lood **o**xygen **l**evel **d**ependent activity. Researchers are still in the process of gaining a fuller understanding of the nature of the BOLD signal. Many had suspected it to be synaptic in origin, while others had assumed it to reflect cell firing. Work at the Max-Planck Institute for Biological Cybernetics in Tuebingen, Germany, used simultaneous electrophysiological recordings and fMRI measurements in non-human primates to reveal that the best correlate of the BOLD signal is local field potential (indicating the size of the electrical input to a population of cells), rather than cell firing (reflecting the area's neural output).[176] Researchers highlight that, without this work, fMRI signals would continue to be misinterpreted.

Respondents drew attention to the fact that fMRI does not permit detection of the difference between excitation and inhibition of brain cells. Any interpretation given to human activation maps derived from fMRI techniques is therefore, at best, incomplete. They argued that non-human primate experiments are required to compare results from single cell responses with the amplitude and time course of the BOLD signal, to determine more precise relationships between the BOLD signal and neuronal activity.

One important corollary to the Max Planck discovery is a deeper appreciation that the fMRI BOLD signal reflects the input to hundreds of cells (or more) and will not, even at higher field strengths, ever provide a measure of the activity of individual cells. This is because it is a haemo-dynamic signal that reflects changes in the oxygenation of blood carried in capillaries near brain cells. As noted earlier, the spatial resolution of the BOLD signal is therefore determined by vascular parameters. However, other MRI approaches can be conceived, such as the activation of genes driving MRI contrast agents that could in principle allow detection of single cells (provided that the network expression of such cells was sufficiently sparse). However, even if such techniques were developed and proved successful, it would not be possible for them to be tested in humans.

176 Logothetis NK, Pauls J, Augath M, Trinath T & Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. *Nature* **412**, 150-7.

Written submissions to the working group commented on the validation and interpretation of results from imaging techniques, particularly where uncertainties exist about what is actually being measured. Respondents argued that imaging results do not show how foci of brain activity relate to the activity of groups of nerve cells. They emphasised that understanding the cellular basis of overall activity is necessary because therapeutic drugs work at the molecular and cellular level (i.e. specific types of receptors on particular nerve cells). Given current limitations and uncertainties, many neuroscience researchers (including those not involved in non-human primate work) argued that non-invasive imaging research should be complemented by invasive cell recording work involving non-human primates for the foreseeable future.

6.5 Neuroscience research tools: observational and interventional techniques

A full understanding of a physiological or biological process requires the establishment of mechanisms of cause and effect. Securing a sound understanding of the relationship between a particular cause and its effect generally requires some sort of intervention in normal operation. Without intervention, it is impossible to conclude definitively whether an identified mechanism is both necessary and sufficient for the process being studied. Determining cause and effect therefore demands procedures that alter the normal workings of the brain. Non-invasive imaging techniques, by definition, preclude such intervention: fMRI does not show that particularly neural systems are necessary for aspects of cognition, only that they are active.

6.5.1 Studying brain-damaged patients

Many fundamental neuroscientific concepts were first elucidated in human patients with brain damage. 'Natural' experiments in which patients incur localised brain damage as a result of stroke, tumour or trauma, have provided many important insights into the anatomical organisation of cognition and localisation of function in the nervous system (e.g. the lateralisation of speech to the left hemisphere).[178,179] Coupled with MRI studies to define the exact location and size of the brain damage, behavioural neurology continues to be an important approach to the study of the human brain.[180]

This approach is most valuable when the nature of the patient's brain damage, or the pattern of the neuropsychological deficit (or both), allow investigation of specific scientific questions in a particularly cogent manner. However, such damage typically affects relatively large areas of the brain, including both grey and white matter, and precludes any detailed localisation of specific functional areas. One witness stated that: *'one could wait a lifetime for a patient who happened to have the exact 'natural' brain lesion of interest'*. Similarly, the damage is different in each patient, making it difficult to repeat and consolidate research findings, in order to confirm that the specific lesion is both necessary and sufficient for the observed outcome.

6.5.2 Lesioning studies in non-human primates

Researchers argue that animal studies permit finely localised lesions to be made in a manner that can be replicated and conducted in sufficient numbers for statistical reliability. Experimental lesions can be made in a number of ways. One way is to remove directly, by aspiration, small regions of brain tissue. This approach is now being replaced by the use of direct injection of chemically specific toxins that target and kill brain cells without necessarily affecting the connections that link one region to another. Alternatively, these fibres may themselves be cut without affecting cells. Reversible pharmacological techniques are also being increasingly used. In all cases, the lesions are made using stereotaxic techniques under full recovery anaesthesia in accordance with the terms of the relevant Home Office Project Licence.

178 Shallice T (1988) *From neuropsychology to mental structure.* Cambridge University Press, New York.
179 McCarthy RA & Warrington EA (1990) *Cognitive Neuropsychology.* Academic Press, San Diego.
180 Vargha-Khadem F, Gadian DG & Mishkin M (2001) Dissociations in cognitive memory: the syndrome of developmental amnesia. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **356**, 1435-40.

The observation of a drug- or lesion-induced behavioural/performance deficit is taken to indicate that the affected area is necessary for the impaired ability. The impact of lesions upon brain cell firing patterns in behaving animals can therefore provide valuable direct information of a causal nature. Several research programs now combine brain lesion techniques and single cell recording with MRI imaging and clinical observations.

Scientists emphasise that lesioning studies involve very selective areas of the brain: gross brain damage would be scientifically valueless. However, such experiments have significant welfare implications, mostly in relation to the surgical procedure (e.g. postoperative pain, the risk of infection; see 6.4.1). There are promising developments in techniques that might offer an alternative to some lesioning studies in non-human primates, such as Transcranial Magnetic Brain Stimulation (see Box 10).

6.6 The use of non-human primates in fundamental neuroscience research

Fundamental neuroscience research seeks to understand normal brain function that may or may not be of immediate relevance to clinical dysfunction, though as discussed earlier, increasingly it is becoming linked to research into neurological disease in a seamless fashion. Several respondents to the call for evidence asserted that understanding the brain is one of the great challenges of our age. The cerebral cortex effortlessly performs many complex real-

Box 10. Transcranial Magnetic Brain Stimulation (TMS)

Human subjects are generally, although not always, inappropriate for interventional research on the brain. The exception is a new technique called Transcranial Magnetic Brain Stimulation (TMS), which offers a safe, painless and reversible method of intervening in human brain function.[181] TMS is carried out using a handheld device that, when positioned outside the skull at different points around the head, can induce a flow of current just inside the brain that interrupts normal brain activity (producing a 'virtual' lesion) or, in repetitive mode, can induce a lasting change in neuronal excitability.

There is considerable optimism about the eventual clinical applications of TMS[182]: it is already widely used in the diagnosis of stroke, epilepsy and spinal cord injury and has potential therapeutic uses in Parkinson's Disease, spinal injury and depression. It has also been cited as an important opportunity to replace invasive research involving non-human primates. It seems likely that future developments in this technique and a greater understanding of its properties and mechanism of action could open avenues of research that replace non-human primate work. However, researchers emphasise that TMS is in its infancy and there is still significant uncertainty about exactly how it works. They also note that TMS is currently limited to those areas of the brain that lie close to the surface. Written evidence received stressed that interpretation of TMS relies largely on non-human primate experiments that first elucidated its action on basic neural elements within the cerebral cortex. Similarly, researchers argued that validation and interpretation of new developments, such as theta-burst repetitive TMS, will depend on further non-human primate research. A key issue is to understand better the relative impact of TMS on excitatory and inhibitory neurons, the proportion of neurons affected, the distance from the brain surface, the impact on neuronal plasticity, and so on. This is particularly important in cases where TMS is used as a long-term therapeutic tool.

181 Walsh V & Cowey A (2000) Transcranial magnetic stimulation and cognitive neuroscience. *Nature Neuroscience* **1**, 73-78.

182 See Academy of Medical Sciences (2003) *'Restoring Neurological Function: Putting the Neurosciences to Work in Neurorehabilitation'.* Academy of Medical Sciences.

world computations that would defeat even the most sophisticated modern computers. One witness put it thus: *'Unlike genetics, where the code has been known for 50 years, neuroscientists have not cracked the code of how the brain encodes, processes and integrates the vast amounts of information coming in from the senses, or how it is combined with current motivational status and memory to ensure adequate motor output'.* Another respondent described the *'challenge of neuroscience': 'to link behaviour and perception to brain mechanism in a fashion that moves from neuron, neurotransmitter systems, synaptic organisation and neural circuitry through to an integrated model at the systems level.'*

Basic neuroscience involves several facets, including neuroanatomy (understanding the brain's neural circuitry), neurophysiology (understanding how the brain processes information and determining the causal role of brain structures in behaviour and cognition), neuropharmacology and experimental therapeutics for cognitive disorders.

Written submissions to the call for evidence cited several examples of fundamental neuroscience findings to emerge from non-human primate research, including:

- The columnar organisation of neurons within cyto-architecturaly defined areas.
- The concept of cortical magnification factor (according to which the area of the cortex devoted to a region of the body is in proportion to its importance and not its size).
- Confirmation of the concept of 'blindsight'.
- The role of cortical-basal ganglia loops.
- The mediation of working-memory and executive function by the prefrontal lobe.
- The interacting roles of the medial-temporal and frontal lobes in long-term memory.

We have examined several areas of fundamental neuroscience research in more detail, including vision, motor control, mirror neurons, learning and memory.

6.6.1 Vision

Respondents to the call for evidence cited several reasons why non-human primates are more suitable than rodents for the study of vision. Primarily, unlike both human and non-human primates, rodents are not visual animals. This stems from the fact that rodents are nocturnal, while most primates are predominantly daytime creatures. Unlike rodents, primates have forward-facing eyes, tri-chromatic colour vision with retinal rods and cones and well developed eye-movements that allow the gaze to be cast onto specific objects. The primate fovea is packed with cone photoreceptors that enable the animal to focus on objects with very high acuity. In contrast, mice possess lateral facing eyes for panoramic vision and a retina that is dominated by rod photoreceptors for optimum night vision. Visual acuity varies across mouse strains, but in even the most visual strain (C57BL/6), it is 100-120 times poorer than that of humans and non-human primates.[183] Unlike mice, primates also have the capacity to focus equally well on both near and far objects.

Differences between rodents and primates in retinal structure and function are reflected in differences in the visual circuitry as the optic nerve enters the brain. For instance, whereas 97% of retinal ganglion cells in rodents project to the contralateral hemisphere, the figure is about 50% in humans and non-human primates. Primates also possess a radically different geniculo-striate pathway, in which information is carried from the thalamus up to the visual areas of the occipital cortex. There are important differences between Old and New World monkeys with respect to colour vision: for the most part, male New World monkeys are dichromatic while females are dichromats or trichromats. In contrast, Old World monkeys exhibit full trichromatic vision.

Neuroscientists, in evidence to the working group, stressed the need for a multiplicity of

methodologies for understanding the biological basis of vision. They argued that the techniques used must be appropriate for the particular question, which will often depend on the scale of the anatomical structure under investigation. These can include visual 'areas' (as identified by Brodman in 1909), 'modules' (such as the modules of the visual area V1-5), synapses and neuronal cells. Researchers emphasised that electrophysiological recording of brain cells complements imaging scans of modules and areas, enabling a circuit level map of brain connections to be transposed on brain regions. This work has established a hierarchy of connections in the visual circuit, with anatomical differences between 'ascending' and 'descending' (feedback) neurons: ascending neurons appear to be involved in the processing of information inputs, while it is hypothesised that descending neurons process visual information according to memory.

Currently, elucidating the visual system in this way is essentially a scientific pursuit, but researchers argued that gaining this type of biological knowledge can have serendipitous applications for human disabilities, e.g. dyslexia (see Box 11). Studies on visual learning in non-human primates have implicated the perirhinal cortex (part of the medial temporal lobe), as critical for visual memory and perceptual processing. Researchers highlight that this finding directly affects our understanding of memory deficits in humans with amnesia or dementia and is forming the basis for the development of neuropsychological tests to be used as diagnostic tools for Alzheimer's disease.

6.6.2 Motor systems, the precision control of the hand and the use of tools

The motor systems of the mammalian central nervous system include:

1. The striatum, which appears to be involved in the initiation of movements.
2. The motor cortex, which contains the cells of origin of the corticospinal tract that project extensively to the brainstem and spinal cord.
3. The cerebellum, which is believed to play a critical role in the learning that underlies precision timing and automisation of actions.

These three structures are evolutionarily conserved between mammals and much useful research has been conducted on 'lower' species, including rodents, without the need for non-human primate work.

However, researchers point out the difficulties of modelling deficits that occur in human movement disorders in quadruped animals such as the rodent. It is also now clear that the organisation of the corticospinal tract is distinctive in primates; only in primates (human and non-human) are there direct projections to the neurons of the ventral horn whose axons leave the spine to innervate the muscles of the limbs. Differences between

Box 11. The magnocellular hypothesis of dyslexia

The 'magnocellular' hypothesis for dyslexia suggests that the cause lies in a problem with the visual system itself. Magnocells detect flickering lights and are located in the lateral geniculate nucleus (LGN). These cells input a visual signal into the V1 modules of the visual area, where it is moved through modules 1 to 5 before transfer to the parietal cortex.

Post mortem analysis of dyslexic individuals has revealed abnormalities in the LGN. Reading is a complex visual process in which the eye flickers across the page in short jumping movements. It has been suggested that deficiencies in the magnocells are amplified as the signal moves through the visual modules, resulting in reading difficulties. In this case, researchers argue that knowledge of the basic neural circuitry is essential for analysing the hypothesis in humans.

rodents and primates in the pattern of corticospinal terminations are both quantitative and qualitative. Careful comparative study of the anatomical connectivity of spinal cord circuits has revealed that, early in mammalian evolution, the corticospinal tract developed to provide central gating of proprioceptive input back to the brain. Later in evolution, and unique to primates, the more ventral portions of the cord became enervated, permitting central control of the digits of the hand. It was this development that enabled the precision grip control between thumb and index finger that characterises primate tool use.[184]

Researchers therefore argue that non-human primates provide the only suitable model for studying precision hand control and subsequent tool use. Neurophysiological studies, in which single brain cell recordings are made in macaques at various stages of tool use training, have revealed that the central representation of the hand region changes systematically - expanding to encompass not only the areas of space in the parietal cortex where the hand itself is represented, but also the region of space that can be reached by the tool.[185]

6.6.3 Mirror neurons

The recent discovery of mirror neurons in rhesus macaques has excited much interest. These cells are located in the F5 area of the pre-motor cortex and are unique in firing action potentials both when the animal itself engages in a specific action (such as picking up a raisin that it is offered) and when the experimenter or another animal is observed performing the same action.[186] These cells show specificity to a particular action and will not fire when the animal or experimenter performs a different action (such as a different hand movement); different cells in this brain region 'mirror' different actions.

Moreover, if the animal is used to seeing the action performed regularly, the mirror neuron will continue to fire at the appropriate moment, even when large parts of the action are obscured behind a screen. Mirror neurons are therefore not visually tuned to match actual movements, but to the intentional action underway. They are therefore likely to be part of a brain network involved in interpreting the actions of others. Some also suspect that, in rhesus macaques, mirror neurons constitute part of a primitive gestural system that later evolved into human language.[187] Following these initial observations in macaques, new research using brain-imaging of human subjects [188] has suggested a link between disturbances in the mirror system and conditions such as autism.

Researchers point out that nothing that might compare to a mirror neuron has ever been identified in a rodent. This discovery has, for the first time, secured a neurobiological tool that allows researchers to investigate issues connected to the theory of mind and knowing the minds of others.[189]

6.6.4 Learning and memory

Research into cognitive behaviours such as perception, learning and memory has had a long history of using human volunteers and has become increasingly amenable to genetic and molecular biology techniques using Drosophila and transgenic mice. However, researchers argue that the need to test complex cognitive behaviours in a stable and reproducible way requires the use of non-human primates. They assert that careful experimental lesions and single-cell recording in the non-human primate have confirmed important clinical neurological findings. For instance, that regions of the medial temporal lobe are important for certain forms of learning and memory, while the striatum and cerebellum are important for other types of learning, such as habits and the precise timing of learned motor skills. In addition to their anatomical and physiological similarities, researchers also highlight that

183 Prusky GT, West PW & Douglas RM (2000) Behavioral assessment of visual acuity in mice and rats. *Vision Res.* **40**, 2201-9.

184 Lemon RN & Griffiths J (2005) Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? *Muscle Nerve* **32**, 261-279.

185 Hihara S *et al.* (2006) Extension of corticocortical afferents into the anterior bank of the intraparietal sulcus by tool-use training in adult monkeys. *Neuropsychologia*.

186 Gallese V, Fadiga L, Fogassi L & Rizzolatti G (1996) Action recognition in the premotor cortex. *Brain* **119,** 593-609.

187 See also http://en.wikipedia.org/wiki/Mirror_neurons

188 Rizzolatti G & Craighero L (2004) The mirror-neuron system. *Annu Rev Neurosci* **27**, 169-92.

189 Rizzolatti G, Fogassi L & Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. *Nat Rev Neurosci.* **2**, 661-70.

only non-human primates are capable of being trained and assessed in the complex tasks necessary to elucidate cognitive function.

An example given in submitted evidence involved 'recognition memory', i.e. when a previously novel stimulus is recognised on second exposure. Understanding the neural basis for this important capacity has required the development of a behavioural test in which a macaque can communicate whether it has seen a stimulus before and the context in which it was seen.[190,191] Researchers hypothesised that there may be at least two ways of solving the recognition problem. Either a repeated stimulus simply becomes 'familiar', or it evokes the explicit recall of a memory associated with a particular time and place ('remembering'). Non-human primate work has revealed a population of neurons in the perirhinal cortex that signal familiarity,[192,193] while activity in other brain networks, including the hippocampus, computes various associations and sequential information that could be the basis of explicit recall.[194]

More recent research has explored the interaction between the temporal and frontal lobes in memory processing, where brain imaging studies in humans have revealed that both areas are active during memory encoding and recall. Further non-human primate work has revealed that the perirhinal cortex may have perceptual functions beyond the domain of memory, such as when humans or non-human primates are required to make 'odd man out' judgements of a set of stimuli viewed from different angles.[195]

Researchers are increasingly using powerful computational techniques to model physiological aspects of learning and memory, with UK teams at the forefront of this work.[196] Evidence to the working group suggested that computational tools will play an increasingly important role in research into cognition and behaviour. However, researchers emphasised that computational models require parameters that must be defined in the real world; in many instances only obtainable from animal experiments. Modelling studies based on existing data make predictions about how the human brain might solve a certain problem. These predictions are then tested experimentally, resulting in further refinement of the model. One respondent put it thus: *'modelling studies are only as informed as the data that feed them and currently a lot of questions about brain function remain unanswered.'*

Studies in monkeys are also proving useful in the search for cognitive enhancing drugs based on the developing understanding of the way that synaptic strength can be modulated, with one study revealing that a class of drugs called AMPAkines can also offset some of the memory loss associated with sleep loss.[197] This work in non-human primates is complemented by studies on rodents, including transgenic mice that have enabled the molecular-genetic 'rescue' of memory impairments,[198] but these in no way supplant the need for further primate research. A constructive dialectic also exists between studies of neural memory mechanisms based on animals and research studies on patients with neurological disorders of memory. Sometimes tasks first tried with humans are 'modelled' in animals, but increasingly there is interest in moving the other way - developing analytic tasks for humans that were first developed with animals. This effort has led to

190 Gaffan D (1974) Recognition impaired and association intact in the memory of monkeys after transection of the fornix. *Journal of Comparative and Physiological Psychology* **86**, 1100-1109.

191 Gaffan D (1994) Scene-Specific Memory for Objects: A Model of Episodic Memory Impairment in Monkeys with Fornix Transection. *Journal of Cognitive Neuroscience* **6**, 305-320.

192 Fahy FL, Riches IP & Brown MW (1993) Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterion and medial inferior remporal and rhinal cortex. *Esperimental brain research* **96**, 457-472.

193 Aggleton JP & Brown MW (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral and Brain Sciences* **22**, 425-489.

194 Rolls ET & Treves, A (1998) *Neural Networks and Brain Function.* Oxford University Press, Oxford.

195 Bussey TJ, Saksida LM & Murray EA (2005) The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. *Q J Exp Psychol B* **58**, 269-82.

196 Treves A & Rolls E T (1994) Computational Analysis of the Role of the Hippocampus in Memory. *Hippocampus* **4**, 1-18.

197 Porrino LJ, Daunais JB, Rogers GA, Hampson RE. & Deadwyler SA (2005) Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. *PLoS Biol* **3**, e299

UK-based 'spin-out' companies that market the available software such as the CANTAB test battery that is now proving so useful in the therapeutic studies of Alzheimer's disease (see Box 14).

6.7 The use of non-human primates in clinical neuroscience research

We discuss three examples of areas of clinical neuroscience research either previously or currently involving non-human primates. These examples – Parkinson's disease, stroke and Alzheimer's disease - have been chosen on the basis of the volume of evidence received. The role of non-human primates in the development of Brain-Machine Interfaces is also outlined in section 6.8.

6.7.1 Parkinson's disease

Parkinson's disease (PD) is a crippling neurodegenerative disorder characterised by altered gait, tremor and progressive slowing of movement developing towards chronic rigidity and loss of motor function. Symptoms of cognitive impairment can also develop in PD patients. There are approximately 120,000 PD patients in the UK. International studies of the health costs associated with PD show a consistent pattern of increasing costs as the disease progresses; in the UK this rises from around £2,000 per patient per year to £11,000 (as measured in 1998).

It is now well established that the proximate cause of the impairment of movement is the progressive death of dopaminergic cells in a region of mid-brain called the substantia nigra. The axons of these cells travel to a number of brain targets, including the striatal region, which is involved in motor control. The death of these cells results in the loss of the neurotransmitter dopamine from the target sites, with devastating effects.

In the early stages of the disease, dopamine loss can be effectively compensated by the provision of the dopaminergic agonist drug, L-DOPA. This is widely used in clinical practice in conjunction with other medication. However, L-DOPA has a number of limitations. First, it is a symptomatic treatment that does not stem the course of the disease. Second, not all patients benefit from L-DOPA and third, the drug becomes less effective with time.

6.7.1.1 The MPTP non-human primate model and Deep Brain Stimulation

Great progress was made in understanding the biomedical basis of PD through a serendipitous human case study in which a drug addict developed PD symptoms after taking contaminated heroin. It was shown that the agent responsible, MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), could selectively kill neurons of the substantia nigra. Both researchers and opponents of animal research have highlighted the value of this clinical case in advancing PD research. However, researchers have stressed that, whilst the discovery was made in a human, understanding the effects of MPTP has depended on further non-human primate studies. MPTP selectively binds to the neuromelanin-containing cells in the substantia nigra and, importantly, neuromelanin occurs only in humans and Old World monkeys. New World monkeys and rodents do not have neuromelanin and MPTP exposure does not induce stable PD symptoms.

Several respondents pointed out the deficiencies of non-human primate models of PD, with comments including:

- *'Animal models of PD differ in onset, type and persistence of PD symptoms.'*
- *'Striatal degeneration [i.e. degeneration of neurons in the corpus striatum] in humans is associated with dyskinesia[199], but striatal lesions in non-human primates do not induce dyskinesia or chorea[200].'*
- *'Topography of neuronal loss in the substantia nigra is different in animals and patients.'*
- *'Animals experience an acute, severe and pre-dopaminergic deficiency,*

198 Schmitt WB *et al.* (2005) Restoration of spatial working memory by genetic rescue of GluR-A-deficient mice. *Nat Neurosci.* **8**, 270-2.

199 Dyskinesia refers to an impairment in the ability to control movements, characterized by spasmodic or repetitive motions or lack of coordination.

200 Chorea refers to any of various disorders of the nervous system marked by involuntary, jerky movements, especially of the arms, legs, and face, and by lack of coordination.

whilst PD is chronic, slowly progressive and more than just dopaminergic.'

- *'Genetic, environmental and age-related factors cannot be studied in animals.'*
- *'Cognitive, emotional and other non-dopaminergic signs are difficult to evaluate in animals.'*

These respondents asserted that, because of these deficiencies, the major breakthroughs in PD research have been via epidemiology, clinical studies, genetic research, human tissue studies and autopsies. Respondents from both sides of the debate agreed that a full understanding of a complex disease such as PD requires a multi-faceted approach. Neuroscience researchers emphasised the value of *in vitro* approaches using cultures and co-cultures (2 or more cell types) of human cell lines, but insisted that animal models are necessary to study and intervene in the dynamic processes of the disease (see section 6.2). Similarly, researchers stressed that rodent models have provided useful information, but they are limited by different mechanisms of motor control and do not provide a reliable model of upper limb tremor or dyskinesia.

Researchers argue that the MPTP non-human primate (MPTP-NHP) model has been instrumental in developing Deep Brain Stimulation (DBS), which is a particularly effective treatment in certain patients (see Box 12). Evidence presented to the working group included a striking demonstration of its effectiveness by a patient with an implanted stimulator. The procedure has also been developed in the US, and there are now reported to be approximately 37,000 patients with PD in the US using DBS to successfully control their ability to move.

6.7.1.2 Future treatments for Parkinson's disease

Despite the success of DBS, many problems remain in the management of PD. For instance, PD-associated tremor is still only poorly controlled. UK researchers have shown that the over-activity observed in the STN is also seen in certain brainstem motor regions, including hitherto obscure brain areas such as the pedunculopontine nucleus (PPN) and zona incerta. Autopsy data has showed that the PPN degenerates in late PD. Further studies using the MPTP-NHP model confirmed that, after reversal of symptoms by STN lesion, inhibition of the PPN is reduced. MPTP-NHP studies have recently shown that DBS delivered to the zona incerta can be very effective at limiting tremor.[201] The stage is therefore now set for a promising series of clinical trials.

All the treatments described above alleviate the symptoms of PD, but have no influence on disease progression. Progress in understanding the cellular and molecular mechanisms of cell

Box 12. The path to Deep Brain Stimulation (DBS) for Parkinson's disease

The first step in the path to DBS involved showing that ibutonic acid lesions of the subthalamic nucleus (STN) reversed PD symptoms in the MPTP-NHP model. Unfortunately, ibotenic acid is extremely toxic and was not a suitable basis for human therapy. In 1991, STN lesions in MPTP-NHP models using surgical radiofrequency electrodes confirmed this effect. This was further confirmed by a clinical case in which a haemorrhagic stroke in the STN of a PD patient was associated with the loss of PD symptoms.

However, lesioning the STN is associated with a risk of hemi-ballism (a wild thrashing movement disorder). In 1993, one group working with the MPTP-NHP model showed that high frequency stimulation of the STN silenced it effectively without the need for lesioning. In the same year, the group transferred the observation to humans, eventually leading to the development of DBS.

death in the substantia nigra is now beginning to impact on the development of novel therapeutics. One new idea is that the loss of dopaminergic neurons from the substantia nigra is associated with the formation of fibrillar intraneuronal inclusions (called Lewy bodies). This idea is an important focus of research worldwide, with two major groups in the UK and the US pursuing the theory that dysfunction of the ubiquitin-proteasome system (and thus impaired proteolysis) could contribute to cell death.[202] Other lines of evidence suggest that a molecular mechanism involving the fibrillisation of a α-synuclein might be central to the aetiology of PD; transgenic mice harbouring mutations in α-synuclein gene have been developed.

Respondents to the call for evidence predicted that the use of transgenic mice would improve understanding of the inherited form of PD (accounting for approximately 5% of all patients). However, researchers emphasised that, while initial studies of the genetic, molecular and cellular factors involved can be performed *in vitro* and in rodents, developing and testing potential therapies requires a system with a level of complexity similar to humans. For instance, the development of effective stem cell therapies will need to ensure that the cells differentiate into specific neural cell phenotypes and generate appropriate synaptic connections.[203] This can only be carried out in a living system of neural complexity comparable to humans.

6.7.2 Stroke

Stroke is caused by either a blockage of the cerebral vasculature or an intracerebral haemorrhage.[204] It can result in severe brain damage or death and, of those that survive, there is often severe disability. In the EU there are approximately 1.1 million new stroke events each year and around 6 million people who have survived a stroke.[205] By any criteria, stroke is a therefore major health issue.

Current acute stroke treatment relies on inducing early vascular reperfusion[206] after the blockage or, in the case of haemorrhagic stroke, stopping the intracerebral haemorrhage. The effectiveness of current treatments depends on them being given very shortly after the stroke, which is not possible in the vast majority of cases. There is an urgent need for drug therapies that can rescue neural dysfunction following a stroke and are effective when applied a realistic time after the event.

Great efforts, in both academia and the pharmaceutical industry, have been exerted in addressing this problem. Researchers have developed experimental animal models of stroke, to better understand stroke pathogenesis and test potential therapies for efficacy and safety. Animal models of global and focal ischaemia (e.g. the endothelin-induced MCI occlusion model[207]) have been developed to allow better assessment of infarct volume and improve monitoring of sensori-motor and cognitive changes following a stroke. Many drugs have shown promise in rodent models, including glutamate antagonists, anti-inflammatory agents, ion-channel modulators, free-radical scavengers, and caspase inhibitors.

However, none of these treatments proved efficacious in human patients. With so many compounds failing, this chapter in the history of neurological drug development has been described as a 'case study in failure'.[208] This failure was highlighted in written evidence to the working group. Respondents asserted the unsuitability of animal, including non-human primate, models of stroke, mostly on the basis that these models cannot replicate the range of factors and co-morbidities that contribute to the incidence of stroke in humans.

201 Nandi D, Aziz TZ, Liu X & Stein JF (2002) Brainstem motor loops in the control of movement. *Mov Disord.* **17 Suppl 3**, S22-7.

202 McNaught KSP, Belizaire R, Isacson O, Jenner P & Olanow CW (2003) Altered proteasomal function in sporadic Parkinson's Disease. *Experimental Neurology* **179**, 38-46.

203 See for example Bjugstad KB *et al*. (2005) Neural stem cells implanted into MPTP-treated monkeys increase the size of endogenous tyrosine hydroxylase-positive cells found in the striatum: a return to control measures. *Cell Transplantation* **14**, 183-192 or Behrstock S *et al.* (2006) Human neural progenitors deliver glial cell line-derived neurotrophic factor to parkinsonian rodents and aged primates. *Gene Therapy* **13**, 379-388.

204 Warlow CP (2001) *Stroke: A Practical Guide to Management.* Blackwell Science, Oxford.

205 Andlin-Sobocki P, Jonsson B, Wittchen HU & Olesen J (2005) Cost of disorders of the brain in Europe. *Eur J Neurol* **12 Suppl 1**, 1-27.

206 i.e. restoration of blood flow

207 Sharkey J & Butcher SP (1994) Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischaemia. *Nature* **371**, 336-9.

6.7.2.1 Animal models of stroke

Researchers also acknowledged the deficiencies of animal models and have pointed to several reasons why potential drug therapies may have been 'lost in translation' between the laboratory and the clinic. For instance, drugs may have appeared to work because they were tested in young healthy rodents, rather than in diabetic or spontaneously hypertensive co-morbid animals that would be more analogous models to most stroke patients. Drugs may also be efficacious in animal models (rodents and non-human primates) when appropriate doses are given within a short period after an experimental stroke, but may fail to work when given at an inappropriately lower dose many hours after a stroke in a clinical trial.[209] Outcome measures for testing drug efficacy may also differ, e.g. histological indicators may be used in animal models, but functional measures will be applied in clinical trials.

Some clinical scientists have expressed scepticism about the quality of many (but not all) past animal studies. One recent systematic review revealed that over 1000 animal studies have been conducted in relation to stroke in the last 25 years.[210] This is a sufficient number for detailed meta-analyses to identify factors that are statistically associated with a positive outcome in a drug trial.[211,212] The identification of unintended, but systematic, bias in many studies of experimental stroke in animals, including some work involving non-human primates, is clearly a matter of concern and these important criticisms of past animal studies have been published by the Research Defence Society.[213]

6.7.2.2 The role of non-human primate models

Debate about the experimental protocols necessary to address these issues, and the role of rodent and non-human primate models, is ongoing. All too often, candidate drugs have been efficacious in animal models because they cause physiological changes, such as a drop in brain temperature, that are themselves neuroprotective,[214] rather than because of any direct pharmacological action on the excitotoxic or apoptotic processes triggered by the stroke. The drug may then be unsuccessful in humans because temperature changes of this kind will not occur (or be much smaller). Using non-human primates, rather than rodents, would not resolve this issue. Respondents to the call for evidence also highlighted the importance of *in vitro* laboratory studies in identifying neurodegenerative mechanisms of likely relevance to stroke, e.g. excitotoxicity, apoptosis, lipid peroxidation and neuroinflammation.

There is widespread agreement that without rigorous, robust and detailed pre-clinical evaluation, it is unlikely that novel stroke therapies will be effective when tested in large, time-consuming and expensive clinical trials. One forum, the Stroke Therapy Academic Industry Roundtable (STAIR),[215] has made a number of recommendations for standards regarding pre-clinical neuroprotective and restorative drug development. These include the need for increased use of behavioural tests and long-term outcome measures in animal models, including non-human primates.

Several reasons have been put forward for the continued role of non-human primate models in stroke research. These mostly centre on dissimilarities between the brains of rodents and primates (human and non-human) that make non-human primates a more accurate and relevant model for some aspects of stroke research. For instance, unlike rats, macaques have a similar microvascular

208 Johnston SC (2006) Translation: Case Study in Failure. *Annals of Neurology* **59**, 447-448.

209 Cheng YD, Al-Khoury L & Zivin JA (2004) Neuroprotection for Ischaemic Stroke: Two decades of success and failure. *NeuroRx* **1**, 36-45.

210 O'Collins VE Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW (2006) 1026 Experimental Treatments in Acute Stroke. *Annals of Neurology* **59**, 467-477.

211 Dirnagl U (2006) Bench to bedside: the quest for quality in experimental stroke research. *Journal of Cerebral Blood Flow and Metabolism* **26**, 1465-1478.

212 van der Worp BH, de Haan P, Morrema E & Kalkman CJ (2005) Methodological quality of animal studies on neurprotection in focal cerebral ischaemia. *Journal of Neurology* **252**, 1108-14.

213 Macleod MR & Sandercock P (2005) Systematic reviews help clinical research design: can they help improve animal experimental work? *Research Defence Society News* Winter Issue**.**

214 Buchan A & Pulsinelli WA (1990) Hypothermia but not the N-methyl-D-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. *J Neurosci* **10**, 311-6.

215 STAIR. (1999) Recommendations for Standards Regarding Preclinical Neuroprotective and Restorative Drug Development. *Stroke* **30**, 2752-2758.

collateral circulation to that of humans,[216] and differences in cerebral blood flow and cerebral glucose metabolism are 2-3 fold greater in rats compared with the non-human primates, which are almost identical to humans.

Researchers point to difficulties in scaling dose regimens from rodents to humans. Differences in recovery times between rodents (weeks) and humans (months) mean that the timing and duration of drug administration is not easily extrapolated from rat models. Similar difficulties apply with drug dosage; while the dosage of some drugs can simply be adjusted by body weight, other stroke therapies (such as proteins and growth factors) must be given intracerebrally, and may therefore need to be scaled up by brain surface area or volume. The shorter distances drugs need to perfuse in a rat or mouse brain to secure access to the stroke site must also be considered. The human brain has roughly a 10:1 ratio of white matter to grey matter, whereas the ratio for rodents is 1:1. If stroke is particularly damaging for neural connectivity,[217] researchers argue that studies limited to rodents could be misleading.

Researchers emphasise that non-human primates are the only models that show the effects of stroke on hand function, arguing that they therefore provide a far better model to investigate the anatomical basis by which rehabilitative training improves long-term recovery.[218] Evidence presented to the working group asserted that research involving non-human primates provided the first evidence of significant plasticity in the cortex after stroke and that this plasticity could be harnessed by intense physical therapy to promote functional recovery. Further evidence was provided on a promising neuroprotective free-radical trapping agent, NXY-059, which has substantial protective effects even when administered four hours after the onset of ischemia in a primate model of stroke.[219] This compound is currently in human clinical trials.

Submissions highlighted the need for non-human primate models in order to translate findings from rodent models of stroke into effective human treatments. They pointed out that only one drug for which behavioural non-human primate studies were conducted has been taken to clinical trial, arguing that, had previous potential treatments been assessed according the STAIR criteria, many failed clinical trials would never have been initiated.

The working group were acutely aware of the ethical dimensions involved in the use of non-human primates in stroke research. In evidence to the group, researchers stressed the importance of considering the cost-benefit equation when using non-human primates, given their likely greater capacity for suffering following an experimental stroke. Refined models are under development, and a promising new intravascular model of stroke has been developed in the US that involves no surgical intervention.[218] However, under any circumstances, such procedures undoubtedly have a significant impact on the animal's long-term welfare.

Clearly, there are many uncertainties regarding the future directions of research into the management of stroke. However, while the frequency of this debilitating condition may be reduced by public health measures such as the control of blood pressure and tobacco smoking, given the dramatic increase in the older population, stroke will continue to be one of our major health problems. As evidenced by the recent demonstration of the neuroprotective action of the hormone erythropoetin in experimental stroke, new and completely unexpected discoveries continue to be made in this field.[220] Due to the many deficiencies in rodent models for the assessment of therapies directed at limiting brain damage after stroke,

216 Gillilan LA (1968) The arterial and venous blood supplies to the forebrain (including the internal capsule) of primates. *Neurology* **18**, 653-70.

217 Dewar D, Yam P & McCulloch J (1999) Drug development for stroke: importance of protecting cerebral white matter. *European Journal of Pharmacology* **375**, 47-50.

218 D'Arceuil HE, Duggan M, He J, Pryor J & de Crespigny A (2006) Middle cerebral artery occlusion in Macaca fascicularis: acute and chronic stroke evolution. *J. Med. Primatol.* **35**, 78-86.

219 Marshall JW, Cummings RM, Bowes LJ, Ridley RM & Green AR. (2003) Functional and histoligical evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. *Stroke*. **34**:2228-33

220 Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A (2000) Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *PNAS* **97**, 10526-31.

there may be an argument for the use of non-human primates in testing new approaches to therapy in the future; each case will have to be carefully assessed.

6.7.3 Alzheimer's disease and other dementias

Alzheimer's disease (AD) is a progressive neurodegenerative condition, with insidious onset, that often begins with a loss of recent memory (sufficient to impair normal everyday activities) and progresses to encompass a broad range of impairments in intellectual function and/or personality.[221,222] It primarily affects older people, but certain familial forms of the disease develop in mid-life. AD is the most common form of dementia and afflicts more than 20 million people worldwide, including approximately 650,000 in the UK. In addition to AD, there are several other neurodegenerative diseases that affect cognition, including fronto-temporal dementia (Pick's disease), semantic dementia, Huntington's disease and Parkinson's disease. The prevalence of dementia is around 2-3% at age 65-74, rising to as high as 30% at age 85. The total cost of care per patient is thought to be between £4,000 to £11,000 per annum.[223] The total cost burden is also set to increase with rising AD incidence.

6.7.3.1 Animal models

Initial research into AD examined end-stage pathology in post-mortem human brains, confirming Alios Alzheimer's original 1906 observation that AD is associated with extracellular amyloid β protein (Aβ) plaques. Such work also revealed other hallmarks of the condition, including intracellular neurofibrillary tangles (NFTs) – protein agregrates found within the neurons of AD patients. NFTs are formed by the hyperphosphorylation of a microtubule-associated protein known as tau, causing it to aggregate in an insoluble form.

AD was shown to be associated with a number of neurotransmitter abnormalities, the most prominent of which is the loss of cholinergic neurons (i.e. neurons using the neurotransmitter acetylcholine) and the enzyme that degrades acetylcholine. Symptomatic non-human primate models of AD were developed in the early 1980s, in which the cholinergic neurons of the nucleus basalis of Meynert were experimentally lesioned or cholinergic transmission shut down with suitable antagonists such as scopolamine.[224,225] These models revealed impairments in attention and memory that could be ameliorated by cholinergic agents such as arecoline, or by inhibitors of the cholinergic degrading enzyme acetylcholinesterase.[226]

Research groups using marmosets have deployed more sophisticated behavioural protocols to investigate the efficacy of potential treatments and have raised the possibility of using cholinergic transplants as a treatment for memory disorders.[227] Researchers assert that both rodent and non-human primate work has contributed to the development of drugs such as Aricept that provide transient relief from the loss of recent memory characterising the early stages of the disease. This compound (along with others) is now licensed for use in the UK in patients with mild to moderate AD, although not without controversy. While many patients benefit from treatment, some do not, and there is little indication that these drugs have much impact on the course of the disease. However, such symptomatic treatments will continue to be valuable and can considerably extend the period of independence for those in early stage of the disease.[228]

221 Albert MS.(1996) Cognitive and neurobiological marks of early Alzheimer disease. *PNAS* **93**, 13547-13551.
222 Hodges JR (2000) In *Oxford Handbook of Memory* (eds. Tulving, E. & Craik, F. I. M.) 441-459. Oxford University Press, Oxford.
223 Andlin-Sobocki P, Jonsson B, Wittchen HU & Olesen J (2005) Cost of disorders of the brain in Europe. *Eur J Neurol* **12 Suppl 1**, 1-27.
224 Bartus RT, Dean RL, Beer B & Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-417.
225 Bartus RT (2000) On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp Neurol* **163**, 495-529.
226 Rupniak NMJ, Tye SJ & Gield MJ (1997) Enhanced performance of spatila and visual recognition memory tasks by the selective acetylcholinesterase inhibitor E2020 in rhesus monkeys. *Psychopharmacology* **131**, 406-410.
227 Ridley RM & Baker HF (1991) Can fetal neural transplants restore function in monkeys with lesion-induced behavioural deficits? *Trends Neurosci.* **14**, 366-70.
228 Bartus RT (2002) On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp Neurol.* **163**, 495-529.

6.7.3.2 Genetic approaches

An important phase of AD research has involved the investigation of genetic factors associated with AD in humans. Researchers have identified mutations in a number of genes, such as amyloid precursor protein (APP), the presenilins (PS1 and PS2) and Apolipoprotein (ApoE) associated with the disease.[229] For example, researchers working at St Mary's Hospital in London first revealed the importance of the APP mutation through work in families with a very high incidence of the disease,[230] an observation that was later confirmed in a family in Indiana.

This important phase of research, involving gene identification and associated molecular mechanisms, has not involved non-human primates. Instead, research has focused on *in vitro* studies of APP processing and work using transgenic mice, guided by the emerging 'amyloid-cascade' theory of AD (i.e. that amyloid plaque formation triggers the disease, and that NFTs are a secondary effect).[231] Mouse models of AD have been developed that reproduce at least part of the condition. In such models, over-expression of 'minigenes' carrying a human APP (hAPP) mutation causes deposition of amyloid plaques and the loss of synaptic connections between neurons.[232,233] Later work has shown that these mice display progressive impairments in learning and memory,[234] suggesting that disruptions in APP processing is sufficient to cause some aspects of the disease phenotype.

There is continuing debate about the adequacy of the 'amyloid-cascade' theory of AD,[235] but it is now recognised that distinct soluble and

Box 13. Neuro-immunisation

An important step forward in developing routes to treating AD was the observation that transgenic hAPP mice could be successfully immunised against the deposition of amyloid plaques.[236] Studies in transgenic mice have also shown that immunisation can protect against hAPP-associated failure to learn simple memory tasks.[237,238] These findings were the basis for an initial Phase II safety trial in humans and then a large-scale Phase III trial conducted in several countries, including the UK.

Unfortunately, the Phase III trial was suspended following the development of meningoencephalitis in 5% of the subjects. This was an extremely distressing outcome for both the affected subjects, the researchers, and for the wider body of patients waiting for an improved treatment. Nonetheless, a cohort of patients vaccinated with Aβ did show promising results, such as a slower rate of decline of cognitive function over an 80-week period.[239] Post mortem analysis of brain sections also revealed decreased Aβ plaques (relative to age-matched untreated controls) in regions of the neocortex associated with activated microglia and T-cell infiltrates.[240] The immunisation route has not been abandoned by the companies involved and remains a focus of new efforts, including a Phase II passive vaccination trial currently underway (i.e. using direct administration of antibodies).

229 Hardy J & Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-6.
230 Goate A *et al.* (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704-6.
231 Selkoe DJ (1999) Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **399**, A23-31.
232 Games D *et al.* (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* **373**, 523-7.
233 Hsiao K *et al.* (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99-102.
234 Chen G *et al.* (2000) A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**, 975-9.
235 Hardy J & Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-6.
236 Schenk D *et al.* (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173-7.
237 Janus C *et al.* (2000) Abeta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979-82.
238 Morgan D *et al.* (2000) Abeta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982-5.
239 Gilman S *et al.* (2005) Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* **64**, 1553-62.
240 Nicoll JAR *et al.* (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nature Medicine* **9**, 448-452.

insoluble epitopes of Aβ protein may play a differential role in memory disturbances and the progression of the disease. The cause and effect relationship between APP mis-processing and tau phosphorylation is also gradually being elucidated.

6.7.3.3 Developing therapies

Neuroscientists across the globe are pursuing a range of therapeutic avenues to AD. While neuro-immunisation is one approach (see Box 13), work is also focused on a more traditional drug approach targeting the enzymatic cleavage of APP by β- and γ-secretases. Much of this work is being done largely in cell-culture, with some lines of enquiry incorporating *in vivo* studies using transgenic mice. Scientific obstacles remain, including interactions with key developmental signal-transduction pathways such as the Notch pathway, but there appears to be widespread optimism about the future outcomes of this work.

One difficulty with the mouse models is that the human gene is transgenically over-expressed (by around 5 times) within a normal mouse genetic background. This has led some researchers to warn that conclusions from mouse studies may be misleading with respect to what might happen when a candidate therapy interacts with the endogenous gene in human patients. Recently, researchers have started to return to non-human primate models of AD, establishing that aged primates naturally show the same pathological hallmarks as humans. For example, Aβ plaques are apparent in marmosets.[241,242] and Aβ immunisation has been successfully demonstrated in both the rhesus macaque [243] and Caribbean vervet.[244] With regard to the latter, detailed biochemical analyses of a small number of aged vervets have shown that neuro-immunisation generates an altered Aβ distribution between cerebro-spinal fluid and blood plasma that is consistent with therapeutic efficacy.

Researchers explain this return to non-human primate research in terms of the need to assess the functional, cognitive and behavioural outcomes of potential treatments, in addition to the physiological effects. The new studies underway involve examining the impact of Aβ immunisation on the age-associated decline in cognitive function.[245] These will use the CANTAB test battery developed at the University of Cambridge (see Box 14).

Researchers point to the history of AD research in asserting the need for science to adopt a multi-faceted and flexible approach. They claim that non-human primate models were invaluable in the early stages of research

Box 14. The CANTAB test for diagnosing Alzheimer's disease

An important part of tackling dementia is the development of effective methods for early diagnosis in patients exhibiting mild cognitive impairment (MCI) and the ability to differentiate between different forms of dementia. The results of traditional clinical tests for memory impairment can be confounded by patients feeling depressed or anxious. Non-human primate work at the University of Cambridge led to the development of the CANTAB battery of tests, which includes a range of different cognitive assessments. One of these tests, called 'object-in-place memory', is highly selective in distinguishing those human patients showing MCI who go on to develop AD from those who do not.[246] These tests also distinguish memory impairments associated with AD from those related to depression.

241 Maclean CJ, Baker HF, Ridley RM & Mori H (2000) Naturally occurring and experimentally induced beta-amyloid depositis in the brains of marmosets (Callithrix jacchus). *Journal of Neural Transmission* **107**, 799-814.

242 Geula C, Nagykery N & Wu C K (2002) Amyloid-beta deposits in the cerebral cortex of the aged common marmoset (*Callithrix jacchus*): incidence and chemical composition. *Acta Neuropathol (Berl)* **103**, 48-58.

243 Gandy S *et al.* (2004) Alzheimer A beta vaccination of rhesus monkeys (Macaca mulatta). *Alzheimer Dis Assoc Disord* **18**, 44-6.

244 Lemere CA Beierschmitt A, Iglesias M, Spooner ET, Bloom JK, Leverone JF, Zheng JB, Seabrook TJ, Louard D, Li D, Selkoe DJ, Palmour RM, Ervin FR (2004) Alzheimer's disease abeta vaccine reduces central nervous system abeta levels in a non-human primate, the Caribbean vervet. *Am J Pathol* **165**, 283-97.

245 Taffe MA, Weed MR, Gutierrez T, Davis SA & Gold LH (2004) Modeling a task that is sensitive to dementia of the Alzheimer's type: individual differences in acquisition of a visuo-spatial paired-associate learning task in rhesus monkeys. *Behavioural Brain Research* **149**, 123-133.

246 Swainson R *et al.* (2001) Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks. *Dement Geriatr Cogn Disord* **12**, 265-280.

to further understanding of AD-associated pathology and disease progression. However, the non-human primate model does not permit the analysis of the underlying molecular pathology of the disease: this requires modern genetic approaches combining *in vitro* techniques with research using transgenic mice. It is these techniques that have provided the most significant advances in AD research in recent years. Nevertheless, researchers argued that testing new treatments will continue to require the use of non-human primates, to provide the necessary functional and physiological assessments.

6.8 Brain-Machine Interfaces

Several respondents to the call for evidence highlighted the potential for research involving non-human primates to inform future directions in artificial intelligence and the Brain-Machine Interface (BMI). BMI research investigates ways in which recorded brain signals, including single neuron activity, local field potentials and electroencephalogram (EEG) signals, can be used to directly control computers, robots and artificial limbs, i.e. the manipulation of devices by pure cognitive effort.

While some progress has been made with BMIs that operate non-invasively using scalp electrodes to record EEG signals, they have low bandwidth and high error rates. Far more effective are neuro-prosthetic interfaces that use signals derived from invasively recorded local field-potentials, or multiple single-unit recording electrodes, deep inside the brain. Using an array of fast signal-processing algorithms derived from studies in the motor cortex of non-human primates, these devices have the potential to translate the 'intention' of an action into the movements of a robot arm. BMIs are one of a number of other ways in which research with non-human primates can, for example, offer help in relation to spinal cord injury.[247]

One protocol developed at the Brain Mind Institute in Switzerland involves first training non-human primates to move a hand-held pole, while simultaneously recording unit activity in multiple cortical areas. By 'locking' the neural patterns to actual movements and studying the statistical predictability of different brain cell patterns, it is possible to train the BMI to predict the measured velocity of the pole. As this is achieved, control of the pole's movements can be gradually transferred from the animal's limb to the robot arm. Eventually, the movements of the pole are 'controlled' more by the animal's brain activity than by its hand: hand-movements no longer become necessary and the animal has only to 'think' the movement.[248]

This technology is at an early stage and some way from being clinically useful. Issues at stake in future developments include: identifying the appropriate input signals for such a device; the optimum number of sources or cells from which such recordings should be taken; whether single or multiple brain areas should be monitored and co-processed; and the role of neural plasticity in fine-tuning the performance of such devices.

Very recent work has developed the notion of 'implantable neuromotor prosthetics'. A report from the US[249] has described the first implantation of electrode arrays into the brain of a paralysed man, which allowed him to use his motor intentions to directly control devices including a computer mouse and television. Parallel work in non-human primates seeks to optimise a new software approach for extracting information about intended actions from the neural activity recorded from multiple single electrodes in the primate brain. Reports indicate that this can dramatically improve the potential speed with which an animal's 'intentions' can successfully control external devices.[250]

These are valuable steps forward towards clinical devices that could benefit patients with

247 Courtine G *et al.* (2006) Can non-human primate experiments expedite translation of potential reparative interventions after a spinal cord injury in humans? *Nature Medicine* in press.

248 Carmena JM *et al.* (2003) Learning to control a brain-machine interface for reaching and grasping by primates. *PLoS Biol* **1**, E42.

249 Hochberg LR, Serruya MD, Friehs GM, Mukand JA, Saleh M, Caplan AH, Branner A, Chen D, Penn RD, Donoghue JP (2006) Neuronal ensemble control of prosthetic devices by a human with tetraplegia. *Nature* **442**, 164-171.

250 Santhanam G, Ryu SI, Yu BM, Afshar A. & Shenoy KV (2006) A high-performance brain-computer interface. *Nature* **442**, 195-198.

motor neuron disease or spinal cord injury. Beyond the role of non-human primates, the use of such devices in human patients raises important ethical questions, which are already under discussion.[251]

6.9 Discussion

This section has reviewed the role of non-human primate and other animal work in achieving both specific and general aims in neuroscience research. While a great deal of research to understand the anatomy and structure of the brain has been carried out in post-mortem human tissue, an understanding of the connections between brain cells and regions requires investigation in living systems. In this respect, the major rationale for using non-human primates in neuroscience research is that they are the only group of animals with brain circuits and networks that are really similar to those of humans.

Both opponents of research using non-human primates and scientists who work in this field are understandably hopeful that non-invasive methodologies carried out in humans, particularly imaging techniques, will supplant the need for invasive experiments using non-human primates. In certain cases they already do; the important field of human cognitive neuroscience has developed in the last 15 years consisting of researchers who are interested in brain processing, but who seek to study it using only human subjects. However, we are convinced of the importance of the controlled, experimental animal model in determining cause and effect relationships between neurological structures/processes and function. Lesions produced experimentally can be controlled, both in anatomical extent and time relative to behavioural experience, making it is possible to conduct detailed assessments of the brain areas involved in different functions. In some cases, without such functional mapping of brain, it may be impossible to know where to look for disease processes that contribute to cognitive impairment in dementia and amnesia.

In this context, and as stressed elsewhere in this enquiry, we do not consider there to be a sharp distinction between research addressing fundamental scientific questions and work directed at more clinical problems. Important discoveries in neuroscience have been made by research that set out to define the basis for particular functions, without any knowledge of their potential relevance to human disease, yet have nonetheless turned out to deepen understanding of complex disease mechanisms. For instance, following the discovery of 'mirror neurons' in non-human primates, EEG studies in humans appear to show an absence of 'mirror' responses in the frontal cortex of patients with autism.

Clinical applications of non-human primate research have already had an impact on healthcare. A notable example drawn on this section is the development of Deep Brain Stimulation, first in non-human primates and then in patients, for the treatment of Parkinson's disease. Similarly, research into the pathogenesis of stroke has also been informed by animal research, although here the use of non-humans has been, until recently, less central to the research effort. The failure to translate potential stroke therapies developed using animal models into successful clinical treatments has led some to question the validity of animal-based approaches. However, a careful review suggests that small-scale studies on non-human primates may still be the only means of providing insurance against the failure of lengthy, expensive human trials of candidate stroke treatments. For this reason, the working group considered that the use of non-human primates in a limited number of future stroke experiments should not be ruled out; each case will have to be analysed individually, based on the lessons learnt from the extensive and often inconclusive results of work in this field.

251 Ackerman SJ (2006) *Hard Sciences, Hard Choices.* Dana Press, New York.

It is clear that a better understanding of the anatomical organisation of memory systems in the non-human primate brain is providing information that relates directly to principles of healthy ageing and to new therapies for Mild Cognitive Impairment and Alzheimer's disease. More than many other fields, this area of research has seen a more seamless integration between studies using humans, non-human primates, other animals and *in vitro* approaches, but effective therapeutics still seem to be some way off.

What emerges from this survey is that non-human primate work has been essential at some stages of research on a particular disease, and not others. Its relevance waxes and wanes, as the emphasis transfers between elucidating underlying pathophysiology, establishing molecular and genetic mechanisms of disease and identifying functional outcomes during pre-clinical testing of potential therapies. In all cases we emphasise that dynamic research requires a flexible approach to methodology; tractable problems are sometimes best addressed in humans, sometimes in non-human primates or other animals, and sometimes *in vitro* or using computational models. The use of non-human primates has provided unique insights into neuroscience research and yielded important scientific findings and clinically relevant developments that would not have emerged from other approaches. While the reduced use of laboratory non-human primates is therefore an important long-term goal, we consider that, to address a number of particularly complex questions in the neurosciences, their use cannot be entirely replaced in the foreseeable future.

7 Other research areas

7.1 Introduction

As well as the topics considered in the previous sections, evidence submitted to the working group covered a variety of other research fields, many of them related either directly or indirectly to ageing, reproduction and development. Clearly, it was not possible to analyse these large fields in detail and hence the working group focused on a few areas in which the case for a continuation of non-human primate research had been particularly emphasised.

7.2 Reproductive biology

Humans and non-human primates share many features of reproductive biology that are not present in other mammals, including mechanisms of gametogenesis, fertilisation, implantation of embryos into the uterus and maintenance of early pregnancy. Similarly, only human and some non-human primate females menstruate (a cycle of 28 days in macaques) and undergo the menopause. Hence, rodents and other non-primates have only limited usefulness as models of human reproductive physiology.

Respondents pointed out that the inaccessibility of the relevant tissues often prevents the study of reproductive processes in humans. For these reasons, researchers argued that data from non-human primates have been a vital component of advances in areas such as infertility, methods to support pregnancy, contraception and the treatment of miscarriage and premature labour, in addition to better understanding the development of blood vessels in the corpus luteum (a feature unique to primates that has important implications for tumour biology and cancer of the uterus). The working group's attention was also drawn to the role of Old World monkeys in reproductive studies and endocrine research in general, since New World monkeys have a generalised steroid resistance that is not recapitulated in humans.

In 2001, the US Institute of Medicine published a report that reviewed the history of women's health issues, discussed the current state of science in this field, and made recommendations for future research.[252,253] The report's summary statement calls for the increased development and utilisation of animal models, notably non-human primates, for the study of female reproductive disorders. In support of this argument it cites important contributions to our understanding of fertility, accelerated coronary artery disease after the menopause, and post-menopausal osteoporosis that have already resulted from work in non-human primate models. It also cites more recent studies on anovulatory infertility and endometriosis. With regard to future research, the report discusses the importance of understanding gender differences inthe occurrence of common diseases, particularly coronary artery disease and insulin-resistant diabetes.

While many of these suggested indications for further research using non-human primates are still speculative, we considered it important to analyse at least one area of past research and another of current work in this field in more depth.

7.2.1 Endometriosis

Endometriosis is a gynaecological disorder characterised by the presence of endometrial tissue, which normally lines the uterus, outside the uterus. It is often manifested by reduced fertility, painful menstrual periods and pain on sexual intercourse. Although pain relief can provide symptomatic help for the condition, very little can be done about the underlying cause or associated infertility. Since the condition can only be diagnosed with laproscopy, the prevalence of the disease is unknown, although it is estimated to affect 10% of all women. The difficulty of carrying out serial laproscopies also means that little is known about the natural history of the disease.[254] Progress towards the management of endometriosis has therefore been hampered

252 Wizemann TM & Pardue M-L. (2001) *Exploring the Biological Contributions to Human Health. Does Sex Matter*? pp. 1-27. National Academy Press, Washington DC.

253 Kaplan JR (2004) Modelling women's health with nonhuman primates and other animals. *Ilar J.* **45,** 83-88.

254 Story L & Kennedy S (2004) Animal studies in endometriosis: a review. *Ilar J.* **45,** 132-138.

by an incomplete understanding of its cause, primarily as a result of the extreme difficulty of studying the disease in women.

Epidemiological and genetic analyses in humans have provided increasing evidence that endometriosis has a strong genetic basis. For instance, twin studies have shown that over 50% of the variance in susceptibility can be attributed to genetic factors. A number of case-control studies have also shown that first-degree relatives of affected women have a 3-9 times increased risk of developing the disease compared with first-degree relatives of controls.[255]

Research into the genetic components of endometriosis will encounter similar problems to any work that attempts to identify susceptibility genes that may be affected by environmental components. First, several genes may be involved, each with a relatively small effect. Second, to obtain sufficient and robust linkage data (that is to identify the genes in affected families by attempting to establish their proximity to particular DNA markers), very large numbers of cases and controls will be required. The need for a surgical diagnosis of endometriosis means that patient groups are often highly selected relative to the general population in terms of their environment, and, possibly, their genetic backgrounds. This has important implications for the validity and generalisability of the resulting data. Such studies are further impaired by the difficulty of being certain that control patients are unaffected.

Unlike rodents, rhesus macaques can naturally develop endometriosis, with a pathology that is identical to the human condition: the lesions that develop are morphologically identical and occur at similar sites. Research in the US has shown a remarkably high prevalence of spontaneous endometriosis in macaques, with about 30% of female animals affected in some colonies. This may be due to the greater genetic homogeneity of such colonies, where the animals might possess unique or high frequency genetic variants for susceptibility to endometriosis. Despite this caveat, researchers argue that macaques represent an important model in which to study the genetics and epidemiology of endometriosis. A recent study confirmed the strong familial aggregation of endometriosis in rhesus macaques, concluding that this model could be used for the investigation of its heredity, the location of potential genetic susceptibility loci and the influence of environmental factors.[256]

Given that so little is known about the cause of endometriosis, and that inheritance seems to play such an important role in its occurrence, it seems reasonable to conclude that the discovery of susceptibility genes might throw considerable light on the underlying disease mechanisms and hence produce some more logical approaches to prevention and management. Currently, it appears that continuing research in macaques, integrating data with that from human studies, is the only really promising approach to a better understanding of this condition. In short, considering the medical importance of endometriosis, the potential value of the genetic approach to its better understanding, and the difficulties that will be encountered in analysing its inheritance further in human populations, there is a case for continuing work towards defining the genes involved in spontaneous endometriosis in non-human primates and for their use in testing potential therapies.

7.2.2 Other reproductive disorders

There are several other examples of studies of reproductive biology involving non-human primates.[257] They range in scope from research related directly to human disease, such as that on soy milk (see Box 15) and angiogenesis, to more basic questions that, although they do not have immediate relevance to a particular disease, are aimed at gaining further knowledge of normal function through which a variety of disease mechanisms could be better understood.

255 Zondervan KT, Cardon LR, Kennedy SH (2001) The genetic basis of endometriosis. *Curr Opin Obstet Gynecol.* **13**, 309-314.

256 Zondervan KT, Weeks DE, Colman R, Cardon LR, Hadfield R, Schleffler J, Trainor AG, Coe CL, Kemnitz JW & Kennedy SH (2004) Familial aggregation of endometriosis in a large pedigree of rhesus macaques. *Hum Reprod.* **19,** 448-455.

257 Kaplan JR (2004) Modeling women's health with nonhuman primates and other animals. *Ilar J.* **45,** 83-88.

Extensive angiogenesis, i.e. the development of blood vessels, takes place in the female reproductive tract, the ovary, uterus and placenta. UK researchers are carrying out studies to quantify the changes in maternal vasculature, cell proliferation and angiogenesis during early pregnancy in the marmoset endometrium. For instance, it has been shown that pregnancy is associated with increasing angiogenesis in the upper zone of the endometrium, which is significantly increased at 3 weeks. Understanding angiogenesis at the time of implantation and in early pregnancy is important because it is likely to be a key component in the effective establishment of implantation by the endometrium, the failure of which may be a cause of early miscarriage.[258]

Non-human primates are also currently being used in the investigation of factors that regulate angiogenesis and the development of compounds that can stimulate or inhibit their action, a field of great importance in the treatment of cancer, as well as in reproductive biology.[259] For example, studies involving macaques and marmosets have focussed on the inhibition of vascular endothelial growth factor (VEGF) to determine the effect on pituitary-ovarian function. Results show that lowering VEGF levels inhibits luteal angiogenesis and follicular angiogenesis. This exerts a potent and dose-dependent, but reversible, inhibitory effect on ovarian function. It is hoped that the development of effective inhibitors of angiogenesis may open new avenues for the treatment of reproductive disorders characterised by pathological angiogenesis, inflammation and increased vascular permeability, e.g. polycystic ovarian syndrome (PCOS), ovarian hyperstimulation syndrome (OHSS) and endometriosis.[260,261,262] The fact that angiogenesis is so active in the normal ovary and uterus suggests that unexplained infertility may also stem from abnormalities in blood vessel development.

7.3 Gender comparative studies relating to common human diseases

Many of the common killers of Western society, including cardiac disease, stroke, and type 2 (insulin-resistant) diabetes, show considerable sex differences, either in their frequency, severity, or complications. For example, in the case of stroke and coronary artery disease women enjoy an age-related epidemiological advantage relative to men. It has been suggested that this pattern of protection indicates that female reproductive

Box 15. The effects of soy milk on the developing male reproductive system

Marmosets have been used to determine whether infant feeding with soy formula milk, which contains high levels of plant oestrogens, poses any immediate or longer-term health risk to the developing testis and reproductive system of the male. An initial study found that testosterone levels were suppressed in animals fed with soy formula milk.[263] A longer term follow-up analysis of these animals indicated that infant feeding with soy formula milk had no gross reproductive effects in male marmosets, but that it does alter testis size and cell composition. The authors concluded that similar changes are likely to occur in adult men who were fed soy formula milk as infants.[264]

258 Rowe AJ, Wulff C & Fraser HM (2004) Angiogenesis and microvascular development in the marmoset (Callithrix jacchus) endometrium during early pregnancy. *Reproduction* **128,** 107-116.

259 For a review of recent progress in protein kinase inhibitors as anti-cancer agents see JS Sebolt-Leopold & JM English (2006) Mechanisms of drug inhibition of signalling molecules. *Nature* **441**, 457-462.

260 Fraser HM, Dickson SE, Lunn SF, Wulff C, Morris KD, Carroll VA, Bicknell R (2000) Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. *Endocrinology* **141**: 995-1000.

261 Taylor PD, Hillier SG, Fraser HM (2004) Effects of GnRH antagonist treatment on follicular development and angiogenesis in the primate ovary. *J Endocrinol.* **183**, 1-17.

262 Fraser HM, Wilson H, Rudge JS & Wiegand SJ (2005) Single injections of vascular endothelial growth factor trap block ovulation in the macaque and produce a prolonged, dose-related suppression of ovarian function. *J Clin Endocrinol Metab.* **90,** 1114-1122.

263 Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS, Walker M. (2002) Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. *Hum Reprod.* **17**, 1692-703.

264 Tan KA, Walker M, Morris K, Greig I, Mason JI, Sharpe RM. (2006) Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. *Hum Reprod.* **21**, 896-904.

hormones play a role in delaying the onset of these diseases. The report from the Institute of Medicine, cited earlier, offers an extensive discussion on the potential use of non-human primates for attempting to determine the mechanism for the difference in the manifestations of these diseases between males and females and, in particular, the way in which they may have a hormonal basis. The same argument is explored in the case of osteoporosis in post-menopausal women and work directed towards better understanding of the cessation of cyclical hormone exposure on the nervous system as the basis for cognitive decline and mood disorders, both of which are more prominent in post-menopausal women than age-matched men.[265]

A word of caution is necessary in considering some of these issues. As emphasised earlier, common diseases such as coronary artery disease, stroke, diabetes and psychiatric disorders are probably the result of a complex interaction between our environment, our genetic make-up and the ill-understood pathology of ageing. The different manifestations of these conditions between males and females, while of considerable interest, are only a small part of the total picture. Furthermore, there is no clear evidence that information obtained from studies in non-human primates will be relevant to these diseases in humans, particularly in view of their different environments, patterns of ageing, and many other factors. Hence, the justification for using non-human primates in work of this kind would have to be examined very carefully from the scientific viewpoint as well as the potential suffering to the animals involved. Currently, it is not clear whether work of this type in non-human primates would have a great deal to add to the major drive to the better understanding of these conditions in humans through the combined efforts of epidemiology, molecular and cell biology, and work directed at the pathophysiology of ageing in other model systems (see below). We urge particular caution in using non-human primates in these fields.

7.4 Fetal development and common disease in adult life

One of the most interesting and potentially important epidemiological observations over recent years is the association between low birth weight and the subsequent development of disease in later life, including high blood pressure, insulin-resistant diabetes, and cardiovascular disease.[266] Whether these associations are between fetal birth weight itself, or the pattern of early growth, or both, is not absolutely clear, but there seems little doubt that early patterns of development are associated with some of the most common diseases of developed countries.

These observations have raised the intriguing possibility that environmentally-induced genetic programming may occur during development, either due to patterns of fetal nutrition or to some form of endocrine response, for example the action of prenatal glucocorticoids.[267] Clearly, the concept that our genetic make-up may be modified by our intrauterine environments is extremely important and is the subject of a great deal of current research. This work makes use of a wide range of experimental subjects including humans, rodents, sheep, and, to a limited degree, non-human primates. Although these studies further underline the extreme complexity of the underlying causes of conditions such as hypertension, coronary artery disease, obesity and insulin-resistant diabetes, it is becoming clear that a better understanding of the intrauterine influences that increase the likelihood of their occurrence in mid-life holds the potential for a much greater understanding of how they might be avoided. A full understanding of these relationships may well require a detailed knowledge of how intrauterine influences work at the cellular and molecular levels. And since the central nervous system also comes under the influence of prenatal hormonal activity, such information may have even far more reaching implications for the understanding of human disease.[267]

265 Kaplan JR (2004) Modeling women's health with nonhuman primates and other animals. *Ilar J.* **45,** 83-88.

266 Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. (1993) Fetal nutrition and cardiovascular disease in adult life. *Lancet*. **341**:938-41.

267 Seckl JR (2004) Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol,* **151 Suppl 3,** U49-62.

Undoubtedly, the further exploration of the relationships between fetal development and disease in later life will require the application of whole animal models. While much will be achieved by research on rodents, as it is at the present time, the major differences in reproductive physiology between rodents and both non-human primates and humans, means that more definitive arguments for the limited use of non-human primates may become apparent. Given the important clues such research may provide for understanding the pathogenesis of some of our major killers, the potential role of non-human primates in this field will have to be examined carefully on a case-by-case basis in the future.

7.5 Ageing

The undoubted association between the biology of ageing and many chronic diseases has led to increasing interest in exploring model organisms for research into the mechanisms of the ageing process. In the past much of this work involved studies in rodents, but more recently attention has turned to the analysis of the genetic regulation of ageing using organisms such as *Drosophila* or *C. elegans*. A number of genes have now been identified in these organisms that, when mutated, have a significant effect on their lifespan. Information about the metabolic functions of the gene products involved is also gradually increasing.

A report from the National Institute on Ageing (part of the US National Institutes of Health) has made a strong plea for the increased use of non-human primates, notably rhesus macaques, for studying the mechanisms of ageing.[268] Rhesus macaques have a median lifespan of about 20 years, with a maximum span of 40 years. The Institute supports colonies of ageing macaques at 5 research centres in the US. Longitudinal studies have suggested that the patterns of diseases of ageing are remarkably similar to those of humans. Since it has long been known from studies in rodents that calorie restriction is associated with longevity, these centres are carrying out long-term studies of regimens of calorie restriction (at 30% below the level of control animals) in an attempt to discover the metabolic basis for this phenomenon. While underlining the expense, organisational complexity and requirements for such multi-centre interactive studies, the authors assert that the rhesus macaque is a genuine model for learning more about the mechanisms of ageing and their relationships with the chronic diseases of old age.

Since calorie restriction is one of the few factors that have been shown quite unequivocally to be associated with increased longevity, and it is undoubtedly easier to control other variables in these non-human primate studies compared with similar work in humans, the American case for this particular programme is scientifically well reasoned. However, since the effects of calorie restriction are conserved across species, it is not clear why it requires the use of primates to answer this important biological question.

Although the current trend in research into ageing involves genetic studies in invertebrate model organisms, it seems very likely that lessons learned from these systems will have to be studied in higher organisms if their true relevance is to be determined. Hence, like so many research fields that are in a state of rapid change, it is difficult to assess the place of non-human primate research into ageing at the present time. Given the relatively long lifespan of rhesus macaques, this system will always present problems for research workers in this field, but as more is learnt about ageing at the genetic and cellular level there may well be further questions that can only be answered through studies in non-human primates. Some aspects of ageing research have also been covered in section 6.

268 Roth GS, Mattison JA, Ottinger MA, Chachich ME, Lane MA & Ingram DK (2004) Aging in rhesus monkeys: relevance to human health interventions. *Science* **305,** 1423-1426.

7.6 Discussion

The central argument for the use of non-human primates in the study of reproductive biology and related fields is the much closer approximation of their reproductive processes to those of humans. While in the past, research on non-human primates has undoubtedly provided important information about the regulation of the menstrual cycle,[269] and while there appears to be a case for continuing work along these lines towards a better understanding of reproductive physiology, particularly in women, a major expansion of work of this type as suggested in the report of the Institute of Medicine, seems to be premature. While there may be a case for the use of non-human primates in a very carefully defined group of human reproductive disorders, endometriosis for example, the arguments for its expansion towards attempting to better define the differences between the manifestations of common diseases between the sexes are far less convincing.

There now seems little doubt that differences in fetal birthweight, and hence intrauterine programming, have an important influence on the development of several common diseases of adult life. The further exploration of this unexpected discovery will undoubtedly require studies of animal models, with some progress already being made in rodent systems. It is too early to predict whether strong cases for the use of non-human primates will be made; they could be required to help to define the relative roles of fetal nutrition, genetic factors and hormonal activity that are likely to underlie this important finding.

The potential role for non-human primates in research on the biology of ageing is also unclear at the present time. The case that has been made for calorie restriction studies is not entirely convincing, but there may be a valid role for non-human primate studies in the future to extend the information that is currently being derived from molecular and cellular studies in small model organisms to assess their relevance to humans.

269 Kaplan JR & Manuck SB (2004) Ovarian dysfunction, stress and disease: A primate continuum. *Ilar J.* **89,** 115.

8 Drug discovery and development

8.1 Introduction

Statistics for UK non-human primate use show that the vast majority occurs within the pharmaceutical industry (see section 3). Whilst the largest proportion is for toxicology and safety testing of medicines, the commercial sector also uses non-human primates during earlier phases of drug discovery and development. The remit of this study, i.e. the use of non-human primates in hypothesis driven research, dictates a focus on use in this early stage. Other reports, most notably the 2002 publications from the Animal Procedures Committee and the Boyd Group, provide in-depth examinations of the use of non-human primates in regulatory toxicology. However, the working group considers that, given the numbers of non-human primates involved, this area warrants some discussion in this report. Similarly, respondents to the call for evidence highlighted that the distinction between early stage drug discovery and regulatory toxicology is not always clear. As one witness put it: *'research to understand the action, distribution and metabolism of candidate medicines provides information on potential efficacy, but can also inform toxicology tests performed to meet regulatory requirements'.*

8.2 Regulations and regulatory agencies

The development and production of new medicines in the UK is regulated by the Medicines & Healthcare products Regulatory Agency (MHRA) and the European Medicines Agency (EMEA). The MHRA is an executive agency of the Department of Health and is responsible for ensuring that medicines and medical devices work, and that they are acceptably safe. EMEA is a decentralised body of the European Union (EU) that coordinates the evaluation and supervision of medicinal products throughout the member countries.

New medicines for distribution in the UK are required to meet the requirements of the Medicines Act 1968. Under the Act, a medicine must be demonstrated as safe (given the seriousness of the condition it is meant to treat), effective and of uniform high quality. Following harmonisation of the rules governing the licensing of medicines throughout the EU, the UK Medicines Act incorporates the relevant EU Directives and guidelines. All UK medicines are directly approved by the MHRA, which then issues a 'marketing authorisation' licence.

UK and EU regulations require that all new prescription medicines are studied in animals before they are tested in humans. Specifically, pharmaceutical product safety tests must be carried out in 2 species of mammal: one rodent and one non-rodent.[270] This requirement is made on the basis that known species variation makes reliance on tests in one species insufficient for approval of clinical trials in humans.[271] A variety of non-rodent species are used by UK companies, including the dog, pig, ferret, rabbit, Old World monkey and New World monkey. The selection of non-rodent species depends on a number of factors, involving regulatory, ethical, scientific, technical and practical criteria.[272]

8.3 Non-human primates and drug safety

Submissions suggested that regulations often appear to lag behind the state of scientific advance. It was suggested that regulators are inherently conservative and that this is part of the reason why retrospective analysis can sometimes show that the number of animals that could have been effective in addressing a toxicological question is actually fewer than was mandated.

While there is no mandatory requirement for the use of non-human primates in safety testing, the APC report acknowledged that *'the real, perceived or anticipated requirements of regulators are an extremely important factor*

270 See EU Council Directive 2001/83/EC and CPMP/ICH 286/95 adopted 1997 and modified 2000.

271 Animal Procedures Committee (2002) *The use of primates under the animals (scientific procedures) Act (1986).*

272 ABPI (2002) *'Non-Rodent Selection in Pharmaceutical Toxicology: A 'Points to Consider' document, developed by the ABPI in conjunction with the Home Office.'*

in species selection.' This issue was also raised in the 2002 Boyd Group report, which stated: *'Non-human primates may be selected out of caution of the risk that choosing another species may later prove unacceptable to the regulators, and thus result in costly delays in bringing a new medicine to market. The 'cost' of delays can be seen in terms of financial expense and time, the potential effects on human health of delaying the new medicine, and also the requirement to use more animals in another round of tests. Growing consumer concerns about safety, together with an increasingly litigious society provide their own pressures to test in non-human primates, on grounds of their similarities with humans'.*[273]

Some respondents to the call for evidence pointed out that non-human primates are often poor models for drug safety in humans. They highlighted the fact that single amino acid differences in protein sequences, small changes in gene expression levels or slight differences in biochemical pathways relating to drug action or distribution can have dramatic consequences in terms of pharmacological action. It was suggested that such differences are the reason why many potential drugs, some of which have been tested in non-human primates, still fail in clinical trials. Instances were also highlighted where a drug was shown to have adverse effects in non-human primate studies, but not in human trials. An example was given of the case of GDNF (Glial cell Derived Neurotrophic Factor) and Parkinson's disease to highlight the problems of transferring research results between non-human primates and humans. In this instance an overlap in trials of GDNF in non-human primates and humans showed that the side effects exhibited in non-human primates were not seen in the human patients; human side effects were avoided due to the greater volume of brain tissue to be penetrated by GDNF, compared with non-human primates.

It is undoubtedly the case that all animal models are limited in their predictability for humans. However, given the serious consequences of wrongly assessing drug safety, companies and regulators will choose to use an imperfect model, rather than no model at all. Companies also point out that most new drug candidates fail toxicology testing for 'off-target' effects, which cannot be predicted without a whole-animal system. Crucially, the limitations of any model, whether animal or non-animal, can only be elucidated through further research.

8.4 Second species selection in regulatory toxicology

Evidence submitted to the working group emphasised that the decision to use non-human primates in the toxicological testing of a new drug may be taken at different stages in the developmental process. For instance:

- It may be evident from the very early stages of product development that: other animal species (mouse, rat and dog) do not respond to the pharmacological action of the drug and that non-human primates are the only suitable species that respond pharmacologically; the molecular target of interest may not have a homologous target in non-primates (this is especially true of antibodies and other biological agents); or the drug target may be a component of a body system that shows distinct physiological differences between primates and non-primates.
- During pharmacokinetic studies it may become apparent that the ADME (Absorption, Distribution, Metabolism and Excretion) profile of the product in non-primate species is different from human and that non-human primates have an ADME profile that is closest to humans.
- A new drug may produce a specific toxic effect in one species. In this case a third species may be used, often a non-human primate, in order to determine if the effect observed was species-specific.

273 The Boyd Group (2002). *The use of non-human primates in research and testing.*

Ultimately, the decision to use non-human primates for regulatory toxicology studies rests with the company developing the product; companies do not need approval from a regulatory body to use non-human primates, although the MHRA, EMEA and Home Office (see Box 16) do advise on this issue. However, companies wishing to use non-human primates (or any animal) must obtain approval through their local Ethical Review Process (ERP) and obtain the necessary licences from the Home Office Animals (Scientific Procedures) Inspectorate.

The decision to use non-human primates can depend on the availability of animals in the country in which the toxicological research is being conducted, especially with regard to prolonged transport or lead-time for breeding and supply. The dog has generally been the default non-rodent species[274] and it is the view of the Home Office that the dog must be actively de-selected before non-human primate use can be authorised.[275] Evidence to the working group suggested that pharmaceutical companies are attempting to shift away from the use of Old World monkeys towards the use of New World monkeys, primarily marmosets, although this claim is not reflected in the statistics (section 3).

A 2002 report from the ABPI highlighted some of the complexities and conflicts involved in non-rodent species selection:

- The conflict between the requirements of product safety legislation, which implies that species selection should be based on the similarity to humans (leading to a tendency to use 'higher' species) and that of animal protection legislation, which requires that 'lower' species are used.
- It may be quicker and may require fewer animals to default to a well-characterised species (such as a non-human primate), rather than use a 'lower', but less familiar, species.
- In the early stages of developing a drug, the amount of the new drug that can be made is often very small. The size of the animal becomes important, as smaller animals require a reduced amount of the drug to test for safety. An example of the ethical issues that could arise is the use of a 400g marmoset monkey compared to a 15kg dog.

8.4.1 Development of biologicals

Biologicals are a class of pharmaceutical drug that includes, amongst others, monoclonal antibodies (MAbs), antibody related products, and therapeutic proteins (such as soluble cytokines or chemokine receptors). Interest in the medical value of biological drugs has increased significantly in recent years; some estimates suggest that over 160 biological drugs are in clinical use with 500 more in development. Biological drugs have a number of characteristics that impact on requirements for the use of non-human primates in research and testing. This area is being actively considered by the NC3Rs and ABPI as part of their initiative into the use of non-human primates in drug discovery and development (see 8.5.1).[276]

Box 16. Home Office guidance on regulatory toxicology

A 2001 Home Office Guidance note, 'The Conduct of Regulatory Toxicology and Safety Evaluation Studies', states general principles for the design and conduct of such studies:

- The cost/benefit assessment performed under the 1986 Act assumes, in the case of regulatory toxicology and safety testing, that the principal benefit is the facilitation of sound regulatory decisions, rather than the utility or profitability of the end product.
- Applicants should not propose 'over-testing' or 'check-listing' approaches.
- Applicants must demonstrate an awareness of the scope and limitations of the available animal-based tests and a knowledge of possible alternative methods.

274 Except in cases where it is considered unsuitable, e.g. testing of non-steroidal-anti-inflammatories (NSAIDs) and drug vehicles such as cremaphor.

275 Animal Procedures Committee (2002) *The use of primates under the animals (scientific procedures) Act (1986).*

276 A workshop was held in March 2006 to discuss opportunities to replace and reduce non-human primate use in the research and development of biological drugs. The report of this workshop, made available to the working group in May 2006, provides a useful introduction to biologicals and biotechnology-derived products. It also discusses factors influencing the use non-human primates in preclinical testing of MAbs and identifies opportunities for alternatives.

As described earlier, factors such as cross-reactivity, immunogeneticity and pharmacokinetics will influence second species choice with regard to MAbs. MAbs are highly specific for their target molecule and accurate prediction of 'on-target' human responses to a particular MAb requires testing in a species in which it cross-reacts. Submissions to the working group stressed that non-human primates are frequently the only species that cross-react with humanised MAbs. An example was given of a fully human MAb that blocks recruitment and tissue extravasation of activated lymphocytes, which was found to bind human and macaque receptors only.

Species choice is usually determined following cell-based tests on binding affinity *in vitro*, followed by confirmation of MAb activity *in vivo*. Researchers emphasised that a lack of MAb binding and activity in non-primate models precludes an assessment of efficacy, safety, toxicology and pharmacokinetics in a non-primate species. The NC3Rs/ABPI paper notes that cynomolgus macaques were used to test the majority of MAbs currently on the market and are viewed by the regulators as the most relevant test species.

While MAbs are generally viewed as being safer than conventional small molecule compounds, severe toxicities can occur, including anaphylactic shock[277] and cytokine release syndrome.[278] The tragic events that occurred during the phase I clinical trial of the MAb TGN1412 in March 2006 were widely reported and have been the subject of several editorials, commentaries and papers. An Expert Scientific Group (ESG) has also been convened by the UK Secretary of State for Health to address issues raised by the trial, which published an interim report in June 2006.[279]

Commentators agree that the case of TGN1412 illustrates the difficulty of testing therapies designed to display a high degree of human specificity. In this respect, it has been noted that cell- and tissue-based approaches confer the significant advantage of using human material and avoiding the need for inter-species extrapolation.[280] However, the severe limitations of existing *in vitro* models of the human immune system are also acknowledged, particularly the problems of modelling the potentially ubiquitous effects of drugs on immune cell activity, mapping the complex interplay between biological systems of the immune system and providing information that can be interpreted to account for potential in vivo effects.[280] In evidence to the working group, researchers joined with calls for greater attention to be given to improving *in vitro* tests for biologicals. Nevertheless, the need for continued animal, and in some cases non-human primate, research and testing is widely recognised. The interim report of the ESG states that:

- *'Animal studies taking due regard of the three 'Rs', (replacement, reduction and refinement of animal testing) remain necessary for many aspects of preclinical development of novel agents including testing of 'off-target' and 'on-target' toxicity and understanding the fundamental biology relevant to a new medicine and its target molecules in the human.'*
- *'In general, agents aimed very specifically at human molecular targets may show much reduced activity in other species such as in mice, rats and rabbits, but have some activity in non-human primates where molecular structures are closer to those in humans.'*

There may be several reasons behind differences in human and non-human primate responses to MAbs. For instance, recent research suggests that the loss of Siglec (Sialic acid-recognising Ig-superfamily lectins) expression on T lymphocytes during human evolution may contribute to the intrinsic hyper-reactivity of human T cells.[281] Nevertheless,

277 Anaphylaxis occurs when drugs form immune complexes with pre-existing antibodies, causing massive activation of the complement cascade. The sudden release of vasoactive complement factors can cause clinical shock.

278 In cytokine release syndrome, Abs binding to cell surface molecules cause excessive release of cytokines by the targeted cells, in some cases inducing a life-threatening shock reaction.

279 Expert Scientific Group on phase one clinical trials. Interim Report July 2006. http://www.dh.gov.uk/assetRoot/04/13/75/69/04137569.pdf

280 Bhogal N, Combes R (2006) An update on TGN1412. *Altern Lab Anim*. **34**, 351-6.

some MAbs have been shown to cause significant toxicity in macaques, a side effect not seen in mice, probably because of the different expression of MHC class II molecules between rodents and primates. Studies into monoclonal anti-Ia antibody therapy in rodents and macaques also showed toxicity in the latter, but not the former.[282] The overall message appears to be that non-human primate studies are an important step in testing toxicity of potential MAb therapies. However, a 'positive' non-human primate result, particularly with regard to therapies designed to stimulate T cell responses, should only be taken into humans with caution. Like the ESG report, we stress that decisions relating to appropriate approaches during pre-clinical development and safety assessment must be based on robust scientific analysis and justified on a case-by-case basis. In this respect we support calls in both the ESG and NC3Rs/ABPI reports for improved accessibility to information on pre-clinical studies and phase I trials.

8.5 Non-human primates, regulatory toxicology and the 3Rs

There have been strong calls for improvements in how the 3Rs principle is applied to the use of non-human primates in regulatory toxicology and both the APC and Boyd Group have published reports making a number of recommendations in this regard. The APC report, 'The use of primates under the Animals (Scientific Procedures) Act (1986): analysis of current trends with particular reference to regulatory toxicology' was published in December 2002. It made 14 recommendations under the categories of 'taking the issues forward', 'development of alternatives', 'species selection and associated issues', 'validity and necessity', and 'regulatory toxicology and the A(SP)A; all with the overall aim of reducing primate use. Under the first category of 'taking the issues forward', the report recommended that a stakeholder's forum be convened to address the issues and questions raised in the report and to review its recommendations. This forum was convened by the Home Office in January 2004, and a report of the event was published in March 2005.[283] The government's formal response to the APC's report was published in June 2006 and draws heavily on the discussions recorded at the stakeholder's forum. Areas discussed in the APC report and subsequent government response, and on which the working group also received evidence, are discussed below.

8.5.1 Priorities and harmonisation

The APC report stated that: *'We believe that the development and implementation of non-animal alternatives to replace the use of non-human primates must be accepted within industry and the international regulatory arena as a high priority goal, which requires immediate and dedicated attention. To achieve this goal, involving the pharmaceutical industry (ABPI, EFPIA and other), regulatory bodies (EMEA, USFDA and Japanese Ministry of Health and Welfare and scientific societies (e.g. the British Toxicology Society and the Society of Toxicology). The International Conference on Harmonisation should adopt a co-ordinating role in the development of this strategy '.*

With regard to the first part of this recommendation, the Government's response noted concern expressed at the stakeholder's meeting that the current focus and momentum on reduction and refinement could be lost if replacement is seen as the sole goal. It also noted that, since publication of the APC report, the NC3Rs was established to provide additional focus for the identification and development of alternatives to animal research.

The working group consider the recent collaboration between the NC3Rs and Association of the British Pharmaceutical Industry (ABPI) to be a particularly timely and welcome development. Through this collaboration, a strategy has been developed *'to review the scientific rationale for the use of non-human primates in drug discovery and*

281 Nguyen D.H. et al (2006) Loss of Siglec expression on T lymphocytes during human evolution. *PNAS* **103**, 7765-7770.

282 McDevitt HO *et al.* (1987) Monoclonal anti-Ia antibody therapy in animal models of autoimmune disease *Ciba Found Symposium* **129**, 184-93.

283 Report by the Animal Procedures Committee on the use of non-human primates under the Animals (Scientific Procedures) Act 1986. Note of Home Office primates stakeholders forum held on 9 January 2004.

development, with the aim of highlighting opportunities and challenges to replacing and reducing primate use in the pharmaceutical industry'.[284] Four main areas for investigation have been identified within this strategy, including toxicology, pharmacokinetics (PK), drug dependency and biologicals (see 8.3.1). Outputs from this initiative will be published in 2007.

The second part of the APC's recommendation concerns effective joint working and collaboration between various stakeholders. Submissions to the working group complained that 'joined-up' approaches are rarely adopted between regulatory agencies, such that a refinement, reduction or replacement in an animal protocol accepted in one jurisdiction does not necessarily apply in the others. Harmonising regulatory toxicology requirements between various regulatory agencies is clearly a complex matter. However, we consider this an area requiring further attention and we hope that UK efforts will be extended to Europe and beyond.

8.5.2 Generic licenses

A great deal of regulatory toxicology comprises standard repetitive protocols and the Home Office licensing policy has therefore been to award what have been called 'generic' project licences to companies carrying out this work. Such licences may include a number of different protocols and procedures. While the severity limits and species used for each protocol will be defined, the actual substance to be tested will not be specified, other than in generic terms. In its 2002 report, the APC stated that it found this system to be unsatisfactory and expressed concern about whether it allows for the *'adequate assessment of harms, benefits and justification for non-human primate use, and for monitoring non-human primate use'.* It recommended that local ERP processes should explicitly review the justification for using non-human primates in all types of procedures for each substance used.

In its response to this recommendation, the government expressed its understanding that the APC has since accepted that the granting of 'generic' licences, within the meaning intended in the report, is not a practice adopted by the Home Office. Attendees at the stakeholder's forum expressed a view that the Home Office already places a suitable framework of controls and restraints on 'generic' licenses, which permit cost/benefit assessments and the fulfilment of legislative requirements at a study-by-study level. It was stated that the Home Office might, for instance, require that ERP approval is obtained for each study, although it is unclear how often this is the case. Evidence to the working group also reflected questions raised in the 2002 Boyd Group report on whether (and how) local ERPs could be expected to distinguish the benefits of different pharmaceuticals so that these can be weighed against the costs to animals of the tests.

8.5.3 Microdosing

The APC report recommended that the use of *'highly sensitive analytical methods to provide human pharmacokinetic data should be further developed. Early ultra-low dose studies in human volunteer [known as 'microdosing'] should be encouraged'.* Developments in microdosing have arisen through improved detection techniques, in which nano- and pico-molar concentrations of drug safety biomarkers can be detected in human blood, tissues and urine. These techniques are extremely promising and have the potential to filter drug candidate selection, leading to a reduction in the number of animals used in testing each compound. However, the government's response highlighted the ongoing debate about the ethical and technical acceptability of this approach; at present, the availability of toxicity data is considered necessary before human studies can begin. Microdosing is discussed further in section 9.4.2.

8.5.4 Re-use of non-human primates

The APC and others have called for further examination of the opportunities for re-use of

284 http://www.nc3rs.org.uk/primatesabpi.

non-human primates for regulatory toxicology testing as a means of reducing the numbers used. Re-use of animals raises complex issues around the balance between reduction and refinement. On the one hand, there is little disagreement that reduction in overall numbers of non-human primates is desirable and that some refinement may occur because animals that are re-used would be accustomed to procedures. However, such re-use has the potential to increase the overall suffering of individual animals. Further discussion on re-use can be found in section 10.5.6.

8.6 Discussion

Pharmaceutical companies emphasise that the complex regulatory environment, public perceptions, and sheer expense of undertaking animal research are natural incentives for industry to develop alternative methodologies. There is no doubt that in recent years commercial organisations have adopted an entirely new approach to drug discovery and development, using advanced computational technologies that complement the development of animal 'alternatives' (see section 9).

Contemporary drug discovery and development begins with the identification and validation of large numbers of candidate drug targets. High throughput screening then allows the most promising compounds to be selected for further biochemical and cell-based analysis, before evaluation in animals and eventually in humans. The initial stages of target identification and screening have changed dramatically in recent years: where in the past chemists could produce and screen between 50 and 100 new compounds a year, new robotic techniques and computational power can increase this to up to a million a week.[285]

High throughput screening, bioinformatics and cell-based assays relate to increasing productivity in early stage development. These advances often replace the use of 'lower' animals, but do not necessarily apply to the use of non-human primates. Similarly, while these technologies reduce the proportion of non-efficacious drug candidates that reach the animal testing stage, they may also increase the number of overall promising candidates that are taken to animals for further development (although this is likely to increase the number of rodents, rather than non-human primates). Overall, it appears that these developments have had a zero net effect on animal use: whereas R&D spending by pharmaceutical companies has almost doubled in the last 10 years, the amount of animal research carried out has remained relatively constant (see figure 3.5).

Beyond the early development stage, novel drugs must be tested in model systems for both efficacy and safety. Given the vast complexity and variability of biological systems, it is not surprising that there are sometimes problems in extrapolating data from model systems to humans. However, we emphasise that these problems are not confined to animal studies, but are also encountered with *in vitro* and even human studies. In short, none of these methods can faithfully reproduce all the features that characterise the wide diversity of genetic and biological processes that occur in a population of humans. The intrinsic limitations of *in vitro* systems in modelling the dynamic and complex processes between cells, tissues and organs means that, in most cases, they will need to be supplemented with animal testing. However, there does appear to be scope for enhancing the role of such systems in testing novel therapies. We support calls for greater efforts in this regard.

Given the numbers involved, any reduction in the use of non-human primates for regulatory toxicology purposes would constitute a significant reduction in overall numbers. However, in very crude terms, there is a proportional relationship between more testing and increased drug safety. For this reason we consider that a blanket removal of non-human primate testing would increase associated

285 ABPI briefing document *'The development of medicines'*

drug risk. Instead, the selection of non-human primates as the mandated 'non-rodent' species must be judged on a case-by-case basis. This selection is clearly based on a complex set of factors. However, we do not consider the lack of availability of other species, e.g. the ferret and mini-pig, to be sufficient justification. As with all fields addressed in this report, the use of non-human primates must be justified on a scientific basis, with due regard to welfare considerations. We also remain concerned about the provision of generic licences and query how local Ethical Review Panels can judge the validity of the use of non-human primates in all cases.

Our short discussion of the emerging field of biological drugs highlights the need for improvements in the availability and accessibility of pre-clinical information, particularly from non-human primate work, before human trials are commenced.

We consider that steps towards making toxicological studies involving non-human primates publicly available would be very valuable in this respect. The publication of such information would also serve a beneficial purpose in preventing any repetition of studies.

We support all efforts for harmonisation in the regulatory requirements between different jurisdictions. Initiatives such as that of the NC3Rs and ABPI have a very significant role in providing a platform for the various regulatory authorities, scientists, pharmaceutical companies and animal welfarists to jointly identify areas where the use of non-human primates might be reduced or replaced. The working group strongly endorses the approach taken by the NC3Rs; it is only through convening groups with appropriate expertise and representation that the issues can be effectively taken forward and real progress made.

9 Alternatives to the use of non-human primates for medical research and toxicology

9.1 Introduction

Remarkable advances in recent years in the fields of molecular and cell biology and the development of non-invasive approaches to studying human physiology in health and disease have raised the expectation that it should be possible to gradually reduce the requirement for non-human primates in medical research and toxicology. In this section we briefly summarise current progress, referring to examples given in other sections. We discuss some of the problems that remain to be solved and, in particular, try to assess the overall extent of the work that is being carried out towards reducing the requirement for non-human primates. There are a number of organisations, both within the UK and internationally, that promote the use of alternatives to animal use in research (see Box 17).

As part of the submissions of written and oral evidence, the working group received a large amount of information on alternatives to the use of non-human primates in research. Examples included the use of 'lower' animal species, *in vitro* methods, *in silico* techniques and human studies. The replacement of invasive non-human primate methods with non-invasive techniques was also discussed.

Many submissions pointed out that humans themselves provide the best model for studying human processes and diseases, but there was little disagreement that many experimental approaches cannot be performed in human subjects. Respondents emphasised that all other experimental models have intrinsic limitations in their applicability to the human condition. All models, both animal and non-animal, are in some a way a compromise between providing an experimental tool that can be controlled and manipulated to address a particular hypothesis and providing data that can ultimately be extrapolated to humans.

The central question is whether alternative techniques can provide, either singly or in combination, data of comparable validity and applicability to those derived from non-human primate studies. Non-human primate research has a relatively long scientific history, over which a considerable body of data has been accumulated. In many instances, the comparatively new techniques described below are at an immediate disadvantage in terms of the ease with which data can be interpreted and applied. Respondents pointed out that this will only be rectified by focussing more research activity on these alternatives. Some respondents criticised researchers for not taking a broader view of whether there might be an alternative approach to a scientific problem (pointing out that this is different from a replacement alternative to a particular experiment, such as cell culture). Nevertheless, many respondents drew attention to the short and longer-term problems and limitations of alternative techniques. It was asserted that these problems mean that, in the near future, alternative techniques might be able to reduce the number of non-human primates used or refine procedures, but will not completely replace them.

9.2 Molecular and cell biology approaches

In addition to the human genome, the complete sequence of the DNA that constitutes the genomes of many pathogens, worms, insects, and animals (including the chimpanzee) have now been obtained, or are in the process of being completed. A start has been made at determining the function of the thousands of proteins, the structure of which is regulated by individual genes, and how they interact with each another to underlie the characteristics of living things. Although it may take many years to understand fully these highly complex biological systems, there seems little doubt that discoveries along

Box 17. Organisations that support the development and implementation of alternatives

The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established in 2004. This centre grants funding for 3Rs-related research, produces and disseminates information about the 3Rs and aims to provide a forum for industry, academia, regulatory agencies and the animal protection community to work together to progress and implement the 3Rs. The use of non-human primates in research and testing is a matter of particular concern to the NC3Rs. It has developed a broad programme of work that is designed to improve non-human primate welfare and lead to greater application of the 3Rs to primate use. This includes funding research, convening and co-ordinating working groups, symposia and workshops, and producing training material. A recent initiative has addressed the use of non-human primates in the development and testing of biological drugs (see section 8.4.1).

In addition to FRAME and the Dr Hadwen Trust, many of the main biomedical and biological research funding bodies have specific funds available for 3Rs research (e.g. Research Councils such as MRC and BBSRC, and charities such as the Wellcome Trust).

The UK Inter-Departmental Group on the 3Rs is led by the Home Office and includes members from the Department of Health, the Department of the Environment, Food and Rural Affairs, the Department of Trade and Industry, the Office of Science and Technology, the Food Standards Agency, the UK Health and Safety Executive, the Medicines and Healthcare Products Regulatory Agency and others. The terms of reference for this group are *'to improve the application of the 3R's and promote research into alternatives, reducing the need for toxicity testing through better sharing of data and encouraging the validation and acceptance of alternatives.'* According to the Home Office this group is also pursuing the issue of species selection and the justification for the use of primates with the relevant regulatory authorities.[286]

The European Centre for the Validation of Alternative Methods (ECVAM) was established in 1991.[287] Its role is to: coordinate the validation of alternative test methods at the EU level; act as a focal point for the exchange of information on the development of alternative test methods; and maintain a database on alternative procedures. Its central role to date has been to validate alternative methods for toxicity studies and to report the results of validation studies in chemical and vaccine testing. In particular, ECVAM works in close cooperation with the European Directorate for the Quality of Medicines on issues related to vaccine testing. At its Workshop in 1994, entitled *Alternatives to Animal Testing in the Quality Control of Immunologicals: Current Status and Future Prospects*, it recommended that animal tests for abnormal toxicity, intended to detect toxic contaminants, should be deleted from a number of vaccine programmes. This was done in 1997, and is estimated to have reduced the number of animals used in Europe by 35,000 per year. A later ECVAM report, *Validation of Alternative Methods for the Potency Testing of Vaccines,* highlighted that vaccine quality control is essentially a means of ensuring consistency of production, and that in vitro tests, introduced alongside established animal tests, could gradually replace the latter to a large extent without the need for extensive, independent validation studies. The early involvement of regulatory control authorities in the validation of these new technologies was identified as a key factor. These issues are discussed further by Huggins and in a report from the Working group of the Associate Parliamentary Group for Animal Welfare.[288,289]

286 Home Office (2006) *Report by the Animal Procedures Committee on the Statistics of Scientific Procedures on Living Animals, Government Response.*

287 http://ecvam.jrc.it/index.htm

Recently a group called the European Partnership on Alternative Approaches to Animal Testing (EPAA) has been formed, which is a consortium of seven European industry trade associations, the European Commission and eight international enterprises.[290] The partners have committed to pooling knowledge, research and resources to accelerate the development, validation and acceptance of alternative approaches over an initial five-year period. The Partnership's work will focus on mapping existing research, developing new alternative approaches and strategies, and promoting communication and education through an annual conference and the publication of reports. Their goal is to ensure that every opportunity is taken to refine, reduce and replace the use of animals in safety assessment tests.

the way will have a major impact on our understanding of human biology and disease processes.

The post-genomic era is characterised by a remarkable and rapidly developing technology for exploring gene action in *in vitro* systems. Using gene chip and related technology it is now possible to explore the expression of genes in a wide variety of tissues, either at the RNA or protein level. Already these approaches are providing an extraordinary breadth of information about gene function and its coordination. It seems very likely that using these increasingly sophisticated *in vitro* techniques it will be possible to learn a great deal about biological function in health and disease.

It has already been possible to isolate the defective genes in the case of many human genetic diseases and a start has been made towards understanding the gene-environment interactions that are responsible for important diseases such as heart disease, diabetes, stroke, and Parkinson's disease. Rapid progress is also being made in the field of comparative genomics and it is becoming apparent that many genes critical for particular biological functions have been highly conserved throughout evolution. This is allowing the analysis of certain processes, such as the biology of ageing and abnormal gene function, in *Drosophila*, *Caenorhabditis elegans*, and rodents (see for example[291,292]).

Another area of rapid development in molecular and cell biology is the increasing ability to manipulate the genetic make-up of fruit flies, worms, and rodents. In the case of the mouse for example, by using embryonic stem (ES) cell or related technology it is possible to knock out or insert genes and hence produce transgenic models of human disease. Similarly, it is possible to manipulate the genetic make-up of human or other cells grown in culture.

There has been another major change in our approach to some important diseases as the result of the studies in the molecular era; it is now clear that at least some disorders, particularly different forms of cancer, arise from genetic changes acquired during our lifetime, which are then passed on to the progeny of affected cells. There is increasing evidence that the ageing process may also involve acquired changes in our genetic make-up.

The examples below illustrate the potential for these approaches to provide the basis for a reduction in the requirement for non-human primates in medical research and toxicology. However, the use of transgenic animals raises significant ethical issues,[293] not least the acceptability of replacing one type of animal research involving non-human primates with work using rodents.

9.2.1 A mouse model for the study of systemic HIV/AIDS infection

Research into HIV/AIDS has recently benefited from the development of a mouse model of HIV infection. Genetic engineering approaches were used to target the HIV-1 virus to mice

288 Huggins J (2003) Alternatives to animal testing: research, trends, validation, regulatory acceptance. *Altex,* **20,** 3-61.
289 The Associate Parliamentary Group for Animal Welfare (2005). *The use of animals in vaccine testing for humans.*
290 See http://ec.europa.eu/enterprise/epaa/index_en.htm
291 Bier E (2005) Drosophila, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet.* **6**, 9-23.
292 Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J (2006) Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature* **441**, 1157-61.
293 Nuffield Council on Bioethics (2005) *The ethics of research involving animals*.

by replacing part of the coding region of a particular gene in HIV-1 with a gene from a murine leukaemia virus (a virus that affects only rodents). The resulting chimaeric virus construct was found to infect murine white blood cells (lymphocytes) but not human lymphocytes. Adult immunocompetent mice were shown to be susceptible to infection by a single inoculation. Further studies of this genetically engineered virus have shown that it produces an illness and immune response that may provide an extremely valuable model of HIV infection, allowing convenient and safe investigation of new forms of therapy, vaccines, and potentially, the mechanisms of the pathology of the disease.[294]

9.2.2 A transgenic-mouse neurovirulence test for oral poliovirus vaccine

Oral Polio Vaccine (OPV) is a mixture of 3 types of poliovirus that are produced and tested separately. The virus used in the vaccine is not killed, but attenuated (i.e. weakened or rendered non-virulent), such that it elicits an immune response without causing disease. Each batch of these 3 types of OPV is tested separately, to ensure that the virus has been properly attenuated and has not reverted to a strain that could result in very serious side effects. Hitherto the safety of OPV has had to be tested in macaques, because only they share receptors with humans for all three types of poliovirus (the agent responsible for poliomyelitis). Over the last 15 years major efforts have been made to develop a transgenic mouse model of poliomyelitis.[295]

In 1999 two groups of scientists engineered a transgenic mouse that was susceptible to poliovirus. After innumerable studies it was finally agreed that a newly developed mouse strain, designated TgPVR21, provided a reliable test for the neurovirulence of OPV that was sensitive, reproducible and compared favourably in all respects to the macaque test. In October 1999, the WHO Expert Committee on Biological Standardization approved this test as an acceptable alternative to the macaque model, at least for testing OPV type 3.

There are now three strains of transgenic mice, each expressing one of the three human polio receptors. In 2003 the WHO Expert Committee approved the mouse virulence test as an alternative to the macaque test for all three OPV types and devised standard implementation processes for laboratories that wished to use it. This study involved extensive collaboration between WHO and institutes in Asia, Europe and the USA and represents the first successful introduction of transgenic animals into the control of biologicals.[296] It was recommended by the regulators that these mice should be used for toxicity testing from January 1st 2006.

Due to the ever-present fear of the disastrous consequences of even one faulty batch of polio vaccine, companies and regulators may still wish to run mouse studies in parallel with a limited number of non-human primate studies. This is particularly important since there are practical problems due to the relatively small size of the mouse and difficulties of the injection technique, which requires very precise positioning of the inoculum into the mouse spinal cord. The UK National Institute for Standards and Biological Control has therefore elected to maintain expertise in evaluation of vaccines in macaques in order both to maintain competence in this approach and to monitor the mouse test.

An entirely non-animal method for testing polio vaccine called MAPREC has also been developed, which detects and quantifies mutations that can cause polio vaccine virus to regain virulence.[297] This test has been accepted by the WHO since 1999 as a method of ensuring consistency of polio vaccine production, but has not been accepted as a full replacement to using non-human primates. In 2005, a WHO report stated that the MAPREC assay had been fully validated and is in routine use for type 3 poliovirus and that studies for

294 Potash MJ, Chao W, Bentsman G, Paris N, Saini M, Nitkiewicz J, Belem P, Sharer L, Brooks AI & Volsky DJ (2005) A mouse model for study of systemic HIV-1 infection, antiviral immune responses, and neuroinvasiveness. *PNAS* **102,** 3760-3765.

295 Levenbook I, Dragunsky E & Pervikov Y (2001) Development of a transgenic mouse neurovirulence test for oral poliovirus vaccine: international collaborative study 1993-1999. *Vaccine* **19,** 163-166.

296 Dragunsky E, Nomura T, Karpinski K, Furesz J, Wood DJ, Pervikov Y, Abe S, Kurata T, Vanloocke O, Karganova G, Taffs R, Heath A, Ivshina A, Levenbook I (2003) Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study. *Bull World Health Organ.* **81**, 251-60.

types 1 and 2 are in progress.[298] While some respondents argued that the MAPREC test could completely replace the macaque test, the WHO report emphasised that it does not assure safety, but can provide good evidence for consistency in manufacture.

While this progress is welcome, commentators have expressed frustration at the slow acceptance and implementation of alternative testing methods, partly due to the need for coordination between a range of bodies with different jurisdictions. These issues are further complicated by the fact that the use of live polio vaccine may soon be unnecessary because of the success in containing poliomyelitis. Indeed, in most countries the live vaccine is already being replaced by an inactivated vaccine, which does not require the same level of batch testing.

9.2.3 Transgenic mice for testing therapeutic monoclonal antibodies

Transgenic mouse models are being developed to reduce the requirement for non-human primates in testing efficacy and safety in potential therapeutic monoclonal antibodies (MAbs). For example, a MAb directed against CD4 subsets of lymphocytes for the treatment of asthma and rheumatoid arthritis has been tested extensively in a transgenic mouse model, with promising results. Several similar systems are under evaluation.[299]

9.2.4 Reporter mice: a new approach to analysis of drug action

Reporter genes encode for an easily detectable protein that can be inserted into cells. They have been used for many years to study the regulatory regions of individual genes or to define proteins encoded in other parts of the genome that help to regulate particular gene function.

It is now believed that the introduction of reporter genes into the mouse genome may provide an opportunity to investigate the activity of regulatory regions in living organisms, particularly if the action of the gene can be quantified or examined by sensitive imaging technology.[300] A promising approach to this new field has entailed a detailed analysis of the ERE-Luc model as a tool to study the activity of drugs and toxic compounds. ERE is the gene for the oestrogen receptor, while Luc (luciferase), acts as an easily identifiable 'reporter'. This model seems to fit many of the requirements for drug-toxicology studies and has already proved to be of use in the analysis of potentially toxic compounds in pregnancy and breast-feeding. Several similar models are in early stages of development.

A major potential advantage of reporter mice is that they may be able to provide measurable end points for the evaluation of drug activity in all the tissues of living animals. However, a number of difficulties will have to be overcome before they are ready for use in routine toxicological studies. For instance, there are still problems in generating mice with generalised expression reporters and it is not clear whether the efficiency of systems needed for this purpose will remain stable in different mouse strains. More research is also required to address the generation of the appropriate viral vectors or delivery systems that are needed for the analysis of the activity of reporters. However, given the potential of this system there is a genuine possibility that it will provide a useful tool for evaluating drug activity at the cellular level.

9.3 Computer modelling and systems biology: *in silico* approaches

The major goal of the post-genomic era will be to understand the functions of genes and their many protein products and how these activities are integrated within cells, organs and whole organisms. At the same time, it is hoped that a much clearer view will be obtained of how the activity of the human genome is related to environmental factors. Systems biology provides a framework for handling the vast quantities of genomic, proteomic, physiological and environmental data necessary to provide

297 Horie H, Miyazawa M, Ota Y, Wakabayashi K, Yoshida H, Doi Y, Hashizume S. (2001) Analysis of the accumulation of mutants in Sabin attenuated polio vaccine viruses passaged in Vero cells. *Vaccine* **19**, 1456-9.

298 WHO (2005) *Final Report IABS Scientific workshop on neurovirulence tests for live virus vaccines.* WHO, Geneva, Switzerland.

299 ABPI & NC3Rs (2006) *Opportunities for reducing the use of non-human primates in the development of biologicals – a workshop report.*

300 Maggi A, Ottobrini L, Biserni A, Lucignani G & Ciana P (2004) Techniques: reporter mice - a new way to look at drug action. *Trends Pharmacol Sci.* **25,** 337-342.

this integrated view, based on powerful computer modelling techniques.[301]

This rapidly moving field is already finding applications in many aspects of cell biology, medical research, and drug discovery.[302] The field is still in its infancy, and many respondents emphasised that models will require verification at the molecular and cellular levels (see section 6.4.2). However, it seems very likely that its predictive potential will, in the long term, play an increasingly important role in reducing the requirement for non-human primates and other animals for medical research and toxicology programmes.

9.4 Human studies

Human studies, including clinical investigation of patients and healthy volunteers and research on post-mortem cells and tissues, have a long history in medical research (see 4.3 and 6.5.1). Epidemiological and population-based studies will also continue to play an important role in elucidating the environmental alterations associated with disease and the molecular pathology of complex conditions. Here, we summarise developments in human tissue culture research, micro-dosing and non-invasive imaging that may impact on non-human primate research.

9.4.1 Human cells for medical research and toxicology

Major advances in cell biology and tissue culture are providing another approach to medical research and toxicology testing.[304] With appropriate consent from individuals or their relatives, human cells can be obtained from a wide variety of sources, including surgical samples, autopsies, biopsy material, small skin strips, smears from the lining of the mouth, blood samples and hair follicles. These cells can be grown in short-term culture, long-term established cultures, or even immortalised. All these techniques are being augmented by the increasing availability of purified growth factors and related regulatory proteins.

There is always the concern that cells maintained in culture may not retain their normal physiological function, or that it may be lost with time. However, there is increasing evidence that, using

Box 18. Pharmaco-metabonomic phenotyping

Quite recently, a new variation on some of the themes described in this section has been developed, which has been named pharmaco-metabonomic phenotyping.[303] This approach to personalisation of drug treatment goes one step further than pharmacogenomics (a description of variation in drug response and toxicity based on an individual's genetic make-up) and attempts to take into account important environmental influences on drug absorption, distribution, metabolism and excretion. In essence, it involves the combination of pre-dose metabolic profiling and chemometrics to model and predict the responses to drugs of individual subjects.

Proof-of-principle for this new approach was provided by a study of paracetamol administration to rats, which allowed pre-dose prediction of the urinary drug metabolite profile and an association between pre-dose urinary composition and the extent of liver damage sustained after paracetamol administration.[303] While the long-term value of this more holistic approach to studies of individual differences in the efficacy and toxicity of drugs is still very much in its early stages, it provides yet another example of an area of research that could, in the longer term, reduce the requirements for non-human primates for both research and toxicology purposes.

301 Church GM (2005) From systems biology to synthetic biology. *Molecular Systems Biology* ***1****,* 2-3.

302 Blundell TL, Sibanda BL, Montalvao RW, Brewerton S, Chelliah V, Worth CL, Harmer NJ, Davies O & Burke D (2005) Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. In: *Bioinformatics: from molecules to systems* (ed. by D. Jones, M. Sternberg & J. Thornton), pp. 413-424. Transactions of the Royal Society B, London.

303 Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, Provost JP, Le Net JL, Baker D, Walley RJ, Everett JR & Nicholson JK (2006) Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* **440,** 1073-1077.

304 Combes RD (2004) The use of human cells in biomedical research and testing. *ATLA* **32,** 43-49

more advanced tissue-culture techniques, and with particular respect to certain cell types such as skin and corneal cells, this may be less of a problem. Recent advances in molecular biology have led to the possibility of immortalisation of cells by the introduction of particular genes; several organotypic models have now been developed along these lines, comprising skin or corneal cells.

Naturally, there are many difficulties and disadvantages in using cultured human cells for potential research or toxicology purposes. Obviously, they lack normal absorption, distribution, metabolism and excretion processes that occur in the complex pathways in the human body, together with the normal background of immune mechanisms and endocrine and nervous system control. Some of these problems are being approached by the use of more complex culture media or by the introduction of genes coding for mammalian metabolising potential.

9.4.2 Stem cells

Stem cells are self-renewing cell populations that have retained the ability to differentiate along different pathways and hence produce progeny that can form different tissues in response to appropriate regulatory stimuli. They can be obtained from early embryos, some adult and fetal tissues, and, theoretically at least, from other adult cells. Embryonic stem cells, which retain the greatest plasticity, are present at an early stage of the developing embryo, lasting from the fourth to seventh day after fertilisation.

While much of the early research in this field was carried out in mice, human embryonic stem cells were grown in the laboratory for the first time in 1998. There has since been some progress in coaxing them to produce specific cell types.[305,306,307] Some adult tissues retain stem cell populations. For example, bone marrow transplantation has been applied for the treatment of a wide range of blood diseases, and it is clear that human marrow contains stem cells capable of differentiating into the full complement of cell types found in the blood. There is also evidence that marrow stem cells can, under certain circumstances, be induced to differentiate into other tissue types. Work involving a variety of fetal and adult mouse and human cells has also suggested that it may be possible to restore their potential to differentiate into diverse cell types.[308]

As well as having the potential for a wide range of therapeutic applications, it has also been suggested that cell populations derived from stem cells may be of value for both medical research and drug toxicology studies in the future. Currently, cells derived in this way would suffer from the same problems and disadvantages as those outlined in section 9.4.1. Of course, the situation would be different if, in the long term, it were possible to derive organised tissues or organs from stem-cell sources.

9.4.3 Microdosing

It has been realised for a long time that any form of animal study has certain limitations regarding the evaluation of the critical features of human drug metabolism, including absorption, distribution, metabolism and excretion (ADME). Recently it has been suggested that in the very early phases of drug trials their evaluation might involve human volunteers exposed to tiny doses of the particular agent under study.[309] A microdose is so small (1/100th of the pharmacological dose) that it is not intended to produce a particular pharmacological effect when administered to humans and therefore is unlikely to cause an adverse reaction. The concept relies on the view that many of the processes controlling the pharmacokinetic profile of a particular compound are independent of dose level and hence that a microdose will provide sufficiently

305 Report of Chief Medical Officer's Expert Group, Department of Health (2000) *Stem cell research: medical progress with responsibility.*

306 Vogelstein B (2002) *Stem Cells and the Future of Regenerative Medicine*. A report from the National Research Council. National Academy Press, Washington DC.

307 De Soussa PA, Gales G, Turner M (2006) The road to providing human embryo stem cells for therapeutic use: the UK experience. *Reproduction* **132**, 681-689.

308 Surani MA, McLaren A (2006) A new route to rejuvenation. *Nature* **443**, 284-285.

309 Rowland M (2006) Microdosing and the 3Rs. *NC3Rs* **5,** 1-7.

useful information to help in deciding whether it is worth continuing to develop a particular drug.

Important advances in determining minute quantities of a drug, or its metabolites, in the body (particularly in plasma or blood) have enabled the development of this approach. In particular, the development of liquid chromatography coupled with tandem mass spectrometry and, more recently, accelerator mass spectrometry (AMS), has made it possible to determine extremely low levels of a drug and therefore assess various aspects of its metabolism. Although the agent under investigation must be isotopically labelled, the dose of radioactivity required is, at most, only double that of the normal body burden of the particular isotope derived from the normal environment.

Results from recent microdosing clinical trials have been promising and have resulted in the establishment of the European Union Microdose AMS Partnership Programme (EUMAPP).[310] EUMAPP, which is coordinated by Xceleron Ltd and funded by the European Commission, involves 10 organisations from 5 different countries (UK, Sweden, The Netherlands, France and Poland). The study partners are working towards the certification of high and low voltage AMS as the most accurate, reproducible and appropriate analytical methodologies for all measurements required by microdosing studies. The project aims to demonstrate the reliability of the microdosing approach and to develop *in silico* modelling applications to predict pharmacokinetic parameters from data derived from microdosing studies. As well as reducing the requirement for animal testing, microdosing is very attractive to industry because of the potential to reduce the time required in pre-clinical testing of drugs; reducing the need for costly pre-clinical development work and enhancing candidate drug selection are clear financial incentives.

Researchers and regulators stress that microdosing is useful in relation to ADME studies, but does not replace the need for full dose safety testing (precisely because the dose is designed to be so low that it does not cause toxicity). The European Medicines Agency has encouraged the development of non-clinical safety studies to support trials with a single microdose.[311] Current information suggests that this approach, or variations of it, is also being taken seriously by the FDA[312] and that it may begin to play a role in helping to reduce the load of animal testing in drug development.

9.4.4 Non-invasive human studies

We received a number of submissions highlighting the potential for the use of non-invasive imaging techniques in humans to replace research currently undertaken in non-human primates. Recent advances in studying the function of the nervous system in humans using non-invasive behavioural techniques, biomarkers of various kinds, and brain imaging, have been discussed in section 6.4.2. In particular, the remarkable advances and potential limitations of neuroimaging techniques, including computer assisted tomography (CAT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) were considered in detail. It was emphasised that techniques such as fMRI assess the increased blood supply to active regions of the brain and cannot measure the firing of individual cells or particular groups of cells. Since a great deal of current research in the neurosciences is directed at the study of neural circuits, particularly during development, this field is becoming of particular interest and has potential clinical importance. The very recent development of transcranial magnetic resonance imaging (TMS), offers a safe, painless and reversible method of actually intervening in human brain function. This an exciting advance, although its ability to study excitatory and inhibitory neurones, the proportion of those affected, the distance from the brain surface, and the impact of neuronal plasticity is still not clear. However, this new technique promises to add a vital new arm to current methods for non-invasive analysis of the nervous system.

310 See http://www.eumapp.com/

311 European Medicines Agency (2004) Position paper on Non-Clinical Safety Studies to Support Clinical Trials with a Single Microdose.

312 Wadman M (2006) Drive for drugs leads to baby clinical trials. *Nature* **440,** 406-407

Particularly in view of the relatively recent development of many of the new techniques for brain imaging, it is difficult to assess the effect they might have on the requirement for research on non-human primates in the near future. In an insightful editorial in *Nature Neuroscience*, Paradiso (1999) points out that the explosive increase in the use of fMRI in humans, together with the use of PET, has virtually defined the way ahead in the field of cognitive neuroscience.[313] In the future we will understand how we see and feel and how our memories are formed and fade. The paper also describes how a fMRI machine designed specifically for non-human primates will make it possible to interpret years of studies with implanted electrodes and throw considerable light on poorly understood aspects of perception and cognition. Many researchers see this new development as the formation of a bridge between human studies and a large body of previous animal research.

All alternative techniques require validation and calibration on animal models. For instance, interpretation of the activity maps from fMRI required fMRI measurements paired with simultaneous single-cell recordings in non-human primates (see 6.4.2)

Overall, recent developments in imaging techniques are extremely encouraging with respect to the use of non-human primates for research. Imaging technology is improving at a remarkable rate; much can now be learnt from its application to the studies of the human brain and it is making important inroads into the requirements for invasive studies in non-human primates. All the signs are that these trends are likely to continue.

9.5 Discussion

This short survey of promising developments that may reduce the use of non-human primates (and other animals) in research and toxicity testing shows that genuine progress is being made on many fronts. While some of the more speculative approaches may take many years to come to fruition, and much will depend on the pace of development of integrative biology, this field holds considerable promise for the future.

There are, however, two areas of uncertainty. First, the current reductionist approach to biological research will ultimately have to move through a phase of integrative biology in an attempt to explain how the thousands of gene products interact with one another in the intact organism. Currently, it is far from clear to what extent present developments in systems biology will achieve this end and hence whether, as seems more likely, studies on animals and humans may be required to validate this complex information. The second area concerns the complexities of translating and validating information gained from new alternative technologies with data from conventional animal toxicology studies. Given recent public concerns about vaccine safety, it is not surprising that regulatory authorities require lengthy and detailed analyses of any new alternative approach, backed up with the availability of more conventional animal testing, before new technologies are accepted as safe and efficacious.

Overall, however, the picture that is emerging is of a potential to move towards the gradual reduction in the requirement of animals for biological research and toxicology studies. What is impossible to predict is the timescale involved. The considerable promise of this field should compel bodies that fund biological or medical research to take every opportunity of supporting research directed at developing alternative approaches. At the same time, regulatory authorities need to be vigilant to innovations and new developments, such that they can fully appreciate the potential and complexities of the science involved. Achieving the undoubted potential of this rapidly moving field in as short a time as possible requires clear and sustained channels of communication between the regulators, researchers of alternative techniques and the wider scientific community.

313 Paradiso MA (1999) Monkey business builds a bridge to the human brain. *Nat Neuroscience* **2,** 491-492.

10 Welfare issues

10.1 Introduction

While the remit of this study primarily concerns the scientific basis for non-human primate research, this cannot be considered in exclusion from the associated welfare issues. These issues have been considered by several other bodies and organisations, most notably the European Commission Scientific Committee on Animal Health and Animal Welfare, whose scholarly 2002 report made 48 recommendations on husbandry, breeding and supply, human–animal interaction, transport and health.[314] In this section we comment on non-human primate welfare issues in light of this report, noting where the recommendations have been fulfilled in the UK and where improvements can still be made. We focus on issues of breeding, housing, transport and welfare during research practice.

Our comments are informed by written submissions to the working group, observations made during visits to four UK non-human primate centres, published reports, scientific papers and oral evidence from a variety of sources, including: the RSPCA; the Dr Hadwen Trust; FRAME; the NC3Rs; the Universities Federation for Animal Welfare (UFAW); scientists; vets; and licence holders at non-human primate research and breeding centres. We have further considered the growing scientific understanding of the cognitive abilities, awareness and social needs of non-human primates that has emerged from laboratory and field studies.

10.1.1 Species and regulations

As discussed in sections 3.1.1 and 3.4.1, non-human primate research in the UK mainly involves macaques, specifically the rhesus (*Macaca mulatta*), macaque (*Macaca fascicularis*) and stump-tailed macaque (*Macaca arctoides*), and the common marmoset (*Callithrix jacchus*). Great apes have not been used in UK research since 1986, and their use was effectively banned in the UK in 1997.

Research with non-human primates must comply with the Animal (Scientific Procedures) Act (1986) (A(SP)A) and its associated codes of practice, which enshrine the principles of the 3Rs: replacement, reduction and refinement. Details on governance procedures for research involving non-human primates are given in section 3.3. Here we note the Home Office guidance, which stipulates, among other measures, procedures for non-human primate acquisition and transport, identification and breeding programmes. Regulations are overseen by visits from the Home Office Inspectorate, which may be unannounced. We also note that each centre housing non-human primates has a named Veterinary Surgeon and a named Animal Care and Welfare Officer.

Changes to the European Convention and Directive (2002) are currently being negotiated. The overall effect of the proposals will be to create a new Directive similar to the UK's legislation, considered by many to be the most rigorous of any EU member state. The closing date for submission of comments on the new proposals from experts and organisations was 18 August 2006.[315]

10.1.2 Provision and measures of good welfare

Housing non-human primates in captivity must take into account their cognitive abilities, complex social relationships, capacity for suffering, and interaction with a large and diverse home range in the wild. Some respondents highlighted that, in certain respects, it may be easier to provide for the needs of non-human primates in captivity than some other species; non-human primates can adapt well to captive housing, be co-operative (especially through training), enjoy human interaction and be provided with a varied diet relatively easily. However, submissions also acknowledged that non-human primates are essentially undomesticated and have spent relatively few generations in captivity, whereas many other laboratory species have a long

314 Morton D C (2002) *The welfare of non-human primates used in research*. Report of the Scientific Committee on Animal Health and Welfare (European Commission).

315 For further details see http://ec.europa.eu/environment/chemicals/lab_animals/ia_info_en.htm

history of co-existence with humans, either as pets or domesticated farm animals.

Providing for non-human primates under laboratory conditions remains a significant challenge. We emphasise that good welfare should encompass living conditions that go beyond the minimum for survival and which, in addition to regular food and water and sufficient space, provide the animal with a quality of life that includes social interactions and stimulation for play. Many respondents quoted Russell and Birch's maxim that *'good science is humane science'*, a view we wholeheartedly support. Laboratory conditions that involve restricted space, insufficient enrichment and social deprivation may prevent non-human primates from exhibiting their natural range and proportion of behaviours. This can produce stress levels that impact on several physiological systems, including immune competence, coronary health, brain structure and function, metabolism, reproduction, growth and cognitive behaviour. In addition to the impact of stress on welfare, there are clearly also implications for the validity of research data.

Objective measures for good welfare (as opposed to poor welfare) are not easily defined. Such measures may include physiological and behavioural indicators, in addition to breeding success. Variability in reporting of welfare indicators is further discussed in section 10.5.3.[316]

10.2 Breeding

10.2.1 Provision of non-human primates

Under current EU legislation, all non-human primates used in biomedical research are bred in captivity specifically for experimental purposes, unless a special exemption has been made.[317] The capture of wild animals for breeding purposes is controlled by regulations implementing CITES (see section 3.4.3) and is limited to cases where it does not put the species concerned at risk. Wild-caught animals can be an important source of new genetic characteristics in a breeding colony, but all guidelines emphasise that their introduction requires careful consideration and management. Some submissions highlighted that several primate species are considered as pests in their native countries and are subject to unauthorised culling (e.g. *M.fascicularis* in Mauritius, *C.aethiops* in Kenya and the Caribbean and *Papio spp*. in Kenya). Submissions acknowledged the ethical implications of using wild caught animals and the possibility of health issues. UK regulations require applications for the use of wild-caught primates for research to be reviewed by the Animal Procedures Committee. Evidence submitted to the working group suggests that this occurs only very rarely.

There are plans for the UK research community to become self sufficient in providing domestically bred non-human primates for academic biomedical research. Currently, macaques are bred at the Centre for Macaques (CFM) (owned by the MRC, the Wellcome Trust and the Universities of Oxford and Cambridge) and at dstl (the Defence Science and Technology Laboratory), both located at Porton Down in Wiltshire. There is also one further small macaque breeding colony in Scotland. There is one dedicated UK breeding colony for marmosets, in addition to colonies at Dstl and within some academic institutions.

The CFM houses around 240 rhesus macaques and supplies about 65-70 animals per year to research laboratories. Animals are kept in groups of about 12-15 females and 1 male. Newborn animals generally remain with their natal group until about 14 months, when they are moved to a weaning group. Occasionally there is a need to hand-rear an infant following rejection by the mother (this has happened on 3 occasions in the last two years), after which infants are reintegrated into their group as

316 We also welcome the forthcoming primate welfare meeting, organised by the NC3Rs, which will address methods for assessing health and psychological well-being, covering behavioural, physiological and immunological measures.

317 Directive 86/609 on protection of vertebrates used for research and other experimental purposes and Council of Europe Convention ETS 123 on protection of vertebrates used for research and other experimental purposes. Article 7 (3) states "Experiments on animals taken from the wild may not be carried out unless experiments on other animals would not suffice for the aims of the experiment". Article 21 states "Non-human primates to be used in experiments shall be bred animals unless a general or special exemption has been obtained under arrangements determined by the authority".

soon as possible. At dstl, about 415 macaques are housed in groups of 25–30 females with 2 males per group. At both facilities, about 65% of the females give birth each year, which is considered to be an acceptable level of breeding success.

Cynomolgus macaques are bred at a facility owned by the Department of Health. These animals are mainly used for polio vaccine testing, HIV research and by industry for safety and efficacy testing of pharmaceutical products. However, data on the numbers of imported non-human primates suggest that these colonies do not meet the demand from pharmaceutical companies for toxicology testing (see 3.4.3).

As part of the revision of the EU Directive 86/609/EEC, there is a proposal to exclude the use of animals from the first generation born in captivity (F1) and to use only animals from the second (F2) or subsequent generations. This is being implemented to reduce the incidence of trapping wild primates to supplement breeding stocks. The implementation of this proposal will have no effect on UK marmoset availability, since marmosets have been bred in captivity for several generations. However, concern has been expressed that the supply of macaques (both cynomolgus and rhesus) may be compromised, given the relatively long time needed to bring the second generation of animals to maturity. In the short term, this proposal could also create a cohort of F1 males that could not be used in research but are surplus to breeding needs. An abrupt introduction of this policy could therefore be deleterious in the short term and should be avoided. In the longer term, the extent of the potential impact on UK academic research (for which most macaques have been bred in captivity for several generations) is unlikely to be significant.

10.2.2 Interactions between breeders and researchers

Animals are generally moved to research laboratories at around 18 months - 2 years of age. The long gestation period and the time needed to rear animals to sufficient maturity requires detailed forward planning by researchers with regard to their animal requirements. For instance, most endocrinology research requires macaques that have passed puberty (>4 years), neuroscience research generally requires animals of 2-3 years, and research on age-related diseases can require animals of 15-20 years.

Supply and demand between breeding centres and researchers requires careful management and breeding facilities have an understandable reluctance to over-supply. Dialogue between the breeding facility and researchers is a vital factor in ensuring that sufficient animals are available at the appropriate time and that the animals supplied are best suited for particular research protocols. Evidence submitted to the working group suggested that such interactions could be significantly improved, both in forward planning by the academic community and in more interaction on the selection of animals for their research use.

For marmosets, some UK universities retain their own breeding colonies. This brings several advantages, most notably in avoiding transport of animals and allowing close involvement of researchers in the development of the colony.

10.2.3 Costs

Non-human primates are valuable and costly animals. The pricing structure for the supply of macaques in UK academic research is complicated by the fact that the two major research funders, the MRC and the Wellcome Trust, also part own the CFM breeding centre. Macaques are currently priced to researchers at £20,000, which represents full cost recovery for CFM (based on the supply of 65 animals per year). Macaques cost £200-400 per week to house (under full economic costing). Marmosets are priced at around £2000, with a further £32 per week in housing costs. The price to import a cynomolgus macaque is around £3000 per animal.

In some other countries, the costs to researchers of using non-human primates appears to be considerably lower. In China for example, monkeys cost less than US$1,000, which is said to be a tenth of what they cost in most of Europe.[318] The price of a non-human primate in the US varies, but reports to the working group suggest an average figure of US $5,000. However, these prices must be weighed against the standards of facilities and care that can be provided for the animals and respondents acknowledged that the expense in the UK is, in part, because of better standards of welfare in the UK than elsewhere.

10.3 Housing

There are about 13 locations in the UK where non-human primates are housed for use in academic research. Approximately six pharmaceutical companies and contract research organisations also house and use non-human primates.

The overall impression given by evidence submitted to the working group is that housing, care and welfare conditions of laboratory non-human primates in the UK have improved significantly in the last decade according to a growing understanding of animal needs. However, there is still considerable work to be done. Standards of housing and care for non-human primates are specified in the Home Office Codes of Practice.[319,320] These include species specifications for minimum cage sizes, temperature range, lighting, humidity, diet and environment.

10.3.1 Cage size

In evidence to the working group, animal care staff emphasised that Home Office cage specifications constituted a minimum standard and that some aspects of the regulations did not conform to current best practice. At all facilities viewed by the working group, cage sizes exceeded the stipulated minimum. We note that the NC3Rs Guidelines on '*Primate accommodation, care and use'* and the joint Laboratory Animal Science Association (LASA) [321] and MRC paper '*Key considerations in the breeding of macaques and marmosets for scientific purposes'*[322] are widely judged to be an improvement on the Home Office Code of Practice.

We also note the lack of guidelines on the use of cages that accommodate particular research purposes. For instance, special conditions or treatment may be required for pathogen-free animals or animals that are used for blood sampling.

10.3.2 Environmental enrichment

There is an extensive literature on best practice in environmental enrichment for non-human primates. Here we note various practices at the centres visited, which for macaques included:

- Housing in groups of between 10 and 25
- Inclusion of windows, allowing the animals to view the outside
- Regular changes of equipment and toys
- Facilities to allow swinging and jumping and engaging in activities from floor to ceiling
- Wood chip substrate on the floor to provide opportunity for foraging
- The use of puzzle feeders and placement of food in different locations in the enclosures
- Access to partially partitioned areas within the enclosures for rest and privacy

Box 19. Home Office housing specifications

For marmosets, the minimum cage height must be 1.5m (with the top of the cage at least 1.8m from floor), with a minimum pen area of 0.55m^2 for a breeding pair. Macaques must be housed in pens with a minimum height of 1.8m (indoor) or 2.4m (outdoor) and a minimum pen area of 6m^2 (for a breeding group) or 2m^2 (for a single animal).[282]

318 News article. China takes steps to secure pole position in primate research. *Nature* **432**, 3.

319 Home Office (1995) *Code of practice for the housing of animals in designated breeding and supplying establishments. Part 2: 9. Non-human primates.*

320 Home Office (1989) *Code of practice for the housing and care of animals used in scientific procedures.*

321 NC3Rs Guidelines: Primate accommodation, care and use, http://www.nc3rs.org.uk/downloaddoc.asp?id=418&page=277&skin=0

322 Medical Research Council (2004) *MRC Ethics Guide: Best practise in the accommodation and care of primates used in scientific procedures.* London.

- Access to adjustable mirrors, allowing the animals to view adjoining corridors and visually identify sources of noise
- Occasional access to a water pool

For marmosets:

- Housing in pairs (female and vasectomised male)
- Tall cages, including a balcony fo viewing other cages and for foraging
- Weekly access to an additional 'play' cage of toys, slides etc.

In some UK facilities, animals have access to outside areas. At one institution visited, the marmosets could access a series of roof gardens by tunnels from the indoor cages. Staff attested to the benefits of access to outside space, which is used by the animals in all weather conditions.[323] Some respondents pointed out that access to outside space can make it more difficult to keep the animals disease free and has associated security issues. However, these problems may be partially addressed by a roof arrangement where a cover can avoid infection from birds.

10.3.3 Single housing

There was a consensus in the evidence submitted to the working group that single housing of non-human primates should be avoided, unless necessary for the animal's welfare or, in some instances, for a specific scientific experiment. This view is strongly reinforced by the European Commission Report and is entirely consistent with research showing that social interactions are one of the most important factors for non-human primate welfare.[324]

In some circumstances, welfare considerations may require an animal to be housed separately over the shorter or longer term. For example, when introducing a primate into a new colony, the animal may benefit from observation of the group without any interaction in the first instance. Similarly, a few animals can have difficulty integrating into a social group.

The working group heard evidence that group housing can occasionally cause stress amongst subordinate animals and increase the likelihood of injuries due to fighting. Further evidence suggested that this can be successfully addressed through the provision of sufficient stimulation and by structuring cages to allow animals to establish social hierarchies using different levels of perches and vantage points. [325]

We emphasise that single housing should be avoided where at all possible. In the few instances where experimental constraints require the separation of individuals, researchers and care staff have particular responsibilities to create acceptable conditions. In such circumstances, guidelines recommend that the animals have sight and sound of other non-human primates. Furthermore, there should be an emphasis on close observation and contact with the animals, and on keeping the period of single housing to a minimum.

10.3.4 Staffing issues

In every respect, recruitment, training and retention of skilled staff are crucial in ensuring good standards of welfare. Working group members were impressed by the care and understanding of the animals shown by the staff at the facilities visited. The ratio of staff to animals is important and submitted evidence suggested that a ratio of 1 staff member to every 30 animals is required to ensure sufficient contact and care in a breeding colony, but a higher staff to animal ratio is required in a unit that carries out surgery and has animals requiring a high level of post-operative care.

We emphasise the importance of continuous development for researchers and care staff to keep abreast of improvements in non-human primate welfare. Frequent contact with staff members from other primate laboratories would facilitate this. The group identified a particular need to increase the training opportunities for

323 A video of outdoor runs for stock and breeding animals can be viewed on the NC3Rs website. www.nc3rs.org.uk

324 Morton DC (2002). The welfare of non-human primates used in research; Report of the Scientific Committee on Animal Health and Welfare (European Commission).

325 Honess P & Marin C (2006). Enrichment and aggression in primates. *Neuroscience and Biobehavioural Reviews.* **30,** 413-436.

researchers and staff, since few have specific training in primate care. The working group was surprised that the Home Office does not require licence holders to undergo any specific non-human primate training. The 3-day course that is undertaken by project licence holders has a primate component of modules 2 and 3, which can last between 0.5 and 3 days, but the focus is often on health and safety and procedures, rather than welfare needs and assessment, human-animal interactions and primate behaviour and impact on science. We therefore welcome the review of the scope of UK licence training currently being undertaken by the APC Education Subcommittee. We note that the Institute of Animal Technology provides training courses for technicians working with non-human primates and welcome the NC3Rs annual meeting for the professional development of staff working with non-human primates.

10.4 Imported animals and transport

10.4.1 Importation of non-human primates

As discussed in section 3.4.3, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) database holds records on all wildlife trade worldwide. These figures show that significant numbers of non-human primates are annually imported to the UK. According to 2005 statistics, 70% of all procedures on non-human primates used animals sourced from outside the EU. This is an increase on the figures for 2004, when 57% of procedures were carried out on animals sourced outside the EU (see section 3). This appears to involve mainly cynomolgus macaques for use in toxicology testing by pharmaceutical companies.

Home Office requirements state that approval for the acquisition of non-human primates from overseas will only be given if conditions at the overseas breeding or supply centre are acceptable and comply with the Guidelines issued by the International Primatological Society (IPS) in 1993. Current criteria for the acceptability of overseas centres include requirements for group housing, protection from adverse environmental conditions and weaning at no less than six months of age.

The APC primate sub-committee has recently published a report *'Acceptance of Overseas Centres supplying non-human primates to UK laboratories'*, which reviews current practises around importation of non-human primates.[326] It makes a number of recommendations on how overseas centres should be assessed and accepted by Home Office Inspectors, to ensure that facilities meet required welfare standards. The report calls for clarification of the criteria used for assessment, improvements in the quality of information supplied by centres and a policy whereby new centres are visited prior to consideration of their application. In addition, the report recommends that the Home Office Inspectorate should monitor all centres at appropriate intervals, usually once every 2 years. We consider all of these recommendations to be appropriate.

10.4.2 Transport issues

Importation of non-human primates raises serious questions about welfare prior to and during transport. Evidence suggests that most of the cynomolgus macaques imported into the UK come from Mauritius, with other primates (almost all macaques) imported from the Philippines, China, Vietnam and Israel. There are regulations in this area (e.g. the International Air Transport Association's Live Animals Regulations) and some improvements have been made to shipment containers and equipment in recent years. However, many respondents highlighted that transport, particularly involving long-distances and multiple stages, can cause considerable stress and suffering in non-human primates.[327,328]

Stress is caused by the separation of the animals from their group, the use of small cages (which can be 30x50x65 cm for young macaques), the length of the journey (possibly over 2 to 3 days) and the distress associated with loading and unloading,

326 Animal Procedures Committee (2006). *Acceptance of overseas centres supplying non-human primates to UK laboratories*. A report by the primates sub-committee of the APC.

327 Prescott M J (2002). *Counting the cost: welfare implications of the acquisition and transport of non-human primates for use in research and testing.* Royal Society for the Prevention of Cruelty to Animals.

328 Wolfensohn SE (1997). Brief review of scientific studies of the welfare implications of transporting primates. *Laboratory Animals* **31**, 303-305.

flights, shipping, customs, veterinary checks and overland journeys. Airlines no longer fly primates to the UK, partly because of difficulties associated with their transport, but primarily in reaction to the actions of animal rights activists. Animals are instead flown to a European destination and further transported by sea and land. These multiple stages add considerably to the length and stress of the journey.

We note a recommendation from Carlsson *et al* for the development of primate research centres in source countries to alleviate animal welfare issues in transport.[329] The Caribbean Primate Laboratories of the Behavioural Science Foundation in St. Kitts provides an interesting example. This centre has provided the location for work on vervets,[330] which are African Green monkeys imported from West Africa between 1630 and 1700. There are currently about 30,000 vervets on St Kitts where they are non-endangered and considered by the local population to be an agricultural threat. They live about 15-20 years in the wild and about 20-30 years in captivity. These monkeys have been used to research Alzheimer's disease. This issue raises important questions about the need for a more strategic approach to non-human primate supply and demand in the UK and internationally (see 12.6).

10.5 Welfare during the practice of research

Evidence supplied to the working group indicated that, in academic fields, neuroscience research generates the most concern about welfare. Procedures involved in neuroscience research are outlined in section 6. Again we emphasise that all procedures involving surgery and invasive methods demand high standards of the research laboratories, anaesthesia, surgery and post-operative care of the animals.

Although outside the direct remit of the working group, several respondents commented on welfare issues in relation to the use of non-human primates in toxicity testing of medicines. This has been considered by a number of groups, including the APC and Nuffield Council on Bioethics.[331] It is also being actively examined by an expert group convened by the NC3Rs and the Association of the British Pharmaceutical Industry (ABPI). Here we note that toxicology testing uses a larger number of non-human primates than any other field of research. A reduction in numbers used in this field would therefore have a significant impact on overall numbers.

10.5.1 Refinements: training, food and fluid control

Developing and implementing refinements that improve the welfare of non-human primates before, during and after experimental procedures is an important area of research. For instance, improving the quality of anaesthetic practices and investigating ways to reduce the risk of infections associated with surgical procedures or implants.[332] With regard to the latter, such infections can have a significant effect on the quality of behavioural data obtained and in some cases may lead to the experiment being terminated before sufficient data is gathered. In this section we focus on positive reinforcement training (PRT) and food and fluid control.

Nearly all guidelines on non-human primate welfare emphasise the importance of training non-human primates to co-operate with scientific protocols and so minimise the stress and impact of the procedures. Training non-human primates to co-operate with procedures can also reduce the need for physical restraint and/or anaesthesia and thus avoids the accompanying risks associated with those events. A reduction in adverse effects can reduce intra- and inter-animal variability in the data obtained, so improving the quality of the

329 Carlsson H-E, Schapiro SJ, Farah I, and Hau J (2004). Use of primates in research: a global view. *American Journal of Primatology* **63**, 225-237.

330 Lemere CA, Beierschmitt A, Iglesias M, Spooner ET, Bloom JK, Leverone JF, Zheng JB, Seabrook TJ, Louard D, Li D, Selkoe DJ, Palmour RM, Ervin FR (2004) Alzheimer's disease abeta vaccine reduces central nervous system abeta levels in a non-human primate, the Caribbean vervet. *Am J Pathol* **165**, 283-97.

331 See also Palmer N *et al*, (2004). *The use of animals in vaccine testing for humans.* The Associate Parliamentary Group for Animal Welfare.

332 For example see project 'Transcutaneous signal transmission without breaching the skin's natural barrier to infection' http://www.nc3rs.org.uk/page.asp?id=282

science and potentially leading to a reduction in the number of animals required.

A special edition of the *Journal of Applied Animal Welfare Science* in 2003 included several studies that looked at the feasibility of positive reinforcement training (PRT). A study by Reinhardt found that it took cumulative total of around one hour to train an adult female or adult male rhesus macaque to present a leg voluntarily and accept venipuncture for a blood sample.[333] Unlike the manual restraint method, which was shown to increase serum cortisol by two fold, the trained method produced no increase. PRT has also been successfully demonstrated in common marmosets. For example, McKinley *et al* trained 12 marmosets in pairs to provide urine samples and to allow weighing in the home cage.[334] Their study showed that the desired behaviours were established in 2-13 training sessions, each of 10 minutes in duration. The authors of this study highlighted the reliability of the trained animals in performing the tasks and the increased speed with which the work could be performed (up to 20 times faster than the manual restraint method). The trained method also needed only one member of staff, while the restraint method required two. These studies indicate a very satisfactory return on the time and staff investment made in PRT.

The working group itself observed instances where non-human primates had undergone PRT (using food treats) to receive injections and undergo blood sampling. These animals required only a minimal amount of restraint during the procedure, similar to a parent holding a child. Evidence to the group cited examples of studies on vision, where macaques had been trained to place their heads voluntarily into a mask so that a remote camera could follow eye movements in response to an object on the screen.[335] In other work primates were trained to carry out cognitive tests (involving selecting an image on a computer screen following certain visual cues), by a reward system involving banana milkshake.[336] A further refinement of this protocol allowed the animals to perform the task in view of the other animals. This avoided the need to separate the animal and also stimulated the interest of animals in the surrounding cages.

A recent UK survey of non-human primate training concluded that there is considerable scope for refinement of common scientific, veterinary and husbandry procedures through the use of PRT. [337] The survey identified real or perceived constraints to training on the part of researchers and animal technicians, including a lack of information on how to train and a lack of available staff. In response, Part 2 of the survey provides a tabulated literature review of primate training, guidance on developing and implementing a training programme and a detailed sample training protocol.[338] This is also an area of NC3Rs activity and we fully support their efforts to facilitate the more systemic and efficient use of training to refine non-human primate use and management.

One area that raised concern amongst respondents is food and fluid control, which is used as a motivational tool in behavioural neuroscience procedures involving macaques. In these circumstances, animals are trained to perform tasks with the use of food or fluid as a 'reward' or 'reinforcer'. This may involve the restriction of the period in which food or fluid is available around the time of experiment or a reduction in the total amount of food or fluid provided per day. Naturally, food or fluid control has important welfare implications. This area is currently being considered by an expert working group that has been convened by the NC3Rs to review current practice, identify potential and actual refinements and make recommendations on best practice.

333 Reinhardt V (2003) Working with rather than against macaques during blood collection. *Journal of Applied Animal Welfare Science* **6**, 189-97.

334 McKinley J, Buchanan-Smith H, Bassett L, Morris K (2003) Training common marmosets (Callithrix jacchus) to cooperate during routine laboratory procedures: ease of training and time investment, *Journal of Applied Animal Welfare Science* **6**, 221-33.

335 Fairhall SJ, Dickson CA, Scott L, and Pearce PC (2006). A non-invasive method for studying an index of pupil diameter and visual performance in the rhesus monkey. *J Med Primatol* **35**, 67-77.

336 Crofts HS, Muggleton NG, Bowditch AP, Pearce PC, Nutt DJ, and Scott EAM (1999). Home cage presentation of complex discrimination tasks to marmosets and rhesus monkeys. *Laboratory Animals* **33**, 207-214.

337 Prescott MJ & Buchanon-Smith HM (eds) (2003). Training non-human primates using positive reinforcement techniques. *Journal of Applied Animal Welfare Science* **6**, 157-261.

338 Prescott MJ, Bowell VA & Buchanon-Smith HM (2005). Training laboratory-housed non-human primates, part 2: Developing and implementing training programmes. *Animal Technology and Welfare*. **4**, 133-148.

We look forward to the publication of this group's findings and urge all those targeted for recommendations to take the necessary action.

10.5.2 Levels of severity

The Home Office defines levels of severity that can be assigned to a research project as 'mild', 'moderate', 'substantial' and 'unclassified' (see section 3.4.4). 'Unclassified' refers to procedures where the animal is under terminal anaesthetic. All applications to use non-human primates in research are reviewed by the HO Inspectorate and a local Research Ethics Committee (REC). The APC Primate Subcommittee further reviews and makes recommendations on all applications for project licences where the work is likely to be classified as 'substantial' or where the use of wild-caught primates is proposed. The Home Office is the final arbiter for permission to allow a scientific project involving non-human primates to proceed.

Examples of procedures include non-invasive ethological observations, immunisation (mild), implantation of microelectrodes in the brain (moderate), or surgery resulting in cognitive or physical impairment (substantial). Submissions to the working group indicated a view that the classification of 'moderate' may be too broad and could be further subdivided. Records indicate that the number of proposals in the 'substantial' category was 2 per year for 2003 and 2004. The working group received evidence on the care of animals during some of the substantial procedures, which used anaesthetics, analgesics and post-operative recuperative facilities to minimise suffering. There are rightly concerns about levels of suffering encountered by animals, both in absolute terms and in terms of the cost/benefit arguments discussed in section 11. While the information received by the working group during their opportunities to directly question scientists was reassuring, this provides only a limited survey and as discussed earlier (section 3.4.4), it is not easy to obtain an overall view.

10.5.3 Retrospective recording of procedures

Several recent reports have highlighted the limitations of defining categories of severity prospectively. The European Commission Report also noted that the level of severity and animal suffering is often poorly recorded.[339] For this reason a retrospective reporting system has been recommended, in which reports by vets and animal welfare officers are collated and reviewed. An important added advantage of improvements to the collection of retrospective data will be in informing methods to alleviate suffering.

LASA and the APC jointly published the results of a pilot study on retrospective reporting of severity levels in December 2005.[340] The study was not restricted to non-human primates but included all animals used in research. The conclusions of the study favoured a reporting system using two grids to indicate severity intensity and duration.[341] The pilot study acknowledged the balance between the need to record average severity over a whole group while at the same time recognising that the experiences of an individual animal may exceed those of others.

10.5.4 Reporting on animal welfare in publications

Evidence to the working group indicated that welfare information provided in published papers involving non-human primates is patchy, and in many cases, unsatisfactory. Although data on the species, number, gender, age and weight of animals are usually given, information on housing conditions, welfare, training and the fate of the animals at the end of the experiment is rarely provided. Submissions from researchers indicated that the limited reporting of factors relating to housing and

339 Morton DC (2002). The welfare of non-human primates used in research; Report of the Scientific Committee on Animal Health and Welfare (European Commission).

340 Laboratory Animals Science Association/Animals Procedures Committee (2005) *Report of a LASA/APC Pilot study to assess the feasibility of collecting and reporting data on the severity of adverse effects caused to animals used in procedures regulated under the A(SP)A 1986.* This study was in response to the recommendations by a number of groups (the House of Lords Select Committee on Animals in Scientific Procedures (2002), the APC review of cost benefit assessment in the use of animals in research (2003), the Boyd Group/RSPCA report on discussion on categorising severity (2004) and the Nuffield Council on Bioethics working party (2005)).

341 The first Grid would indicate maximum severity over the short (<1 day), medium and long (>7 days) term under the headings mild, moderate and substantial. The second grid would indicate the severity over the rest of the procedure.

husbandry makes it difficult to evaluate and/or replicate the research. While the researchers state that such information is included in the project licence application, this information is not in the public domain.

We explored these statements further through a database search, using the Primatelit database[342] to search for experimental papers published by UK laboratories in 2005. Reviews and reports, in addition to observations on zoo or wild animals, were excluded. The search returned 56 publications (see section 3.5). More detailed analyses of a selection of these publications showed that less than 25% gave details of the care of the animals during the procedures, or the steps taken to alleviate suffering (where necessary). Notable examples that did provide a sensitive description of the care taken to minimise suffering included a paper where procedures were likely to be classified as substantial[343] and an earlier paper from a pharmaceutical company.[344]

10.5.5 Species choice

Species selection is an important issue both from a scientific and welfare perspective. Even within genera, different non-human primate species show behavioural and ecological differences such that their responses to laboratory environments can be quite different. For example, rhesus, long-tailed bonnet and pig-tailed macaques show distinct and consistent differences in responses to stress as a result of transport, cage confinement and room change.

Special justification must be made under A(SP)A for research involving Old World, as opposed to New World, non-human primates. Several respondents questioned the biological evidence for this distinction, a point also made by the Boyd Group.[345] It is certainly less expensive to use marmosets and, in some respects, it may be easier to provide for their needs in captivity due to their small size and preference for living in small family groups. This contrasts with macaques, which are larger, live in larger natural troops, have a greater home range and tend to exhibit complex dominance relationships and aggressive behaviours. On the other hand, macaques are easier to train for taking blood samples for example, and because of their larger size, suitable aliquots can be removed with minimal physiological side effects.

As discussed in section 6, the closer similarities between macaques and humans in terms of perceptual and emotional behaviour and neural systems usually means that they are preferred over marmosets. However, while it is generally true that Old World monkeys are better at cognitive tasks, some New World monkeys, such as capuchins species (e.g. *Cebus spp*.) can out-perform many Old World monkeys.[346] Evidence to the working group emphasised that sensitivity to species differences can aid in judgements about the suitability of particular species for research programmes. In every case where non-human primates are used, the species should be justified from both a scientific and welfare point of view.

10.5.6 Reduction and re-use of non-human primates

Submissions emphasised the need to increase efforts to maximise knowledge gained from primate work to refine and inform future research. Respondents expressed the need to move forward with the development of databases, detailed genomic sequencing and comparisons of archived tissues for detailed characterisation and pathology research, as well as more comprehensive studies over the lifespan of captive and wild primates.
It was asserted that a reduction in numbers would be assisted by the use of more imaginative experimental designs, e.g. factorial designs to evaluate two (or more)

342 Available at: http://primatelit.library.wisc.edu

343 Clarke HF, Walker SC, Crofts HS, Dalley JW, Robbins TW, and Roberts AC (2005). Prefrontal serotonin depletion afects reversal learning but not attempted attentional set shifting. *J Neuroscience* **25**, 532-538.

344 Virley D, Hadingham SJ, Roberts JC, Farnfield B, Elliott H, Whelan G, Golder J, David C, Parsons AA, and Hunter AJ (2003). A new primate model of focal stroke: endothelin-1-induced middle cerebral artery occlusion and reperfusion in the common marmoset. *Journal of Cerebral Blood Flow and Medicine* **24**, 24-41.

345 Smith JA, and Boyd KM (2002). *The use of non-human primates in research and testing.* The British Psychological Society.

346 McGrew WC, and Marchant LF (1997). Using the tools at hand: manual laterality and elementary technology in Cebus spp. and Pan spp. *Intertnational J Primatology* **18**, 787-810.

procedures at the same time. However, this must be balanced by the recognition that sufficient animals need to be used in order to give a statistically valid result. It is clear that careful consideration must be given to sample size calculations for non-human primate studies.

Different types of animal experiment have different endpoints. Many neuroscience experiments end with euthanasia and histological analysis of the brain. Other research, particularly in immunology, does not generally require the death of the animal. This raises the question of whether the animal is used in subsequent experiments, is retired (perhaps for breeding), or is culled. In view of the long life span of non-human primates and the aim to reduce the number of animals used within the framework of the 3Rs, it may be desirable to re-use animals. Commentators have argued that re-use of non-human primates is currently not dealt with either consistently or rationally.[347] However, re-use may have significant implications for welfare and suffering and may, in some instances, compromise scientific validity. For instance, participation in, and the cumulative effects of, a first protocol (e.g. a hepatitis C vaccine test) may make subjects unsuitable for additional protocols. In some circumstances re-use can improve welfare as the animal continues to be trained.

We note that re-use of non-human primates is much higher in the US than the UK, one reason being that the A(SP)A places strict restrictions on re-use. Good experimental design, closer interactions between primate centres and consideration of centres of excellence (see below) may offer opportunities to re-use animals. However, we emphasise that re-use must always be considered on a case-by-case basis.

10.6 Discussion

The submitted evidence supports a view that the welfare of non-human primates used in UK biomedical research has improved in recent years, but more can be done. This process has been guided by developments in understanding how best to meet the needs of non-human primates in captivity. The contribution of local experience has been considerable: observations and innovations around housing, environmental enrichment and welfare emerging from UK primate facilities have provided new insights into the animals and their behaviour.

The working group observed no behavioural abnormalities, such as repetitive pacing or cage circling, at the non-human primate facilities visited. The animals were interested in their human visitors and, although cautious of strangers, were relaxed with staff. We are conscious that we are a non-expert group and saw only a few centres, probably among the best. Even here, staff reported a need for further improvement of facilities, both in terms of space and the resources available. We focus our discussions on the areas of: reporting procedures; housing and dissemination of good practice; training; transport; and specialised centres.

10.6.1 Reporting procedures

It is clear that implementation of retrospective reporting systems across animal research requires further exploration, e.g. tackling reporting for the large numbers of animals involved in rodent studies. However, non-human primate experiments involve relatively few animals (the literature suggests between 1 and 50 monkeys per research programme), all of which should be well known to the researchers. Such information would record adverse events that had not been anticipated in the project licence, so better informing future studies. Furthermore, such a system would increase transparency by ensuring that accurate information on severity levels is in the public domain. For these reasons it is the working group's view that the implementation of a retrospective reporting system for non-human primates, as described in the recent APC/LASA report, should be considered immediately.

Our study of the quality of information relevant to welfare included in published papers was

347 Carlsson H-E, Schapiro SJ, Farah I, and Hau J (2004) Use of primates in research: a global view. *American Journal of Primatology* **63**, 225-227.

only very preliminary and a more thorough analysis should cover several years and include output from countries such as US and Japan. However, the analysis provided useful indications. We understand that authors in the UK are reluctant to give details because of security threats from animal rights extremists. We also note that Project Licences include strict protocols to minimise suffering. However, we suggest that, for research using non-human primates, editors should require authors to provide comprehensive information concerning origin, training, caging conditions, husbandry, care, welfare, severity of treatment and fate of the animal after the experiment, in addition to the experimental procedures. Such information could readily be incorporated as supplementary online material. Editors should further consider rejecting papers if these details are not satisfactory.

10.6.2 Housing and dissemination of good practice

Given the disparity between stipulated Home Office guidelines and currently accepted best practice, consideration should be given to improving the current regulations to include: recommendations on minimum cage size (in accordance with best practice); an emphasis on the need to avoid single housing where at all possible; indications for changing cage fittings to accommodate the purpose of the experiment; and an emphasis on the advantages of outside access and visual stimulation.

Evidence to the working group suggested that improvements could be made in the speed with which innovations in housing and welfare are translated into practice at all establishments in the UK. There is a need for education, access to information and increased funding. Respondents argued that some research institutes are lagging behind current best practice, e.g. grid flooring in marmoset cages that make foraging difficult and insufficient training of animals to co-operate in procedures. It is crucial that experiences leading to improvements in housing conditions and welfare practices are shared amongst the non-human primate community. In this respect, the working group welcome the work of the UK NC3Rs, the European Marmoset Research Group, the European Federation of Primatology and the European Primate Resources Network, all of which aim to promote co-operation and collaborations.

10.6.3 Training

A strong case can be made for increasing the training period of scientists and technicians working with non-human primates, beyond the short course currently given. This should be supported by continual professional development for all those involved with primate research. Improved training could help to facilitate exchange between researchers and breeders so that the animals could be better matched for particular procedures. At the same time such courses should highlight the importance of non-human primate training to co-operate with procedures and so reduce stress and improve welfare.

10.6.4 Transport

The working group consider that improvements could be made in the care of non-human primates during transport. A 2004 report by Prescott & Jennings reviewed worldwide conditions for the acquisition and transport of non-human primates for use in research and the associated ethical and welfare implications.[348] The recommendations of this report could serve as a starting point for improvements in welfare during transport. Given that the majority of imported non-human primates are used for regulatory toxicology purposes, we note that reducing the numbers of animals used in this area would bring significant welfare advantages.

10.6.5 Specialised centres

It may be beyond an individual university's resources to give the required space and enriched environment to ensure optimum non-human primate welfare; this requires specialised centres that are well equipped in terms of facilities and

348 Prescott MJ & Jennings M (2004) Ethical and welfare implications of the acquisition and transport of non-human primates for use in research and testing. *ATLA* **32,** 323-327.

expertise. For this reason, we believe that a case for focusing UK non-human primate research at specialised centres should be seriously considered. Such a system could bring significant advantages. The centre would be led by a specialist primatologist who would have significant input to welfare, training (staff and animals) study design and husbandry developments. For the animals, there would be a reduction in transport-induced stress, continuity of housing and groups, the potential for improved retirement arrangements and the possibility of a reduction in overall use due to a more rational programme of re-use. For researchers, there are advantages of technical expertise, improved co-ordination with animal technicians, shared equipment and economies of scale. There could also be advantages for training both researchers and non-human primates at such centres. The drivers for such a centre should come from primatologists, scientists, veterinarians and core staff.

However, we are conscious of the disadvantages of potentially distancing non-human primate research from researchers' home institutions. Several researchers stressed the importance of being close to their animals and the logistical difficulties of travelling to, or remaining at, a centre to carry out long-term research programmes. There are also advantages in multidisciplinary approaches to science that flourish in a university environment, in which non-human primate research is integrated with clinical studies on humans, *in vitro* methodologies and rodent work. Such a multi-faceted approach, and the development of non-primate alternatives, might be diminished if non-human primate research is confined to specialised centres. We also realise the potential for specialised centres to become a focus of attention from animal rights groups, as the proposal for the Cambridge non-human primate centre showed, and there is an enhanced security risk. An advantage of the centres at Porton Down is that they are well patrolled and guarded and therefore quite different from an academic institution.

At the very least, consideration should be given to alliances and networks between UK centres of non-human primate research, perhaps collectively organised into a 'virtual Centre of Excellence' covering the entire UK. Such a network could also form useful links with non-human primate research institutes elsewhere in the world to improve sharing of knowledge, resources and expertise and to ensure that consistently high standards are implemented.

11 Ethics

11.1 Introduction[349]

Human interactions with animals have fascinated humankind for millennia and the ethics of such interactions have been considered for almost as long.[350] Much recent work relevant to this study has concentrated on what are now often called 'animal rights' approaches to the ethics of using animals in research.[351] Very recently, the Nuffield Council on Bioethics published a detailed report on the ethics of animals in research, which considered, but did not focus on, research with non-human primates.[352] In this section we set out the basis of our approach to the ethical issues raised by research involving non-human primates.

11.1.1 The dilemma

Animals are used in research in the expectation that the results of the work will benefit humankind. Justification of research depends on comparing any suffering caused to animals with the probable benefits to our own species. This means comparing two variables, neither of which can be measured with precision. Many respondents to the call for evidence argued that non-human primates are the best model to address particular research questions because of their close phylogenetic relationship with humans, with whom they share many anatomical, physiological and behavioural features. Other respondents argued that it is precisely this evolutionary link that causes them to object to non-human primate research, since their similarity implies that non-human primates can suffer in the same way as humans. Respondents exhibited a range of moral and ethical stances in response to this inevitable tension.

11.2 Moral status and legal personality

In its thoughtful report the Nuffield Council on Bioethics (NCOB) reviews the ethical issues associated with animal research and the scientific facts as they bear on ethical issues. It contains brief and informative summaries of the relevant moral theories and approaches and is a useful resource for all those who are interested in the ethics of animals in research. Two further considerations, 'moral status' and 'consistency' are discussed, which are important for the approach taken by this working group.

The NCOB report states a belief that *'the debate is not best characterised in terms of the relative moral status of humans and animals but in terms of what features of humans and animals are of moral concern, in the sense of making certain forms of treatment morally problematic'.*

In our law and in our morality, human beings offer different protections to different living beings. The basis of these differences depends on two linked, but connected ideas, namely those of 'moral status' and 'legal personality'.

- **Moral status** refers to an individual's entitlement to the concern, respect and protection of the moral community expressed in behaviour, laws and regulations.
- **Legal personality** refers to the ways in which differences in moral status are reflected in law.

Of course, neither moral status nor legal personality are concepts that can be established empirically. Rather they are theoretical constructs, and judgements about them will always be subject to review. Creatures of the highest moral status are believed to be entitled to the highest level of concern, respect and protection (effectively this applies to competent human individuals). The NCOB report defines the basis of differences in moral status in a similar way[353] and notes *'Beings differ in their moral status if differences in their entitlement*

349 We are grateful to Dr Lisa Bortolotti and Dr Sarah Chan for comments and criticism throughout.

350 Aristotle for example extensively studied animals in relation to humans. He believed humans and animals, in a number of "psychical qualities… differ only quantitatively". (History of Animals, Book VIII. 588a) *The Basic Works of Aristotle,* Richard McKeon Ed. Random House. New York 1968. 634ff. The Stoic philosophers showed related interests; see Richard Sorabji *Animal minds and human morals: the origins of the Western debate* Duckworth, London 1993. See also *Pliny's Natural History,* Harvard University Press, Harvard Loeb Classical Library, Hardcover edition 1940.

351 Regan T (1983) *The case for animal rights* Routledge and Kegan Paul, London.

352 The Nuffield Council on Bioethics *The ethics of research involving animals*.

353 See Box 3.1 on page 39 of the Nuffield Report.

to certain liberties or goods can be justified in a morally valid way'. These morally valid justifications for differential entitlements to certain liberties or goods, when applied to whole classes of beings such as 'animals' or 'humans', are expressive of differences in moral status.[354]

Differences between humans and animals in moral status and legal personality do not necessarily reflect species prejudice (sometimes termed 'speciesism'), since they are applied by the moral community differentially to both human and non-human individuals. For example, most human societies regard it as morally acceptable to kill other humans in war, and to imprison them in various circumstances. In such cases several moral arguments justify overriding individual people's entitlements to life or liberty. In the UK it is considered legitimate, and is legally permissible, to terminate human pregnancy and to experiment on human embryos up to 14 days into development. The justification for treating human embryos or fetuses differently from newborn humans, children or adults is largely due to the fact that such individuals are considered, for reasons believed to be morally valid, to have different moral status and legal personality. Indeed, UK law holds that, at all stages of gestation, the emerging human individual lacks what is termed 'full legal personality', i.e. it lacks the sort of moral and legal status that gives it rights and interests comparable to such individuals after birth. This difference is also accepted in most jurisdictions and has been repeatedly (though somewhat controversially) upheld in the European Court of Human Rights. Some who argue against animal experimentation maintain that a central difference between the use of animals and humans is that animals cannot consent; but nor can human embryos and fetuses (see 11.4.7).

11.3 Moral intuitions

In setting out to discover the elements of an ethical approach to research involving non-human primates, it is important to be aware of what people in general believe about differences between animals and humans (as well as between some human individuals and others) and about how they believe these differences should be reflected in law and practice. The following imaginary scenario helps to reveal these beliefs and to further explain what is meant by moral status:[355]

The 'Hospital Fire' thought experiment

Suppose a major teaching hospital is on fire. As well as the full range of medical specialities treating patients of different ages (with differing life expectancies, quality of life and many other distinguishing features), the hospital also contains other life forms: visitors, health professionals, an animal house (including non-human primates), a maternity and assisted reproductive technology unit with stored embryos and gametes, and – inevitably - the hospital pet cat. For the very fastidious there are also live plants on many of the window ledges and live bacteria and viruses, both *in vitro* and in the bodies of patients and staff. How are we to prioritise rescue for all these different life forms with differing needs and capacities? And more precisely, how can we work out morally defensible priorities for rescue?

The 'hospital fire' thought experiment shows that without knowing (or needing to know) the theoretical basis or ethical justification, almost all humans intuitively make important distinctions about the moral importance of different living things. Most moral theories and traditions provide a combination of evidence and argument that supports and explains these intuitions, purporting to demonstrate why it is morally right and rationally required to make such distinctions, and morally wrong, or even culpable, not to do so.[356]

1. Humans generally, and almost universally, accord a lower priority to all animals than

354 In: The European Court of Human Rights Case of Vo v. FRANCE (application no. 53924/00) Strasbourg 8th July 2004. And most recently in EVANS V. THE UK (Application no. 6339/05) Judgement, Strasbourg, 7 March 2006.

355 In philosophical ethics so-called 'thought experiments' are often employed to sharpen the focus of an argument and to reduce the complexity to a level at which the relevant principles and issues can be clearly identified. We hope this thought experiment is helpful, because it performs the following tasks with economy and clarity: it shows that we all accept principles, of which we may not be explicitly aware, concerning the relative moral importance of different sorts of living creatures.

356 We give examples in 11.5.1. – 11.5.6 below.

they accord to any humans (which means, *inter alia*, that they believe it right to save humans before animals).
2. Humans think it is morally required to sacrifice the lives of animals to save human life (consistency then requires that they should do so – other things being equal – in medical research, as well as in hospital fires).

Humans do not always make these distinctions based on species prejudice, i.e. in favour of members of our own species, but based on an analysis or theory about what justifies such distinctions, which is race, gender and species neutral.

The hospital fire experiment is of course not a perfect analogy for animal research, but it does illustrate two important points. The first is that there are differences in value or in moral importance between different types of individual. The second is that ideas of value or moral importance (however analysed) make intuitive sense. The example assumes that competent human beings are a paradigm case of individuals with the highest moral importance, i.e. that if *any* types of beings have value, then such humans do. Of course it may be that there is some degree of special pleading to this argument and that any species, if it could, would give itself the highest moral priority. Be that as it may, the fact is that almost all human beings do see humans as having a special moral status.

11.4 Costs and benefits

There would be no point in performing a scientific experiment if the result were known with certainty beforehand. Thus there is always an element of uncertainty in predicting whether a particular experiment or series of experiments will benefit humans. Neither the costs of an experiment in terms of animal suffering, nor its potential human benefits, can be measured precisely. However, as enshrined in the Animals (Scientific Procedures) Act, researchers must ensure that suffering is minimised and that the results are likely to justify the costs to the animal.

We cannot have certain knowledge of the feelings of another human being, let alone those of a non-human primate. However, there are a number of criteria by which we can make a reasonable judgement. There is an extensive literature on this issue with regard to animals in general, and the following discussion draws on points raised by the NCOB Report and also by Bateson.[357] Here we emphasize the situation with respect to non-human primates.

1. *Pain*. Pain is a heterogeneous category; deep pain, visceral pain and cutaneous pain have different properties and individuals differ in their responsiveness to them. Some of the factors affecting responsiveness are mentioned below.

2. *Behaviour.* A variety of behavioural indices can be used to assess an animal's response to potentially painful stimuli.[358] Such criteria must be used with caution because of the possibility of analgesic gating: a zebra pulled down by hunting dogs exhibits few signs of pain to the uninitiated. However, efforts to withdraw from the stimulus are a potent source of information, and experiments in which animals are required to choose between two situations can be used to indicate suffering or unpleasantness.[359]

3. *Cognitive abilities and learning capacities*. The more closely an animal's abilities approach those of humans, the more likely is it to experience pain and suffering in the way that we do.[360] In terms of cognitive abilities and learning capacities, the great apes are superior to other non-human primate species, and most Old World primates are better than most, but not all, New World primates. The literature here is

357 Bateson P (1991) Assessment of pain in animals. *Animal Behaviour* **42**, 827-839
358 Thorpe WH (1965) *The assessment of pain and stress in animals*. Report of the Technical Committee to Enquire into the Welfare of Animals Kept under Intensive Livestock Systems
359 Dawkins MS (1980) *Animal suffering: The science of animal welfare.* Chapman and Hall, London
360 McFarland D (1989) *Problems of Animal Behavior.* Longman, Harlow, Essex

extensive.[361] While non-human primates as a group exhibit greater capacities for learning and demonstrate more sophisticated cognitive abilities than almost any other animal, none equals humans.

4. *Anatomy*. Similarity to humans in terms of neuroanatomy and pain receptors indicates a human-like experience of pain. However, as discussed in section 6.3, it is precisely these anatomical similarities that make non-human primates especially likely to yield data relevant to humans.

5. *Reflective self-awareness; sentience*. The issue here is not just response to pain, but the ability to anticipate and reflect upon pain, as well for painful memories to endure after a painful episode. Adult humans' ability to reflect on what they are doing and feeling emerges gradually in the course of development, and the evidence suggests that full self-awareness is not present before two years of age.[362] It is thus probable that there are species, as well as individual, differences in this ability. However, while apes are likely to resemble humans in this regard, both Old World and New World monkeys may differ in degree.[363]

6 *Relatedness*. Relatedness to humans is considered by some to be an important consideration in its own right. Relatedness is used in two senses. The first concerns genetic relatedness: the importance of this is presumably mediated through the similarities in anatomy and cognition mentioned above. The other issue concerns familiarity which, as mentioned above, is an important criterion of the in-group. It is probably for that reason that horses and domestic animals were accorded special rights in earlier laws concerning animal research. It may be that the dependence of domestic animals on human care, and the possibility of individual relationships with them, are also issues. More recently the emphasis has been placed on cognitive and behavioural similarity to humans – hence the special protection for non-human primates and especially the great apes.

11.5 Ethics and research involving non-human primates

The justification that has been most widely used for the use of non-human primates in research depends on comparing the costs in terms of animal suffering against the expected benefits to humans; whether it would be justifiable to sacrifice human lives or welfare for the sake of animals or vice versa. As we have seen, the outcome of this moral calculus involves the assumption that the suffering caused to each animal is less heinous than similar suffering caused to a human being, since if they suffered to the same extent (assuming the numbers involved to be equal) the 'trade-off' between animal suffering and human benefit (or vice versa) would be unacceptable in most circumstances. However, there are of course examples of cases in which we inflict non-consensual harm and even suffering on some humans in order to secure benefits for others. Mandatory quarantine, jury service, compulsory military service and compulsory vaccination programmes are obvious examples.

There is a considerable body of evidence[364] and argument that supports the idea that non-human primates generally, and the great apes in particular, resemble humans more closely than any other living creatures.[365] There are many different reasons and types of theory that purport to explain and justify the relative moral status of humans and animals. Some of the main theories are listed below; we note their relative strengths and weaknesses without endorsing any of them in particular.

361 See for example, Sommerhoff G (1990) *Life, brain and consciousness.* North Holland, Amsterdam.

362 Kagan J (1981) *The Second Year.* Harvard University Press, Cambridge, Massachusetts.

363 Visalberghi E and Fragaszy D (2006) "What is challenging about tool use?" The Capuchin's Perspective. In Wasserman and Zentall (eds) *Comparative Cognition*, Oxford University Press, Oxford 529-554.

364 See for example de Waal F (1996) *Good natured: the origins of right and wrong in humans and other animals* Harvard University Press, London; Cambridge, Mass. ; de Waal F (2002) *The Ape and the Sushi Master: Cultural Reflections of a Primatologist.* Penguin Press Science 2; Peter Singer, ed. *In Defense of Animals*. Blackwell, Oxford 1985; Peter Singer and Paula Cavalieri, eds. *The Great Ape Project: Equality Beyond Humanity*. St Martin's Griffin, New York 1995; Peter Singer, ed. *In Defense of Animals: The Second Wave*. Blackwell, Oxford 2006.

365 With the possible exception of dolphins and some whales.

11.5.1 Personhood theory

This type of theory holds that the highest moral status is possessed by persons (a type of being that includes most humans with legal personality) by virtue of certain capacities they possess.[366] Normally we use the term 'person' as a synonym for 'human being'. However, we are also familiar with the idea that there might be humans who may not be persons or full persons. Human non-persons (in addition to humans who are not fully fledged persons or humans that lack legal personality) include zygotes and embryos, or human individuals who are 'brain-dead', and may include anencephalic infants, or controversially, individuals in a permanent vegetative state.[367]

Persons can be defined in different ways, but most contemporary accounts can be traced back to John Locke, who wrote in his *'Essay Concerning Human Understanding'* (Chapter 27):[368] '*We must consider what person stands for; which I think is a thinking intelligent being, that has reason and reflection, and can consider itself the same thinking thing, in different times and places; which it does only by that consciousness which is inseparable from thinking and seems to me essential to it; it being impossible for anyone to perceive without perceiving that he does perceive.'*

Using this theory, non-human primates, and perhaps other creatures, will be persons if they possess a set of capacities analogous to those identified by Locke. Great apes may be examples of a *'thinking intelligent being, that has reason and reflection, and can consider itself the same thinking thing, in different times and places'.* If so, they would clearly possess interests comparable to human persons and may perhaps also be entitled to similar rights. Insofar as serious doubts remained about whether or not great apes were persons in Locke's sense (or in some other defensible sense), we would perhaps have reasons to 'err on the safe side' and accord them personhood status, and the moral and legal protections that go with it.

Personhood is considered by many to be important because it identifies as crucial those capacities that create some of the most important interests we know of: an interest in continued existence, in autonomy and choice, in freedom and in quality of life. Personhood is not an objective property in the same way as sentience (see below), which is detectable scientifically or empirically. It is a moral and legal term, with a theoretical basis. Importantly though, the class of beings we think of ethically or legally as persons can be modified on the basis of evidence and argument. As John Locke emphasised, the term 'person' is a 'forensic term',[369] which does not identify persons with human beings. This leaves open the possibility that we might find reasons to include some animals as morally, legally and constitutionally protected 'persons'.

11.5.2 Sentience or awareness

In discussions of the moral status of animals, sentience is perhaps the capacity most frequently appealed to as important. Sentience is literally *'the set of capacities that permit awareness through the senses'*, as distinct from, for example, the self-awareness identified by Locke. Although self-awareness comes partly through the senses, it also requires *'reason and reflection'*, which sentience *per se* does not. Sentience can also be said to create interests, in that a creature with the capacity to feel discomfort or pain may be said to have an interest in avoiding those sensations, and a creature with the capacity to see, hear and smell also has an interest in retaining those capacities; certainly it is harmed to the extent that these capacities are damaged or denied. Sentience is then important because it may be said to create interests in animals to which we have moral reasons to respond. It also identifies a range of harms that we may have moral reasons to avoid inflicting on animals. Whether or not animals are harmed by being painlessly killed is a complicated question that depends,

366 See for example John Harris *The Value of Life.* Routledge, London 1985; Mary Ann Warren *Moral Status* Clarendon Press, Oxford 1997; Michael Tooley *Personhood*, in *A Companion to Bioethics*, Helga Kuhse and Peter Singer, eds. Blackwell Publishers, Oxford 1998, 117-127.

367 See for example *Airedale NHS Trust v Bland* 1993 1 All ER 821, 894 HL.

368 John Locke *An Essay Concerning Human Understanding,* First published 1690. We have used the Clarendon Press Edition: A.S. Pringle Pattison Ed. Oxford, Clarendon Press 1924. Book II. Chapter 27.9 p188.

369 Locke *op cit.* Chapter 27.26 p.198.

in part, on how the concept of harm is analysed.[370]

Degrees of sentience are sometimes discussed as if they correlate with the capacity for suffering or with tolerance of pain. There do appear to be differences both within the human community and between humans and other creatures with respect to these things. However, there are no reliable measures that could support prioritising some animals, or indeed some humans, as research subjects on the basis of alleged degrees of sentience defined in terms of capacity to experience pain and pleasure and other responses dependent upon a central nervous system.[371]

Sentience can be relevant to moral status,[372] since sentient creatures are generally regarded as holding higher moral status than non-sentient creatures (such as plants or life forms without a central nervous system) because they have interests that cannot be possessed by non-sentient creatures. However, there appears to be an element of sentimentality as well as rationality in the way that humans apply this logic to practice. As we have observed previously, non-sentient humans, such as those in a permanent vegetative state, tend to be accorded higher status than fully sentient animals.

11.5.3 Intelligence and sociability

While non-human primates are undoubtedly highly intelligent and adaptive, there does not seem to be any compelling evidence (except in the case of the great apes and a few other species) that they may possess 'reason and reflection' of a sort required for personhood and hence for a moral status comparable to most humans. However, their intelligence and ability to learn are resonant of human capacities and incline us to consider their interests more closely than animals that lack such intelligence. Above a certain minimum threshold, intelligence does not appear to confer any enhancement in moral status, the obvious illustration being a universal disinclination to regard highly intelligent persons as possessing more in the way of rights or interests than other humans.

The sociability of non-human primates is often taken as something that makes them more akin to humans than other creatures. This is a suggestion of powerful intuitive force and it suggests the importance of avoiding the single-caging of non-human primates. Sociability is also important as evidence of other capacities that are also required for personhood, and is not morally relevant except as further evidence for the possession of personhood.

11.5.4 Genetic relatedness

Some seem to regard the degree of genetic relatedness to humans as important. Thus chimpanzees, which share 98.5% of their genome with humans[373], are regarded as more morally important than bananas, with which humans share only around 50% of their genes. This seems implausible as a measure of moral status for two reasons. The first is that there are more important differences between bananas and chimpanzees than degrees of genetic relatedness to humans, or to each other (which is only contingently related to intuitively morally relevant differences between bananas and chimpanzees). Secondly, degrees of genetic relatedness within the human community are rightly regarded as irrelevant to moral status or value.

11.5.5 Vulnerability

Protection of so-called vulnerable groups is an established ethical principle that finds expression in some of our leading Human Rights documents and protocols.[374] Some people feel that the principles protecting vulnerable humans should apply even more

370 In such a case there are no interests that can be harmed or compromised but if damage to the organism is a harm then certainly even creatures which lack self-consciousness can be harmed.

371 As argued in Bateson P (1991) Assessment of pain in animals. *Animal Behaviour* **42**, 1991 827-839; Bateson P (1992) Do animals feel pain? *New Scientist* **134**, 30-3; Bateson P (2004) Do animals suffer like us?--the assessment of animal welfare. *Vet J*. **168**, 110-1.

372 Bortolotti L and Harris J, (2005) Stem cell research, personhood and sentience. *Reprod Biomed Online* **10 Suppl 1**, 68-75.

373 Wasserman EA and Zentall TR (eds) (2006) *Comparative Cognition.* Oxford University Press, Oxford. Varki A (2000) A chimpanzee genome project is a biomedical imperative. *Genome Res*. **10**, 1065-70; Olson MV and Varki A (2003) Sequencing the chimpanzee genome: insights into human evolution and disease.*Nature Reviews Genetics* **4**, 20-8.

374 For example UNESCO's *Universal Declaration on Bioethics and Human Rights* Article 8. Respect for Human Vulnerability and Personal Integrity. Adopted by acclamation 19 October 2005 23rd Session of the General Conference of UNESCO. http://portal.unesco.org/shs/en/file_download.php/46133e1f4691e4c6e57566763d474a4dBioethicsDeclaration_EN.pdf Accessed 7th January 2005.

strongly to animals, given their inability to adequately protect themselves from humans and indeed from other predators, parasites and diseases. Applying the vulnerability criterion leaves open the question of entitlement to protection and it makes no distinction between humans, primates, mammals, insects, plants, viruses and so on.

11.5.6 Ethical awareness

Some commentators have noted the capacity of some non-human primates to show behaviour suggestive of awareness, concern, sensitivity to ethical concepts or the capacity to have ethical reactions.[375] As with other capacities that admit of degrees, ethical awareness raises threshold issues. Is it simply ethical awareness beyond a certain minimum threshold that raises questions of moral status or is it 'the more the better', with the implication that creatures (including humans) possessing greater ethical awareness have the greater moral status? In the case of humans, most ethical systems (and indeed most theories of human and legal rights) operate with an egalitarian threshold approach: this holds that all individuals who come within the scope of the principle are equal from the perspective of rights and interests.

Applying such an approach to animals would require threshold issues to be addressed. For example, a feature of most moral principles is that they are both of universal application (within the class of individuals to whom they apply) and generalisable (i.e. that those who show ethical awareness of other creatures recognise that the reasons for applying such ethical sensitivity extend to all similar individuals). Ethical awareness is thus a dimension of personhood (see above).

11.5.7 Consent

Since consent is a decisive factor in medical and biological research with humans, objectors to animal research make a reasoned case. If the prime concern with humans is the interests of the individual, why, they ask, should this not equally apply to other animals? Informed consent - the almost universally respected principle of research ethics - is apparently violated in animal research.

While it is true that animals cannot give informed consent, they cannot give informed refusal either. This is not to say that they cannot forecast danger, apparently communicate their fear or disapproval, or struggle to avoid compliance. On the other hand, for almost all experiments involving conscious non-human primates it is essential that the animals are calm and co-operative; while this may be taken to indicate that they are not significantly distressed this cannot be taken as equivalent to informed consent.

The same is true of those humans who cannot give informed consent, i.e. young children and adults who lack competence. In the case of incompetent humans where consent is impossible, we use another test for the legitimacy of research or treatment, that of 'best interests'. We ask: is the proposed course of action in the interests of the individual who cannot consent? If it is, then even though consent is not possible, the action may be permissible and sometimes even mandatory. The key issue is thus not one of consent. In the case of non-human primates, if it is right to claim that they have a different moral status to human beings then a balance must be struck between their interests and the interests of the humans who might benefit from the proposed research. The test of the justifiability of research with non-human primates is therefore whether (or not) the research is justifiable when the costs to the non-human primates are set against the benefits to humans; informed consent is simply not the relevant issue.

11.6 Discussion

We accept, for reasons identified elsewhere in this report, the importance of well-founded scientific research that will, with high probability, be of serious benefit to human

375 Frans de Waal for example is prominent among theorists who emphasize this capacity. See his *Good natured : the origins of right and wrong in humans and other animals* Harvard University Press, London; Cambridge, Mass. 1996; Frans de Waal *The Ape and the Sushi Master: Cultural Reflections of a Primatologist* Penguin Press Science, 2002; Jane Goodall *Through a Window: Thirty Years with the Chimpanzees of Gombe* Weidenfeld and Nicolson, London 1990.

beings. The justification for the continued use of non-human primates in research is that their use is required lest greater harm occur. This is an application of perhaps the most basic ethical and medical principle of 'do the least harm possible'. This reasoning depends upon a difference in self-awareness, cognitive awareness, cognitive capacities and sentience between most non-human primates and most humans, certainly between the non-human primates that are used in UK research and the humans that will benefit.

One issue often neglected here is the fact that the numbers of non-human primates used in any medical experiment are very small, and that the number of humans whose suffering is ameliorated is often very large. So the equation to be made is not simply between suffering caused and benefits to humans: both sides must be multiplied by the number of individuals involved. In the case of AIDS research, for instance, the number of macaques used may be measured in dozens, the number of humans who stand to benefit could be measured in millions. Suffering or harm caused in animal experiments, both in terms of numbers and in terms of degree, is likely to be less than the benefits to humankind from properly licensed research carried out with meticulous care.

Jeremy Bentham famously observed, of all who share the same moral status: *"each is to count for one and none for more than one"*. His utilitarian approach could be held to allow for trade-offs, by which some individuals can be made to suffer in order to improve conditions for others. We accept such trade-offs frequently, for example in imprisoning offenders, or even in requiring citizens to undertake jury duty. But medical research chooses an altogether different approach. The universal standard is that no individual may be harmed for the betterment of others.

How then, can this be applied to research involving animals, and how can it be reconciled with Jeremy Bentham's stricture? The answer to this question depends on two issues. First the evidence indicates (though it cannot prove) that a given procedure carried out on a non-human animal would result in less suffering than a similar procedure carried out on a human being. Second, the number of animals used in experiments is much less than the number of humans expected to benefit. However, since non-human primates have more sophisticated cognitive capacities than other animals, we should be exceptionally careful to reduce the numbers of non-human primates that are subjected to discomfort or distress so far as is compatible with the alleviation of human suffering.

The members of this working group share the view that the continued use of non-human primates in research is therefore morally required, so long as such research is directed towards significant human benefit and there are no plausibly more effective ways of pursuing such research. The alternative is to permit continued suffering to very large numbers of humans which might be alleviated or indeed removed by a careful, well monitored and meticulously regulated programme of animal research, including research with non-human primates.

12 Discussion

12.1 A polarised debate

The central goal of this enquiry has been to examine the scientific case for the continued use of non-human primates for research into the treatment or prevention of common diseases, or for more fundamental research that has the long-term potential of achieving the same end.

One of the main problems encountered during the preparation of this report has been the fragmentation and diversity of the evidence. Conflicting statements were received from different sources, including organisations or individuals that are opposed to either non-human primate research in particular or to any form of animal research, as well as from individual scientists, academic and regulatory bodies, government agencies, industry, and others. Opinions were often offered without background references, and in some cases, the same references were interpreted very differently by different constituencies. Hence, to achieve anything approaching a balanced view of this information, it was essential to refer to the original source references to try to interpret these diverse views.

12.2 The pace and unpredictability of biomedical research

For a variety of reasons, it was not felt appropriate to try to assess the overall requirement for the use of non-human primates in biological and medical research in the future. There is clear evidence that a good deal of research is moving from the study of intact humans or animals to the analysis of biological function and disease at the cellular and molecular level. However, the likely need to take a more holistic and 'intact-organism' approach to these problems, and the pace and unpredictability of research, means that new requirements for non-human primates in the future cannot be excluded. For example, the development of methods for non-invasive imaging of the human brain have moved so rapidly that their role in replacing invasive non-human primate procedures is not yet clearly defined.

In short, the biological sciences are moving so quickly and in so many directions that it is simply not possible to predict the requirement for non-human primates and other animals for research in the immediate future. What is clear is that it would be unwise at the present time to take any blanket approach to preventing the use of non-human primates for biological and medical research; a move of this type could have a deleterious effect on current areas of critically important research.

In this context, although overseas work on great apes has been described in several places in this report, the working group saw no reason to change the current policy regarding research of this type in the UK. It did seem important, however, to clarify our current position. What would happen, for example, if an outbreak of a devastating infection caused by a newly emerging organism could only be studied in great apes? Would this country have to rely on expertise in the use of these animals for research that had been retained in other countries? Is the UK's position entirely logical, and indeed, ethical in this respect? This clearly requires further discussion and clarification.

12.3 A lack of integration in non-human primate research

There were other reasons for the fragmentation and complexity of the evidence assessed during this study. Although the efforts of individual scientists in explaining the role of non-human primates in their work were often impressive, it did not appear that their associated learned societies were active in discussing, in more general terms, how changing research technologies might affect the need for continued

non-human primate research. There seem to have been few efforts to determine whether the closer integration of research groups within different fields, or centralisation of their activities, might have the same effect. There also appeared to be scope to improve interactions between the various international regulatory bodies, the scientists involved, and those who promote alternatives to animal research.

Probably for historical reasons, the lack of coordination of the diverse players within different research fields appears to hinder the most efficient (and therefore most limited) use of non-human primates in research and, to some extent, slows the evolution of improved approaches to the welfare of the animals involved. There is clearly a need for rationalisation and much better integration of these activities.

Considering the complexity of these issues, we also wondered how it would be possible for the public at large, regulators and government to become sufficiently well informed so that a more rational level of debate could be both achieved and maintained in this emotive field.

12.4 The anecdotal nature of some of the issues in this report

The working group is well aware of some of the statistical deficiencies in this report. For while it was possible to cite individual cases for the benefits that have come from research using non-human primates, and there was a clear scientific case for the continued use of these animals in many of the current research areas that were analysed, through lack of appropriate data it has not been possible to provide an overall picture of the outcomes of research of this kind over recent years.

The differences inherent to retrospective analyses of the origins of research leading to important scientific or medical discoveries were outlined briefly in section 4 with respect to the very limited number of studies that have attempted to define the relative roles of basic and applied science in major medical advances. Particular problems include lack of objectivity in deciding the merits of a particular discovery, obtaining accurate retrospective information about the many scientific routes to a particular discovery, and numerous other difficulties. For example, in the classical investigation of Comroe and Dripps, cited in section 4, an investigation of the scientific origins of electrocardiograms involved the analysis of work stretching back to ancient Greece!

Given the sensitive nature of research involving non-human primates, however, and allowing for the intrinsic difficulties involved, the working group did conclude that a more systematic study of the outcome of research of this type over the last 10 years would be an extremely valuable addition to the complex debate about the future of this work. A starting point for a study of this type could be the final reports handed into the major granting bodies who support this work from the scientists involved in the particular projects. A study of this kind would also require interaction between the funding bodies and the regulatory bodies, particularly with respect to the amount of suffering caused to the animals in each research project.

Particularly in view of the much greater use of non-human primates in drug toxicology, the group also considered that a similar study of the outcome of toxicological studies should be initiated.

12.5 The need for wider public debate

The subject of this report is only one facet of the much broader question of how the many ethical and social issues posed by modern biomedical science can best be transmitted to the public and government. Essentially, there are two major pathways involved. First, there is the interaction between scientists and the media and hence with the public. Second there

are the more direct forums whereby scientists can discuss their work directly with the public. The main links between scientists and the media are through bodies such as the Science Media Centre and the public relations staff of universities, learned societies and medical Royal Colleges. There are very few organisations that ensure regular meetings between scientists and the media. In this respect, thought should be given to the reinstitution of bodies such as the BBC Science Council, an organisation through which scientists met regularly with representatives of the media that was disbanded in the 1980s.

The more direct links between the scientists and the public include the Office of Science and Innovation's Science and Society and Sciencewise programmes, the British Association for the Advancement of Science, lectures and demonstrations organised by the learned societies, and local arrangements between individual universities for events such as open days or public lecture series.

In view of the very rapid developments in the biological sciences, and the continued concern about animal research in general and research in non-human primates in particular, it would be particularly helpful if the bodies concerned focussed on this particular problem in planning their public engagement programmes over the next few years.

12.6 The future of UK non-human primate research

It is clear that UK non-human primate research is held in high international regard. However, throughout this study, we heard claims that the future of such research in the UK is threatened by a number of factors, including a climate of intimidation created by some opponents to animal research, a shortage of available animals and the high costs of the research compared with other countries.

The intimidation, and sometimes violence, associated with the activities of some animal rights activists is likely to be a significant disincentive to younger researchers who might enter the field. Anecdotal evidence indicates that recruiting postdoctoral scientists or more senior academic staff is becoming increasingly difficult, with very few recruitments into UK academic non-human primate research in the recent past. We note that this problem is not unique to the UK, with recent reports of a US neuroscientist abandoning his work on non-human primates after years of terrorisation by opponents of animal research.[376] The working group strongly condemns any actions of harassment, violence or intimidation. In addition to the personal costs to those who are targeted, fears of attacks from extremists have detrimental effects on non-human primate welfare by restricting the environments in which animals are housed and transported, the prevention of outdoor housing and air travel, for example.

Throughout the course of the study, researchers asserted that there is a critical shortage of non-human primates for use in research in general and for HIV/AIDS studies in particular. Evidence from the Home Office Animal Inspectorate confirmed that, while the supply of New World monkeys is relatively satisfactory, the current demand for Old World monkeys outstrips supply. This may have several undesirable knock-on effects, for example in reducing the number of animals used in research programmes to the point were the results are statistically underpowered.

UK researchers claim that these factors are driving non-human primate research overseas. While it is difficult to show this quantitatively, the working group shares the view of many respondents to the call for evidence that, if non-human primate research is deemed to be important, the skills and capacity to conduct such research should be retained in the UK. There are several reasons for this. First, it retains control of welfare standards in accordance with what is widely thought to be the most stringent regulatory framework

376 Editorial (2006) Resisting terrorism. *Nature* **443**, 122

anywhere in the world. Second, research can be carried out according to priorities set by the UK public and scientific community. Third, evidence from pharmaceutical companies testifies to the value they place on the strength of academic collaborations with UK non-human primate researchers; retaining strength in this area therefore gives the UK a clear research and commercial advantage.

We believe that, if the UK considers non-human primate research to be important, we must address how it can be supported in terms of supply, methods and cost effectiveness. Such research cannot be carried out on a short-term, needs-driven basis; successful non-human primate work requires skilled teams with years of experience of the science, husbandry and welfare of the animals. There is an urgent need for all the stakeholders involved in non-human primate research to work together in formulating a national strategic plan for non-human primate research, addressing issues of supply and demand over the nearer and longer term. This could incorporate the establishment of specialised centres or virtual networks, as proposed (see 10.6.5).

12.7 Future action based on this report

Finally, during our investigation, we became increasingly convinced that, although important work directed at the control of human suffering would not be possible without the use of non-human primates, current approaches to reduce the numbers of animals involved and raise standards of welfare could be improved. Hence, as well as providing what we hope is a better documented basis for future debate on the use of non-human primates in research, **we urge the bodies that sponsored this study, through their connections with the appropriate specialist scientific societies, government, and regulatory agencies, to work to activate the recommendations of this study and, importantly, to monitor progress in achieving these ends over the next few years.**

13 Conclusions and recommendations

Is the continuing use of non-human primates in fundamental biological and medical research justified?

Conscious of the extreme complexity and polarisation of the arguments for the use of non-human primates in research, and sceptical about the claims of the protagonists at both extremes of this discussion, this study has focused on whether the continued use of these animals for research is required to solve some of the most important scientific and medical problems that face us in the 21st Century.

As a background to these investigations, the distinction between fundamental (or basic) science and research applied directly for the control of human disease was discussed. While this distinction may have been useful in the past, the cross-disciplinary developments in the biological sciences over recent years leads us to conclude that it is becoming less valid. Rather, the biological sciences now form a continuum ranging from basic to applied research, with one continuously feeding off the other. Knowledge of normal biological function is essential if abnormal function in disease is to be understood.

After considerable initial uncertainty on the part of the working group, it became clear that there is a valid scientific case for the continued use of non-human primates in order to make progress in fields of research that have the potential to improve human health in both developing and developed countries. In short, the use of a relatively small number of animals has the potential to save many thousands of lives. Whilst this analysis has been extremely complex (as in all fields of science, there is always disagreement about the most effective way forward), members of the working group are convinced that, for some areas of biological and medical research, the continued use of non-human primates is essential, at least for the foreseeable future. We emphasise that, as enshrined in the Animals (Scientific Procedures) Act, all cases must be judged individually according to a rigorous assessment of the costs and benefits.

Recommendation 1
There is a strong scientific case for the carefully regulated use of non-human primates where there are no other means to address clearly defined questions of particular biological or medical importance.

The role of non-human primates in communicable disease, neuroscience, reproductive biology and ageing research.

Excluding the use of non-human primates in regulatory toxicology, which was outside our terms of reference, the conclusions of this report are based on a study of the role of non-human primates in those fields of research where their use is currently greatest, i.e. neurosciences, communicable disease, reproductive biology and ageing. Particular attention has been paid to the biological or medical importance of non-human primate research programmes in relation to other available approaches.

There is widespread agreement among the international health community that the long-term control of some of the major communicable diseases, including HIV/AIDS, malaria and tuberculosis, will only be achieved by the development of vaccines. However, this is proving to be extremely difficult. Currently, the pattern of many vaccine development programmes consists of extensive experimental work in rodents, followed by exposure of candidate vaccines to a limited number of non-human primates to investigate efficacy and potential toxicity, before moving to human trials.

The limitations of immunogenicity testing in rodents mean that testing in non-human

primates is often an important step in avoiding unnecessary large-scale clinical trials in humans with non-immunogenic vaccines. In addition, although pilot studies are carried out with human volunteers whenever possible, until the ideal immunogens, virus vectors and adjuvants are identified, in many cases it will remain important to assess safety in non-human primates before progressing to clinical trials in humans.

Given the vital role of non-human primates in some aspects of infectious disease research, particularly with regard to vaccine development, we therefore consider there to be a strong case for maintaining the use of non-human primates in this field. Furthermore, as evidenced by the recent appearance of SARS, there is a constant risk of devastating epidemics of infection by other newly emerging organisms. It is vital that expertise in the use of non-human primates is maintained, if rapid responses to these new infectious agents are to be effective.

A better understanding of the mechanisms that underlie the normal functions of the brain is one of the major goals of current biological research. As well as its intrinsic importance for the better understanding of human biology, such research has the proven potential to identify causes of neurological disease and elucidate better approaches to diagnosis and management. We are convinced of the importance of the controlled, experimental animal model in determining cause and effect relationships between neurological structures/processes and function (or dysfunction in disease). Given that non-human primates are the only group of animals with brain circuits and networks that are really similar to those of humans, we consider there to be a strong case for their continued use in some aspects of fundamental neuroscience research.

Non-human primate research has contributed to progress in understanding the causes of common neurological diseases such as Alzheimer's disease, Parkinson's disease and stroke. Rodent models and non-invasive imaging studies in humans are leading to major advances in understanding the pathogenesis of these disorders, but it would be premature to draw definitive conclusions about the adequacy of these techniques in defining the complex mechanisms of neurological function. There is increasing evidence that some of this research will require validation in non-human primates in the future, particularly in demonstrating functional, as well as physiological, outcomes during testing of potential therapies. Similar to vaccine development, in some cases non-human primates may still be the only means of providing insurance against the failure of lengthy and expensive human trials of new therapies for neuro-psychiatric diseases.

The working group identified important areas of research in the fields of reproductive medicine and developmental biology where there is case for the continued use of non-human primates, at least for the immediate future. However, the group found arguments for expanding non-human primate research to better define differences in common disease incidence between the sexes to be less convincing. In the field of ageing research, the working group did not find there to be a strong case for using non-human primates in long-term calorie restriction studies. However, there may be a valid future role for non-human primate studies in extending the information currently being derived from small model organisms of ageing to assess its relevance to humans.

Recommendation 2
In the fields of research considered in this study, namely communicable disease, neuroscience and reproductive biology, there is a strong scientific case for maintaining the use of non-human primates in some aspects of this work, at least for the immediate future.

Assessing the case for the use of non-human primates in biological and medical research

The biological and medical sciences are passing through a period of unprecedented technological development. With regard to obtaining a fully integrated view of biological function, in most fields it is too early to assess the relative roles of molecular and cell biology, non-invasive human investigation and mathematical/systems approaches, compared with whole animal studies. Hence, we have concluded that it would be unwise to make any blanket decisions about the future requirements for non-human primates for research; each case has to be examined individually against this background of rapid change.

All those involved in non-human primate research must ensure that their decisions are supported by an ongoing assessment of the biological or medical importance of the work, including approaches that do not require the use of non-human primates, together with consideration for every aspect of the welfare of the animals involved. However, the remarkable speed of development in biological research, particularly the major advances in molecular and cell biology, will make it increasingly difficult for individual ethical, peer review and regulatory bodies to maintain the breadth of knowledge required to make fully informed judgements about individual cases of non-human primate research. We therefore consider that specialist research societies, funding agencies and regulatory bodies should increase their efforts towards coordinating and constantly reviewing the need for non-human primate research.

Although it has been possible to identify the scientific or medical value of many individual pieces of research involving non-human primates, it has been difficult to assess the overall efficiency and impact of research of this kind. There are many pitfalls and difficulties involved in assessing the outcome and value of scientific research, but there is no doubt that an ongoing debate on the use of non-human primates in research would benefit from more systematic information on its overall impact on scientific and medical advances.

Recommendation 3
The major specialist organisations involved in research fields that utilise non-human primates, particularly neuroscience, communicable disease, and reproductive and developmental biology, should regularly collate information about evolving research technology in their fields, with particular respect to the need for non-human primates. This information should be disseminated to funding bodies, ethics committees and regulatory agencies.

Recommendation 4
As part of their ongoing programmes to assess the outcomes of their research, the major funding organisations should undertake a systematic review of the outcome of all their research using non-human primates supported over the last decade.

Working towards non-human primate alternatives

There is an impressive body of work directed at finding alternatives to non-human primates and other animals for both medical research and toxicology studies. While some of it has already borne fruit, it is too early to assess the time that will be required for many of these projects to achieve their goal. Although we were impressed and encouraged by the numerous approaches to evolve non-human primate alternatives for both research and toxicology studies, it is clear that many of them are at an extremely early and tentative stage of development. Sustained support and funding on the part of government and other research funders will be necessary to ensure that these approaches come to fruition.

There is also a growing body of work on the development of refinements for non-human primate research practices. However, the working group consider there to be scope for a significant expansion in such research, particularly in relation to behavioural neuroscience procedures.

Recommendation 5
UK research funding organisations, both governmental and charitable, should continue to take every opportunity to encourage and fund research into developing alternatives to the use of non-human primates for both research and toxicology. Funders should expand their support for research into refining non-human primate research practices, particularly in the behavioural neurosciences.

Improving non-human primate welfare

The study's limited examination of issues relating to the welfare of non-human primates in biological and medical research suggests that there are still a number of areas that require examination and improvement. This is an area of active investigation on the part of several organisations, notably the APC and NC3Rs. We restrict our recommendations to reporting procedures, training, publication of welfare information and housing.

Recommendation 6
Retrospective reporting on the severity of procedures for non-human primates, as recommended by the LASA/APC pilot study, should be introduced as soon as possible.

Recommendation 7
Improvements in the supervised continuous training of research workers in non-human primate research should be instituted.

Recommendation 8
Scientific journals should include details of animal welfare and steps taken to ameliorate suffering in all published papers that involve non-human primate research

Recommendation 9
Work should be accelerated towards improving and applying current best-practice regarding housing of non-human primates, including minimum cage size, an emphasis on the avoidance of single housing, how cage fittings and conditions can be accommodated to the purpose of individual experiments, and a better assessment of the advantages of outside access and visual stimulation.

The role of non-human primates in regulatory toxicology

Although not strictly in the remit of this report, the use of non-human primates in regulatory toxicology is closely associated with their use in hypothesis-driven research. The working group therefore considered it important to investigate a few aspects of the use of non-human primates in drug discovery and development.

We consider that interactions between regulatory bodies at national and international levels and co-ordination of the activities of regulators and scientists could be improved. Efforts to co-ordinate regulatory toxicology requirements and guidance, particularly in validating and standardising non-animal alternatives are welcome, but more could be done. We support calls from the Nuffield Council on Bioethics for this issue to be prioritised by the UK National Coordinators at the Organisation for Economic Cooperation and Development (OECD).

We also support the recommendations of the 2002 APC report, noting that the call for greater re-use of non-human primates in toxicology

testing raises complex questions around the balance between the level of suffering of individual animals and the overall number of animals involved. Despite the government's assurances with regard to generic licences, we wonder how local ethical review panels can properly assess the use of non-human primates in these circumstances.

It is clear that maintaining drug safety whilst reducing the number of animals used in toxicological testing is a challenge for pharmaceutical companies and regulators, particularly in the context of an increasingly litigious climate. While we have no evidence of unnecessary overlap and repetition of non-human primate safety tests, it is difficult to draw firm conclusions on whether the numbers of non-human primates used are justified. In this regard we strongly support the current study being undertaken by the NC3Rs and the Association of the British Pharmaceutical Industry (ABPI), which aims to identify means of minimising primate use in drug discovery and development.

The debate around the availability of information on pre-clinical testing of potential drugs and therapies is complex and extends much further than the use of non-human primates. However, we consider that improving the accessibility and availability of pre-clinical information from non-human primate studies would be of significant social and economic benefit. Not only would it would serve as a safeguard against any unnecessary repetition of non-human primate toxicological studies, but it would greatly improve the basis on which decisions to progress to human trials are taken and could prevent trials of non-efficacious or worse, unsafe, medicines. This reflects recent initiatives to improve access to information about ongoing, completed and published human clinical trials and the WHO's widely supported objective to ensure that all clinical trials are registered at inception and that results are made publicly available. The wider availability of pre-clinical information from non-human primate studies would also serve a valuable purpose in aiding the ongoing assessment of their role in toxicological studies.

Recommendation 10
Further efforts should be made to improve interactions between regulatory bodies at national and international levels and between regulatory bodies and the scientific community. Given the current speed of research in the biological sciences, new approaches to improve these interactions are urgently required.

Recommendation 11
Steps should be taken to make the results of toxicological studies involving non-human primates publicly available, in the same way as initiatives to register and publish the results of all human clinical trials.

Recommendation 12
It would be premature to make firm recommendations on how a reduction in the number of non-human primates used in regulatory toxicology might be achieved before the completion of the NC3Rs/ABPI study. However, we urge government and other stakeholders to act on the recommendations of this study, and in the light of its findings, to re-examine responses to the 2002 APC report.

Promoting a strategic and integrated approach to UK non-human primate research

The relatively high cost of non-human primate research, together with harassment from extremists and administrative problems, are perceived to be causing considerable difficulty for non-human primate scientists working in the UK. We believe that, if non-human primate research is considered to be important, the UK must address how it can be supported in terms of supply, methods and

cost effectiveness. Such research cannot be carried out on a short-term, needs-driven basis.

All the stakeholders involved should work together in formulating a national strategic plan for non-human primate research. This should address issues of supply and demand in the short and longer term and include a re-evaluation of the organisation of non-human primate research facilities. In this respect, we urge consideration of the creation of UK non-human primate research centres of excellence, perhaps starting with the development of 'virtual' networks between existing facilities.

Recommendation 13
Concerns that costs and harassment by activists are forcing scientists and research companies to pursue non-human primate work overseas require urgent examination by the relevant UK research funding and regulatory bodies.

Recommendation 14
The major funding bodies, together with government, other stakeholders, scientists, primatologists, vets and welfare specialists, should give careful consideration to the creation of UK centres of excellence for non-human primate research.

Promoting constructive debate

The evolutionary proximity of non-human primates to humans leads to particular concern and uncertainty about the acceptability of their use in biological and medical research. Unfortunately, views on this issue have become polarised; abolitionists often present their case as though no harmful consequences would result from abandoning this work, while scientists sometimes claim that prohibiting non-human primate research would entail the sacrifice of an unacceptable amount of knowledge and potential improvement in human health.

Clearly, a much better-informed and ongoing debate is needed by all the parties involved. For instance, while the working group saw no reason to change the current UK policy regarding research in great apes, the potential emergence of a devastating infection that could only be studied in these animals raises questions that require a mature level of debate.

It is beyond the scope of this report to recommend in detail how a better-informed public debate might be achieved, and even more importantly, sustained. It will need to involve the media, groups attempting to develop closer relationships between the public and universities, animal welfare groups, anti-vivisection organisations, research funders, bodies that govern both science in general and its different specialities, the British Association for the Advancement of Science, the Science Media Centre, and many more. While interaction between the media and scientists is satisfactory, it works on an ad hoc basis and there are relatively few forums for regular discussion such as the successful, but now disbanded, BBC Science Council.

The extra investment announced in the government's in 2004-2014 Science & Innovation Investment Framework promises to energise public engagement around science and technology. We hope that issues around the use of animals, specifically non-human

Recommendation 15
All bodies involved in engaging the public around issues of science and medicine, including the UK government, should ensure that the whole field of research utilising animals, including non-human primates, has a major place in their future programmes. Given the extremely rapid pace of development in the biological sciences, mechanisms for regular meetings between scientists and the media should be further explored

primates, in research are prioritised in the Office of Science & Innovation's Science and Society and Sciencewise portfolios.

Next steps

We welcome this initiative on the part of the sponsoring bodies to review the current and future use of non-human primates in biological and medical research. We strongly encourage future activities of this kind. Although important work directed at the control of human suffering would not currently be possible without the use of non-human primates, this must be subject to ongoing review against emerging alternative approaches and technologies, together with sustained efforts to reduce the numbers of animals involved and raise standards of welfare.

Recommendation 16
The bodies that sponsored this study should establish a mechanism for monitoring progress in achieving the aims of these recommendations over the next few years.

Appendix I Report preparation

Working group

This report was prepared by a working group drawn from outside the active non-human primate research community. Members participated in a personal capacity, rather than as representatives of their organisations.

Chair

Sir David Weatherall FRS FMedSci (Chair)
Regius Professor of Medicine Emeritus, University of Oxford; Chancellor, Keele University
Molecular medicine, haematology, tropical medicine

Members[377]

Dr Peter Goodfellow FRS FMedSci
Senior Vice-President, Discovery Research, GlaxoSmithKline
Mammalian development, sex determination, drug discovery and development

Professor John Harris FMedSci
Sir David Alliance Professor of Bioethics, University of Manchester
Ethics of genetics, transplantation, reproduction

Professor Robert Hinde CBE FRS FBA
Sub-Department of Animal Behaviour, University of Cambridge
Ethology, psychology

Professor Dame Louise Johnson FRS
David Phillips Professor of Molecular Biophysics, University of Oxford
Structural and cellular biology, protein crystallography and electron microscopy

Professor Richard Morris FRS FRSE FMedSci
Professor of Neuroscience, University of Edinburgh
Neuroscience of learning and memory, neurodegenerative disease

Mr Nick Ross
Broadcaster
Health and bioethics, science policy, community safety, environment

Sir John Skehel FRS FMedSci
Director, MRC National Institute for Medical Research
Virology, microbiology, influenza

Sir Crispin Tickell
Director of the Policy Foresight Programme at the James Martin Institute for Science and Civilization, University of Oxford
Environment, climate change, world affairs, sustainable development

377 The Chair and members are grateful for the contribution of Professor Anthony Nash FRSE FMedSci, who withdrew from the working group for health reasons after the fifth meeting.

Secretariat

Dr Helen Munn
Policy Manager, Academy of Medical Sciences

Dr Aileen Aherne
Policy Officer, Academy of Medical Sciences

Dr Simon Edwards
Policy Manager, Royal Society

Review group

This report was reviewed by an independent group appointed by the Academy of Medical Sciences, Royal Society, Medical Research Council and Wellcome Trust. Reviewers were asked to consider whether the report met the terms of reference and whether the evidence and arguments presented in the report were sound and supported the conclusions. Reviewers were not asked to endorse the report or its findings.

The Earl of Selborne KBE FRS (Chair)
Chancellor, University of Southampton

Professor Brian Greenwood CBE FRS FMedSci
Professor of Clinical Tropical Medicine, London School of Hygiene & Tropical Medicine

Professor Tom Jessell FRS FMedSci
Professor of Biochemistry and Molecular Biophysics, Columbia University

Professor Peter Lipton FMedSci
Head of Department of History & Philosophy of Science, University of Cambridge

Professor David Perrett FBA FRSE
School of Psychology and Centre for Social Learning & Cognitive Evolution, University of St Andrews

Professor Torsten Wiesel ForMemFRS
President Emeritus, The Rockefeller University, New York

Appendix II List of consultees and respondents to the call for evidence

Organisations

Animal Science Group, Biosciences Federation
Association of the British Pharmaceutical Industry
Biotechnology and Biological Sciences Research Council
Biomedical Sciences, Defence Science and Technology Laboratory
British Heart Foundation
British Union for the Abolition of Vivisection
Department of Health
Dr Hadwen Trust
Europeans for Medical Progress
Foundation for Comparative Biology, USA
Fund for the Replacement of Animals in Medical Experiments
General Medical Council
Genetic Interest Group
Health Protection Agency
Institute of Neurology, University College London
International Brain Research Organisation
London School of Hygiene and Tropical Medicine
Medicines and Healthcare products Regulatory Agency
National Centre for the Replacement, Refinement and Reduction of Animals in Research
National Primate Research Centre, Wisconsin, USA
National Institute for Biological Standards and Control
Parkinson's Disease Society
Pfizer
Royal College of Pathologists
Royal College of Physicians
Royal Society for the Prevention of Cruelty to Animals
Society for Accountability of Animal Studies in Biomedical Research and Education
Universities Federation for Animal Welfare
University of Cambridge
University of Oxford
Universities UK

Individuals*

Dr Ian Addison
Dr Alistair Buchan, Professor of Clinical Gerontology, University of Oxford
Dr Hannah Buchanan-Smith, Department of Psychology, University of Stirling
Dr Tim Crow FMedSci, MRC External Scientific Staff, Department of Psychiatry, Oxford
Paul Dean, University of Sheffield
Dr Barry Furr FMedSci, CEO, Astra Zeneca
Professor J Hau, Department of Experimental Medicine, University of Copenhagen
Sir David King FRS, Office of Science and Innovation
Professor Roger Lemon FMedSci, Director, Institute of Neurology, University College London

*A further 8 individuals who submitted evidence asked for their name to be withheld.

Professor John Martin, Professor of Cardiovascular Biology, University College London
Dr Philip Minor, Deputy Director and Head of Virology, NIBSC
Professor John Newsom-Davis, Emeritus Professor of Clinical Neurology, University of Oxford
Professor Andrew Parker, Professor of Physiology, University of Oxford
Dr Mark Pettigrew, Associate Director, MRC Social & Public Health Sciences Unit
Dr Christopher Pryce, Behavioural Neurobiology Laboratory, Swiss Federal Institute of Technology, Zurich
Dr Rosalind Ridley, University of Cambridge
Professor Trevor Robbins FRS FMedSci, Professor of Cognitive Neuroscience, University of Cambridge
Professor Jonathan Seckl FMedSci, Professor of Molecular Medicine, University of Edinburgh
Dr Jayastree Sengupta, Primate Implantation Biology Laboratory, All India Institute of Medical Sciences, New Delhi
Dr Stuart Shipp, Department of Anatomy, University College London
Professor Adam Sillito, Department of Visual Science, University College London
Professor John Stein, University Laboratory of Physiology, University of Oxford
Dr Bert 't Hart, Chairman of Immunobiology, Biomedical Primate Research Centre, Netherlands
Professor Hermann Waldmann FRS FMedSci, Head of Department, Sir William Dunn School of Pathology, University of Oxford
Paul Watkins, Director of Biological Services, Kings College London
Professor Andrew Whiten, University of St. Andrews
Dr Sue Wilson, Psychopharmacology Unit, University of Bristol
Ms Sarah Wolfensohn, Supervisor of Veterinary Services, University of Oxford

Oral evidence[378]

Professor Tipu Aziz, Consultant Neurosurgeon, Radcliffe Infirmary, Oxford
Professor Michael Balls CBE, Trustee, Fund for the Replacement of Animals in Medical Experiments
Professor Oliver Braddick FMedSci, Head of Experimental Psychology, University of Oxford
Dr David Buist, Animal (Scientific Procedures) Inspectorate
Professor Richard Frackowiak FMedSci, Principal Investigator, Functional Imaging Laboratory, University College London
Dr Derek Fry, Animal (Scientific Procedures) Inspectorate
Dr Robert Hubrecht, Deputy Director, Universities Federation for Animal Welfare
Dr Leslie Iversen FRS, Department of Pharmacology, University of Oxford
Dr Maggy Jennings, Head of Research Animals Department, RSPCA
Dr Tomas Hanke, Weatherall Institute of Molecular Medicine, University of Oxford
Professor John Hodges FMedSci, MRC Professor of Behavioural Neurology, MRC Cognition and Brain Sciences Unit, Cambridge
Mr David Holmes, Registrar, University of Oxford
Dr Leslie Iversen FRS, Professor of Pharmacology and Director, Wolfson Centre for Research on Age-Related Diseases, Kings College London
Mr Stephen Kennedy, Nuffield Department of Obstetrics & Gynaecology, University of Oxford
Professor Barry Keverne FRS FMedSci, Professor of Behavioural Neuroscience and Director of Sub-Department of Animal Behaviour, University of Cambridge
Dr John Landers, Chair of Central Research Ethics Committee, University of Oxford
Dr Gill Langley, Scientific Adviser, Dr Hadwen Trust for Humane Research
Professor Roger Lemon FMedSci, Director, Institute of Neurology, University College London
Professor Andrew McMichael, Director, Weatherall Institute of Molecular Medicine, University of Oxford

378 Including those who gave oral evidence during site visits.

Professor Alan McNeilly FRSE, Deputy Director, MRC Human Reproductive Sciences Unit, Edinburgh and Chairman of the Animal Procedures Committee Primates Sub-Committee
Dr Anne Moore, Wellcome Trust Centre for Human Genetics, University of Oxford
Dr Tim Morris, Head of Animal Ethics and Welfare, GlaxoSmithKline
Professor Andrew Parker, Department of Physiology, University of Oxford
Superintendent Steve Pearl, Head of the National Extremism Tactical Co-ordination Unit
Dr Mark Prescott, Programme Manager, National Centre for the Replacement, Refinement and Reduction of Animals in Research
Professor Nick Rawlins, Department of Experimental Psychology, University of Oxford
Dr Rosalind Ridley, Head, MRC Comparative Cognition Team, University of Cambridge
Mr Mike Robbins, Parkinson's disease patient
Professor Trevor Robbins FRS FMedSci, Professor of Cognitive Neuroscience, University of Cambridge
Dr Vicky Robinson, Chief Executive, National Centre for the Replacement, Refinement and Reduction of Animals in Research
Dr Matthew Rushworth, Department of Experimental Psychology, University of Oxford
Dr Leah Scott, Biomedical Services, dstl Porton
Professor Jonathan Seckl FRSE FMedSci, Head of School, Molecular & Clinical Medicine, University of Edinburgh
Dr Stewart Shipp, Senior Research Fellow, Institute of Cognitive Neuroscience, University College London
Professor David Smith FMedSci, Deputy Head, Division of Medical Sciences, University of Oxford
Dr Mark Tricklebank, Head of In Vivo Biology, Eli Lilly & Co
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