nextofkin...

A REPORT ON THE USE OF **PRIMATES IN EXPERIMENTS**

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BUAV
BRITISH UNION FOR THE ABOLITION OF VIVISECTION

EUROPEAN COALITION TO END ANIMAL EXPERIMENTS

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Since 1979, Gill has worked as a scientific consultant to the BUAV and other animal protection organisations, in Europe and internationally. She is also the scientific adviser to the Dr Hadwen Trust for Humane Research, which funds the development of replacements for animal experiments in biomedical research.

Gill served as a member of the British government's Animal Procedures Committee for eight years and has also advised the government on the introduction of the new EU chemicals legislation, REACH. She has been an invited expert in several initiatives of the European Commission and of the Organisation for Economic Co-operation and Development (OECD). Gill is currently a member of the Replacement Advisory Group of the British National Centre for the Three Rs.

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THE BODY SHOP FOUNDATION

The British Union for the Abolition of Vivisection (BUAV) is the world's leading anti-vivisection campaigning organisation. The BUAV is dedicated to using all peaceful means possible to end all animal experiments, both nationally and internationally.

The BUAV is chair of the European Coalition to End Animal Experiments (ECEAE), in which it works with animal groups across Europe to co-ordinate campaigning initiatives and ensure that laboratory animals are high on the European political agenda.

Through the ECEAE, the BUAV led the Europe-wide campaign to raise awareness of the animal testing implications of the new European Union chemical control legislation (REACH). As a result, measures have been included in the proposals that will greatly reduce the numbers of animals who will suffer and die in chemical testing.

As a founding member of the International Council for Animal Protection in OECD Programmes (ICAPO), the BUAV also collaborates with animal protection groups across Europe, the United States and Japan to ensure that the interests of laboratory animals are represented within the Organisation for Economic Co-operation and Development which co-ordinates international testing guidelines.

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Note *The citing of results of animal experiments in this report does not imply approval of them by the BUAV, which is opposed to all animal experiments*

EXECUTIVE**SUMMARY**
and Call for Action

It is now clear that other primates share with us many morally-relevant capacities that were once thought unique to humans. A number of initiatives around the globe, involving the public, politicians and scientific and ethical experts, have been calling for a new relationship with our close primate relatives (Chapter 1). The pressure to protect all primates, not just the apes, from laboratory experiments is strong and growing.

Complementary avenues of scientific study demonstrate beyond doubt that there is no biological rationale for morally discriminating between all humans and all other primates. Chapter 2 explains in depth why and how the notion that humans are unique has broken down under the weight of new knowledge. A review of the way we use other animals in laboratories is urgently needed.

Despite concerns about research on primates from many quarters, official statistics show that they are not decreasing (Chapter 3). Existing legislative and administrative controls that attempt to reduce harms to primates are essentially unenforceable, as they rely on the good intentions of staff - some of whose commitment or expertise is inadequate, and some of whom have become desensitised to the suffering of primates in their care (Chapter 4).

...there is ample evidence that it is, in practice, impossible to keep primates in laboratories without compromising their physical and psychological health...

It is widely agreed that the trapping or breeding of primates overseas, their confinement at breeding centres, and their transport to European laboratories, cannot be accomplished without causing immediate as well as longer term stress and suffering (Chapter 5). Equally, there is ample evidence that it is, in practice, impossible to keep primates in laboratories without compromising their physical and psychological health (Chapter 6). Other primates, like humans, are stressed by social isolation, malaise, boredom, frustration, fear and anxiety. Numerous physiological and pharmacological changes, both obvious and hidden, occur as a result. As well as damaging the well-being of primates, such changes have the potential to confound research data. The adoption of best practices can reduce but not eliminate harms to primates; however few laboratories are willing - or have the funding or facilities - to pursue these standards.

The major laboratory use of primates is for pharmaceutical development and testing (Chapter 7). These tests cause substantial suffering and sometimes deaths, yet they cannot be relied upon to predict accurately human responses. The development and application of alternative approaches, including *in vitro, in silico*[1] and ethical volunteer studies, could replace these primate experiments.

Another main use of primates is in fundamental research, which is inherently more difficult to justify on a cost/benefit basis (Chapter 8). Using primates in such research, most especially when similar studies can be undertaken without their use, is insupportable. At best such experiments may provide some information that can be extrapolated to humans. At worst, the results may cause misconceptions that will delay a clearer understanding of human conditions.

The BUAV believes that the time for talking has passed, and action is urgently needed. Together with the European Coalition to End Animal Experiments, the BUAV calls for the end of primate experiments on ethical and scientific grounds.

For the reasons set out in this report,

- We call for a legislative or administrative prohibition on all primate experiments in Britain and throughout Europe. In Britain, the government already has the power to refuse licences for primate experiments, and they should use it. In the European Union, the present review of Directive 86/609 may offer one opportunity to achieve the end of primate experiments;

- We urge leading individuals and organisations within the research community to take a public stand and support our proposals;

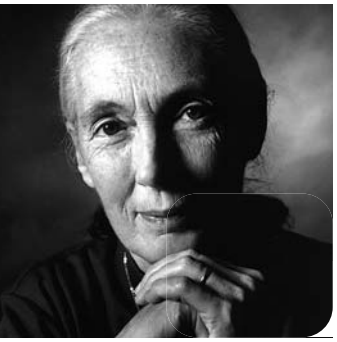
- We also urge the competent authorities of those EU member states who currently permit primate experiments to take action at a national level to end them;

- We call on research funding agencies to end the provision of grants for primate experiments, and to redirect funds to develop and implement replacement strategies.

1 i.e. by computational methods and models.

FOREWORD

by Dr Jane Goodall, DBE



Over the past decades increasing numbers of scientists have studied non-human primates in the wild, and in semi-natural conditions in captivity. These studies have provided a great deal of information that proves that these animals, along with many others with complex brains and nervous systems, have complex minds, live in complex societies and show emotions that, in some cases, may be very similar to some of our own. As a result of the dissemination of this understanding, in publications that target both scientists and the general public, there has been an increasing concern for the many ways in which they are traditionally exploited by humans, whether for entertainment, as pets, or as subjects in medical and scientific research. The public debate is most heated when non-human primates are subjected to intense physical or psychological suffering; and we are learning that this is all too often the case with regard to vivisection

While the public appears to be growing increasingly concerned about the use of non-human primates in medical research, the population of laboratory animals has not decreased. In reality, most people do not know - and do not want to know - what happens to non-human primates and other animals in medical research labs. That is why I am so glad that BUAV has produced this report to raise awareness about the daily realities for primates held captive in medical and pharmaceutical facilities.

Not only are many experiments on non-human primates unethical, many are unnecessary, and their results may be misleading. Experiments are still used today that were developed in a time when scientists knew little about the effect of stress on the immune system. Today this is well understood, and it is recognized, also, that the conditions in the typical medical research laboratory are psychologically stressful and that this may affect the physical health of the primates in question. It may thus throw doubt on the validity of many experiments. There are also significant differences between the immune systems of human and non-human primates; for example, even chimpanzees, a species with which we share more than 98% of the structure of our DNA, and which can keep the HIV-1 and HIV-2 retroviruses alive in the blood, do not develop typical symptoms of full blown AIDS. We certainly cannot assume that laboratory experiments involving other primates, less like us, will yield reliable results for humans.

The evidence in the BUAV's report reveals the true level of suffering of many primates used in animal experimentation, and the scientific pitfalls of using primates to study human diseases and drugs. It also describes many viable alternatives to the use of non-human primates.

We must pressure scientists to use all new and proven technologies that make the use of non-human primates in experiments obsolete; and we must strongly encourage additional research into yet other alternative methods. At the same time, we must educate parliamentarians and legislators on the importance of banning primate experiments in Britain, Europe - and, indeed, around the world.

I hope this report will persuade many people to reconsider the issue of vivisection and to join BUAV in helping to stop the unethical and often unnecessary use of primates in experiments. Too often I hear proponents of animal experimentation assuring us that, while it will always be necessary to use some animals for some areas of research and testing, efforts will be made to use as few as possible and treat them humanely. We need a new mind set: recognizing that the use of sentient beings for this purpose is essentially unethical we must set the human brain, linked with the heart, to find new ways forward without the use of any of these animals. We can - and must - create more humane and more effective methods of alleviating human suffering.

Jane Goodall

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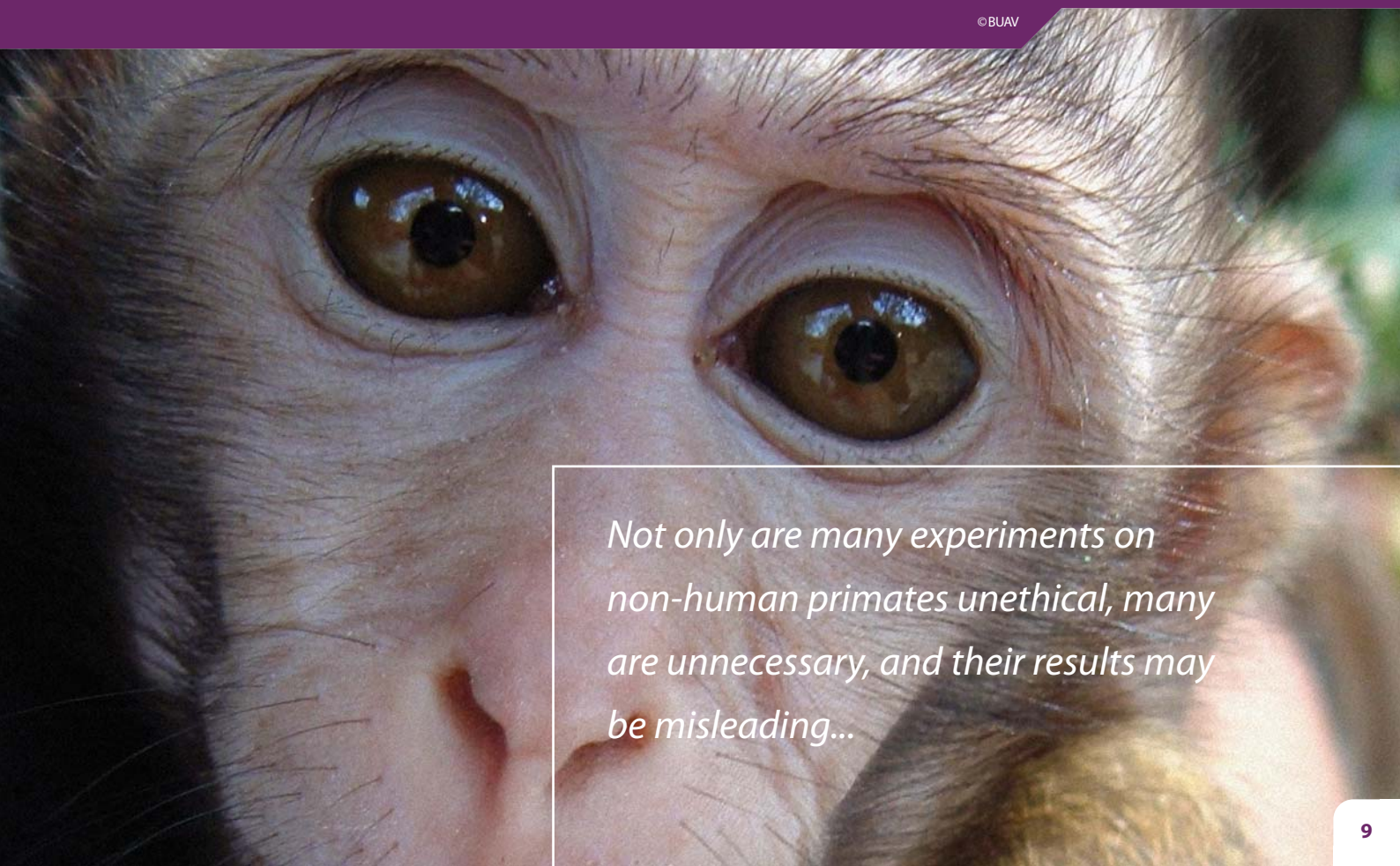


Not only are many experiments on non-human primates unethical, many are unnecessary, and their results may be misleading...

CHAPTERONE

Concern about primate experiments

The BUAV is opposed to all animal experiments, on ethical and scientific grounds, and has campaigned against them for more than a century. Because other primates have particular capacities to suffer extensively in captivity and when used in experiments, the BUAV has led many informed and closely-argued campaigns against such experiments[2]. Our undercover investigations in Britain and Germany and in overseas primate supply centres have revealed the daily realities of life for primates in laboratories.

BUAV *Primates* hand in a 163,000 signature petition at Downing Street in August 2005 calling for a total ban on primate testing in the UK. © BUAV

1.1 Public and political concern about primate experiments

Public opinion polls have their limitations, but they are a rough gauge of people's views on controversial topics. NOP polls commissioned by animal rights group Animal Aid in 2003 indicated that 52% of British respondents regarded experiments on primates as morally unacceptable[3] . Only 40% agreed with them.

In 2002, MORI carried out a survey for the pro-vivisection Coalition for Medical Progress. 46% of respondents were opposed to primate experiments for medical purposes, with only 39% in favour. However, one of the 'reassurances' incorrectly[4] given to respondents was that *"80-90% of experiments using animals are classified as "mild" e.g. they involve taking a temperature or a blood or urine sample only".* Without that false reassurance, it is safe to assume that the number of respondents opposed to primate experiments would have been higher.

The 1999 MORI opinion poll carried out for the New Scientist magazine is the most unbiased and comprehensive survey ever undertaken of British public opinion on animal experiments. It showed a majority of the public opposed to experiments on primates if they involved pain, illness or surgery. For example, research into hearing causing suffering to primates was opposed by 75% of respondents; primate experiments to develop a new painkiller were opposed by 61%; and even research on primates to develop an AIDS vaccine was opposed by 52%[5] .

Against this background of public concern it is unsurprising that Cambridge University's plans to build a new primate research centre faced strong opposition, when announced in 2000. The University claimed that the primate research to be undertaken there would assist the understanding and treatment of human neurological disorders.

In 2002 a public enquiry resulted in the government's planning inspector Stuart Nixon ruling against the plans[6]. He concluded that the University had failed to show a national need for the laboratory and recommended that permission to proceed be denied. Indeed, he went further, suggesting that more efforts should be made to develop alternatives to animal experiments:

"On the basis of the technical input, I could not conclude that need in the national interest is demonstrated insofar as this pertains to the scientific/medical research and procedures undertaken by the University.

In fact, if one accepts the premise that wherever possible research should not involve animals, it would be a stronger argument to say that it is nationally important to keep together and service the excellent and acknowledged research expertise in Cambridge to catch up on alternative forms of research to that employing animals."

2 E.g. Paradise Lost: A review of UK primate research (1994). London, UK: BUAV; and Next of Kin: The use of primates in animal experiments (2005). London, UK: BUAV.
3 See <http://www.animalaid.org.uk> **4** This was wrong, as the Market Research Society has acknowledged. **5** Animal experiments: Where do you draw the line? New Scientist, 22 May 1999, p. 26-31.
6 See <http://www.animalaid.org.uk/viv/history.htm>

CHAPTERONE
Concern about primate experiments

Deputy Prime Minister John Prescott overruled his own inspector and gave permission to proceed; but in 2004, Cambridge University announced that it had abandoned its plans due to spiralling costs. At the time, an Early Day Motion in Parliament calling for the end of all primate experiments, on the grounds that they cause suffering and are not medically reliable, was signed by more than 130 MPs.

In political terms, several countries have taken steps to prevent experiments on our closest primate cousins. New Zealand, Great Britain (since 1998), Sweden, Austria and the Netherlands have already introduced bans on the use of great apes (chimpanzees, bonobos, gorillas and orangutans) in research and testing.

Sweden's 2003 regulations banned research on great apes and gibbons; only non-invasive behavioural studies are permitted. And following a unanimous vote of the Austrian Upper Chamber in December 2005, all apes in Austria, including all eight species of gibbons, are now protected from research unless conducted in the interests of the individual animal.

In 2002, the Belgian Minister responsible for animal welfare announced that Belgium would be working towards a ban on all primate experiments. Furthermore, the British Animal Procedures Committee's remit includes *"...how to minimise, and eventually eliminate, primate use and suffering"*[7].

The European Commission, in response to growing criticism, commissioned an analysis of primate use throughout the European Union. The subsequent report acknowledged, in considerable detail, the cognitive complexity of these animals and their capacity to suffer in laboratories[8].

1.2 Expert concern about primate experiments

According to Britain's Nuffield Council on Bioethics[9], primates are used in many areas of neurobiology because their brains share structural and functional features with ours, but *"While this similarity has scientific advantages, it poses some difficult ethical problems, because of an increased likelihood that primates experience pain and suffering in ways that are similar to humans".*

The Boyd Group, comprising researchers (including primate experts), research-funding agencies, animal welfarists and philosophers, has called for a global prohibition on the use of all great apes in research and testing[10].
The Group also agreed that experiments on other primates should require very strong justification, while some members supported a total ban on the use of monkeys as well as apes.

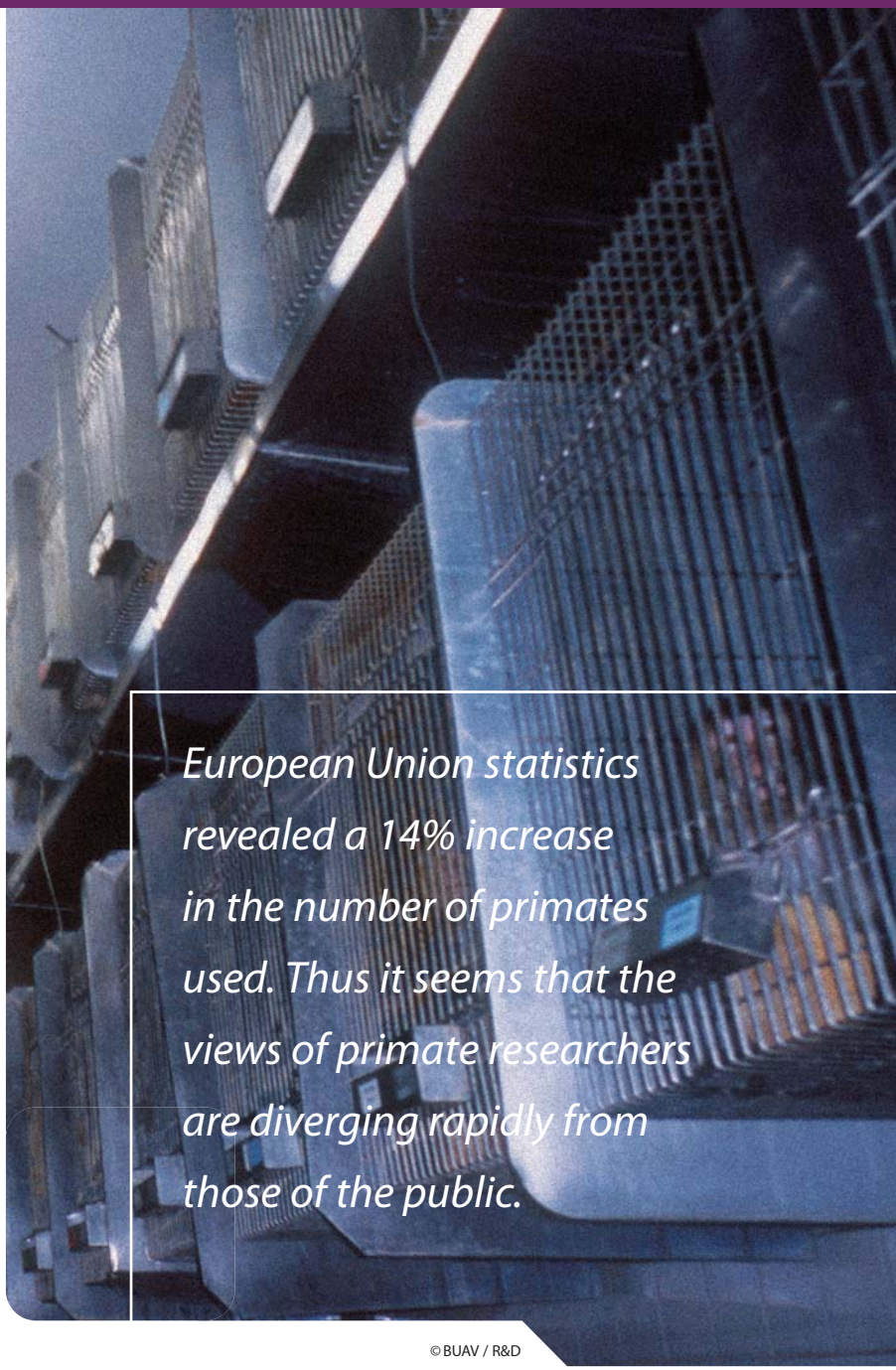
Most recently, a British consortium of four scientific and research-funding organisations[11] established a working group to examine the recent, current and future scientific bases for research involving primates. The group, set up

7 Report of the Animal Procedures Committee for 1997 (1998), Annex 2, p 78. The Stationery Office. **8** Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG. **9** Nuffield Council on Bioethics (2005). The Ethics of Research Involving Animals. London, UK: The Nuffield Council. See <http://www.nuffieldbioethics.org> **10** Boyd Group (2002). The Boyd Group Papers on the use of Non-Human Primates in Research & Testing. Leicester, UK: British Psychological Society.
11 The Royal Society, Medical Research Council, Wellcome Trust and Academy of Medical Sciences.

in 2005 and chaired by clinical geneticist David Weatherall, is also assessing the status of alternatives to using primates in research.

At the Fifth World Congress on Alternatives and Animal Use in the Life Sciences in August 2005, Jane Goodall was joined by 57 individuals and organisations from 19 different countries in signing a resolution calling for an end to the use of all primates in biomedical research and testing (see Appendix 1).

Despite the growing weight of opinion against primate experiments, there is no evidence that they are decreasing, either in Europe or the USA. In fact, the most recent European Union statistics revealed a 14% increase in the number of primates used (Chapter 3). Thus it seems that the views of primate researchers are diverging rapidly from those of the public on whose behalf they conduct their experiments.



European Union statistics revealed a 14% increase in the number of primates used. Thus it seems that the views of primate researchers are diverging rapidly from those of the public.

CHAPTER TWO

The moral status of primates and their evolutionary relationship with humans

The moral status of primates[12] is at the centre of the ethical debate about their use in biomedical research and testing.

The key issue with experimentation on primates, as for all animals, is the capacity to suffer. If an animal does not consciously experience pain, for example because it lacks the faculties to generate feelings of suffering and distress (i.e. it is not sentient), then we need not be concerned about causing individual harm (although there may be other concerns).

The vertebrate animals used in research and testing are sentient. This is accepted throughout the European Union as evidenced by the legally-binding protocol of 1997 annexed to the Treaty of Amsterdam, by which acknowledgement of animal sentience - the *"ability to experience pleasure and suffering"*[13] - was written into EU law. Animal sentience is also recognised in the wording of European Directive 86/609/EEC[14] as well as the UK Animals (Scientific Procedures) Act 1986, which regulate scientific procedures on animals with the capacity to experience *"pain, suffering, distress or lasting harm"*. The UK Act also covers one invertebrate species, *Octopus vulgaris*, for which the scientific case for sentience has been considered sufficiently compelling.

The fact that sentient animals feel pain and distress is sufficient reason, on moral grounds, to avoid inflicting them. However in the case of experimentation, it is often argued that the potential benefits to humans justify keeping animals in unnatural and highly confined conditions and causing them pain, suffering and distress. We disagree with this anthropocentric viewpoint.

In addition to pain sensation, issues of mental complexity also impact on the moral status of animals. There is persuasive evidence that many animals - certainly mammals, probably birds and possibly other species - have thoughts, feelings, memories and intentions[15].

Modern studies in ethology, genetics, neurophysiology, neuropharmacology and psychology have shown that there is no abrupt discontinuity between humans and all other primates in terms of ability to feel pain, distress and suffering; or in their morally-relevant cognitive, social and emotional faculties. Rather, there is a spectrum of capacities throughout the animal kingdom (including humans), with considerable overlap between species. This biological continuity offers no support for moral positions that discriminate absolutely between all humans and all other animals.

As Britain's Animal Procedures Committee report on the laboratory use of primates acknowledged[16]:

"...there are serious ethical and animal welfare concerns regarding the use of primates in experiments, and considerable public disquiet with regard to such use. These concerns are also

12 Throughout this report, we refer to "non-human primates" as "primates" or "other primates". **13** Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG. **14** Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of Member States regarding the Protection of Animals Used for Experimental and other Scientific Purposes. Official Journal of the European Communities 1986 L358:1-29. **15** Edelman DB, Baars BJ & Seth AK (2005). Identifying hallmarks of consciousness in non-mammalian species. Conscious. Cogn. 14:169-187. **16** Animal Procedures Committee (2002). The use of primates under the Animals (Scientific Procedures) Act (1986): Analysis of current trends with particular reference to regulatory toxicology.

likely to increase as more is discovered about their advanced cognitive faculties, complex behavioural and social needs, and the difficulties of satisfying these in a laboratory environment".

Many primates share with humans the ability to remember past events, to have desires, to anticipate and plan for future events, to communicate, form concepts and have complex emotional and social experiences, as this chapter describes. These attributes are morally significant because they show that other primates are harmed not only by physical pain, but also by mental and emotional distress - such as is caused by a barren environment, frustration, restraint or social isolation and the presence, or anticipation, of something fearful or painful.

Globally, the Netherlands, New Zealand, Great Britain, Sweden and Austria have already introduced some form of prohibition on the use of great apes in laboratories. In the case of Sweden, gibbons are also protected from invasive experiments. In other countries such as Germany, Italy and Norway, great apes have not been used in research and testing for some years. However, most importantly, in the USA there are currently more than a thousand chimpanzees in research facilities, and with the publication of the chimpanzee genome, there are plans to increase research efforts using these animals[17].

In this chapter we review the evidence for levels of consciousness and cognition in other primates that were previously thought to be unique to humans. As well as the primates most commonly used in European laboratories, we also deal with the great apes (chimpanzees, bonobos, gorillas and orangutans). This is not only to illustrate the continuity of mental and emotional function among primates, but also because experiments on great apes continue in the USA, with the possibility of an increase in the near future. Finally, we make the case that there is no biological rationale for distinguishing between the moral status of humans and all other primates.

2.1 What is consciousness?

There is no universally accepted definition of consciousness. Most concepts of consciousness include qualities of mind such as subjectivity, sentience, self-awareness and/or the ability to perceive the relationship between oneself and one's environment.

Consciousness is often differentiated into several levels, for example:

- Primary consciousness refers to the ability to generate a mental 'scene', in which information of different kinds, including sensory and motor events, can be integrated to enable individuals to direct their behaviour. This kind of consciousness creates internally experienced states of emotion and feeling and is now believed to be shared by all mammals, and birds, reptiles and large-brained invertebrates as well[18].

17 VandeBerg JL et al (2005). A unique biomedical resource at risk. Nature 437:30-32. **18** Seth AK, Baars BJ & Edelmann DB (2005). Criteria for consciousness in humans and other mammals. Conscious. Cogn. 14:119-139.

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- Secondary consciousness reflects the ability to have thoughts about experiences, especially about how external events relate to internal ones. Monkeys as well as great apes have this level of consciousness, although it is more likely to function by means of perceptual images than linguistically.

- Tertiary consciousness involves higher-order cognitive abilities such as a sense of self, in which individuals can 'think about their thoughts' and are 'aware of being aware'. Chimpanzees[19] and at least some monkeys appear to have these capacities[20]. This level would also include the faculty to reason about what other individuals think and want and see, and an ability to construct past and future scenes.

2.2 The blinkers of behaviourism

In the last several years there has been a resurgence of interest in understanding mind states. A better understanding would contribute to important ethical debates about suffering involving questions of consciousness in humans (such as fetuses or those in a persistent vegetative state) as well as other animals.

René Descartes' 17th-century concept of mind-body dualism, and decades of classical behaviourism in the early 20th century, led to a sustained disregard for - indeed a virtual denial of - consciousness in other animals. Animals were permitted bodily reflexes, complex instinctive behaviours and an ability to learn, but experiential states were ignored because they were considered inaccessible to scientific study.

Over time, animals came to be seen by many researchers as simply devoid of significant conscious faculties, such as emotions (e.g. pleasure, fear, distress), purpose, mental imagery, thinking, inner speech, or even conscious perceptions[21]. Remnants of this view of animals persist and still influence interpretation of modern data (see below).

Similarly, cognitive functions that were once thought the unique preserve of the human species are being discovered in a range of other animals. Monkeys know that they know; Caledonian crows creatively design and use tools; great apes use keyboards and parrots use human words to ask for what they want and to answer complex questions[22]. Even honeybees have now been shown to exhibit learning abilities formerly ascribed only to vertebrates, going beyond simple stimulus-stimulus or response-stimulus associations[23]. New information about other animals must prompt an urgent review of how we understand and treat them.

Chimpanzee in the wild ©RSPCA

19 Hauser MD (2005). Our chimpanzee mind. Nature 437:60-63. **20** Hampton RR (2001). Rhesus monkeys know when they remember. Proc. Natl. Acad. Sci. 98:5359-5362. **21** Baars BJ (2005). Subjective experience is probably not limited to humans: the evidence from neurobiology and behaviour. Conscious. Cogn. 14:7-21. **22** Griffin DR & Speck GB (2004). New evidence of animal consciousness. Anim. Cogn. 7:5-18. **23** Giurfa M (2003). Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. Curr. Opin. Neurobiol. 13:726-735.

2.3 Primate field studies

Decades of painstaking observations of primate behaviour in natural habitats have fuelled the revolution in our understanding of the mental, social and cultural complexities of primates. A very important body of work was generated by scientists such as Jane Goodall, Dorothy Cheney and Robert Seyfarth. Their field studies demonstrated in primates a sophisticated social intelligence, behavioural parallels with humans, evidence of local 'cultures' and complexity of vocal communications, all previously unrealised.

Jane Goodall and others have reported extensive similarities between chimpanzee and human behaviours such as[24,25]:

- emotional capacities
- affectionate family bonds
- long-term social relationships
- conscious awareness of self as separate from others
- altruism
- group aggression
- communication by gestures, body posture, facial expression and sound
- learning by observation
- making and using tools
- using medicinal plants to treat illness
- understanding and using abstract symbols for communication
- manipulating social situations for their own purposes.

Prior to this body of research, some of these behaviours - including altruism, tool design and use, and semantic communications - had formed the cherished boundaries considered to separate humans from all other primates. Laboratory research has, of course, made use of chimpanzees as 'models' for humans in a range of subject areas.

As Jane Goodall wrote a decade ago[26]:

"In view of these physiological and anatomical similarities, it is sad to find that the equally striking similarities between ourselves and these apes in the sphere of behaviour, emotional expression and intellectual performance have been largely disregarded or even denied by many of the researchers who use the living bodies of chimpanzees in their laboratories".

Since then there has been some progress, in that some countries have banned or restricted experiments on chimpanzees. However they are still widely used for research and testing, particularly in the USA, with a risk of this use increasing.

24 Goodall J (1971). In the Shadow of Man. London: Collins. **25** Russon AE, Bard KA & Parker ST (Editors) (1996). The Minds of Great Apes. Cambridge: Cambridge University Press. **26** Goodall J (1995). Why is it unethical to use chimpanzees in the laboratory? ATLA 23:615-620.

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2.3.1 Culture and communication

As well as the similarities between chimpanzees' behaviour and our own, there are cultural similarities too[27, 28]. Culture is the transfer of knowledge between individuals by a learning process involving observation and imitation. It requires an ability to understand the concept of 'do as I do', and to recognise and test that one's own actions are being imitated by others[29]. Dozens of learned behaviours have been documented in chimpanzees and there are distinct cultural variations between different populations: different types of learned (rather than genetically programmed) behaviours based on the social ties and shared history of the group.

A recent review of 151 combined years of chimpanzee field studies has revealed extensive cultural variations on a scale never before recognised - except in humans[30]. Thirty-nine different behaviour patterns or traditions, including tool use, grooming, greeting and courtship behaviours, showed cultural variations indicating that these behaviours are learned by observation and apprenticeship.

For example, chimpanzees in Tanzania design and use a different tool for extracting various insects for food and are four times more efficient at this than chimpanzees elsewhere. Drumming by males has varied meanings in different populations - in one troop for example, drumming codes instructions about how long the group should rest and where to move to next. More recently, applying the same method to orangutans has identified 19 clearly defined cultures with five more tentatively identified[31].

Compared to human cultures, ape culture is relatively simple but, even so, illustrates that a shared 'cognitive heritage' between humans and other great apes underlies these examples of social learning.

Other primates have not been shown to practise local cultures to the same extent as the great apes. Nevertheless, field studies have revealed that monkeys engage in social behaviours requiring a considerable complexity of mind, once denied them.

For instance, vervet monkeys recognise social relationships within their group[32], and are able to compare types of social relations and make 'same/different' judgements about them. In the wild, if a monkey sees a fight between a relation and a member of another family, this increases the likelihood that s/he will be aggressive towards another member of that other family. This demonstrates that they can mentally represent the properties of social relationships.

Twenty-five years ago it was assumed that primate vocalisations merely reflected the individual's emotional state. Today, numerous studies have shown that the calls of many primates (including vervets, baboons, diana monkeys, and rhesus, pig-tailed and toque macaques), as well as other mammals (e.g. meerkats), also give their companions information about external events such as food, predators and social relationships[33].

Rhesus monkeys have five distinct calls known as 'recruitment screams' for getting help from allies during aggressive encounters. The particular call used relates to the class

27 Povinelli DJ & Preuss TM (1995). Theory of mind: evolutionary history of cognitive specialisation. Trends Neurosci. 18:418-424. **28** Byrne RW & Whiten A (Editors) (1988). Machiavellian intelligence. Oxford: Oxford University Press. **29** Whiten A (2005). The second inheritance system of chimpanzees and humans. Nature 437:52-55. **30** Whiten A et al (1999). Cultures in chimpanzees. Nature 399:682-685. **31** van Schaik CP et al (2003). Orangutan cultures and the evolution of material culture. Science 299:102-105. **32** Cheney DL & Seyfarth DM (1990). The representation of social relations by monkeys. Cognition 37:167-196. **33** Ghazanfar AA & Hauser M (1999). The neuroethology of primate vocal communication: substrates for the evolution of speech. Trends Cogn. Sci. 3:377-384.

of opponent and the level of aggression. Different kinds of calls are also given for different kinds of food. Other monkeys respond to the information in the call, and not simply in a conditioned way to the sound signal itself.

Like baboons, rhesus monkeys also recognise the caller's identity and use this information to guide their responses. Baboons recognize the calls of individuals and have a detailed understanding of family relationships, not only within their own family but in unrelated families too. This awareness of their own and others' social position suggests a sophisticated sense of self. Baboons also eavesdrop on calls exchanged between other individuals and thereby acquire information about socially relevant factors such as dominance ranking, age and competitive ability.

The alarm calls of wild vervet and diana monkeys signal the threat of different predators (leopard, snake, eagle), and other group members respond in ways appropriate to the type of predator indicated. Field experiments show that listeners compare these vocalisations according to the information they convey, as well as their acoustic properties[34].

Marmosets are new world monkeys with complex social interactions, and their vocalisations are specific to context and gender. They call to maintain contact with other group members and they change subtle aspects of their calls when they encounter new social groups or acquire a new mate.

Studies of this kind have provided ample evidence that primate communications, like human speech, encode both semantic (meaning) and emotional information. Of course, the ability of great apes including chimpanzees and gorillas to communicate by human sign language and computers, not only with humans but also in private between each other, effectively demolishes the old idea that language is a dividing line between humans and other animals.

2.4 Consciousness and cognition

Field studies have been considered by some researchers, especially neuroscientists and behaviourists, as insufficiently rigorous to prove consciousness and assess cognitive functions in other animals. This is partly because of the historical bias in interpreting the results of animal behaviour research (see above); and partly because the standard index for consciousness in humans is the ability to report events accurately ('accurate' or 'verifiable' report) - harder to achieve in other species.

Therefore in recent years, more controlled but, sadly, sometimes highly invasive methods[35] to analyse consciousness in other primates have been devised.

These approaches include experiments into the neural activity underlying perceptual awareness, to distinguish differences between conscious and unconscious events. For example, studies of cortically blind (blindsight) patients[36] have revealed that they can perform certain visual tasks, but cannot

34 Seyfarth RM & Cheney DL (2003). Meaning and emotion in animal vocalizations. Ann. NY Acad. Sci. 1000: 32-55. **35** The BUAV is opposed to all animal experiments. Throughout this report, the citing of data from invasive procedures on animals does not imply approval of any kind. **36** In cortical blindness, the first cortical projection area (VI) of the primary visual pathway is damaged. Blindsight patients report a loss of awareness of aspects of visual stimuli, but their behaviour shows that they can still discriminate them.

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acknowledge this because they are consciously unaware of the visual event. Tests have been devised that elicit the differences between conscious and unconscious visual events.

Parallel experiments have been conducted by Alan Cowey and Petra Stoerig at Oxford, using rhesus macaques blinded by lesions in the visual cortex. Tested similarly for their visual awareness[37], the 'blindsighted' monkeys signalled whether their visual responses involved a conscious qualitative experience. In lay terms, the experiments showed that not only do rhesus monkeys 'see the world', but they are also consciously *aware* of what they see - a higher level consciousness shared with humans.

Another example involves using binocular rivalry to study visual consciousness and unconsciousness. When sensory input from one eye 'clashes' with that from the other[38], the individual only becomes conscious - or aware - of one of the stimuli. However, both stimuli activate cells in the visual cortex of the brain. In tests with humans, changes in brain activity have been recorded when subjects reported their consciousness of a visual object[39].

Using the binocular rivalry paradigm, invasive electrode experiments on rhesus macaques measured neuronal activity in different visual areas of the brain, while the macaques reported their perceptions by pulling levers. The experiments identified brain cells that respond to conscious vision and others that respond to unconscious visual input[40].

Other parallels between humans and animals, including primates, provide evidence of consciousness. In humans, the brain's characteristic electrical activity is distinctly different between waking consciousness and deep sleep, a feature shared by all mammalian species.

All mammals have a highly developed thalamocortical system which, in humans, is essential for consciousness. In human subjects, consciousness involves widespread, fairly fast, low-amplitude interactions in the thalamocortical pathways of the brain, driven by current tasks and conditions. The underlying brain activity is considered to be so similar in humans, monkeys and cats that these species are studied interchangeably in experiments on states of consciousness[41]. The thalamocortical system appears not to have changed greatly in 100-200 million years of evolution, suggesting that brain structures supporting consciousness are very ancient and probably biologically fundamental to mammals.

On the basis of these complementary lines of evidence - behavioural, neurophysiological and neuroanatomical - many scientists now believe that consciousness is a major biological adaptation for the planning of behaviour whose origins go back tens of millions of years. Jaak Panksepp at Bowling Green State University, Ohio, argues that it is evolutionarily more coherent to accept the working hypothesis that all other mammals, and probably other animals as well, have experiential states that help guide their behaviour.

37 Cowey A & Stoerig P (1995). Blindsight in monkeys. Nature 373:247-249. **38** That is, two incompatible visual stimuli are presented, one to each eye. **39** Srinivasan R, Russell DP, Edelmann GM et al (1999). Increased synchronization of neuromagnetic responses during conscious perception. J. Neurosci. 19:5435-5448. **40** Sheinberg DL & Logothetis NK (1997). The role of temporal cortical areas in perceptual organization. Proc. Natl. Acad. Sci. USA 94:3408-3413. **41** Baars BJ (2005). Subjective experience is probably not limited to humans: the evidence from neurobiology and behaviour. Conscious. Cogn. 14:7-21.

Chimpanzee at Dutch Research Centre © CEECE

Douglas Watt, of Boston University School of Medicine, added[42]:

"If we are truly prepared to dismiss consciousness in mammals, it seems only a step away from dismissing it in very young children and infants".

2.5 Objective self-awareness and theory of mind

The capacity for objective self-awareness means an individual has the conscious and cognitive abilities to be aware of its own state of mind, and to 'know that it knows, and remember that it remembers'. This reflective capacity represents the tertiary level of consciousness, and there is indisputable evidence that chimpanzees and orangutans are objectively self-aware.

In Gordon Gallup's classic studies[43], a spot of paint was applied to the brow or ear of anaesthetised chimpanzees who, later recognising themselves in a mirror, removed the paint. The standard mirror self-recognition test has also been passed by bonobos, orangutans and gorillas. Appropriate use of mirrors (such as the ability to recognise that another animal is approaching from behind) has been demonstrated in various species including parrots, monkeys and elephants[44]. Objective self-awareness is believed to require an ability to reflect on one's knowledge state in a certain situation and reason by inference about another individual's knowledge state in the same situation. It should also be accompanied by the capacity to anticipate what other individuals might do and to influence what other individuals do[45].

These underlying capacities have now been illustrated in a number of primate species, for example by demonstrating causal understanding and deception. Studies with captive chimpanzees have provided, by analogy with human infants, clear evidence of an understanding of causality[46].

Capuchin monkeys have the highest encephalisation quotient[47] of monkey species. Wild capuchins in the Brazilian forest have been shown habitually to use tools such as stones for digging out tubers, cracking seeds and probing crevices in trees and rocks. This suggests that capuchins are far more skilled at understanding cause and effect than had previously been realised[48].

O'Connell and Dunbar[49] argue that experiments demonstrating rhesus and tamarin monkeys' understanding of numerosity also indicate an understanding of causal relationships. Taken together, these findings support the claim that monkeys as well as apes have an implicit understanding of cause and effect.

42 Watt DF (2005). Panksepp's common sense view of affective neurosience is not the commonsense view in large areas of neuroscience. Conscious. Cogn. 14:81-88. **43** Gallup GG (1970). Chimpanzees: self recognition. Science 167:86-87; and Gallup GG (1977). Self recognition in primates - a comparative approach to the bidirectional properties of consciousness. Am. Psych. 32:329-338. **44** Suddendorf T & Whiten A (2001). Mental evolution and development: evidence for secondary representation in children, great apes and other animals. Psychol. Bull. 127:629-650. **45** Sedikides C & Skowronski JJ (1997). The symbolic self in evolutionary context. Personal. Social Psychol. Rev. 1:80-102. **46** O'Connell S & Dunbar R (2005). The perception of causality in chimpanzees (Pan spp.). Anim. Cogn. 8:60-66. **47** This is a ratio representing brain mass to body mass in a species. **48** Moura AC & Lee PC (2004). Capuchin stone tool use in Caatinga dry forest. Science 306:1909. **49** O'Connell S & Dunbar R (2005). The perception of causality in chimpanzees (Pan spp.). Anim. Cogn. 8:60-66.

CHAPTER TWO

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Theory of mind is the capacity to reason about what others think, want, believe and see and to act on that knowledge. Known in lay terms as 'mind-reading', theory of mind is part of a tertiary level of consciousness, being dependent on the ability for objective self-awareness. There has been considerable interest in finding evidence of theory of mind in other primates.

Whether or not great apes have theory of mind is a debate that has swung both ways in the last 25 years. Some primates certainly have some of the core capacities required by theory of mind, such as understanding the relationship between seeing and knowing. Experiments with chimpanzees illustrate that individuals know what others can and cannot see, and that they use this knowledge to devise effective social-cognitive strategies, for example when competing for food[50]. Untrained chimpanzees behaved differently depending on whether a human is unwilling or unable to give them food[51].

Cumulatively, such studies show that chimpanzees can infer what others know on the basis of what they see[52]. In the words of the European Union's Scientific Committee on Animal Health and Animal Welfare wrote[53], "*There is strong evidence that the Great Apes, at least both chimpanzee species, can view a situation from the perceptual perspective of others*". Such capacities enable them to feel empathy, act altruistically, negotiate with others and manipulate them[54].

One of the building blocks for theory of mind is the ability to observe and interpret the gaze of other individuals. Chimpanzees share with other animals (including dogs), the ability to infer the location of hidden food by following the direction of another individual's eye gaze. This has also been demonstrated in old world monkeys. Pig-tailed macaques (*Macaca nemestrina)* followed the gaze of an experimenter by using head/eye cues, and eye cues alone[55].

In another study, stump-tail macaques *(Macaca arctoides)* engaged in visual co-orientation with a human trainer, that is, they turned to look in the same direction as another individual whose focus of attention changed[56]. In a task where rhesus macaques could 'steal' a contested grape from one of two human competitors, they selectively retrieved the grape from the experimenter who could not see it - rather than one who was visually aware. This suggests that macaques also possess an essential component of theory of mind: the ability to deduce what others perceive on the basis of where they are looking[57].

Although theory of mind is often considered to be a solely human attribute, the necessary skills do not develop fully in human children until the age of about four years, and some autistics do not have theory of mind at any age[58]. Theory of mind is also compromised in symptomatic patients suffering from schizophrenia and bipolar affective disorder. Additionally, several human studies have shown that gaze-following problems are associated with deficits in cognitive and social abilities, such as autism. These difficulties are not considered to adversely affect the moral status of these patients. The fact that humans, great apes and macaques can follow and interpret the gaze of other individuals corroborates the notion of cognitive continuity across primate species[59].

50 Hare B et al (2000). Chimpanzees know what conspecifics do and do not see. Anim. Behav. 59:771-785. **51** Call J et al (2004). 'Unwilling' versus 'unable': chimpanzees' understanding of human intentional action. Dev. Sci. 7:488-498. **52** Hauser MD (2005). Our chimpanzee mind. Nature 437:60-63 **53** Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG. **54** de Waal, FBM (1982). Chimpanzee Politics. London, UK: Jonathan Cape. **55** Ferrari PF et al (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. Proc. Natl. Acad. Sci. 97:13997-14002. **56** Anderson JR & Mitchell RW (1999) Macaques but not lemurs co-orient visually with humans. Folia Primatol. (Basel). 70:17-22. **57** Flombaum JI & Santos LR (2005). Rhesus monkeys attribute perceptions to others. Curr. Biol. 2005 15:447-452. **58** Hauser MD (1999). Perseveration, inhibition and the prefrontal cortex: a new look. Curr. Opin. Neurobiol. 9:214-222. **59** Ferrari PF et al (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. Proc. Natl. Acad. Sci. 97:13997-14002.

Practising deception is considered to arise from, and depend on, theory of mind, since it requires an individual to understand and reason about what others do and do not know, and to plan actions accordingly. Chimpanzees and gorillas have been observed to shift their gaze away from a half-hidden food delicacy when a competitor appears, and even to groom nonchalantly, until the competitor left the scene. Only then did the animals look again at the food and retrieve it[60]. Capuchin monkeys have used communicative and deceptive pointing in experiments where they benefited by indicating, accurately or falsely, the location of hidden food. One human-reared monkey pointed without any training. Another withheld pointing when beneficial, while the third learned to obtain the hidden food by pointing deceptively in the presence of a 'competitive' trainer[61].

As Andrew Whiten of the University of St Andrews writes[62],

"Parsimonious phylogenetic reconstruction suggests that great apes and humans share some sophisticated representational skills due to our common ancestry - even though a fully representational theory of mind may have evolved in our ancestors only after the split from the line that led to modern chimpanzees."

Other scientists shrug off the parsimonious explanation for the possession by primates of core abilities (such as objective self-awareness, understanding the relationship between seeing and knowing, and the practise of deception) in favour of alternative explanations[63]. However, the evidence for theory of mind in other primates, mainly the great apes, remains compelling. Some of the difficulty in obtaining evidence may have been related to a less than appropriate choice of tasks. Problems that are more ecologically relevant to the species involved (e.g. competition rather than co-operation) have yielded more useful data.

2.6 Learning and memory

Several studies have looked at different types of learning and memory in primates. Declarative learning and memory is what we consciously remember and can describe to others. Non-declarative learning and memory changes our actions and perceptions without our being consciously aware of what caused the change.

Some researchers believe either that animals are incapable of declarative memory, or that they may know important facts but do not 'know that they know'. Others think these topics cannot be researched because animals cannot tell us what they remember[64]. However, this is no longer true, and substantive evidence for declarative learning and memory in other animals, particularly primates, has emerged.

For example, a rigorously designed study of rhesus monkeys revealed that they were able consciously to distinguish between remembering and forgetting. The task required them to deliberately decline, in advance, to undertake a memory test when they knew they were unlikely to succeed. The experiments demonstrated that they could make flexible decisions about future behaviour depending on the knowledge they currently had - or, that they knew when they remembered and when

60 Whiten A & Byrne RW (1988). Tactical deception in primates. Behav. Brain Sci. 11:233-273. **61** Mitchell RW & Anderson JR (1997). Pointing, withholding information, and deception in capuchin monkeys (Cebus apella). J. Comp. Psychol. 11:351. **62** Suddendorf T & Whiten A (2001). Mental evolution and development: evidence for secondary representation in children, great apes and other animals. Psychol. Bull. 127:629-650. **63** Heyes CM (1998). Theory of mind in nonhuman primates. Behav. Brain Sci. 21:101-114. **64** Griffin DR (2001). Animals know more than we used to think. Proc. Natl. Acad. Sci. 98:4833-4834.

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they forgot[65]. Similarly, the performance of rhesus monkeys in cognitive tasks involving numerical sequencing satisfied two criteria for declarative memory: rapid acquisition of new knowledge and flexible application of existing knowledge to a novel problem[66].

Laboratory-kept capuchins can associate a coloured plastic chip with a particular tool and then ask for the tool by presenting the correct chip. Clearly, they can understand and use abstract symbols, representing higher brain functions. Further evidence includes capuchins communicating by pointing and practising deception (see above) by refusing to point[67].

Humans are possibly unique in having the capacity to represent mentally large numbers precisely. However, chimpanzees share with monkeys a natural sense of number, involving an understanding of small numbers (<5) of objects or events in parallel, and an approximate representation of larger numbers that depends on their ratios.

In extensive studies, a chimpanzee called Ai was trained to learn numbers one to nine, acquiring an understanding of the place of a number in a series (ordinality) and in terms of counting (cardinality), although Ai exploited a different way of learning than do human children. Chimpanzees use their sense of number in social activities such as foraging, group hunting and sharing[68].

Cotton-top tamarins[69] have also demonstrated a spontaneous, untrained ability to discriminate large numbers[70]. Well-controlled tests with untrained, wild rhesus monkeys, using the same methodology as studies with human infants (a preferential looking paradigm), showed that monkeys understood that one plus one equals two, and does not equal three[71]. Clearly, these animals have a relatively sophisticated arithmetical representational system.

Laboratory experiments have confirmed that rhesus monkeys can rank numbers of items in numerical order, passing tests which 10-month-old human infants generally fail[72]. They were able not only to understand number sequences, but could also rank numbers larger than those they had been trained with.

Thus, great apes and monkeys have a mental representation of numerical order, and also understand a numerical rule and can apply it to novel tasks, in the absence of spoken language - the possession of which was once thought to be a prerequisite for numeracy.

2.7 Tool design and use

Tool design and use represent a creative approach to problem-solving that indicates complex cognitive functions. Once thought to be a solely human ability, today we know that not only the great apes but also other animals, including capuchin monkeys and crows, can prepare and use tools.

In a recent study, captive gorillas *(Gorilla gorilla)* and orangutans *(Pongo pygmaeus)* had to use a tool to retrieve an out-of-reach food reward in a variety of situations[73]. They fashioned tools, chose tools of the right length, used one tool to reach another tool to gain the reward and

65 Hampton RR (2001). Rhesus monkeys know when they remember. Proc. Natl. Acad. Sci. 98:5359-5362. **66** Terrace HS, Son LK & Brannon EM (2003). Serial expertise in rhesus monkeys. Psychol. Sci. 14:66-73. **67** Mitchell RW & Anderson JR (1997). Pointing, withholding information, and deception in capuchin monkeys (Cebus apella). J. Comp. Psychol. 11:351. **68** Hauser M (2005). Our chimpanzee mind. Nature 437:60-63. **69** Uller C, Hauser M & Carey S (2001). Spontaneous representation of number in cotton-top tamarins (Saguinas oedipus). J. Comp. Psychol. 115:248-257. **70** Hauser MD et al (2003). Evolutionary foundations of number: spontaneous representation of numerical magnitudes by cotton-top tamarins. Proc. Biol. Soc. 270:1441-1446. **71** Hauser MD et al (1996). Numerical representations in primates. Proc. Natl. Acad. Sci. 93:1514-1517. **72** Brannon EM & Terrace HS (1998). Ordering of the numerosities 1 to 9 by monkeys. Science 282:746-749. **73** Mulcahy NJ, Call J & Dunbar RIM (2005). Gorillas (Gorilla gorilla) and orangutans (Pongo pygmaeus) encode relevant problem features in a tool-using task. J. Comp. Psychol. 119:23-32.

refused to use tools that were too short. The gorillas thus used mental representation to encode and compare two key features of the task: the length of the tools (in absolute and relative terms) and the distance to the reward. The studies provided evidence that these apes both represent a problem mentally, and plan a solution involving a sequence of actions. Evidence of tool use by *wild* gorillas was not available until very recently when, in the Republic of Congo, adult female gorillas were observed using a branch as a support while wading in water and to test the deepness of the water; and a detached tree trunk as a support while food processing and as a self-made bridge to cross a deep patch of swamp[74].

Wild capuchins (new world monkeys) in Trinidad use leaves as sponges and as simple water containers[75]. Most recently, Cambridge researchers recorded wild capuchins in the Brazilian forest habitually using stone tools for digging out tubers, cracking seeds and probing crevices in trees and rocks. The authors commented that the wild capuchins were far more skilled at understanding cause and effect than had previously been realised[76].

In a US study, laboratory-kept but untrained capuchin monkeys spontaneously made use of branches to unearth food. Some of the monkeys broke off smaller side branches and removed leaves and bark, to produce an implement suitable for digging up buried peanuts[77]. Younger animals were more proficient in learning tool use, and early disruption of mother/infant relationships had deleterious effects on the acquisition of such skills. In another study, laboratory capuchins in one group transferred stones to subjects in a second group, who used the stones as cutting tools and then transferred food back to the first group[78].

2.8 The genetic basis for a primate continuum

Underlying the cognitive, emotional, neurophysiological and behavioural similarities seen in humans, other apes and monkeys is a shared genetic heritage. Chimpanzees and humans are now classified as members of the same super-family of primates, the hominoids. Chimpanzee and human lineages diverged between five and seven million years ago, and chimpanzees *(Pan troglodytes)* and bonobos *(Pan paniscus)* diverged one to two million years ago. By comparison, the ways of humans and rodents separated an estimated 80 million years ago.

Chimpanzees are thus our closest living relatives: we are closer than the genetic relationship between two species of mice, such as *Mus musculus* and *Mus spretus*; or between donkeys and horses. A draft of the chimpanzee genome, published in 2005, confirmed that human and chimpanzee genomes are 98.77% identical in terms of base pairs (the building blocks of DNA)[79].

After the great apes, the animals most similar genetically to humans are the old world monkeys. The human lineage diverged from that of the old world monkeys about 25 million years ago; and from the new world monkeys 35-40 million years ago. It is estimated that the

74 Breuer T, Ndoundou-Hockemba M & Fislock V (2005). First observation of tool use in wild gorillas. PLoS Biol. 3:e380. <http://www.plosbiology.org> **75** Phillips KA (1998). Tool use in wild capuchin monkeys (Cebus albifrons trinitatis). Am. J. Primatol. 46:259-261. **76** Moura AC & Lee PC (2004). Capuchin stone tool use in Caatinga dry forest. Science 306:1909. **77** Westergaard GC et al (1998). Why some capuchin monkeys (Cebus apella) use probing tools (and others do not). J. Comp. Psychol. 112:207-211. **78** Westergaard GC & Suomi SJ (1997). Transfer of tools and food between groups of tufted capuchins (Cebus apella). Am. J. Primatol. 43:33-41. **79** The Chimpanzee Sequencing and Analysis Consortium (2005). Initial sequencing of the chimpanzee genome and comparison with the human genome. Nature 427:69-87.

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overall DNA sequence of rhesus macaques and vervet monkeys differs only by 5 to 7.5% from the human sequence[80].

It is clear that the sequencing of the chimpanzee genome will increase the pressure to use chimpanzees in experimentation, especially in the USA. Some scientists see the sequence completion as placing *"the chimpanzee in a position to play an increasingly critical and invaluable role in future biomedical advances"*, including in disease research and in testing drugs for their toxicity and pharmacokinetic profiles[81]. Proposals have now been made to sequence the genome of the rhesus monkey, which will similarly increase experimentation on this primate. These are deeply worrying developments, and must prompt a serious re-appraisal of the scientific and ethical bases of primate research and testing.

2.9 Interpreting the data: them versus us?

There has been an explosion in knowledge about the mental and emotional lives of primates arising from rigorous non-invasive studies as well as from laboratory experiments. The evidence supports the suggestion that for a whole range of significant mental and other capacities, there is no fundamental morally-relevant discontinuity between different species of primates, including humans.

The literature also reveals that, historically and still today, the standards of proof required to demonstrate any continuity of mind with other primates are set extremely high.

The evidence is routinely interpreted without giving any benefit of the doubt to great apes and monkeys. This is partly due to remnants of the classic behaviourist mindset persisting within science today; but also reflects a wider, deep-seated need for humans to find or defend absolute dividing lines between their species and all others[82].

Frans de Waal, an expert in primate behaviour, wrote[83]:

"I attribute opposition to [anthropomorphism] to a desire to keep animals at arm's length rather than concerns about scientific objectivity. ...I propose anthropodenial for the a priori rejection of shared characteristics between humans and animals when in fact they may exist. Those who are in anthropodenial try to build a brick wall between themselves and other animals. They carry on the tradition of French philosopher René Descartes, who declared that while humans possessed souls, animals were mere machines".

Neuroscientist Bernard Baars, in discussing evidence for conscious cognition in other mammals, points out that it is an inferential leap for one person to believe in the consciousness of another person, yet such inferences are made routinely when brain-injured patients are tested for impaired responsiveness. He asks, *"But if we make such inferences to other humans, then why not to other creatures, if the objective basis is the same?"*[84]

Similarly, the late Donald Griffin argued[85], *"There is no need for a double standard by which evidence of animal consciousness is accepted only if it provides perfect proof, whereas in other*

80 Page SL & Goodman M (2001). Catarrhine phylogeny: Noncoding DNA evidence for a diphyletic origin of the mangabeys and for a human-chimpanzee clade. Mol. Phylogenet. Evol. 18:14-25. **81** VandeBerg JL et al (2005). A unique biomedical resource at risk. Nature 437:30-32. **82** Midgley M (2003). The Myths We Live By, pp. 135-145. London & New York: Routledge. **83** de Waal F (2002). The Ape and the Sushi Master. Penguin. **84** Baars BJ (2005). Subjective experience is probably not limited to humans: the evidence from neurobiology and behavior. Conscious. Cogn. 14:7-21. **85** Griffin DR (2001). Animals know more than we used to think. Proc. Natl. Acad. Sci. 98:4833-4844.

areas of science we are accustomed to weighing and evaluating imperfect or ambiguous data."

There is very powerful evidence that animals throughout the order of mammals, at the least, are conscious of their pain, pleasure, appetites and emotions, as well as being conscious of the outside world[86]. For a long time perceptual awareness was denied to other animals because they were considered to have no language, but now both those barriers have been broken. Yet some scientists seem willing to interpret these data in every way except the parsimonious - or even just the commonsense - way. Interestingly, in commenting on the resistance (or 'agnosticism') of some behavioural experts to the idea that many animals have conscious feelings and awareness, psychologist Jaak Panksepp suggested that[87]:

"One obvious advantage of agnosticism is that the stance can be efficiently deployed to keep animal rights advocates at bay (and this may currently be the biggest implicit reason for equivocating about animal emotions, especially in laboratories that stress their animals in ways that would be deemed morally reprehensible in humans)".

There is an unresolved paradox, too, in the use of monkeys (and other animals) in experiments that probe consciousness, cognition, meaning, emotions, mind states and mental illnesses, unless it is granted that animals have these experiences. If marmosets do not experience anxiety in the 'marmoset anxiety model', why would GlaxoSmithKline test anxiolytic drugs on them?[88] If psychological research is conducted on macaques because the structure and functions of their brains are considered similar to those of humans, how can their mental experiences be so very different? If marmosets do not experience emotions, why study their emotional responses?[89]

The evidence shows that great apes, like humans, have objective self-awareness and that the underlying capacities for this, such as anticipating and influencing what other individuals might do, are also present in monkeys. Monkeys, as well as apes and humans, 'know what they know and remember' and also 'know when they forget'. Monkeys and apes communicate meaning as well as emotion in their vocalisations; understand and use abstract symbols; mentally represent numbers; undertake problem-solving; comprehend cause and effect; observe and interpret the gaze of other individuals, and practise deception (building blocks for theory of mind). There is thus a continuum of consciousness and cognition throughout the primate order and most likely throughout the whole animal kingdom, in ways that we have only recently begun to understand.

2.10 Reasons to end primate experiments

The aim of this chapter is not to claim that primates have levels of consciousness or cognitive abilities *identical* to those of humans. This would be unlikely, since problem-solving tends to be species specific, so that while human children can learn a large verbal vocabulary in a few months, pigeons are able to navigate the airways by means that we still cannot understand[90].

86 Seth AK, Baars BJ & Edelmann DB (2005). Criteria for consciousness in humans and other mammals. Conscious. Cogn. 14:119-139. **87** Panksepp J (2005). Toward a science of ultimate concern. Conscious. Cogn. 14:22-29. **88** Micheli F (2001). Lesopitron (Esteve). IDrugs 4:218-224. **89** Braesicke K et al (2005). Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. Eur. J. Neurosci. 21:1733-1740. **90** Baars BJ (2005). Subjective experience is probably not limited to humans: the evidence from neurobiology and behaviour. Conscious. Cogn. 14:7-21.

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But there is no evidence for a gulf that definitively separates humans from all other primates, or that separates great apes from monkeys. Increasingly, attributes once considered unique to humans, such as consciousness, tool-making and use, and semantic communications, exist in other primates from our close cousins the chimpanzees to the humble capuchin monkey.

A single line of investigation into the similarities of other primates' minds to our own could be criticised as inconclusive, but the weight and variety of evidence now available argues very strongly indeed for a continuity of mind among all primates, including humans. As Thomas Suddendorf and Andrew Whiten wrote[91], *"The gap between human and animal mind has been narrowed."*

The BUAV contends that the key capacity that should protect animals from experimentation is the ability to experience pain, suffering and distress. But it is also important to acknowledge that other primates have the capacity to experience joy and pleasure. Jonathan Balcombe, in his forthcoming book on animal pleasures, notes that great apes laugh like humans do, and in similar situations, such as when playing with one another or with other species (including humans) and when tickled[92].

Other primates possess cognitive and emotional abilities of great moral significance that increase their susceptibility to suffering and distress. Animals who can reflect on pain, and can remember and anticipate it, will suffer mental distress in addition to the actual experience of physical pain. Animals who form strong emotional bonds with others suffer when these are broken.

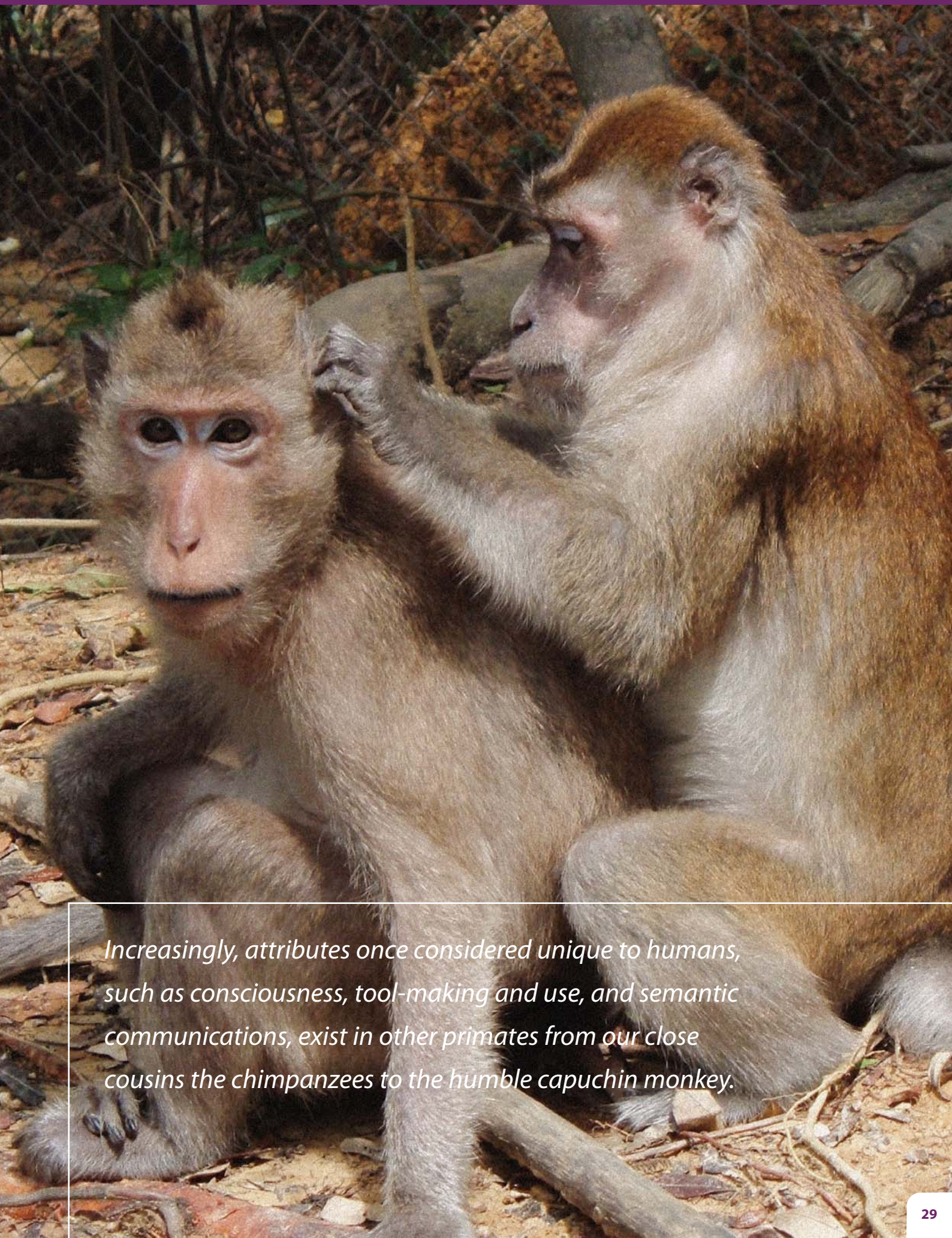
Douglas Watt, of Boston University School of Medicine, in considering the implications of consciousness in other animals, asked[93]:

"What would it do to... our ability to justify experimenting on animals? For our treatment of animals more generally? What if an increasing consensus about a mammalian form of consciousness made certain types of animal experimentation appear unethical?"

It is now known that other primates, at least, do share many morally-relevant capacities with humans: capacities which humans have always considered to underlie our uniquely human moral community. There are people who, by accident of birth, illness or age, perform more poorly at some or all cognitive and emotional levels compared to some non-human primates. Yet such people are not generally considered suitable subjects for forcible experimentation, but the converse has always been the case with other primates.

The notion that humans are unique has broken down under the weight of new knowledge, and we must urgently review the way we treat other animals[94]. In the case of primates, the scientific evidence itself demonstrates that there is no sufficient biological rationale for morally discriminating between all humans and all other primates. Consequently, in this report the BUAV contends that forcible experimentation on all primates, human and otherwise, is wrong.

91 Suddendorf T & Whiten A (2001). Mental evolution and development: evidence for secondary representation in children, great apes and other animals. Psychol. Bull. 127:629-650. **92** Balcombe J (2006). Pleasurable Kingdom: Animals and the Nature of Feeling Good. Basingstoke, UK: Macmillan. **93** Watt DF (2005). Panksepp's common sense view of affective neurosience is not the commonsense view in large areas of neuroscience. Conscious. Cogn. 14:81-88. **94** Bekoff M (2002). Minding Animals: Awareness, Emotions, and Heart. Oxford, UK: Oxford University Press.



Increasingly, attributes once considered unique to humans, such as consciousness, tool-making and use, and semantic communications, exist in other primates from our close cousins the chimpanzees to the humble capuchin monkey.

CHAPTER THREE

Statistics of experiments on primates in britain and the european union

Even today, with high levels of public concern about primate experiments, it is still difficult to obtain a clear and full picture of primate use in laboratories within member states of the European Union (EU). The official EU statistics provide relatively superficial and partial information, and only a proportion of experiments are published in scientific journals. For example, regulatory toxicity tests are frequently not published at all, and these tests account for the majority of primate use.

The main source of information about numbers and types of experiments on primates in Britain are the official Statistics published annually by the Home Office[95]. Within the EU, member states are required to submit their national statistics of animals used to the European Commission in a standardised format, although the extent to which different states have complied has varied.

3.1 Primate experiments in Britain

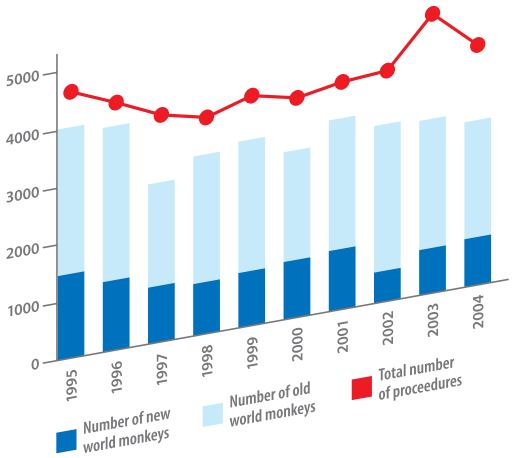
The British Home Office Statistics represent experiments or procedures[96] regulated under the 1986 Act[97], that is, if they may cause *"pain, suffering, distress or lasting harm".* Therefore primates in laboratories who are not used in experiments but are killed by an approved humane method do not appear. There are other exclusions too, as explained below, and for these reasons the official Statistics do not provide the full picture of primate experimentation in Britain.

Britain rivals France as the EU member state conducting the most experiments on primates. It is deeply regrettable that, despite widespread public concern about the use of primates in Britain, there has been no significant or lasting decrease in their use over the last 10 years. As summarised in Chart 3.1, although fewer primates overall were used in 2004 compared to 1995, there has not been a steady decline over the last decade and no clear evidence of a downward trend.

Additionally, the chart shows that the fall in numbers of *individual animals* has come at the cost of re-using individuals in more than one procedure. Numbers of procedures continue to fluctuate. There is always a difference between numbers of *procedures* and numbers of primates used, because a proportion of animals are used in more than one procedure.

95 Home Office: Statistics of Scientific Procedures on Living Animals, Great Britain. London, UK: The Stationery Office. **96** The terms 'procedures' and 'experiments' are here used interchangeably. **97** The Animals (Scientific Procedures) Act 1986.

Chart 3.1 Numbers of new world and old world monkeys and of procedures, Britain, 1995 - 2004



3.1.1 Why statistics underestimate primate experiments

The Home Office Statistics do not reflect all the experiments conducted or all the animals used annually in Britain. Procedures are only ever counted officially in the year that they start, so that any procedure lasting longer than one year will not appear in subsequent annual Statistics.

If an animal survives a first procedure (experiment) and is considered suitable for re-use in a second one, the second use will be counted officially as another procedure in the year it begins; but the animal will not be counted again. Each animal is only ever counted once, in the year when the first procedure begins. This is the reason that the total number of procedures shown in Chart 3.1 exceeds the total number of primates used - the difference reflects re-use of primates in a second or further procedure.

On the question of the re-use of primates, the British government's advisory committee, the Animal Procedures Committee, had this to say[98]:

"...the 1998 Statistics record that eight squirrel, owl or spider monkeys were used and that 37 procedures were performed on animals of these species. In every year since then (up to 2003, the most recent data) procedures involving squirrel, owl or spider monkeys are reported (ranging between 20 and 67 procedures per year), but in each of these years the Tables reporting the number of animals used include a nil return for these species. This (presumably) is because the procedures reported in the five years 1999 to 2003 were all re-uses of the animals first used in 1998."

The same is seen in the subsequent Statistics, for 2004, where 40 procedures on squirrel, owl or spider monkeys appear but no animals are shown as being used. This suggests that some primates are held in laboratories and re-used in experiments, for years - eight years in this case - at a time.

In fact, the way the Home Office applies the 1986 Act allows several procedures on an animal within a single protocol. This means there is already considerable 're-use' of animals, as the term would be understood by members of the public. An example from the BUAV's investigation of neuroscience research at Cambridge University gives a graphic insight into what is involved for the animals. Under a particular protocol in the research licence, a marmoset could be given acute brain lesions under general anaesthetic, followed by tissue implantation under another general anaesthetic, followed by central cannula

98 Animal Procedures Committee (2005). Report of Statistics Working Group. See <http://www.apc.gov.uk/>

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implantation under yet another general anaesthetic. In the licence, the protocol sheet recorded: *"Consequently most animals receive multiple interventions as part of the whole lesion/graft repair procedure".*

The Home Office here walks a fine line with its terminology, allowing multiple interventions within one protocol because some are preparatory to others and must be completed in the same animal. It could well be concluded that the official number of procedures conducted on primates - or other animals - is therefore a significant underestimate of the reality.

Thus the national figures for Britain are incomplete, both for the total number of procedures actually underway, and the total number of animals being experimented upon, in any one calendar year.

3.1.2 Primate species in British laboratories

No great apes have been subjected to experimentation since the 1986 Act was introduced, and there has been a policy ban - although not a legislative prohibition - on their use since 1997, on ethical grounds. No prosimians (e.g. bushbabies, lemurs, tarsiers) have been used in British laboratories for several years.

Research and testing are conducted both on new world and old world monkeys. In the Home Office Statistics, new world monkeys are not identified to the species level but are represented in three groups: marmosets and tamarins; squirrel, owl and spider monkeys; and other new world monkeys. Old world monkeys are also split into three groups: macaques; baboons; and other old world monkeys. More old world monkeys (about 80% of all primates) are used in British laboratories than new world species (about 20%), as seen in Chart 3.1.

According to the published literature the marmoset, *Callithrix jacchus*, is the most commonly used new world species in Britain. Few experiments on tamarins (*Saguinus* species) are reported in the literature and the Statistics show that, over the last decade, procedures on squirrel, owl and spider monkeys (grouped together) accounted for fewer than 6% of procedures on new world monkeys as a group. The actual number of procedures using these species varied between 13 and 80 during the period 1995-2004 (but see above regarding re-use). The remaining group of new world monkeys, described as "Other" species, probably included capuchin monkeys (*Cebus* species); but none have been reported in the Statistics since 1998. Numbers of new world monkeys have fallen gradually over the last decade (Chart 3.1)

Cynomolgous macaques (*Macaca fascicularis*) followed by rhesus macaques (*Macaca mulatta*) are the most commonly reported species in the literature, with a small number of pigtailed macaques (*Macaca nemestrina*) having been used a few years ago.

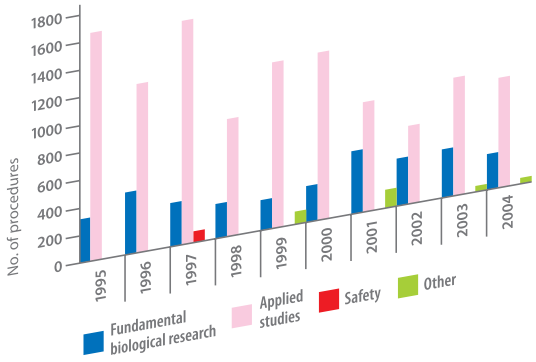
Baboons (*Papio* species) have not been used in British laboratories since 1998 and their numbers had declined for several years before then. This may reflect the ending, in the mid-

1990s, of a 20-year programme of epilepsy research on baboons conducted in a London laboratory; as well as a partial policy ban on the use of wild-caught primates, which was introduced by the government in the mid-1990s. This restriction requires specific and exceptional justification for the use of wild monkeys; baboons are almost always captured from the wild because they do not breed successfully in captivity.

3.1.3 Categories of procedures on primates in Britain

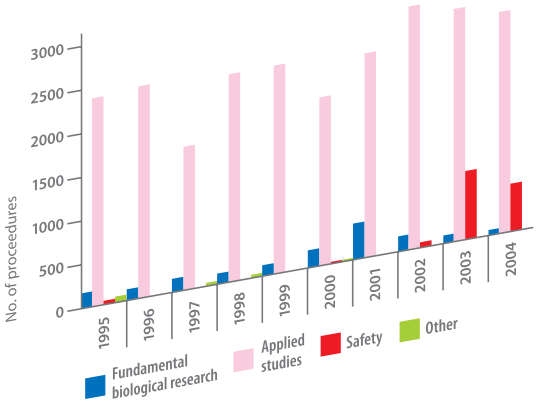
Charts 3.2 and 3.3 illustrate the four main categories of procedures on primates, according to the use of new world and old world monkeys respectively.

Chart 3.2 Numbers and types of procedures on new world monkeys, Britain, 1995 - 2004



For explanation of abbreviated category titles in the legend, see text.

Chart 3.3 Numbers and types of procedures on old world monkeys, Britain, 1995 - 2004



For explanation of abbreviated category titles in the legend, see text.
After 1998, all animals were macaques.

3.1.4 Applied studies: primate species used in medicines testing in Britain

By far the largest category of primate experimentation is called "Applied studies in human medicine or dentistry and veterinary medicine". In practice, all the primate studies relate to human rather than veterinary medicine. As defined by the Home Office, applied studies are those conducted for the purpose of developing and testing commercial products, i.e. *"consisting of research into, development of and quality control of products or devices, including toxicological evaluation and safety or efficacy testing".*

The last decade has seen a decline of about 50% in experiments on new world monkeys in applied studies (Chart 3.2), but an increase in

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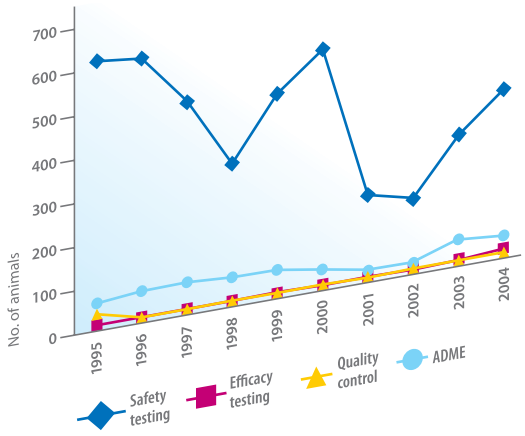
experiments on old world monkeys (Chart 3.3). The Home Office Statistics allow a further analysis of the kinds of tests conducted on primates for applied studies, i.e. the development and quality control of medical and dental products or devices, including their toxicological evaluation and safety or efficacy testing. 99% or more of these are conducted with a view to satisfying regulatory authorities.

Charts 3.4 and 3.5 show four types of experiments conducted on new world and old world monkeys respectively, over the last ten years. Of the new world monkeys, marmosets and tamarins[99] were the only species used; and after 1998 macaques were the only old world species.

Chart 3.4 Applied studies: numbers of new world monkeys in medicines testing, Britain, 1995 - 2004



In this category of applied studies, safety testing is by far the largest use and has fluctuated widely over the last decade, with a generally downward trend for new world monkeys (Chart 3.4) but no overall change for old world species (Chart 3.5).

Chart 3.5 Applied studies: numbers of old world monkeys in medicines testing, Britain, 1995 - 2004

Charts 3.4 and 3.5 show that tests to study the absorption, distribution, metabolism and excretion (ADME) of medicines comprise the next largest group of applied studies. These account for fewer than 200 primates of all species annually, and the numbers have not changed significantly in the last ten years. Similarly, tests for the efficacy of new medicines and for quality control account for relatively few monkeys each year.

3.1.5 Applied studies: types of medicines safety tests on primates in Britain

Over the last five years, primates have been used in eight types of safety tests of new pharmaceuticals:

i. **Acute limit-setting toxicity tests or dose-ranging lethal toxicity tests** - short-term tests which need not use death as an endpoint, but in which animals may nevertheless die due to toxic effects.

99 These species are grouped together in the official Statistics, but virtually all the animals used in this category are likely to be marmosets.

ii. **Acute non-lethal toxicity tests** - short-term tests whose endpoint is based on clinical signs of poisoning rather than a lethal endpoint.

iii. **Subacute limit-setting or dose-ranging toxicity tests** - repeat doses over 14-28 days.

iv. **Subacute fully quantitative toxicity test** - repeat doses over 14-28 days.

v. **Subchronic or chronic toxicity tests** - repeat doses over 90 days or more.

vi. **Toxicokinetics** - tests for the absorption, distribution around the body, metabolism and excretion (ADME) of medicines.

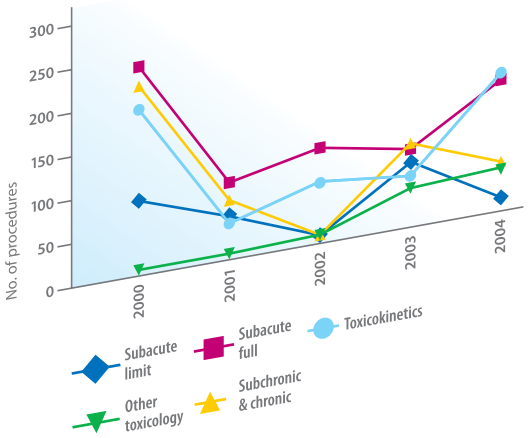
vii. **"Other" tests** - include safety pharmacology studies[100] to characterise the side effects that may be caused by the pharmacological action of a medicine.

viii. **Immunotoxicity tests** - to assess new pharmaceuticals for toxic effects on the immune system.

In the period 2000-2004, only one (new world) monkey was used in tests of type [i], in 2003. Also, in the year 2000 only, 12 procedures concerned with immunotoxicology [viii] were conducted on new world monkeys. The numbers of acute non-lethal tests [ii] on monkeys were few.

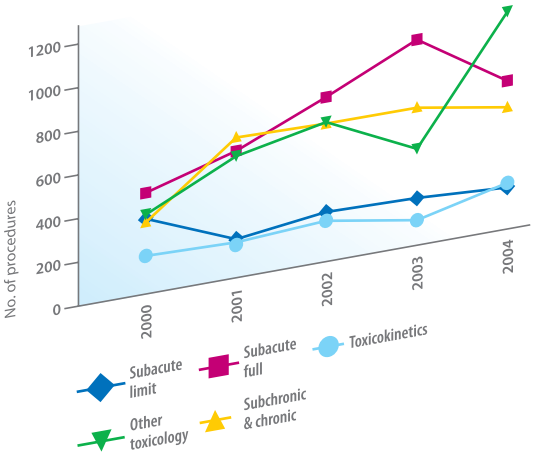
Therefore Charts 3.6 and 3.7 summarise only the most common tests, [iii] to [vii], conducted on new world and old world monkeys, respectively, over the period 2000-2004. For new world monkeys there has been an overall decline in all types of tests since 2000, but for old world monkeys, i.e. macaques, there have been no obvious trends up or down.

Chart 3.6 Procedures on new world monkeys for medicines toxicology by type of test, Britain, 2000 - 2004



See text for full description of test types.

Chart 3.7 Procedures on old world monkeys for medicines toxicology by type of test, Britain, 2000 - 2004



See text for full description of test types.

100 Animal Procedures Committee (2002). The Use of Primates Under the Animals (Scientific Procedures) Act (1986): Analysis of Current Trends with Particular Reference to Regulatory Toxicology, p14. London, UK: Home Office.

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3.1.6 Fundamental biological research in Britain

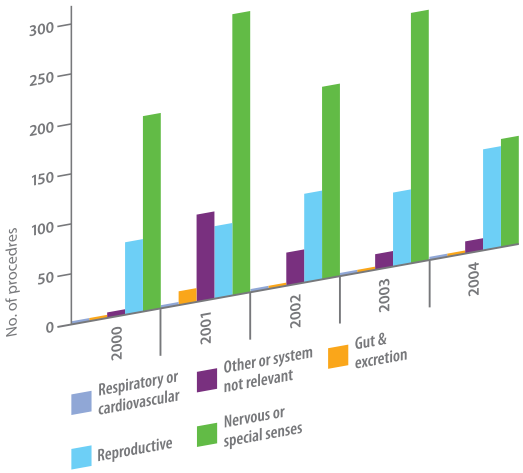
The second largest category of primate research and testing is "Fundamental biological research". This comprises experiments whose main purpose is to increase knowledge of the structure, function and malfunction of humans and other animals. Some of this research is curiosity- or knowledge-driven, in which practical applications are not foreseen or are beyond the scope of the experiments at the time they are done.

Some of this research, such as fundamental medical research into human diseases, is intended to contribute to solutions for human health problems, at some date in the future. Unfortunately it is not easy to distinguish this fundamental medical research from curiosity-driven research in the Home Office Statistics. Neither do they provide details of specific human diseases that are researched at the fundamental level using primates, although the fields of psychology, physiology, microbiology, immunology and pharmacology usually involve the most primates.

Numbers of fundamental biological experiments have varied slightly but not significantly over the last ten years, for both new world and old world monkeys. As Charts 3.2 and 3.3 show, fewer than 400 new world monkeys and 250 old world monkeys, on average, are used each year in this category.

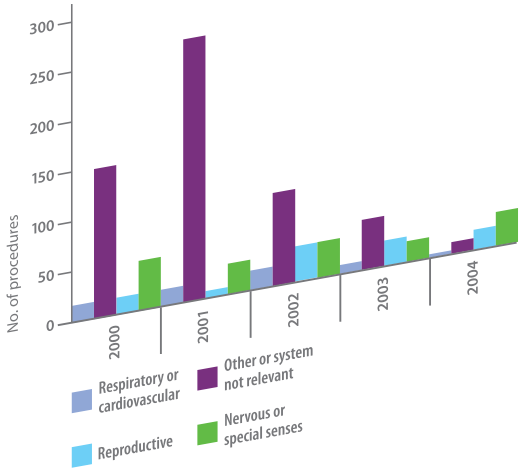
This category is sub-divided according to the primary body systems targeted by the research[101], as shown for new world and old world primates in Charts 3.8 and 3.9 respectively.

Chart 3.8 Fundamental biological research: procedures on new world monkeys by target body system, Britain, 2000 - 2004



"Other or system not relevant" includes immune system.

Chart 3.9 Fundamental biological research: procedures on old world monkeys by target body system, Britain, 2000 - 2004



"Other or system not relevant" includes immune system.

101 See Tree Tables 18d and 18e in the Home Office Statistics for the years 2000 to 2004.

Charts 3.8 and 3.9 illustrate the differences between the uses of new world versus old world monkeys in fundamental research. The major use of new world monkeys is for research into the brain and nervous system or the special senses (sight, hearing, touch, taste and smell).

The scientific literature reveals that some of these experiments involve brain-damaging marmosets with the intention of studying human Parkinson's disease[102]. Others include research into visual[103] and auditory functions[104], fundamental cognitive research[105], and marmoset 'models' of human multiple sclerosis[106]. Fewer such experiments use macaques, although they are subjected to Parkinson's disease research in some British laboratories.

Reproduction is the next most common body system studied in new world monkeys, such as experiments to study the control of cyclical changes in blood capillaries in the ovaries[107]. Macaques are also used in these types of studies, but not so extensively.

For several years, many fundamental research procedures carried out on macaques were simply not specified, being described by the Home Office as either involving unnamed body systems or being experiments where the system is not relevant. Latterly the Statistics have clarified that more than half of these involve the immune system (Chart 3.9).

3.1.7 Non-medical toxicology and research into toxicology in Britain

This third grouping of experiments comprises mainly, but not solely, toxicity or other safety tests of non-medical substances, such as pesticides, industrial chemicals and food additives. The Home Office calls this category "Protection of man, animals or environment - safety". No primates have been used in Britain to test these kinds of substances since the late 1990s.

However, some procedures in this category are conducted for research into toxicology or method development. Very few of these have involved new world monkeys, but since 2002 there has been a notable increase in this use of old world monkeys (i.e. macaques). It appears that in 2003, at least, this reflected the re-use of monkeys where the first use involved the taking of a blood sample for *ex vivo* studies. Thus the increase in non-medical "safety" *procedures* for 2003, seen in Chart 3.3, did not involve a concomitant increase in numbers of *animals* used (as can be seen from Chart 3.1).

3.1.8 Other studies on primates in Britain

The final category of experiments on primates consists of "Other" kinds of studies, mainly diagnosis of disease, which have generally used fewer than 100 monkeys per year.

102 Hurley MJ et al (2005). Immunoautoradiographic analysis of NMDA receptor subunits and associated postsynaptic density proteins in the brain of dyskinetic MPTP-treated common marmosets. Eur. J. Neurosci. 21:3240-3250. **103** McLoughlin N, Cotton P & Schiessl I (2005). A continuous smooth map of space in the primary visual cortex of the common marmoset. Perception 34:967-974. **104** King AJ (2005). Auditory plasticity: vocal output shapes auditory cortex. Curr. Biol. 15:R503-505. **105** Braesicke K et al (2005). Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. Eur. J. Neurosci. 21:1733-1740. **106** Pomeroy IM et al (2005). Demyelinated neocortical lesions in marmoset autoimmune encephalomyelitis mimic those in multiple sclerosis. Brain 128:2713-2721. **107** Fraser HM & Duncan WC (2005). Vascular morphogenesis in the primate ovary. Angiogenesis 8:101-116.

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3.2 Primate experiments in the European Union

Member states of the European Union (EU) collectively used approximately[108] 10,362 primates (including prosimians) in 2002, representing an increase overall of 14% since 1999[109].

In 2002, France (3,840) and Britain (3,173) accounted for the largest numbers of primates, with Germany (1,844) a distant third. Other main users were Belgium (567), Italy (420) and the Netherlands (270). Fewer than 100 primates were used each by Austria, Denmark, Spain and Sweden; and none at all by Finland, Greece, Ireland, Luxembourg and Portugal.

Table 3.1 Primate use in the EU, 2002

Country	Number of primates	% of EU total
France	3,840	37%
United Kingdom	3,173	31%
Germany	1,844	18%
Belgium	567	5%
Italy	420	4%
The Netherlands	270	3%
Sweden	91	<1%
Austria	78	<1%
Spain	74	<1%
Denmark	5	<1%
EU total	**10,362**	**100%**

Most member states do not categorise animal procedures on the basis of the levels of pain and suffering experienced by animals. Britain and Switzerland (non-EU) prospectively allocate expected severity levels to research projects. In Britain there is no information on *actual* levels of suffering and no specific data for primates. Both Switzerland and the Netherlands use scoring systems that allow actual levels of severity to be assessed, but neither country produces statistics specifically for primates.

3.2.1 Primate species in EU laboratories

Old world primates accounted for 8,075 (78%) of all primates used in the EU in 2002; 1,192 (11.5%) were new world monkeys and 1,095 (10.5%) were prosimians. The latter species were used only in Germany and France.

Between 1999 and 2002, experiments on prosimians more than doubled (up by 141%), and those on old world monkeys increased by 56%. Procedures on new world monkeys decreased by 11%. No great apes (chimpanzees, bonobos, orangutans or gorillas) were recorded as being used in the EU in 2002.

The European Commission's Scientific Committee on Animal Health and Animal Welfare (SCAHAW) described the range of primates being used in the EU[110]. Old world monkeys include cynomolgus, rhesus, pig-tailed and stump-tailed macaques, baboons *(Papio* species) and vervet monkeys *(Chlorocebus or Cercopithecus aethiops)*. New world species include marmosets, squirrel and owl monkeys,

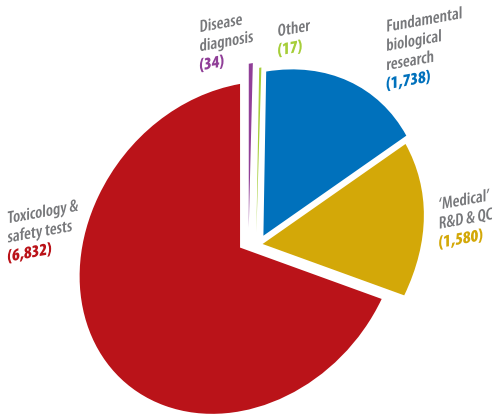
108 The figures are approximate partly because France provided statistics for 2001 instead of 2002. **109** Annex to the Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union in the year 2002. SEC(2005) 45. See <http://europa.eu.int/comm/environment/chemicals/lab_animals/statistics_reports_en.htm>
110 Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG.

cotton-top and saddle-back tamarins, and tufted and brown capuchin monkeys. Prosimian species include mouse lemurs and tarsiers.

3.2.2 Categories of primate use in the EU

Chart 3.10 shows the breakdown of primate use in the EU for 2002 by main categories of purpose. These categories are slightly different from those detailed for Britain in the preceding section.

Chart 3.10 Numbers of primates used in main experimental categories, European Union, 2002



3.2.3 Toxicology in the EU - includes lethal tests on primates

This category represented 66% of primates used in the EU in 2002, compared with 53% in 1999. All the tests were for medicines or other products for human medicine and dentistry, or for veterinary use, with the exception of 101 old world monkeys used in "other", undefined safety evaluations. No other types of products, including industrial, household, cosmetic or agricultural chemicals, or food additives, were tested on primates in 2002.

Shockingly, 603 old world monkeys and 123 prosimians were used in lethal poisoning tests, either the LD50 or LC50 test. 1,592 old world monkeys were involved in acute or subacute non-lethal toxicity tests based on clinical signs of poisoning rather than using death as an endpoint. These tests were also conducted on 129 new world monkeys and 152 prosimians.

2,417 old world monkeys (plus 78 new world monkeys and 172 prosimians) endured repeat-dose toxicity tests lasting 90 days to nine months. Other, unspecified tests were conducted on 1,490 old world monkeys, 41 new world monkeys and 35 prosimians. No primates were used in skin or eye toxicity tests, or in carcinogenicity tests, or in tests for toxicity to reproduction or fetal development.

3.2.4 Fundamental biological research in the EU

The next most common type of experiment was for fundamental biological research which used 1,738 primates, split evenly into prosimians, new and old world species. In 2002 this category accounted for 17% of primates used in the EU, compared to 18% in 1999.

As with the British Statistics, it is impossible to extract from the EU statistics a deeper analysis of the kinds of fundamental research being conducted. There is some information on the human and animal diseases researched using

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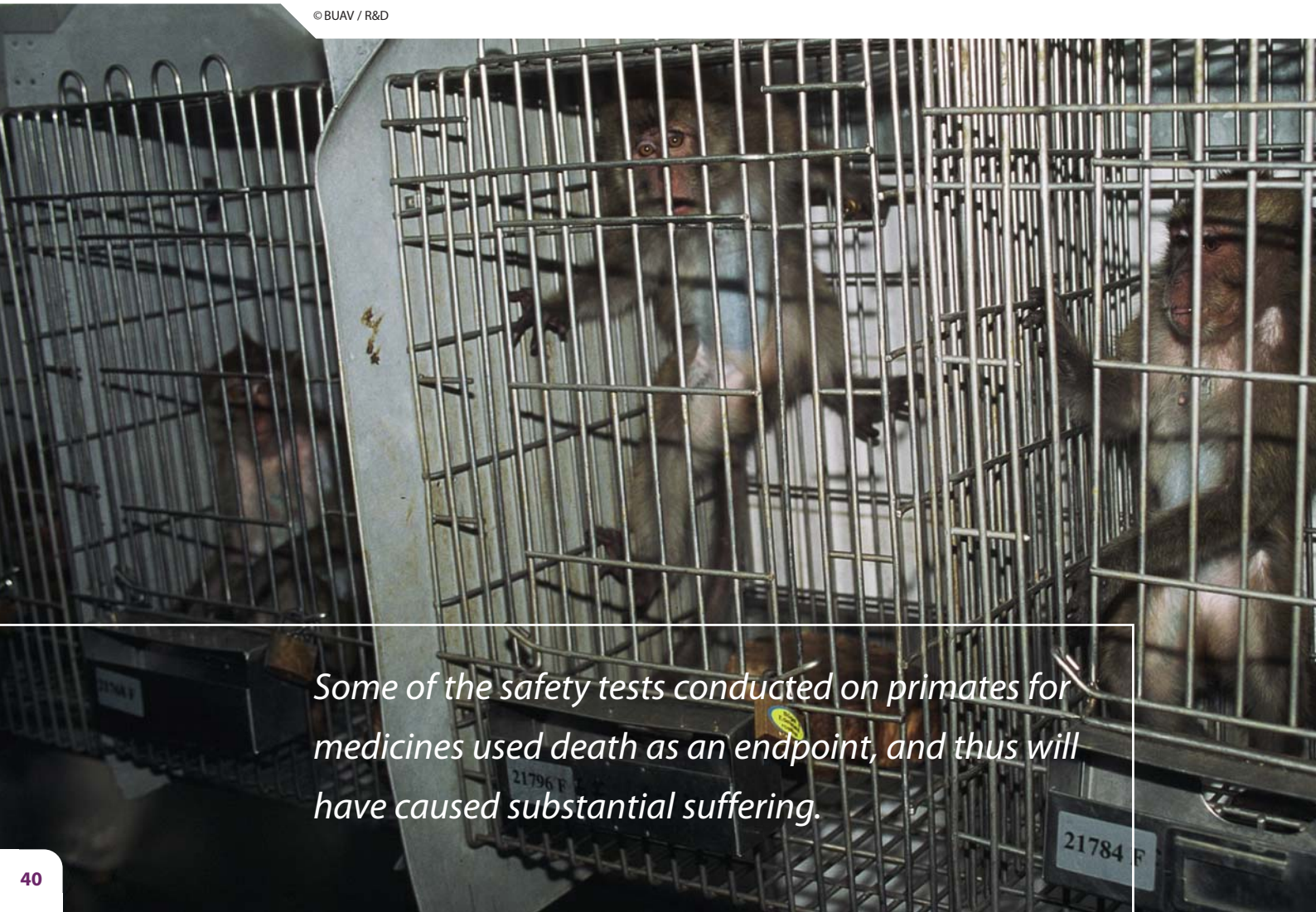
primates[111], but the total number of individuals used in diseases research exceeds the number used in fundamental biological research. This suggests that the figures for diseases research include some applied studies as well.

However, some indication of the main diseases being studied at a fundamental level using primates can be gleaned. This work used more old world than new world monkeys. Most primates (963 animals) were used to study "Human nervous and mental disorders", followed by research into human cancer (189 animals) and cardiovascular diseases (186 animals). Research into other, unspecified human diseases used 3,591 primates, and studies of unspecified animal diseases involved 17 animals.

3.2.5 Medicines research, development and quality control in the EU

Research, development, production and quality control of products and devices for human medicine and dentistry, and for veterinary medicine, was the next largest use of primates, involving 1,580 individual animals in 2002. Chapter 7 discusses this area in detail. The category is further sub-divided in the



Some of the safety tests conducted on primates for medicines used death as an endpoint, and thus will have caused substantial suffering.

statistics into research and development (1,243 primates); and production and quality control (337).

Research and development is an earlier stage in medicines development than safety testing and almost all the products tested, primarily on old world monkeys (899) and new world monkeys (330), were human drugs and vaccines rather than veterinary products.

The same is true for the production and quality control of medicines, mainly vaccines. This used 31 old world monkeys and 22 new world monkeys. The routine safety testing of batches of oral polio vaccine probably accounted for many of the old world monkeys used in this category (see Chapter 7).

Overall, these kinds of experiments involved 15% of all primates used in 2002, as opposed to 26% in 1999.

3.2.6 Other primate experiments in the EU

In 2002, 34 primates were used to diagnose disease, seven were used in education and training and 171 were unclassified by purpose of the experiment.

3.3 Main findings

Official statistics tend to underestimate numbers of procedures and/or numbers of primates used in laboratories each year. In Britain, as in the EU, there is no clear evidence of a downward trend in primate experiments; indeed, in the EU the numbers rose by 14% between 1999 and 2002. France, Britain and Germany between them accounted for 80% of all primate experiments in the EU. More old world monkeys are used in laboratories than new world species. No experiments on great apes were reported in the EU in 2002. The range of species found in British laboratories is narrower than that used in some other EU countries.

The largest category of primate experiments is for the purpose of developing and testing new medicines. Non-medical products such as pesticides or household chemicals have not been tested on primates in the last few years. Some of the safety tests conducted on primates for medicines used death as an endpoint, and thus will have caused substantial suffering. Most were longer-term repeat-dose studies relying on clinical signs of poisoning rather than lethality. Probably most medicines tests on primates are conducted to provide data to regulatory authorities (see Chapter 6); in Britain this is certainly so.

Curiosity-driven fundamental research and basic medical research into human diseases use fewer, though significant, numbers of primates, the key area being neurological function. Some primates are used to diagnose diseases and others, although not in Britain, in unspecified experiments for the purposes of education and training.

111 For example, Table 4.1 in the EU statistics for 2002. Document SEC(2005) 45. See <http://europa.eu.int/comm/environment/chemicals/lab_animals/statistics_reports_en.htm>

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Animal experiments conducted in member states of the European Union (EU), where regulated at the European level, are done so according to European Directive 86/609/EEC[112]. The Directive provides a minimum legal framework: member states were obliged to implement it by November 1989, but they may control animal experiments more stringently, if they wish, through their national measures.

4.1 Directive 86/609/EEC

Broadly speaking, the Directive controls the purposes for which animal experiments may be conducted; sets some guidelines for appropriate housing and care; requires that the harm done to animals should be minimised; and has provisions relating to the sources of laboratory animals, the notification in advance of proposed experiments, the education and training of persons who conduct experiments and the registration of laboratories which perform experiments.

The Directive is 20 years old and no longer represents a modern consensus on animal experiments, or on animal housing and husbandry. It is currently undergoing revision and is expected, in the future, to provide more stringent protective measures for animals. Several of these have a bearing on the use of primates. For example, the Technical Expert Working Group[113] set up a sub-group to advise on whether the Directive should include the requirement for a cost/benefit assessment of animal experiments.

The sub-group recommended that a cost/benefit assessment should be compulsory for each research project using animals, and that this should be conducted both prospectively and retrospectively[114]. This could have a significant impact on the licensing of primate experiments in most EU countries, as few have such a system at the moment.

Another sub-group, tasked with considering the revised scope of the Directive, advised that it should extend to all scientific procedures on animals, not just those narrowly defined as experimental[115]. For the first time this would bring under the Directive all routine 'non-experimental' procedures on primates, such as their regular use in the production of oral polio vaccine in Belgium.

The general guidelines for animal housing and handling set out in Annex II to the Directive are very out of date and have been under revision for many years. The process of updating them to reflect current understanding of the needs of different animal species is being conducted under the auspices of the Council of Europe Convention ETS123[116]. When finally agreed, the

112 Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of Member States regarding the Protection of Animals Used for Experimental and other Scientific Purposes. Official Journal of the European Communities 1986 L358:1-29. **113** The TEWG was tasked by the European Commission to provide scientific and technical background information for updating Directive 86/609. **114** The Technical Expert Working Group's Sub-Group on Cost-Benefit (2003). Final Report.
See <http://europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm> **115** Technical Expert Working Group Sub-Group on Scope (2003). Final Report.
See <http://europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm> **116** Council of Europe Convention ETS123 for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

guidelines are also likely to be adopted under Directive 86/609. However, it is important to remember that these are not best-practice standards. Under Article 5 of the Directive there is an over-arching duty to provide animals with housing and care *"appropriate to their health and well-being"*, but this is often not met in practice.

4.2 The Three Rs in legislation

The Directive requires anaesthetics to be used unless their administration causes more suffering than if they were not applied; or unless they are incompatible with the purpose of the experiment. Analgesics must be used to ensure, as far as possible, that pain and suffering do not occur or are limited to the absolute minimum; and certainly to ensure that no animal suffers severely.

Most primates are used in harmful and sometimes lethal toxicity tests, and analgesics are virtually never provided because they might interfere with the conduct of the tests (e.g. by interacting with the test chemical). So although the wording of the Directive reads reassuringly, some pain, distress or suffering are common and, in reality, almost inevitable features of primate experiments.

The Directive requires that animals are provided with housing and some freedom of movement. Any restriction on the extent to which animals can satisfy their physiological and behavioural needs should be *"limited to the absolute minimum"* (Article 5).

The principles of the Three Rs are enshrined in the Directive (Articles 7[2], [3] and [4] and 23[1]). Accepted worldwide[117], the Three Rs place on those who plan, conduct and regulate animal experiments a responsibility to implement valid Replacement methods (those which do not use living animals); to Refine experimental protocols and housing in order to minimise animal suffering; and to Reduce the numbers of animals used in each experiment.

The following Articles from Directive 86/609 are particularly relevant to primates:

- **Article 4:** experiments on animals listed as endangered under Appendix I of CITES (including all apes) are prohibited with only very limited exceptions.
- **Article 7[2]:** experiments shall not be conducted on animals if a scientifically satisfactory non-animal method is reasonably and practicably available.
- **Article 7[3]:** animals taken from the wild may not be used in experiments unless other animals would not suffice (see Chapter 5).
- **Article 18:** primates, cats and dogs shall be individually identified before weaning with a permanent mark, and records of their identity and origin kept.
- **Article 19[4]:** laboratories may only use animals from breeding or supplying establishments unless a special or general exemption has been obtained. Bred rather than wild-caught animals are to be used whenever possible (see Chapter 5).

117 Anon (2000). The Three Rs Declaration of Bologna and Background to the Three Rs Declaration of Bologna, as adopted by the Third World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, on 31 August 1999, In: Developments in Animal and Veterinary Sciences, 31A:15-22.

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- **Article 21 and Annex I:** primates (and certain other species) shall be purpose-bred unless the national authority gives a special or general exemption (see Chapter 5).

- **Annex II:** sets out as recommendations some minimal guidelines for housing for laboratory animals.

4.3 Regulation in EU member states acknowledges the special status of primates

Having transposed Directive 86/609 into national legislation, each EU member state is free to apply stricter requirements either in its own national laws or by administrative measures. Interestingly, several have done so in the case of primates, acknowledging the widespread and strongly felt concern about these species.

Thus, for example, Austria, Britain, the Netherlands and Sweden have already introduced some form of prohibition on the use of apes in laboratories. In the case of Germany (since 1991) and Italy (and Norway, a non-EU country), great apes have not been used for some years although there is no official national restriction. Similarly, In Ireland it has been the practice not to license any experiments on primates, but this is not written into law.

In another example, for some years Britain has had an administrative system for assessing overseas suppliers of primates according to their standards of housing and husbandry (see Chapter 5). The Home Office, although it has no jurisdiction overseas, does not authorise the import of consignments of primates by any laboratory from an overseas source that falls below certain minimal standards. A similar system has been recommended for the revised Directive 86/609 (see above).

In Italy, experiments on primates (and dogs and cats) require authorisation from the Ministry of Health, while for most experiments on other species researchers need only provide a communication to the Ministry. Similarly in Britain, primates (and dogs, cats and *equidae*) have 'special protection' under the 1986 *Animals (Scientific Procedures) Act*, meaning that they may only be used in procedures if animals of no other species will suffice or are reasonably available.

The fact that so many countries have made special provisions for primates is a clear acknowledgement of their unique moral status. Unfortunately, even with the best will in the world, the regulation of experimentation does not prevent thousands of primates experiencing pain, suffering and distress in EU laboratories every year.

Marmosets at Cambridge University © BUAV

4.4 Are primate experiments properly regulated in Europe?

In the last five years the BUAV has conducted two undercover investigations in laboratories using primates in research and testing, one in Cambridge, England, and one in Münster, Germany. Both revealed serious problems in the implementation either of the Directive itself, of the relevant national law or of administrative measures.

4.4.1 A British undercover investigation

A BUAV investigator undertook an undercover investigation at a department at Cambridge University during 2001. Marmosets were used in a series of research projects to study basic brain function, and in applied medical research looking for novel treatments for strokes and Parkinson's disease.

All the projects involved inflicting brain damage, either by ablation, toxin injection, surgical transection of fibrous tracts or blockage of the cerebral artery (the latter two procedures involved extensive surgery including lifting of the upper half of the skull). Some animals were brain damaged up to four times.

In the months before surgery, marmosets were trained to perform behavioural and cognitive tasks. Afterwards, they repeated the tasks to assess the specific effects of the brain damage and the ability of experimental therapies to ameliorate it. Water deprivation (for 22 out of 24 hours, five days a week, for up to two and a half years), and/or food restrictions were often used to motivate the monkeys to perform the tasks. Of the sixteen different experiment protocols authorised in the project licences for this research, not a single protocol was considered to exceed a 'moderate' severity limit, i.e. none of them was designated 'substantial'. Yet the Home Office's *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986* defines substantially severe protocols as:

"Protocols that may result in a major departure from the animal's usual state of health or well-being. These include: ...major surgery; and some models of disease, where welfare may be seriously compromised..."

It further says, *"If it is expected that even one animal would suffer substantial effects, the procedure would merit a 'substantial' severity limit."*

Immediate post-operative effects of brain damage in these experiments could include pain, swelling or bruising, bleeding from head wounds, epileptic seizures, vomiting, tremors, hypothermia, failure to eat and drink, abnormal body movements (e.g. head twisting and body rotation), loss of movement in one limb or one side of the body, loss of balance, and visual disturbances.

Longer-term effects included physical disabilities, difficulties in making intentional visually guided movements, learning and memory impairments, mood alterations, emotional disturbances and weight loss. Many marmosets appeared confused, with blank expressions and uncoordinated movements. Several were found dead or had to be euthanased, which is strong evidence of substantial or even severe suffering.

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It was clear to the BUAV and to independent experts that, by the Home Office's own definitions and by basic standards of common sense, some substantial suffering must have occurred and, in many cases, continued for a considerable period of time. By not according any protocol a severity limit of more than 'moderate', the Home Office was effectively saying that *no* animal, for *any* period, would suffer more than moderately. We believe this judgement to be completely unacceptable.

In particular, the BUAV feels that proper account was not taken of the suffering caused by mental and emotional disturbances - such as confusion, hallucinations, Kluver-Bucy syndrome[118], social disruption, frustration due to physical incapacity, the stress clearly caused by some training procedures and the effects of deliberate damage to emotional centres in the brain. In this we are supported by David Morton, Professor of Biomedical Science and Ethics at the University of Birmingham, England. Professor Morton saw video footage of the marmosets at Cambridge University and commented in a written statement to the Animal Procedures Committee (APC)[119]:

"I take it that I do not need to re-iterate my views that assessment of psychological/mental and physiological distress, as opposed to overt pain, is an important and under-estimated factor in the assessment of harms done in research projects."

The significance of the 'moderate' severity classifications is threefold. First, it meant that the applications for project licences to conduct the experiments avoided additional scrutiny by the APC, the Government's independent advisory body. With regard to severity, only primate research anticipated to be 'substantial' was, at that time, referred to the APC. Second, it inevitably skewed the cost/benefit test which lies at the heart of the 1986 Act[120]: the lower the level of suffering that is envisaged, the easier it is to satisfy the test. Third, it distorts Britain's annual statistics, one of the few sources of public information about animal experiments.

An additional concern with the Cambridge University experiments on marmosets was that, although measures were proposed for treating expected pain, psychosis and seizures, in a laboratory not staffed at night and with only a skeleton staff at weekends and on public holidays, the animals' suffering often could not be alleviated promptly. Several marmosets only received a single pain-killing injection post-operatively (according to the laboratory records) and were usually left overnight without attention, sometimes for as long as 15 hours. This included animals with low body temperature, bleeding head wounds, tremors, seizures and collapse.

These are highly significant issues pertaining to the implementation of legislation and regulation by the Home Office and by a prestigious academic laboratory conducting primate experiments. They prompted the BUAV to start proceedings to take the Home Office to Judicial Review. In April 2005 we were given permission to proceed on two grounds, and two further grounds were granted on appeal. The four grounds are:

118 Kluver-Bucy syndrome is caused by certain brain lesions and is a psychological condition, lasting one to four weeks, which prevents self-care because marmosets fail to eat and drink. They appear to experience hallucinations, are sometimes in a trance-like state, have psychic blindness and display inappropriate and abnormal social interactions. **119** Animal Procedures Committee (2005). Final Report of the Cambridge/BUAV Working Group, Annex E. 16 June 2005. See <http://www.apc.gov.uk> **120** Section 5[4] of the Animals (Scientific Procedures) Act reads: "In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the licence". The greater the likely suffering, the greater must be the likely benefit, and vice versa.

Brain damaged Marmoset at Cambridge University © BUAV

i. Assessment of the severity of procedures
The BUAV believes that the Home Secretary under-estimated the likely adverse effects that marmosets would experience in some protocols and, in any event, should have revised his assessment of the severity of these protocols in light of the experience that accrued as they proceeded.

ii. Arrangements for care were inadequate
We believe that arrangements were inadequate to care for brain-damaged marmosets, particularly post-operatively and outside normal working hours. [i] and [ii] are closely linked in that, even if suffering was properly described as only 'moderate' assuming early intervention if animals deteriorated, the fact that there was often no-one on site for several hours even after brain surgery meant that such intervention was not possible.

iii. Death as an 'adverse effect'
The BUAV claims that death, a possible if unintended outcome of certain experiments but also the likely ultimate fate of all the animals used, should be counted an 'adverse effect' under section 5[4] of the national legislation, the *Animals (Scientific Procedures) Act.*

iv. Guidance on food and water restrictions
We believe the Home Secretary should have consulted the government's advisory committee, the Animal Procedures Committee, under section 21 of the national legislation, over the guidance notes issued on food and water restrictions for laboratory animals.

The court case, which may set important precedents regarding animals used in experiments and how their suffering is judged, is expected to take place in late 2006.

In the meantime, the Animal Procedures Committee undertook a detailed examination of the issues raised by the BUAV's investigation. As a result, the committee identified a number of areas which should receive further scrutiny[121], including:

- Levels, training and competency of laboratory animal staff.
- The roles and responsibilities of relevant bodies involved in setting, reviewing and maintaining standards of animal welfare, and initiating improvements.
- The publicity issued by funding bodies and medical charities regarding the benefits of the research they fund and, especially, the 'costs' to animals used.
- A re-working of the methods used to indicate the severity of animal suffering, to build a more detailed picture and a more generally agreed understanding of what animal research involves.

121 Animal Procedures Committee (2005). Final Report of the Cambridge/BUAV Working Group, 16 June 2005. See <http://www.apc.gov.uk>

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- The sources of disagreement about the nature and degree of severity of suffering.
- The implications of food and water deprivation and deferral.
- What levels of detection should be in place in order to satisfy the requirements of legislation.

These serious proposals provided a large measure of support for the BUAV's key concerns about the research at Cambridge.

4.4.2 A German undercover investigation

During 2003, the BUAV conducted an investigation at a contract testing facility called Covance GmbH, in Münster, Germany. This laboratory carries out a range of animal toxicity tests for client companies, almost all on primates. Covance is owned by a US-based multinational contract testing company of the same name. Facilities operated by subsidiary companies exist in 18 countries, including Germany, the UK and Switzerland. Covance has more than 6,900 employees worldwide and claims to be *"the global leader in safety assessment testing" and "one of the world's largest and most comprehensive drug development service companies"*. Its 2002 revenues were US$883 million and its customers include the top 50 global pharmaceutical and biotechnology companies in the world.

The BUAV believes that the conditions in which macaque monkeys were kept at Covance were appallingly inhumane and contrary to Directive 86/609/EEC. There were also occasions of inappropriate, occasionally brutal, treatment of monkeys by staff, as evidenced by video footage.

The preamble to the Directive makes clear that member states should ensure that:
"...animals are adequately cared for, that no pain, suffering, distress or lasting harm are inflicted unnecessarily and ensure that, where unavoidable, these shall be kept to the minimum."

Further, Article 5[a] requires that:
"all experimental animals shall be provided with housing, an environment, at least some freedom of movement, food, water and care which are appropriate to their health and well-being."

Article 5[b] states:
"any restriction, on the extent to which an experimental animal can satisfy its physiological and ethological needs, shall be limited to the absolute minimum."

In a report by the Scientific Committee on Animal Health and Animal Welfare, commissioned and adopted by the EU Commission, the following conclusions were reached regarding the housing of primates[122].

13.3 *"Most primates are highly social and intelligent animals and their cognitive skills have been shaped by evolution to find and handle food, and to relate to other individuals in a social group. Having social partners is one of the most significant needs*

122 Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG.

of primates and they develop abnormal behaviour patterns when socially deprived. Providing social partners is an important way to provide stimulation to animals and to enrich their environment...".

13.4 *"Primates need an enriched and stimulus-enhanced environment in captivity to explore, manipulate, play, forage and search for food; merely satisfying minimum space requirements is inadequate...".*

13.8 *"When primates cannot express their normal behaviour and satisfy their needs to show certain behaviours, either because of a lack of environmental diversity, or an insufficient amount of space, they develop abnormal behaviour patterns (e.g. stereotypies)...".*

13.9 *"Since primates are usually social animals, single housing is always detrimental to their welfare, and placing them in cages in double tiers impairs their natural vertical flight reaction and contributes to poor illumination of cages...".*

At Covance, prior to the start of an experiment the macaques were held individually in tiny quarantine cages, 60cm (h) x 40cm (w) x 40cm (d), for approximately two to four weeks. These cages were completely barren and empty. The monkeys were then housed singly in experimental cages of dimensions 80cm x 60cm x 60cm. The rhesus macaques and some of the larger cynomolgus males were housed in slightly larger cages (90cm x 70cm x 70cm). Many of the cages did not even meet the limited cage sizes recommended by Table 9 of Annex II[123] to the Directive. For example, the smaller 'quarantine' cages, where new macaques were kept for a few weeks, did not meet the Annex II minima. The wording of the Directive indicates that the extent to which the macaques could satisfy their physiological and ethological needs should be compared with contemporary standards, not those existing in 1986 when the Directive was passed. The issue, therefore, is whether Covance met standards generally accepted as representing the needs of macaques in 2003, at the time of the BUAV investigation. The evidence uncovered by the BUAV strongly suggests that they did not.

Contrary to good practice, the cages were in two tiers and all the experimental macaques (and the vast majority of the stock macaques) at Covance were housed singly. Both of these conditions should be avoided. There were no shelves or perches in the cages of most cynomolgus monkeys. The cage floors comprised metal bars, the rooms had no natural lighting and there were no opportunities to *"explore, manipulate, play, forage and search for food"*, in the words of the Scientific Committee on Animal Health and Animal Welfare. Some cages were completely barren; others had merely a small wooden block that the monkeys could sit on, and the occasional plastic bone. Even pregnant females were kept in this manner and had to give birth on the metal bars of the cage floor.

123 Whether they do depends on the weight of the macaques.

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These appalling conditions led to serious stereotypical behaviours in some monkeys, including repetitive rocking, circling and back-flipping. As Dr Jane Goodall said, in her expert witness statement for the BUAV:

"In my opinion it is not at all surprising to see monkeys at Covance exhibiting stereotypical behaviour because the conditions in which they are being kept are such that mental decline is a likely outcome. Isolation and loneliness, cramped conditions, barren cages, boredom and underlying fear - woefully inadequate conditions in which opportunities to express natural behaviour are inhibited - are the perfect recipe for mental decline and all of these factors are present at Covance."

In addition to the inadequate housing, the BUAV investigator witnessed and filmed various physical and psychological abuse of macaques, demonstrating a laboratory culture lacking care. Pertinent incidents include forcing a monkey to 'dance' to background music; at least one macaque had its arm broken; a minimum of three other macaques had to be put down following severe injuries suffered during handling or transportation; and anaesthetised animals were carried by just one limb.

Consequently, the BUAV lodged a formal complaint with the German authorities against Covance, citing several concerns including the inadequate nature of the housing facilities and the abuse of primates by staff. However, the principal German national law (the *Tierschutzgesetz*) requires a high level either of animal suffering or of cruelty to be demonstrated before a prosecution can occur. Even so, the German authorities failed to take appropriate action, so the BUAV has taken a complaint against Germany to the European Commission.

We argue that Directives must be transposed into national law in a way that ensures compliance. We said that the sanctions contained in German law are insufficient to ensure that the suffering of laboratory animals is kept to the minimum, as required by the Directive. In essence, the BUAV has accused the German government of failing to adequately transpose Directive 86/609 into domestic law and failing to implement it properly, whether through legislative means or administratively. At the time of writing the BUAV was awaiting the final decision of the Commission.

It is contemptible that such housing conditions and treatment of primates should be considered acceptable in the laboratory of a multinational contract research company. Even before monkeys started on experimentation, they would have suffered weeks of distress, frustration, fear and deprivation. If standards are this poor in the laboratory

Two tier barren cages at Covance Laboratory Germany ©BUAV / R&D

of an industry leader, the public can have no reassurance that the situation is any better in the laboratories of smaller companies and universities.

These examples are graphic illustrations that guidelines on standards of housing can be ignored; and that no legislation, however well intentioned, can ever ensure that individuals behave appropriately. We are told that standards of behaviour of individual staff are better ensured by establishing a 'culture of care' in each laboratory, yet undercover investigations have always revealed failures here too. Animal care staff almost inevitably become desensitised to the suffering or distress of the animals they look after, and in some individuals this results in callous or brutal treatment that is inexcusable.

Therefore, even the minimal standards required by laws, regulations and administrative measures cannot be ensured; neither can the 'appropriate' treatment of primates in laboratories, even as currently defined (and excluding the pain and suffering caused by experiments).

CHAPTER FIVE

The supply and transport of primates to laboratories

The acquisition and transport of primates to the world's laboratories causes these animals extensive distress, suffering and loss of life. Old world monkeys such as cynomolgus and rhesus monkeys are bred and housed in source countries (e.g. China, Mauritius, Vietnam) in conditions that would not normally be acceptable in the European Union (EU). They are then packed in small crates and transported over very long distances, by lorry and by air.

When monkeys are captured from the wild, as many are, the suffering is greatly increased, as the conditions of capture are frequently inhumane, sometimes resulting in injuries and deaths. Keeping wild primates in confinement inevitably leads to psychological and sometimes physical suffering.

5.1 Controls of the supply of primates to the EU

The supply of primates to European laboratories is controlled and monitored under three main legislative provisions:

- the international Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as applied by the EU Wildlife Trade Regulations[124];
- the EU Directive 86/609/EEC[125] on the protection of animals used for experimental and other scientific purposes; and
- the Council of Europe Convention[126] ETS123 addressing the same subject.

All wild primates are protected under CITES (either under Appendix I or Appendix II) because their capture depletes natural populations and may endanger their conservation. The trade in primates is controlled and parties to CITES produce annual statistics of exports and imports, although these exclude transfers between EU countries.

Both Directive 86/609 and European Convention ETS123 refer to the source of animals, particularly primates, used in experiments.

5.1.1 Directive 86/609/EEC

Article 4 of the Directive prohibits research on animals considered as endangered under Appendix I of CITES (which includes all the apes), unless it meets CITES provisions; and is aimed at preserving the species or is for essential biomedical purposes where no other species would suffice.

The Directive also forbids the use of wild-caught primates in laboratories unless no other option is available, in which case an exemption is required. Articles relevant to the supply of primates include:

124 Convention on International Trade in Endangered Species of Fauna and Flora. Official Journal of the European Communities 1982 L384:1-61. **125** Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of Member States regarding the Protection of Animals Used for Experimental and other Scientific Purposes. Official Journal of the European Communities 1986 L358:1-29. **126** Council of Europe Convention ETS123 for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986).

Article 7.3: *"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".*

and

Article 21: *"Animals belonging to the species listed in Annex I* [which includes primates] *which are to be used in experiments shall be bred animals unless a general or special exemption has been obtained under arrangements determined by the authority".*

Under the process of revision of the 86/609 Directive, the Technical Expert Working Group set up by the European Commission to provide scientific and technical advice, established a sub-group on authorisation of animal experiments in the EU. The sub-group's report made clear that[127]:

"In the case of primates used in research and testing, the acquisition of some species may involve capture from the wild, inadequate husbandry, and/or lengthy, multi-staged travel from the country of origin to the user establishment. Thus primates may endure considerable 'harms' even before they reach the laboratory. In view of their advanced cognitive capacities and highly social nature compared to other laboratory animals, these harms may cause primates a great deal of psychological distress."

The sub-group also stated that there is now an important opportunity to improve significantly standards at breeding and supplying establishments, including those in non-EU countries. For primates, the report recommended a detailed, advanced authorisation process at the national level for each consignment of primates imported, as is presently the case in Britain. This would include ensuring, for example by visits from the national authority, that any overseas centre operates to a sufficiently high standard. This would be an improvement on the present situation, but however well-run such centres may be, the process of supplying primates to laboratories will inevitably cause suffering, as we argue below.

5.1.2 European Convention ETS123

Some 46 countries belong to the Council of Europe and 21 have signed the 1986 Council of Europe Convention ETS123, indicating their intention to adhere to its provisions. The Convention permits the use of wild-caught primates, but recommends that efforts are made to develop the supply of purpose-bred primates so that the use of wild animals becomes exceptional.

The Convention says:

Article 21[1]: *"Animals of the species listed below* [which excludes primates] *which are for use in procedures shall be acquired directly from or originate from registered breeding establishments, unless a general or special exemption has been obtained under arrangements to be determined by the Party...".*

but also

Article 21[2]: *"Each Party undertakes to extend the provision of paragraph 1 of this article to other species, in particular the order of primates, as soon*

127 Technical Expert Working Group Sub-Group on Authorisation (2003). Final Report. See <http://europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm>

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as there is a reasonable prospect of a sufficient supply of purpose-bred animals of the species concerned".

Subsequently, the Council of Europe's 1997 *Declaration of Intent concerning Animals used for Scientific Purposes*[128], signed by sixteen member states and twelve professional organisations, included a section specifically on primates which went further than the Convention:

"Furthermore in relation to primates [the signatories agreed]:

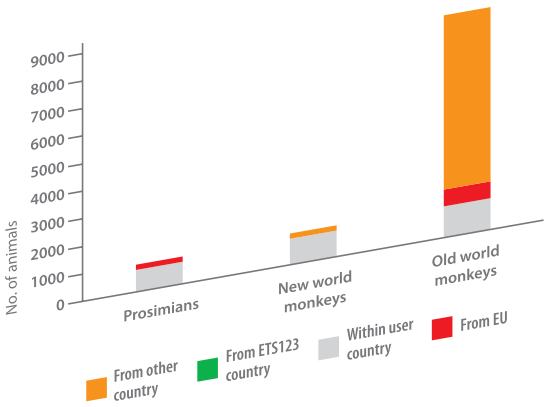
- *to require precise information on the origin and the provenance of the animals with the objective of limiting the use of animals to those which are purpose bred;*
- *to encourage initiatives and measures to end the use of wild-caught primates".*

5.2 Sources of primates used in EU laboratories

Some information about the sources of primates used in EU member state laboratories can be found in the official European Union statistics, collected under Directive 86/609. Chart 5.1, which summarises the latest available figures (for 2002)[129], shows that 91% of prosimians and 89% of new world monkeys were obtained either from breeders or suppliers within the user state, or from other EU countries.

However the reverse is true for old world monkeys, 78% of whom were acquired from non-EU and non-Council of Europe states. This implies much longer journeys, mainly from supplying source countries such as Vietnam, China and Mauritius, where standards of housing and care are considerably lower than in Europe. Some animals, once imported from the original supplying country, are later re-exported to another EU member state.

Chart 5.1 Origins of primates used in EU laboratories, 2002



"From other country" refers to non-user, non-EU and non-ETS123 countries.

"ETS123 country" refers to supplying countries that are not EU member states but are members of the larger Council of Europe, working under Convention ETS123

Within these broad EU statistics are differences between the member states due to varying patterns of species use. For example, Britain has not imported wild-caught primates for several years while France and Belgium still import considerable numbers, especially vervet monkeys from the Caribbean and cynomolgus monkeys from Mauritius.

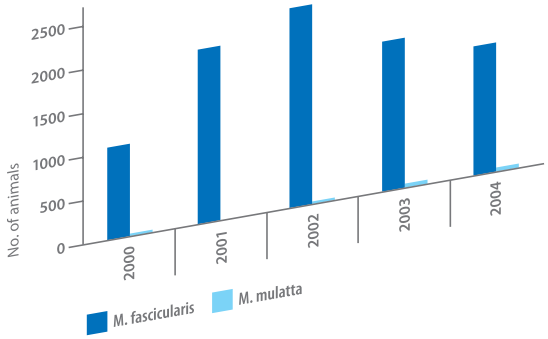
128 Council of Europe (1997). Declaration of intent on the Use, Transport, Care and Accommodation of Laboratory Animals. CONS 123 (96) 5. **129** Annex to the Report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union in the year 2002. SEC(2005) 45. See <http://europa.eu.int/comm/environment/chemicals/lab_animals/statistics_reports_en.htm>

5.3 Imports of primates by Britain

Statistics published under the Convention on International Trade in Endangered Species of Fauna and Flora (CITES)[130] show that, with few exceptions, most of the primates imported by Britain for use in medical or scientific research are old world monkeys, by far the majority being cynomolgus macaques *(Macaca fascicularis)*. Only small numbers of rhesus macaques *(Macaca mulatta)* are imported, others being bred at British centres.

Import statistics for old world monkeys are illustrated in Chart 5.2, which shows that there has been a decline in British imports of cynomolgus monkeys since 2002, although it is too early to say whether this represents a significant trend.

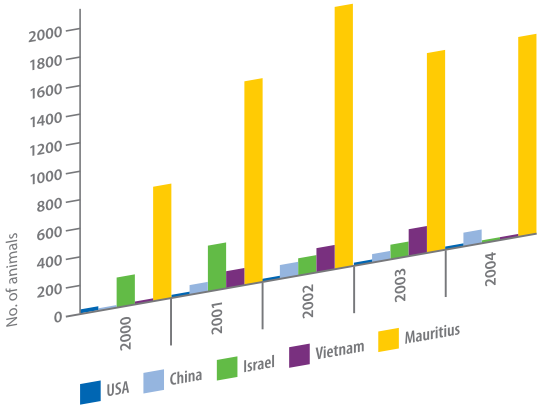
Chart 5.2 CITES figures for imports of old world monkeys to Britain for medical/scientific use, 2002 - 2004



CITES statistics exclude primates traded between European Union member states

The source countries supplying old world monkeys to Britain have varied slightly over the five-year period 2000-2004, as can be seen in Chart 5.3. Mauritius is Britain's consistently largest supplier, imports from Israel have fallen, while numbers from China and Vietnam have fluctuated. Small numbers are very occasionally imported from the USA (e.g. 17 rhesus monkeys in 2000).

Chart 5.3 CITES imports of old world monkeys to Britain by supplying country, 2000 - 2004



CITES statistics exclude primates traded between European Union member states

130 CITES trade statistics derived from the UNEP-WCMC CITES Trade Database, UNEP World Conservation Monitoring Centre, Cambridge, UK. See <http://sea.unep-wcmc.org/citestrade/trade.cfm>

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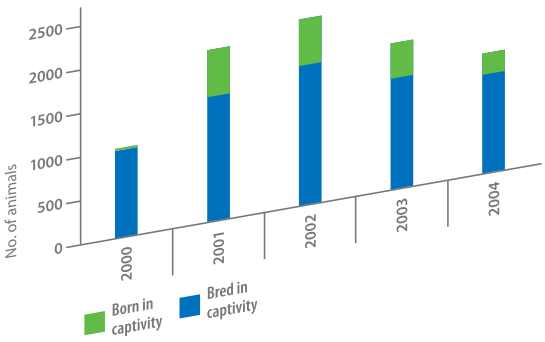
Britain's policy restriction on the use of wild-caught primates was introduced in 1995, and none have been imported for several years. Chart 5.4 shows that between 2000 and 2004, 78% - 99% of the cynomolgus monkeys imported by Britain for medical and scientific research were bred in captivity (according to the CITES definition, see below).

Chart 5.4 CITES figures for imports of M. fascicularis to Britain by captive status, 2002 - 2004



"Born in captivity" and "Bred in captivity" refer to CITES definitions, see below.

All the rhesus macaques imported (from China or Mauritius) during the same period were bred in captivity. Marmosets used in British laboratories have either been obtained from British captive-bred sources, or occasionally from other European countries such as the Netherlands, France or Switzerland.

5.4 The captive status of primates

CITES statistics provide information about the captive status of primates traded between EU member states and other countries. The CITES' definition of 'bred in captivity' means animals who:

- were born in captivity, and
- are the offspring of parents who were mated in captivity,

in a centre where the breeding stock:

- was established according to the provisions of CITES and without detriment to survival of the species in the wild,
- is maintained without the routine introduction of animals from the wild, and
- is managed in a way that reliably produces second-generation offspring.

Thus, the CITES definition of 'captive-bred' includes primates produced at centres that sometimes augment their breeding stock with wild-caught animals, with the stress, ill-health and loss of life which this entails (see below). Animals born in conditions that do not meet these requirements (but are F1[131] or subsequent generations) are described under CITES as 'born in captivity'. These will include some animals whose parents were wild-caught.

5.5 Overseas breeding centres

Breeding centres for old world monkeys in source countries such as Mauritius, China, the Philippines and Vietnam are not controlled by legislation equivalent to that of the EU. The International Primatological Society published international guidelines[132] for the acquisition, care and breeding of primates, but these are only recommendations. In future, European

131 i.e. first generation offspring. **132** International Primatological Society (1993). IPS international guidelines for the acquisition, care and breeding of nonhuman primates. Primate Report 35 (special issue).

Mother and her offspring at an overseas breeding centre ©BUAV

Directive 86/609 may be updated to require EU countries importing primates to ensure, for example by visits from the national authority, that overseas centres operate to adequate standards before they are authorised.
The British Medical Research Council agrees with the importance for primates of their accommodation and environment. It states in its ethics guide[133] that:

"Primates must be provided with a complex and stimulating environment that promotes good health and psychological well-being and provides full opportunity for social interactions, exercise and to express a range of behaviours appropriate to the species."

It is unlikely that any overseas breeding centres, and very few European ones, achieve these goals. For example, there is frequently insufficient species-specific enrichment in group pens, which are often relatively barren, sometimes in poor structural condition, and may not provide opportunities for foraging or play, or for animals to withdraw from social interactions or aggression.

Single housing is unacceptable (unless temporarily required for welfare or veterinary reasons), yet is still used by all overseas suppliers other than for those reasons, for example for 'cage conditioning' prior to export. Cage conditioning involves holding animals in solitary confinement in small cages for between six and 90 days, and it causes considerable distress. In part, cage conditioning is justified by centres because of the expectation of purchasing laboratories, especially in the USA, that monkeys will be accustomed to single caging. Some centres also say that they need to isolate monkeys for health screening of individuals before export.

A centre's breeding programme can involve hardship and stress for breeding animals, who endure many years in confinement. For economic reasons, successful breeding females may be bred too frequently to maintain their condition, while others lacking good mothering abilities (e.g. they may reject their infants) may continue to be used for breeding. Infant mortality can be high. Infant macaques are weaned much earlier than is natural, sometimes at six months or less, rather than the 14-18 months usually seen in the wild. Infants are sometimes separated at weaning into nursery groups without adults, again a very unnatural situation. Inappropriate weaning has short- and long-term welfare implications for infants, including stress and sleep disturbances, behavioural abnormalities including heightened aggression, abnormal social development and poor parenting skills[134].

The British Royal Society for the Prevention of Cruelty to Animals (RSPCA) undertook an investigation at an overseas trapping and breeding centre in Mauritius, called the Centre de Recherches Primatologiques (CRP)[135].
At the time, the CRP exported primates to

133 Medical Research Council (2004). MRC Ethics Guide: Best Practice in the Accommodation and Care of Primates used in Scientific Procedures. London, UK: MRC. **134** Baskerville M (1999). Old World Monkeys. In: The UFAW Handbook on the Care and Management of Laboratory Animals. Vol. 1. 7th edition. Ed: T Poole. **135** RSPCA (2001). Caged and Cruel. Supplement to RSPCA report, Counting the Cost, by MJ Prescott. Horsham, UK: RSPCA

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laboratories in Europe (although not to Britain). Video footage showed a wild macaque caught in a makeshift wooden trap, and wild female macaques in a wire holding cage, one of them with a suckling infant, without food or water.

Most of the cages at CRP provided little or no enrichment, and many were in a squalid state, dirty and in poor repair. Some animals had diseased teeth, and in two cages dead monkeys were found on the floor in full view of other animals. Some breeding females spent 20 years in these conditions. Infants were weaned too young into groups without adults, causing anxiety and delayed behavioural development.

5.6 Wild-caught primates

The capture of wild monkeys inevitably causes fear, distress, anxiety and deprivation, as well as physical injuries and fatalities. For animals who have enjoyed a wild and free existence with little, if any, experience of humans, separation from their social groups and confinement in pens or cages causes immense psychological suffering. This is one reason why the use of wild-caught primates is discouraged by both the European Directive 86/609 and the European Convention ETS123.

The RSPCA which, like most expert groups, opposes the capture of wild primates for research, summarised the high costs borne by wild-caught primates[136]:

"The capture of primates from the wild and their subsequent confinement carries a high price in terms of capture-related deaths. The trapping of free-ranging primates results in the highest incidence of mortality and serious injury of all stages involved in primate acquisition... In some instances mothers may be killed to obtain young... Animals captured but subsequently deemed unsuitable for use may also be killed rather than be released. Where trapping takes place over a large area, monkeys may be left in traps for several days or even weeks before a sufficient number are caught for moving to a holding centre."

France, the main user of primates in the EU, imports the largest number of wild-caught monkeys, including cynomolgus macaques from Mauritius, vervet monkeys from Barbados[137] and, at least until 2000, baboons from Africa[138]. The CITES figures for France show that the number of primates taken directly from the wild fell between 2001 and 2003, but wild-caught primates still accounted for 15% of all primates imported.

Trap to capture primates in the wild ©BUAV

136 Prescott MJ (2001). Counting the Cost: Welfare Implications of the Acquisition and Transport of Non-human Primates for Use in Research and Testing. Horsham, UK: RSPCA.
137 Langley G & Langley C (2005). Primate Experiments in France: A One Voice report. France: One Voice. **138** CITES trade statistics derived from the UNEP-WCMC CITES Trade Database, UNEP World Conservation Monitoring Centre, Cambridge, UK. See <http://sea.unep-wcmc.org/citestrade/trade.cfm>

Baboons (e.g. *Papio hamadryas*) are indigenous to Africa, and there are no captive-breeding centres. In 2000, the BUAV conducted an undercover investigation of the trapping and confinement of wild baboons in Tanzania[139]. We revealed that the animals were caught in crude bamboo traps and moved to holding stations, some of which were run-down and dirty. In the holding centre visited by the BUAV, baboons were kept singly in dilapidated wooden crates for several weeks. They could not stand at full height and could hardly turn round.

In Belgium, GlaxoSmithKline's use of vervet monkeys has accounted for most of the primate experiments conducted in that country each year. The vervets (*Cercopithecus* or *Chlorocebus aethiops*) are acquired from the wild in the Caribbean and used in the production and routine safety testing of oral polio vaccine.

On Barbados, trapped wild monkeys have often been kept singly in cages with little or no environmental enrichment. Long-standing and sometimes fatal health problems have occurred in the monkeys taken from the wild and kept in captivity before shipping abroad. Malnutrition, as well as chronic gum and tooth disease in some 1000 monkeys over several years, led in some cases to gangrene and death. Cage conditioning on Barbados involves single housing wild-caught vervets for 90 days[140]. A study of the stress experienced by wild vervet monkeys when captured and put in single cages showed that their immune systems were severely suppressed, making them extra vulnerable to infection and illness[141].

5.7 Transport of primates

All EU member states are parties to the Convention on International Trade in Endangered Species of Fauna and Flora (CITES), and the EU has implemented CITES by means of the wildlife trade regulations. The regulations apply to the export and import of all primates, and require that animals transported into, from or within the EU should be *"prepared and shipped so as to minimize the risk of injury, damage to health or cruel treatment"*[142]. The regulations are interpreted as only applying from the moment that animals are packed for international transport, and not to prior events such as internal transport between the supplying centre and its national border.

The European Union Council Directive 91/628/EEC, as subsequently amended, lays down conditions for the transport of animals, including primates, within the EU. These include the provision of food and water at regular although unspecified intervals. However, no special provisions are made for primates with regard to maximum journey times, route plans, ventilation or bedding, as is the case with farm animals[143]. The Directive does require compliance with the International Air Travel Association's (IATA) regulations, which include minimum sizes for travel containers; but these are very small, in some cases hardly allowing a macaque to stand or turn easily. The RSPCA states that the current EU legislation does not ensure the welfare of primates during transport.

139 Primates in Peril. See BUAV website <http://www.buav.org/primates/index.html> **140** Prescott MJ (2001). Counting the Cost: Welfare Implications of the Acquisition and Transport of Non-human Primates for Use in Research and Testing. Horsham, UK: RSPCA. **141** Suleman MA (1999). Peripheral blood lymphocyte immunocompetence in wild African green monkeys (Cercopithecus aethiops) and the effects of capture and confinement. In Vivo 13:25-27. **142** Council Regulation EC 338/97 on the Protection of Species of Wild Fauna and Flora by Regulating Trade Therein, Article 9.5. **143** Prescott MJ (2001). Counting the Cost: Welfare Implications of the Acquisition and Transport of Non-human Primates for Use in Research and Testing. Horsham, UK: RSPCA.

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The journey to a laboratory starts at the breeding centre, when monkeys are placed into small transport crates, approximately 30cm x 50cm x 65cm for young macaques, in which they remain until arrival. The journeys last from a minimum 14 hours (e.g. from Israel to Britain) up to 56 hours or more (from some centres in China to Britain), but flight cancellations, mechanical failures or other delays can considerably increase the duration. Wild-caught vervet monkeys are transported from the Caribbean to France and Belgium by air on journeys that last about 48 hours.

Transport includes internal travel, which itself can take many hours, loading into the cargo hold of an aircraft at the airport, flight to a European destination, unloading and onward transport by road (and ferry in the case of Britain). During the whole process, adverse effects on the animals can arise from the following[144]:

- separation from familiar social grouping
- close confinement in a small, unfamiliar container
- handling by unfamiliar humans
- food and drink restrictions
- exposure to changes of temperature, humidity, lighting, pressure, noise and vibration
- physical stress from efforts to maintain balance, including during acceleration and deceleration
- unusual sights and smells
- motion sickness and loss of body weight

Fatalities happen in transit, two well-described cases occurring during the transport of cynomolgus monkeys from the Philippines in 1997 and 1998. In the former, a monkey was found dead on arrival in Britain, probably from a head trauma. In the latter, three monkeys in transit to Britain were found dead at Paris airport. Contributing factors included the larger than normal size of the animals, who were unable to stand up or turn round freely in the transport crates, even though these met the IATA minimum dimensions[145]. Furthermore all the dead animals were in the central compartment of a three-part crate, which was less well ventilated than the outer two compartments.

In the words of the EU's Scientific Committee on Animal Health and Animal Welfare[146], *"One of the main threats to good welfare regarding importing non-human primates from overseas is their prolonged transport."* Surprisingly, less is known about the specific effects of transport on primates than is the case for rodents and farm animals, although it is well accepted that the process is stressful.

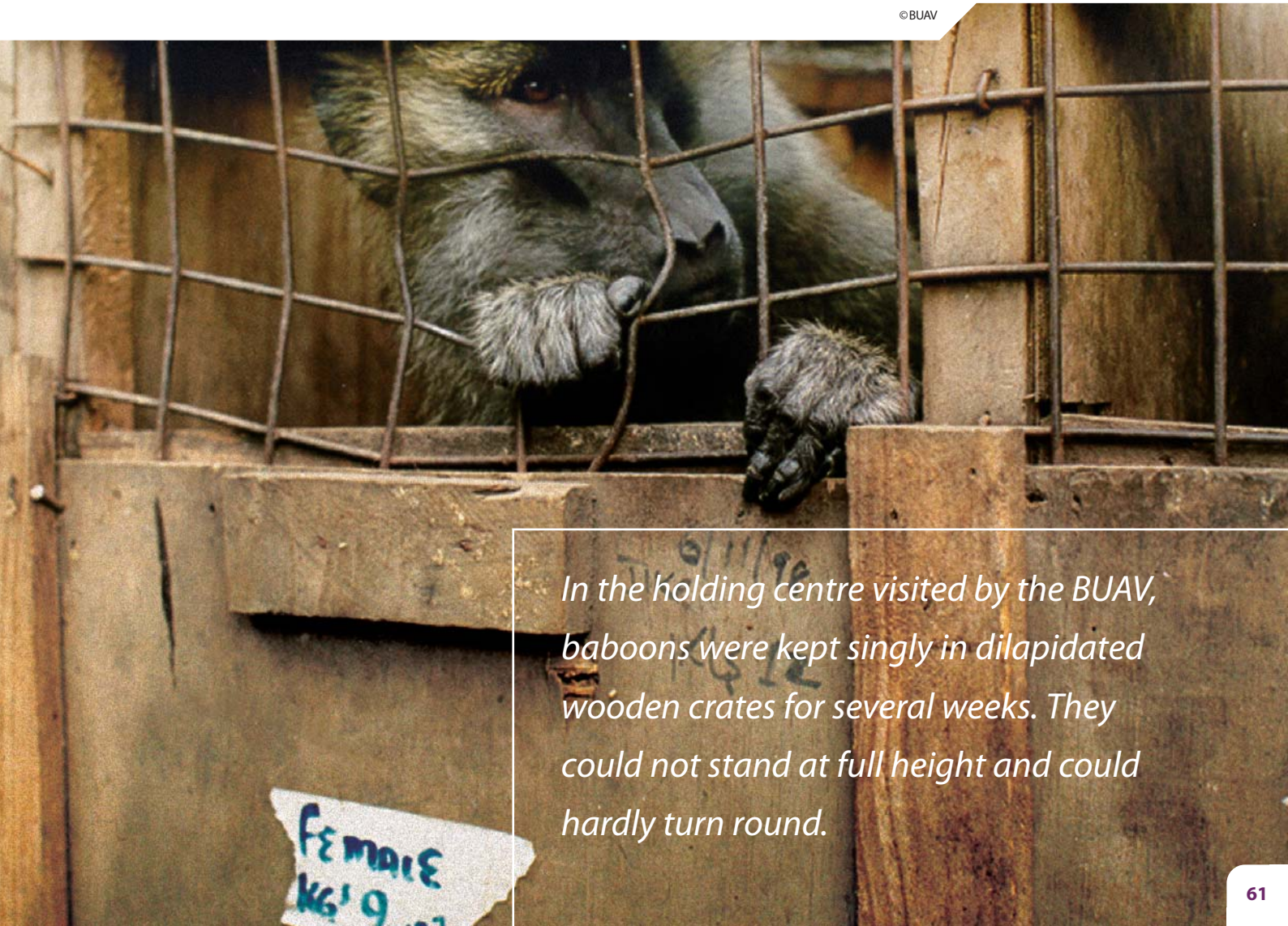
However, a study was published recently of the behavioural responses of cynomolgus monkeys (*Macaca fascicularis*) transported by air to Britain and re-housed in laboratory conditions[147]. The activity patterns and social status of five juvenile male monkeys were studied before their journey, immediately after their arrival and

144 Nuffield Council on Bioethics (2005). The Ethics of Research Involving Animals, page 75. London, UK: Nuffield Council on Bioethics. See <http://www.nuffieldbioethics.org>
145 Animal Procedures Committee (1999). Report of the Animal Procedures Committee for 1998. London, UK: The Stationery Office. **146** Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission: Health & Consumer Protection DG. **147** Honess RE, Johnson PJ & Wolfensohn SE (2003). A study of behavioural responses of non-human primates to air transport and re-housing. Lab. Anim. 38:119-132.

three weeks later. The behaviours recorded included play, play fighting, grooming (self and others), aggression, hugging, eating/foraging and resting.

The results suggested that the combined process of international air transport and re-housing compromised the welfare of the animals, causing behavioural changes indicative of *"heightened levels of stress"*. Although the behaviour of the five monkeys adjusted after an initial change on arrival, after a month it had still not returned to the pattern seen at the original breeding facility.

It is widely agreed that the trapping or breeding of primates overseas, their confinement at breeding centres, and their transport to European laboratories, cannot be accomplished without causing immediate as well as longer term stress and suffering. In the light of what we now know about the mental, social and emotional complexities of other primates, continuing to capture, breed, confine and transport them to laboratories halfway around the world is simply unacceptable.



In the holding centre visited by the BUAV, baboons were kept singly in dilapidated wooden crates for several weeks. They could not stand at full height and could hardly turn round.

CHAPTER SIX

Causes of primate suffering and its impact on research

Many experts believe that it is impossible, in practice, to confine primates in laboratory conditions whilst maintaining their physical and mental health. This is because, like their human kin, other primates have complex psychological and behavioural needs, and cannot be institutionalised in a laboratory without harming their welfare. In the words of the Scientific Committee on Animal Health and Welfare[148]:

"Most primates are highly social and intelligent animals and their cognitive skills have been shaped by evolution to find and handle food, and to relate to other individuals in a social group. Having social partners is one of the most significant needs of primates and they develop abnormal behaviour patterns when socially deprived...

When primates cannot express their normal behaviour and satisfy their needs to show certain behaviours, either because of a lack of environmental diversity, or an insufficient amount of space, they develop abnormal behaviour patterns (e.g. stereotypies)...".

This chapter explores in detail how the laboratory environment harms primates, and how this might adversely affect the quality of research data. The suffering imposed by the conduct of scientific procedures and their outcomes is discussed in Chapters 7 and 8.

6.1 Primates in their natural environments

A consideration of the natural environments and lifestyles of different primates gives some insight into how deeply impoverished the laboratory milieu is for these animals. Their natural environments provide complex sensory stimuli: such as rain, even snow and thunder; sun, heat and cold; subtle changes in light through dawn and dusk; the vegetation that they see, feel and smell; the sounds of other animals; the range of foods that can be obtained throughout the year, and so on.

Of the old world monkeys, wild baboons (*Papio* species) roam furthest, their home range being as large as 39 km^2. They live in social groups and are mainly terrestrial but sleep off the ground, in rocks or trees. Their diet comprises grass, fruit, leaves and roots.

Wild vervet monkeys (*Chlorocebus* or *Cercopithecus aethiops*) are both arboreal and terrestrial, living in groups in woodland, savannah and sub-desert habitats. Fruit- and

148 Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG.

leaf-eating animals, they communicate vocally and by facial expression.

Cynomolgus monkeys (*Macaca fascicularis*) may travel as far as 1.5 km in a day. Arboreal, group-living animals, they inhabit warm, humid regions and natural activities include feeding, travelling, resting and social grooming. They are mainly frugivorous.

Rhesus monkeys (*Macaca mulatta*) have a home range of up to 16 km^2 and live in a range of climates. Arboreal and terrestrial, they are social animals and are mainly frugivorous.

The new world species most commonly used in laboratories is the marmoset (*Callithrix jacchus*): a small, tree-living primate with a wide range of behaviours. In natural conditions marmosets' home range is about 0.5 km^2 of swamp forest, tree plantations and scrub. They live in stable groups with complex social interactions, and communicate vocally and by scent. Marmosets are monogamous, and group members share the care of offspring.

Squirrel monkeys (*Saimiri sciureus*) are also active tree-dwelling animals who feed on fruits, insects and flowers. They normally live in single sex groups and their home range can extend to 13 km^2.

6.2 Laboratory conditions

The contrast between the natural environments of primates and the laboratory situation could hardly be more stark. Few establishments in Europe provide breeding groups of primates with large enclosures, swings, shelves, mirrors, foraging material and other features to add complexity to their environment. Even where they do, this is still a poor substitute for the dynamic, complex and spacious landscapes these animals have evolved to inhabit. The report of the Nuffield Council on Bioethics on the ethics of animal research made this clear[149]:

"Animals such as primates or dogs have evolved to form social groups with defined compositions and hierarchies. In their natural environment these animals usually have sufficient space to perform their social behaviours and maintain appropriate social distances. However, in the laboratory they find themselves in artificially composed groups and the cage or pen size that is provided in research facilities differs significantly from the space available in their natural habitats".

Conditions for experimental (rather than breeding) primates are even less expansive; in some European and US laboratories they are still kept in solitary confinement in small, relatively barren cages that limit free movement and prohibit normal locomotion.

The Animal Welfare Institute in Washington DC discusses five key primate-specific characteristics which are very difficult to satisfy in the laboratory[150]:

- Like human primates, other primates have intense social needs. When deprived of companionship for an extended time, they develop unmistakable signs of depression and frustration. Social deprivation is so

149 Nuffield Council on Bioethics (2005). The Ethics of Research Involving Animals. London, UK: The Nuffield Council. See <http://www.nuffieldbioethics.org> **150** Reinhardt V (2002). Comfortable quarters for nonhuman primates in research institutions. In: Comfortable Quarters for Laboratory Animals, 9th edition. Eds. V & A Reinhardt. Washington DC, USA: Animal Welfare Institute. <http://www.awionline.org/pubs/cq02/Cq-prim.html>

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distressing that approximately 10% of single-caged monkeys develop the serious behavioural pathology of self-injurious biting[151].

- Primates are physiologically and anatomically adapted to live in complex, dynamic environments. Any healthy primate becomes apathetic or restless and develops stereotypical behaviours when deprived of basic species-specific stimulation.

- With only one exception, primates are diurnal animals and need sufficient lighting. Many laboratory monkeys are permanently deprived of natural light and, although few studies have been conducted, primate technicians comment that where natural light is available animals invariably take advantage of it.

  In fact, bone disease has been reported in laboratory rhesus monkeys, causing bowed bones in the arms and legs. The animals were reluctant to climb and jump, had an abnormal gait and poor growth. The multifactorial aetiology included insufficient daylight for vitamin D production[152].

- Primates show physiological and behavioural distress reactions when exposed to threatening situations over which they have no control. This occurs during many scientific procedures.

- All primate species, even those who are largely terrestrial, seek elevated places as refuges from ground predators and as resting sites for the night. When kept in low-level cages, the animals are cornered and perceive the presence of humans above them as particularly threatening[153].

At Cambridge University - one of the most prestigious universities in the world - a BUAV investigation revealed that the marmoset cages only met some minimal, but not optimal, standards. Marmosets undergoing experimental procedures were kept in two-tier caging, so that some were at ground level. This is a source of chronic stress to these almost completely arboreal primates. Some marmosets were housed individually for periods of time and on occasion stock animals were kept in cages deemed acceptable only for experimental animals. Not surprisingly, stereotypical behaviours such as circling and back-flipping were expressed by some marmosets.

The Universities' Federation for Animal Welfare, which publishes good-practice guidelines, recommends against housing marmosets in tiers, at ground level, or singly[154]:

"...cages should be sufficiently high for them to flee upward, preferably above human eye level, so that they can look down on staff...

Marmosets should not be housed in tiers, as this restricts the vertical flight response for both upper and lower cages, and those below are effectively trapped on the ground."

and

"Single caging is unsatisfactory for these highly social animals as they soon lose condition, appear nervous, and are often more susceptible to disease, and may even die."

151 Jorgensen MJ, Kinsey JH & Novak MA (1998). Risk factors for self-injurious behavior in captive rhesus monkeys (Macaca mulatta). Am. J. Primatol. 45:187. **152** Wolfensohn SE (2003). Case report of a possible familial predisposition to metabolic bone disease in juvenile rhesus macaques. Lab. Anim. 37:139-144. **153** National Research Council (1998). The psychological wellbeing of non-human primates, p. 118. USA: National Academy Press. **154** Poole T, Hubrecht R & Kirkwood JK (1999). Marmosets and tamarins. In: The UFAW Handbook on the Care and Management of Laboratory Animals, vol. 1, 7th edition. Ed: T Poole. Oxford, UK: Blackwell Science Ltd.

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The Medical Research Council, which funded some of the experiments at Cambridge University, confirmed the deficiencies in the caging and handling of marmosets[155]:

"The MRC recognised that the two-tiered caging and the level of environmental enrichment were not examples of best practice, but that the standards of husbandry have improved since the exposé. The MRC rarely inspects husbandry standards outside of its own facilities and only when the investment is considerable."

At Covance GmbH, in Münster, Germany (the site of another BUAV investigation, see Chapter 4), the cages of cynomolgus monkeys were also stacked in tiers. Primate expert Stephen Brend pointed out that this was a management decision to economise on space at the cost of the animals' welfare. He also wrote that perhaps the most significant violation of the monkeys' mental welfare was caused by solitary housing:

"All macaque species are highly social. Physical contact with another monkey is hugely important for them. Long-tailed [i.e. cynomolgus] *macaques huddle together when sleeping. They groom, play and maintain social bonds through physical contact. To deny them this - once again a decision taken purely for ease of management - can not fail to be anything other than a source of chronic stress".*

6.3 Scientific procedures

In addition to the stresses of laboratory housing, primates are used in experimental procedures.

As Chapters 7 and 8 deal in detail with the suffering inflicted by methods of research and testing, only a short review is provided here.

Some procedures involve distressing or painful practices, such as oral gavage, repeated venepuncture, or restraint in a primate chair. A recent review of common laboratory practices concluded that

155 Animal Procedures Committee (2005). Final Report of the Cambridge/BUAV Working Group, Annex C. 16 June 2005. See <http://www.apc.gov.uk>

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"...significant fear, stress and possibly distress are predictable consequences of routine laboratory procedures", in the case of primates including room entry by unfamiliar staff and changes of caging[156].

Animals undergoing procedures are usually separated from their cage-mates, which also has a welfare impact. Marmosets, for example, do not like being caught, restrained or handled, and taking them from their family or social groups is stressful. Temporary separation of caged pairs and exposure to an unfamiliar cage leads to substantial stress, indicated by an increase in blood pressure and heart rate[157].

Thus, apart from the deleterious effects of laboratory housing, primates are faced with a range of stressful and/or painful experiences including:

- Separation of pair- or group-housed animals during procedures;
- Pain, discomfort or malaise caused by surgery, gavage, and drug or chemical effects;
- Immobilisation, restraint, loud noises, bright lights, unsympathetic handling;
- Unfamiliar environments such as a new cage, room or test chamber;
- Restrictions on food and water intake.

Most experiments have directly harmful effects, such as toxicity caused by test chemicals, post-operative pain or cognitive damage caused by ablation of part of the brain.

Primates have the cognitive abilities to anticipate further pain or discomfort on the basis of past experiences, a capacity that adds to their suffering. This was evidenced by the 1998 report on primates by the US National Research Council, which stated that marmosets *"...appear to have long memories and respond with fearful behaviour to hearing the voice or footsteps of someone who has captured them several months earlier"*[158].

6.4 Behavioural and physiological evidence of primate suffering

Stereotypical behaviour, such as circling, pacing, somersaulting, self-biting and hair-pulling, is a reaction to the abnormal conditions endured by captive primates. For example, behavioural assessments of 362 singly housed rhesus monkeys showed that 321 exhibited at least one abnormal behaviour[159].

According to Viktor Reinhardt of the Animal Welfare Institute in Washington DC[160], *"Many stereotypies are signs of frustration, with the subject being chronically thwarted from expressing basic activities such as taking a few free steps in one direction, climbing and perching, retreating to a secluded place, foraging, and interacting with another conspecific."*

Reinhardt also disputes the myth that captive rhesus monkeys are naturally aggressive, pointing out that poor husbandry and handling practices that ignore basic ethological principles are the most likely cause of aggressive behaviour[161]. Self-injurious behaviour in monkeys, such as self-wounding and biting, is another stress response and is estimated to occur in 10%

156 Balcombe JP, Barnard ND & Sandusky C (2004). Laboratory routines cause animal stress. Contemp. Topics Lab. Anim. Sci. 43:42-51. **157** Gerber P et al (1996). Sociophysiological aspects of short-term separation in common marmosets. EUPREN/EMRG workshop: The implications of housing and husbandry for scientific quality and wellbeing of non-human primates, Rome. **158** National Research Council (1998). The psychological wellbeing of non-human primates, p. 77. USA: National Academy Press. See also details on the needs of marmosets in: Smith JA & Boyd KM (2002). The Boyd Group Papers on the use of non-human primates in research and testing. Leicester, UK: British Psychological Society. **159** Lutz C, Well A & Novak M (2003). Stereotypic and self-injurious behavior in rhesus macaques: a survey and retrospective analysis of environment and early experience. Am. J. Primatol. 60:1-15. **160** Reinhardt V (2004). Stereotypical behaviour: a Laboratory Animal Refinement and Enrichment Forum discussion. Lab. Primate Newsletter 43(4):3-4. **161** Reinhardt V (2002). The myth of the aggressive monkey. J. Appl. Anim. Welf. Sci. 5:321-330.

of single-caged primates[162]. For example, in socially reared rhesus monkeys subsequently kept singly in cages, bouts of self-harming were accompanied by complex changes in the function of the hypothalamic-pituitary-adrenocortical axis[163].

Another study of rhesus monkeys suggested that individuals exhibiting self-injurious behaviour and unusual aggression may have abnormal 5-HT (serotonin) function in the brain. This is thought to be only one aspect of a more profound disorganisation of brain function involving many neurohormonal and transmitter systems[164]. Whether the cause is isolation, peer aggression, frustration or fear, self-injuring reflects psychological distress and causes physical pain and discomfort.

Behavioural indications of stress in primates are thus underpinned and/or accompanied by physiological abnormalities. Prolonged single confinement of wild vervet monkeys caused suppression of their immune systems, making them more prone to infections and illness[165]. Monkeys subjected to mild psychological and social stresses developed psychological, hormonal and metabolic abnormalities including early signs of diabetes and cardiovascular disease[166].

A rise in plasma cortisol levels, indicative of acute stress, was seen in restrained baboons and in rhesus monkeys when held in a primate restraint chair[167]. In another study, many days of confinement stress in rhesus monkeys led to changes in kidney function with effects on sodium excretion and a ten-fold increase in urinary excretion of growth hormone[168].

Sex hormone changes occurred in male rhesus monkeys held in primate restraint chairs[169]. Vervet monkeys exposed to sustained social stress developed stomach ulcers as well as degeneration in a brain region called the hippocampus, involved in learning and memory[170].

Adult marmosets were housed in either a laboratory cage or in a complex habitat. A month in the complex environment increased the length and complexity of brain cell dendrites[171], and increased other functional features[172] in the hippocampus and prefrontal cortex[173]. The structure of the adult primate brain thus remains *"highly sensitive even to modest levels of experiential complexity"*, and normal laboratory conditions may induce reversible changes in cellular communication in brain regions important for cognition.

6.5 Primate suffering and data validity

According to the EU's Scientific Committee on Animal Health and Animal Welfare, in its recent report on the welfare of primates used in research[174]:

"The unnatural restrictive environments and husbandry practices in research laboratories have raised a concern about the possible negative welfare aspects, both for reasons of ethics and experimental validity".

Ironically, many primates are used in experimental areas related to those systems most obviously affected by stress, i.e. the central

162 Platt DM et al (1996). Factors affecting the expression of self-injurious behavior in rhesus monkeys (Macaca mulatta). XVIth Congress of the International Primatological Society/XIXth Conference of the American Society of Primatologists, Madison, USA, Abstr. 768. **163** Tiefenbacher S et al (2004). Altered hypothalamic-pituitary-adrenocortical function in rhesus monkeys (Macaca mulatta) with self-injurious behavior. Psychoneuroendocrinol. 29:501-515. **164** Kraemer GW, Schmidt DE & Ebert MH (1997). The behavioral neurobiology of self-injurious behavior in rhesus monkeys. Current concepts and relations to impulsive behavior in humans. Ann. NY Acad. Sci. 836:12-38. **165** Suleman MA et al (1999). Peripheral blood lymphocyte immunocompetence in wild African green monkeys (Cercopithecus aethiops) and the effects of capture and confinement. In Vivo 13:25-27. **166** Bjorntorp P (1997). Body fat distribution, insulin resistance, and metabolic diseases. Nutrition 13:795-803. **167** Hayashi KT & Moberg GP (1987). Influence of acute stress and the adrenal axis on regulation of LH and testosterone in the male rhesus monkey. Am. J. Primatol. 12:263-273. **168** Gauquelin-Koch G et al (1996). Hormonal response to restraint in rhesus monkeys. J. Med. Primatol. 25:387-396. **169** Norman RL & Smith CJ (1992). Restraint inhibits luteinizing hormone and testosterone secretion in intact male rhesus macaques: effects of concurrent naloxone administration. Neuroendocrinol. 55:405-415. **170** Uno H (1989). Hippocampal damage associated with prolonged and fatal stress in primates. J. Neurosci. 9:1705-1711. **171** Dendrites are microscopic processes that branch out from the 'body' of brain cells. They pass electrical signals into the cells and are essential for interactions between large numbers of neurons forming networks in the brain. **172** Including dendritic spine density and synaptic protein levels. **173** Kozorovitskiy Y et al (2005). Experience induces structural and biochemical changes in the adult primate brain. Proc. Natl. Acad. Sci. USA 102: 17478-17482. **174** Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG.

Causes of primate suffering and its impact on research

nervous and immune systems. Brain research areas include studies of vision, cognition, stroke, schizophrenia, sensory-motor function, Parkinson's disease and multiple sclerosis. In the case of immune function, stress could be affecting the quality and reliability of research into vaccines and treatments for HIV/AIDS, malaria, hepatitis C and other infectious diseases, as well as cell and organ transplantation studies.

Functional abnormalities caused by laboratory conditions must impact on the reliability of research data. Brain research on chronically stressed primates aimed at extrapolation to the human situation will normally not be comparing like with like. Additionally, the different reactions of individual animals to similar stressors will introduce confounding variables into some experiments.

For example, in research on marmosets into cognition at Cambridge University there were inter-animal variations in the behavioural effects of brain transections[175]. There are many potential causes, but one that is seldom acknowledged, monitored or addressed is the individual variation in responses to laboratory experiences such as housing, handling, separation, and food and water restriction.

Primates are distressed when exposed to life-threatening situations over which they have no control. When this occurs during an experiment, such as immobilisation during a sample collection, the validity of the findings will be jeopardised because the stress is a data-biasing variable[176].

As Viktor Reinhardt also pointed out[177], behavioural and physiological abnormalities caused by the laboratory environment *"...are likely to introduce uncontrolled variables into research data, thereby jeopardising the validity of science that is done with the affected subjects".* Sadly, this problem is usually dealt with by increasing the number of experimental primates to minimise data variability and achieve statistical significance, as happened in the marmoset experiments at Cambridge.

Charles Bowers and colleagues at the University of Washington Regional Primate Research Centre discussed the likely impact of primate stress on research findings. They explained that individual primates who react strongly to anxiety-provoking stressors (such as sudden noises or an unfamiliar technician wearing capture gloves) may experience reduced psychological well-being *"...and would be inappropriate for some experiments"*[178].

A report from the US-based Covance laboratory described how fear can cause rapid alterations in numbers of white blood cells in macaques, a change which can jeopardise the analysis of blood samples during toxicity studies[179]:

"...excited or frightened animals may exhibit a physiological leukocytosis secondary to catecholamine release during blood collection procedures. This is especially common for untrained, unanaesthetized macaques. The shift of leukocytes from the marginal pool to the circulating pool can double the white blood cell count in minutes."

175 Maclean CJ et al (2001). Visual discrimination learning impairments produced by combined transections of the anterior temporal stem, amygdala and fornix in marmoset monkeys. Br. Res. 888:34-50. **176** Brockway BP, Hassler CR & Hicks N (1993). Minimizing stress during physiological monitoring. In: Refinement and Reduction in Animal Testing, eds. SM Niemi & JE Willson, p. 56-69. Bethesda, USA: Scientists Center for Animal Welfare. **177** Reinhardt V (2004). Stereotypical behaviour: a Laboratory Animal Refinement and Enrichment Forum discussion. Lab. Primate Newsletter 43:3-4. **178** Bowers CL, Crockett CM & Bowden DM (1998). Differences in stress reactivity of laboratory macaques measured by heart period and respiratory sinus arrhythmia. Am. J. Primatol. 45:245-261. **179** Hall RL & Everds NE (2003). Factors affecting the interpretation of canine and nonhuman primate clinical pathology. Toxicol. Pathol. 31(suppl.):6-10.

Frightened Macaque at the German Covance laboratory ©BUAV / R&D

According to that report, macaques commonly have muscle enzymes in their bloodstream as a result of muscle trauma due to handling or injection injury. Macaques normally have very low fasting levels of glucose in their blood, but if they are stressed or frightened during handling, dosing or blood sampling, their blood sugar elevates rapidly to levels normally only seen in diabetes. These stress-induced changes may further complicate data analysis.

Evidence for the impacts of stress on the health and welfare of primates has been described by several authors and was reviewed in Caroline Manser's report for the RSPCA[180] . The effects span several physiological systems and include:

- heart damage in baboons used in experimental surgery, and in squirrel monkeys in primate restraint chairs or subjected to electric shocks;
- stomach and duodenal ulcers in monkeys experiencing stress, anxiety and psychological conflict;
- changes in brain neurotransmitters, which could confound studies of brain function;
- abnormalities in heart rhythm and increased blood pressure which may affect the results of tests on drugs and chemicals;
- chair restraint of rhesus monkeys induces a 2.5-fold acceleration of gastrointestinal transit time, which persists in the post-restraint period;
- metabolic changes, including a rise in insulin levels, which may influence the outcome of ADME[181] studies of new chemicals and drugs;
- depression of the immune system, including reductions in natural killer cell activity, which could affect research into infectious diseases, vaccine development, and organ transplantation and cell therapies.

The evidence demonstrates that it is exceedingly difficult, if not impossible, to keep primates in laboratories while maintaining them in good physical and psychological health. The adoption of best practices can reduce but not eliminate harms to primates; but few laboratories are willing - or have the funding or facilities - to pursue these standards.

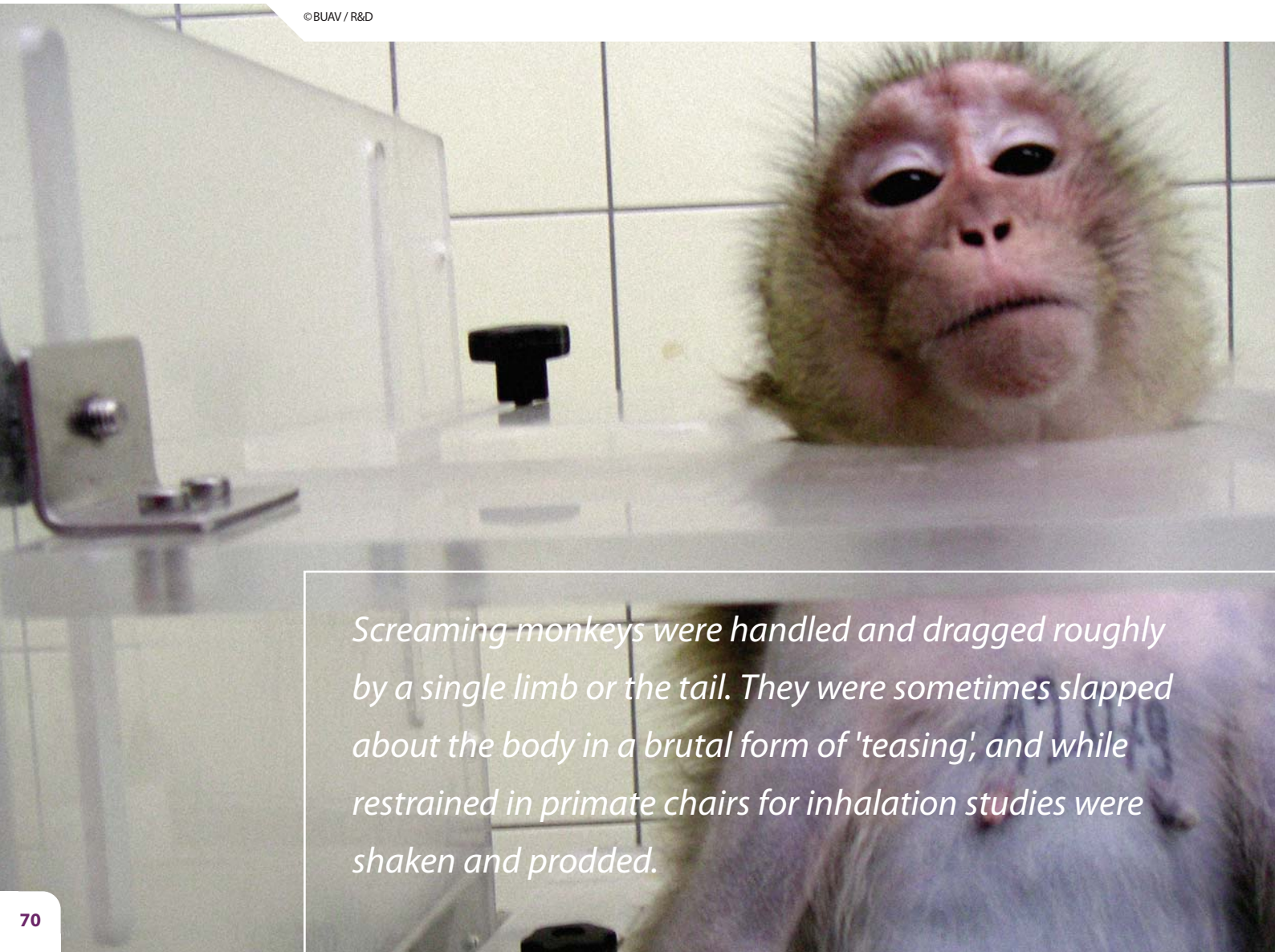
Stressed primates make poor research subjects. Other primates, like humans, are distressed by social isolation or aggression, separation from their peers, handling, pain, discomfort, malaise, boredom, frustration, fear and anxiety. Changes in hormone and neurotransmitter levels; blood pressure and heart rate; lowering of immune function; and gastrointestinal ulcers and other lesions may result. As well as damaging the well-being of our fellow primates, such changes have the potential to seriously confound the interpretation of research data.

180 Manser CE (1992). The Assessment of Stress in Laboratory Animals. Horsham, UK: RSPCA. **181** ADME studies measure the Absorption, Distribution, Metabolism and Excretion of drugs and chemicals.

CHAPTER**SEVEN**

Primates in medicine testing

Throughout Europe, the major use of primates is in the testing of new medicines for safety and efficacy, and for their absorption, distribution, metabolism and excretion (ADME) characteristics. The tests are conducted by pharmaceutical companies, or on their behalf by contract research laboratories, to provide data to the medicines regulatory authorities. The authorities use the primate data, along with information from a wide range of other studies (*in silico*, *in vitro*, *in vivo* from other species, and clinical trials) to approve or reject new medicines.



Screaming monkeys were handled and dragged roughly by a single limb or the tail. They were sometimes slapped about the body in a brutal form of 'teasing', and while restrained in primate chairs for inhalation studies were shaken and prodded.

7.1 Efficacy and ADME testing

Primates are used to test the efficacy of some medicines, especially those intended to treat disorders of the central nervous system. For example, strokes and parkinsonian symptoms are artificially induced in marmosets to study the effectiveness of new drugs or cell or gene therapies for humans. Rhesus monkeys are often selected for efficacy studies of drugs for diabetes and disorders of lipid metabolism, as well as of vaccines against AIDS. Anti-anxiety drugs are tested on marmosets in the human threat test, in which the effect of the drug is tested on the anxiety levels of marmosets in the presence of humans[182].

Drug company Therabel Research s.a., based in Brussels, Belgium, published a review in 1997 of the animal experiments conducted on its anti-schizophrenia drug, code-named JL13[183]. The review described tests comparing JL13 with an existing anti-psychotic drug, clozapine, using three squirrel monkeys (*Saimiri sciureus*) already trained to discriminate clozapine from saline.

During training and testing, the monkeys were isolated in a sound-proofed room, exposed to white noise and held in restraint chairs. They were given injections of JL13 and had to learn to discriminate between these and injections of saline, by pulling a lever. Five milliamp electric shocks were applied to their tails if they pulled the wrong lever. JL13 had already been tested for its behavioural effects in four standard tests with rats and five different standard tests on mice, as well as in tests on dogs.

Studies of the absorption, distribution, metabolism and excretion of pharmaceuticals use dogs or monkeys (macaques or marmosets) as a second species, in addition to tests on rodents. The studies aim to characterise the time course and effects of a drug's movement into, through and out of the body, as well as its metabolic conversion to other substances. The requirement to use rodent and non-rodent animals acknowledges that no single species will reliably and consistently predict ADME characteristics in humans.

Single or repeat doses are administered orally, by inhalation or by injection/infusion. Frequent blood samples are taken to monitor changing drug levels in the bloodstream and to identify metabolites. These procedures usually involve catheterisation of a blood vessel and restraint for several hours in a primate chair during drug administration and blood sampling. For absorption and excretion studies, animals are commonly isolated in metabolism cages for collection of urine, faeces and other biological samples. After the tests primates may be re-used, or they may be killed to examine target organs in the body.

7.2 Medicines toxicology and regulatory requirements

European Union statistics on animal experiments (Chapter 3) show that drug toxicity tests on primates range from acute, single-dose lethal poisoning studies (i.e. LD50 or LC50 tests), through 28-day (sub-acute) and 90-day (sub-chronic) repeat-dose tests, to chronic studies lasting up to nine months or a year, and

182 E.g. Micheli F (2001). Lesopitron (Esteve). IDrugs 4:218-224. **183** Bruhwyler J et al (1997). JL13, a pyridobenzoxazepine compound with potential atypical antipsychotic activity: a review of its behavioural properties. Pharmacol. Res. 36:255-264.

Primates in medicine testing

sometimes several years. The number of monkeys used in each repeat-dose study varies from six to 52, depending on the laboratory and the specific purpose of the test.

Old world monkeys were traditionally selected for these tests, but new world species are now involved as well. For example, marmosets are used in drug discovery and development by pharmaceutical companies in Britain, France, Germany, Japan, the Netherlands, Switzerland and the USA[184].

Again, similar drug toxicity tests are also conducted in rodents, being repeated in a non-rodent species, usually dogs or primates, in efforts to increase confidence in the relevance of the results for humans. Drugs developed to treat conditions of the central nervous system, such as anti-depressants, are also tested on primates to discover whether they cause addiction.

Because of commercial secrecy, regulatory toxicity data are often not published in the scientific press and, when publication does occur, it is frequently several years after the tests were conducted (usually when the product has been patented). This means it is impossible to gain a comprehensive view of these kinds of primate tests.

The regulatory authorities in the three global trading blocs (the EU, USA and Japan) explicitly call for the safety testing of drugs on a rodent species and a non-rodent species. However, as discussed by the British Animal Procedures Committee[185], no regulations specifically require the testing of new drugs on primates. Dogs are commonly selected, and occasionally pigs or ferrets.

Annex I of Directive 2001/83/EC on the EU community code relating to medicinal products[186] specifies that acute toxicity studies *"...must be carried out in two or more mammalian species ...unless a single species can be justified".* It states that repeat-dose toxicity tests *"...shall be carried out on two species of mammals one of which must be a non-rodent".* The Directive does not specify a particular species. Neither do the guidelines of the International Conference on Harmonisation[187], representing the harmonised approach of regulators in the USA, EU and Japan. The general assumption is that the second species should be selected on a case-by-case basis for its expected predictivity for humans, although often this is not really known in advance.

Directive 86/609/EEC on the protection of laboratory animals requires animal species of the *"lowest neurophysiological sensitivity"* to be used. Under British legislation, primates may not be used unless the Home Secretary is satisfied that other species are either not suitable or not practicably available. At present the dog is considered to be the 'default' second species for these tests, but primates are also used.

Medicines regulators, especially in the USA, have considerable influence, but their decisions lack any transparency and it is almost impossible to find out exactly how they operate. In practical terms, the selection of a second species is based on the real, perceived or anticipated requirements of regulators; global pharmaceutical companies

184 Smith D et al (2001). The selection of marmoset monkeys (Callithrix jacchus) in pharmaceutical toxicology. Lab. Anim. 35:117-130. **185** Animal Procedures Committee (2002). The Use of Primates under the Animals (Scientific Procedures) Act (1986): Analysis of current trends with particular reference to regulatory toxicology. See <http://www.apc.gov.uk>
186 Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal of the European Communities L 311, 28/11/2001 p. 67-128. **187** ICH Harmonised Tripartite Guideline: Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals, M3(M), 2000.

After tests primates maybe reused, or they may be killed to examine organs in the body.

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want to conduct tests for new drugs which satisfy as broad a range of global regulatory demands as possible.

7.2.1 Primate suffering in medicines toxicology

At Covance laboratories in Münster, Germany, a BUAV investigation gained evidence that staff sometimes held monkeys' noses closed to force them to open their mouths for oral gavage. Other methods involved pushing the monkey's face against the metal bars of a cage, shaking the monkey's head and even using a fist on the bottom row of teeth.

Professor Nedim Buyukmihci, at the time a veterinarian at the University of California, commented:

"Oral gavage is a very stressful method of administration. It can cause inflammation or ulceration, or even rupture of the esophagus and stomach. The tube should be inserted and withdrawn gently to avoid injury or vomiting. Before anything is sent through the tube, there must be verification that the tube is actually in the gastrointestinal tract and not in the trachea. The video shows that this was not being done routinely. The consequence of inserting material into the lungs would be severe injury, illness and pain followed by death.

Furthermore, the video shows non-human primates being mistreated both prior to and during oral dosing and blood sampling."

Staff at Covance may not be typical in their abusive handling of monkeys but there has been abundant evidence, over many years, that a 'culture of care' is often not found in laboratories. Two further examples may suffice. A BUAV investigator, an experienced primate technician, worked at the then Hazleton UK in Harrogate, Yorkshire, in 1992. He witnessed many instances of monkeys being roughly handled, teased, taunted and physically abused by some animal technicians. Screaming monkeys were handled and dragged roughly by a single limb or the tail. They were sometimes slapped about the body

in a brutal form of 'teasing', and while restrained in primate chairs for inhalation studies were shaken and prodded.

Thirdly, in 1997, a Channel 4 television documentary (*It's a Dog's Life*, based on an undercover investigation at Huntingdon Life Sciences, UK) showed technicians punching beagles in the face and screaming at them. This led to a successful prosecution for cruelty.

Primate toxicity studies last weeks, months or years, during which animals are dosed daily, either in their food, by gavage or inhalation, or by injection/infusion. Groups of monkeys are given different doses of the test drug, the higher dose being expected to have some toxic effects. The gavage procedure itself can result in physical distress. In a British test published in 2000, 12 marmosets were force-fed either an artificial sweetener or a known neurotoxin, by daily oral gavage for 28 days[188]. The gavage procedure caused some marmosets to salivate excessively or vomit.

In fact most methods of dosing cause distress and may result in physical harm, especially if conducted inexpertly or carelessly. For example, monkeys are held in restraint chairs in highly unnatural 'sitting' positions for intravenous drug infusions; while frequent intravenous injection can cause bruising; and repeated blood sampling can lead to muscle damage or anaemia, especially in smaller species such as marmosets.

In 1992, a BUAV investigator witnessed a repeat-dose toxicity study at the contract research organisation Hazleton UK. Fifty-two cynomolgus monkeys were put in restraint chairs and fitted with a full-face mask for inhalation studies of a new drug. The investigator saw the extreme distress of the animals, some of whom screamed and struggled while being placed in the chairs and having the face mask attached. Bruno, a large male cynomolgus, used to scratch his body until he bled, and others pinched themselves or tried to hold hands with the monkey restrained beside them.

It is clear that procedures such as these cannot be conducted without causing suffering, and the accompanying physiological effects have the potential to confound the test results.

In addition to the effects of the test procedures themselves, monkeys also suffer from the toxic effects of drugs, especially at higher doses. According to Wolfgang Scharmann of the Institute for Consumer Health Protection and Veterinary Medicine in Berlin[189],

"...intense pain and suffering are at present also unavoidably linked to the process of the risk assessment of drugs and other chemicals..."

In some EU countries primates are still used in acute lethal dose tests, in which death is an endpoint. Non-lethal acute toxicity testing of pharmaceuticals often involves administration of a maximum tolerated dose, following which effects such as vomiting and convulsions may occur, according to the Nuffield Council on Bioethics report on animal experiments[190]. The Nuffield Council also described an extraordinary catalogue of suffering that can be caused by toxicity tests:

188 Finn JP & Lord GH (2000). Neurotoxicity studies on sucralose and its hydrolysis products with special reference to histopathologic and ultrastructural changes. Fd. Chem. Toxicol. 38(Suppl. 2):S7-S17. **189** Scharmann W (1999). Physiological and ethological aspects of the assessment of pain, distress and suffering. In: Humane Endpoints in Animal Experiments for Biomedical Research. Eds. C Hendriksen & DB Morton. UK: Laboratory Animals Ltd. **190** Nuffield Council on Bioethics (2005). The Ethics of Research Involving Animals. London, UK: The Nuffield Council. See <http://www.nuffieldbioethics.org>

Cynomolgus monkeys used for inhalation studies at Hazleton, UK

"Other signs that can be observed during acute, sub-acute and chronic toxicity testing include both external and internal bleeding, diarrhoea, loss of appetite, vomiting (in non-rodents), aggression, salivation, changes in blood pressure, coma, convulsions, lateral recumbancy and tremors, loss of fur and hair, dehydration, or nasal discharge."

In Britain, project licences that authorise pharmaceutical safety testing on primates are categorised by the highly conservative Home Office as being likely, overall, to cause mainly mild or moderate degrees of suffering. However, as the Animal Procedures Committee agreed in its report to the government, this overall 'severity banding' of whole testing programmes is not only prospective (the reality may be different), but can also can mask the severity of suffering that individual animals experience. This is because a severity band represents an 'average' level of suffering and distress; the Home Office accepts that a project licence banded overall as mild or moderate may include protocols causing substantial suffering.

Further, although all animal experiments in Britain must be licensed in advance, regulatory toxicity studies are authorised under a 'generic' licence which permits a wide range of protocols used in standard tests. Such generic licences may cover the use of many hundreds of animals, including primates, but the identity of the drugs to be tested is not known in advance. Therefore the severity of the drugs' toxic effects is not known at the time the tests are licensed.

7.3 Other medicines tests on primates

As mentioned earlier, drugs intended to treat disorders of the central nervous system may be tested additionally in primates for their potential to cause addiction. In one example that came to light in 1999, a Danish drug company called H Lundbeck A/S tested on baboons a novel drug to treat anxiety and depression.

The baboons were trained to inject themselves with cocaine to which they became addicted. This is usually achieved by implanting catheters in the jugular or femoral veins, and during tests baboons are restrained either in a primate chair or by tether. The cocaine was then substituted with the novel drug, Lu 28-179, for several days, to see if the baboons continued to self-administer. In this case they did not, which means that they would have experienced cocaine withdrawal symptoms likely to include fatigue, craving, mental disturbance and depression.
In some laboratories, such as Covance in Germany, primates are used to test for toxic effects of new drugs to reproductive processes and fetal development. These tests on pregnant cynomolgus monkeys are not required by regulatory authorities, being more usually done on rodents and rabbits. Unless

there was a particular scientific reason why monkeys were used rather than the more usual species, these studies may contravene the requirement in Directive 86/609/EEC that *"...animals with the lowest degree of neurophysiological sensitivity"* are selected.

In a 2003 publication[191], Covance GmbH in Germany actively promoted the use of primates in reproductive and developmental toxicity tests, on the basis that reproductive physiology in cynomolgus monkeys closely resembles that of humans. However, according to the International Conference on Harmonisation, all species of animals have scientific and practical disadvantages when it comes to reproductive toxicity testing. For primates, the ICH[192] describes the following limitations:

"Kinetically[193] they can differ from humans as much as other species, insufficient historical background data, often numbers too low for detection of risk. They are best used when the objective of the study is to characterize a relatively certain reproductive toxicant, rather than detect a hazard."

From a practical point of view, because cynomolgus monkeys have a long gestation period and are slow to mature (compared to rodents or rabbits), reproductive and developmental toxicity tests are of long duration and costly. Ethically, the long duration makes the tests more distressing for the pregnant females. Also, because monkeys only give birth to one or occasionally two infants, the tests are not statistically reliable unless large numbers of pregnant females are used.

7.4 Interpreting primate data for humans

There is a perception that, because primates are our close evolutionary cousins, results from tests on them will almost invariably be predictive of human responses. This is not the case: there are highly significant differences between the species in terms of genetics, molecular biology, pharmacology, physiology, absorption, distribution, metabolism and excretion and in reactions to drugs and chemicals.

Whilst some drug tests on primates will predict human responses, this can only be confirmed - or contradicted - when novel drugs proceed to human clinical trials. The US Food and Drug Administration (FDA), in a recent review of problems facing the development of safe and effective new drugs, pointed out that after many years of pre-clinical testing, a novel drug entering a phase I clinical trial stands only an 8% chance of reaching the market[194]. The main causes of the 92% failure-rate are safety concerns and lack of effectiveness in humans, despite tests on primates and other animals. Indeed, the FDA refers specifically to the limitations of animal toxicology and animal models for assessing drug efficacy.

In a review of 25 cytotoxic cancer drugs, toxicity data from primate (and dog) studies *"grossly overpredict*[ed] *hepatic and renal toxicity"* in patients[195]. In fact the primate tests for hepatic, renal and respiratory toxicities yielded high rates of false positive results when compared with subsequent human data[196].

191 Buse E et al (2003). Reproductive/developmental toxicity and immunotoxicity assessment in the nonhuman primate model. Toxicol. 185:221-227. **192** ICH Harmonised Tripartite Guideline: Detection of toxicity to reproduction for medicinal products. 1993. **193** This refers to ADME properties: how much and how quickly the drug enters the body, circulates to the organs, is metabolised and excreted. **194** Food and Drug Administration (2004). Innovation and Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. **195** Olson H et al (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Reg. Toxicol. Pharmacol. 32:56-67. **196** Reviewed in Greaves P, Williams A & Eve M (2004). First dose of potential new medicines to humans: how animals help. Nature Rev. Drug Discov. 3:226-236.

Discussing whether primates or dogs are the more predictive species for hepatic toxicity in humans, a researcher at AstraZeneca Pharmaceuticals in Cheshire, England, commented[197]:

"Although there is an inbuilt prejudice that the primate will more closely mimic subsequent effects that might occur in man in the clinic, insofar as the liver is concerned, there are many instances where the dog has been more representative of human exposure and metabolism and there is little evidence to show that the nonhuman primate is consistently better than dog in predicting human liver toxicity".

It is relatively common for a drug to be converted in the body to different patterns of metabolites and at varying rates in different species of animals. Some metabolites may be toxic, and some may even be more potent than the parent drug, so accurate predictions of the human response are important. However, it cannot be assumed that such studies in primates will yield reliable results.

For example, in humans the liver constitutively expresses the cytochrome P450 enzyme CYP1A2. This is true for marmosets too, but the enzyme is expressed at very low levels in cynomolgus macaques[198]. Although there are some similarities in the glucuronidation[199] of drugs by liver enzymes in humans and other primates, glucuronidation of morphine only occurs at the 3-position in marmosets, but at the 3- and 6-positions in humans and cynomolgus monkeys[200]. At Merck Research Laboratories in New Jersey, an experimental drug was metabolised more extensively by monkeys than by humans, dogs or rats. The drug and its metabolites were excreted into the bile and urine of monkeys, but only in the bile of rats and dogs[201].

The international forum for drug regulation, the International Conference on Harmonisation, acknowledges that in terms of the way a drug is handled by the body, monkeys can differ from humans as much as any other species[202]. Because comprehensive retrospective analyses have not been done, the overall accuracy of primate tests in predicting drug effects in humans is simply not known[203].

There are numerous documented cases of specific drugs causing different effects in primates compared to humans, and there are inevitably many more residing, unpublished, in the confidential files of drug companies.

Published examples include:

i. Indinavir, a protease inhibitor drug used against HIV, underwent ADME tests in rats, dogs and monkeys. Results revealed significant differences between the three species. Drug absorption was 14% in monkeys, 23% in rats and 72% in dogs, and rates of metabolism also varied. Until volunteer studies were conducted, the equivalent values for humans were unknown. In fact the livers of monkeys generate a unique metabolite of indinavir not seen at all in humans, and a later report concluded that monkeys were not a suitable surrogate species for humans[204].

197 Foster JR (2005). Spontaneous and drug-induced hepatic pathology of the laboratory beagle dog, the cynomolgus macaque and the marmoset. Toxicol. Pathol. 33:63-74.
198 Smith D et al (2001). The selection of marmoset monkeys (Callithrix jacchus) in pharmaceutical toxicology. Lab. Anim. 35:117-130. **199** Glucuronidation is an important enzyme-catalysed process that detoxifies drugs and other substances. **200** Soars MG et al (2001). Evaluation of the marmoset as a model species for drug glucuronidation. Xenobiotica 31:849-860. **201** Kochansky CJ et al (2005). Species differences in the elimination of a peroxisome proliferator-activated receptor agonist highlighted by oxidative metabolism of its acyl glucuronide. Drug Metab. Dispos. 33:1894-1904. **202** ICH Harmonised Tripartite Guideline: Detection of toxicity to reproduction for medicinal products, 1993. **203** Animal Procedures Committee (2002). The Use of Primates under the Animals (Scientific Procedures) Act (1986): Analysis of current trends with particular reference to regulatory toxicology. See <http://www.apc.gov.uk> **204** Chiba M et al (2000). Comparative in vitro metabolism of indinavir in primates - a unique stereoselective hydroxylation in monkeys. Xenobiotica 30:117-129.

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ii. The anti-inflammatory drug fenclofenac was tested on a wide range of species including rhesus and patas monkeys. No adverse effects were observed but in humans it caused acute cholestatic jaundice[205].

iii. The anti-viral drug fialuridine caused liver failure (with additional damage to the pancreas, nerves and muscles) in some patients. This had not been seen in animal tests even at very high doses. The patients' reactions were characterised by lipidosis, abnormal mitochondria and lactic acidosis - none of which was seen in monkeys given the drug at 100 times the human dose[206].

iv. The duration that a drug circulates in the bloodstream is critically important to its efficacy and toxicity. In the case of benoxaprofen (opren), the duration in rhesus monkeys was only half that later seen in humans[207]. Mice and rats proved more similar to humans than monkeys in this respect. Benoxaprofen was eventually withdrawn due to unforeseen side effects.

v. The metabolism of losartan was found to be species specific: the livers of rats and humans produced a major metabolite in large amounts, but not the livers of monkeys[208]. This was important because the metabolite was more potent than the actual drug. Monkeys metabolised the drug to a different substance, which was much less active.

vi. The heart drug amrinone underwent a comprehensive programme of animal studies, including tests on rhesus monkeys, none of which predicted the blood cell changes which later occurred in one-fifth of patients given the drug[209].

vii. During development of the anti-cancer drug 5FU, significant species differences were found in its metabolism by an enzyme called dihydropyrimidine dehydrogenase. In this respect, rats and dogs were similar to humans; but monkeys were quite different. Indeed rhesus monkeys even differed from cynomolgus monkeys in the activity of the enzyme in their livers, a result that was *"unexpected as they are both members of the Macaca genus"*[210].

viii. When recombinant human insulin was being tested for safety, rats and mice tolerated doses 100 times those which would be used by humans. But rhesus monkeys developed severe, life-threatening low blood sugar levels at doses which turned out to be safe and effective in diabetic patients[211].

7.5 Replacing primates in medicine tests

In its report on regulatory toxicity tests on primates, the British Animal Procedures Committee recommended[212] that their replacement should be recognised *"as a high priority goal, which requires immediate and dedicated attention. A coherent appropriately resourced strategy must be developed to achieve this goal".*

205 Gad SC (1990). Model selection in toxicology: principles and practice. J. Am. Coll. Toxicol. 9:291-302. **206** Morton DM (1998). Importance of species selection in drug toxicity testing. Toxicol. Lett. 102-103:545-550. **207** Morton DM (1998). Importance of species selection in drug toxicity testing. Toxicol. Lett. 102-103:545-550. **208** Kling J (1996). In vitro models for in vivo drug profiles. Nature Biotech.14:1655-1656. **209** Eason CT et al (1990). The importance of pharmacokinetic and receptor studies in drug safety evaluations. Regul. Toxicol. Pharmacol. 11:288-307. **210** Sludden J et al (1998). Liver dihydropyrimidine dehydrogenase activity in human, cynomolgus monkey, rhesus monkey, dog, rat and mouse. Pharmacol. 56:276-280. **211** Morton DM (1998). Importance of species selection in drug toxicity testing. Toxicol. Lett. 102-103:545-550. **212** Animal Procedures Committee (2002). The Use of Primates under the Animals (Scientific Procedures) Act (1986): Analysis of current trends with particular reference to regulatory toxicology. See <http://www.apc.gov.uk>

Apart from neurovirulence testing of polio vaccine (see section below), the main uses of primates for regulatory purposes include repeat-dose tests of drugs; drug absorption, distribution, metabolism and excretion studies; and studies of drug efficacy. In most of these areas tests are also carried out on other species of animals, but many useful non-animal methods, such as physicochemical tests, *in vitro*, computer-based and ethical clinical studies, are available and more are under development.

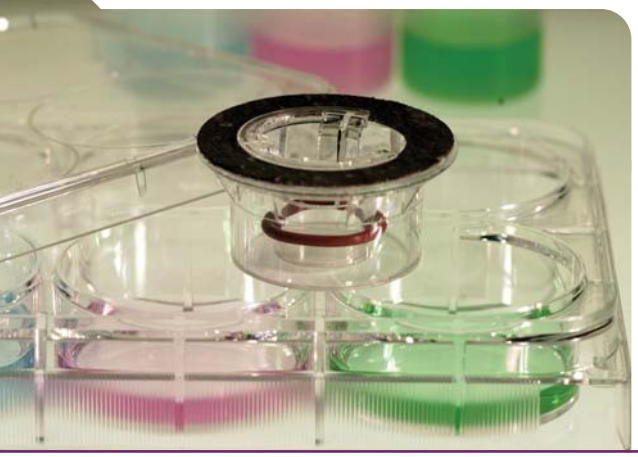
The pharmacology of a novel drug is the basis of its efficacy. Normally, regulatory authorities expect to see *in vivo* as well as *in vitro* data supporting efficacy. But in cases where there is no suitable animal model, such as AIDS and hepatitis C, companies have progressed their drug leads on the basis of *in vitro* efficacy models[213].

In efforts to reduce the high level of attrition in drug development, researchers are turning increasingly to *in vitro* ADME tools[214]. Caco-2 cells cultured on semi-permeable membranes are used to assess likely oral absorption; clearance from the circulation is directly related to the structural characteristics of the drug; *in vitro* metabolism studies provide data on metabolic stability and metabolite identification, as well as likely drug-drug interactions. The use of human cells and sub-cellular components in such strategies will avoid the complications of species differences. Hoffman-La Roche in Basel have developed a modelling and simulation strategy for predicting drug ADME properties, based on currently available *in vitro* and *in silico* prediction tools[215].

A new EU-backed research project called PREDICTOMICS is developing short-term *in vitro* assays for predicting long-term toxicity[216]. The project is identifying early markers of toxin-induced cell damage using genomic and proteomic analysis; and establishing and prevalidating cell systems predictive of toxin-induced chronic kidney and liver damage.

Safe and ethical volunteer studies of new developmental drugs can follow at 'microdose' levels. Quite early in the development of a drug intended to act on the central nervous system, positron emission tomography (PET) studies on volunteers can be conducted at sub-toxic and sub-therapeutic doses[217]. Such studies will identify whether and how much of a drug reaches its target in the brain and which receptors are affected; and will yield predictions of optimal doses for subsequent clinical trials. For ADME data, highly sensitive analytical methods such as accelerator mass spectrometry (AMS) increasingly allow safe microdose studies in fully consenting volunteers[218] before normal

Caco - 2 cell tests ©IFR



213 Combes RD et al (2003). Early microdose studies in human volunteers can minimise animal testing; Proceedings of a workshop organised by Volunteers in Research and Testing. Eur. J. Pharmacol. 19:1-11. **214** Shearer T et al (2005). The role of in vitro ADME assays in antimalarial drug discovery and development. Combinat. Chem. High Throughput Screening 8:89-98. **215** Theil F-P et al (2003). Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection. Toxicol. Lett. 138:29-49. **216** See the European Commission research website: <http://fp6.cordis.lu/fp6/home.cfm> **217** Grasby P (1998). Psychiatric illness: a PET subject. MRC News 77:28-32. **218** Combes RD et al (2003). Early microdose drug studies in human volunteers can minimise animal testing: Proceedings of a workshop organised by Volunteers in Research and Testing. Eur. J. Pharm. Sci. 19:1-11.

phase I clinical trials. The effectiveness of AMS was recently shown in an independent trial with drugs selected for their particularly difficult ADME properties. The trial demonstrated a very promising 70% correspondence between microdose predictions and the outcome of pharmacological doses[219]. The EU is now backing this technology in a 2.1-million-euro research project called the EU Microdose AMS Partnership Programme (EUMAPP). The project involves 10 laboratories from five countries, and aims to boost expertise in microdosing and the application of AMS to developing new candidate drugs[220].

Techniques like AMS, functional magnetic resonance imaging, PET and similar methods provide important data on how novel drugs are handled by the human body, without the difficulties which arise from species differences. These human data include drug bioavailability; distribution throughout the body; receptor-binding; target tissue distribution and elimination; and the identity and levels of drug metabolites.

Novel strategies using imaging, biomarkers, metabonomics, data-mining and analysis[221], are opening the door to safe, ethical and predictive studies in human volunteers.

7.6 Neurovirulence testing of oral polio vaccine

The major manufacturer of oral polio vaccine is GlaxoSmithKline at Rixensart in Belgium[222]. The vaccine is made from attenuated polio virus, which retains the ability to replicate but does not have neurological effects. Neurovirulence tests (NVT) are carried out routinely on monkeys to establish that it has not regained virulence.

The test method is set out in the European Pharmacopoeia (EP) and complies with the requirements of the World Health Organisation (WHO). Each of the three strains of polio virus (types 1, 2 and 3) used in vaccine production is tested separately by injection into the spinal cord of monkeys. For comparison, a reference vaccine is similarly injected into another group of monkeys. Vervet monkeys or macaque monkeys are acceptable to the WHO and the EP and GlaxoSmithKline has habitually used wild-caught vervet monkeys from the Caribbean.

Many monkeys endure cramps and paralysis during the 22-day test, which is considered to cause severe distress[223]. At least 80 monkeys are used to test each combined (trivalent) vaccine batch. It has been estimated that, worldwide, more monkeys are used for this test than for any other single biomedical purpose[224]. However, the relevance and reliability of the results for vaccine safety in use by humans have never been formally validated. In fact the monkey NVT does not prove polio vaccine safety: vaccine which passed the NVT has resulted in vaccine-associated cases of polio. It is a production consistency test - although it is often regarded as confirming safety.

Another concern about the NVT is that it may be unnecessarily duplicated if, having been conducted once by the manufacturer, it is carried out again by more than one country's medicines control laboratory[225]. The British

219 See <http://www.xceleron.com/n25.shtml> **220** See <http://europa.eu.int/comm/research/headlines/news/article_06_01_20_en.html> **221** E.g. Cselenyi Z et al (2004). Joint explorative analysis of neuroreceptor subsystems in the human brain: application to receptor-transporter correlation using PET data. Neurochem. Int. 45:773-781. **222** Langley G (2002). Phasing Out Primate Use in Belgian Laboratories: A GAIA Report. Brussels, Belgium: GAIA. See <http://www.gaia.be/pdf/rapport_langley-EN.pdf> **223** Weisser K & Hechler U (1997). Oral poliomyelitis vaccine: neurovirulence test. In: Animal Welfare Aspects in the Quality Control of Immunobiologicals. Nottingham, UK: FRAME. **224** Hendriksen CFM (2002). Refinement, Reduction, and Replacement of Animal Use for Regulatory Testing: Current Best Scientific Practices for the Evaluation of Safety and Potency of Biologicals. ILAR Journal 43 (Suppl.):S43-S48. **225** Bottrill K (2000). A Report on the Use of Non-human Primates in the European Union. Publ. European Commission 2003.

Associate Parliamentary Group for Animal Welfare was also worried that Britain might be responsible for duplicate animal testing of vaccine batches. It recommended that the Home Office should review its current practice of licensing animal tests, to ensure that any legal basis for repeat testing is examined[226].

7.6.1 Replacing primates in neurovirulence tests of polio vaccine

An entirely non-animal method called MAPREC[227] detects and quantifies mutations which can cause polio vaccine virus to regain virulence. It is now available for all three strains of polio vaccine.

MAPREC has been accepted by the WHO since 1999 as a method of ensuring consistency of polio vaccine production[228, 229] . WHO says that any type 3 vaccine batch which fails MAPREC should not be tested further in monkeys[230], but if the vaccine is normal (i.e. negative) this must still be confirmed in a monkey NVT. Thus it has been used as a screening technique for this vaccine component for more than five years, but not yet as a complete replacement for the monkey NVT. Like the NVT, MAPREC is a consistency test - but a more sensitive one, according to Dr Rezapkin and colleagues at the US Center for Biologics Evaluation and Research (the US national control authority)[231].

For types 1 and 2 vaccines, no batch has ever consistently failed the monkey test. The virus used in these vaccines is extremely stable and is unlikely to revert to virulence in the laboratory. Moreover, it has never been proved that the monkey NVT reliably detects mutations in types 1 and 2 polio virus; in fact, monkey tests failed to detect vaccine batches with deliberately-induced mutations[232, 233].

WHO has been evaluating the use of MAPREC[234] for types 1 and 2 for several years. In the late 1990s Dr Rezapkin recommended that each manufacturer should apply MAPREC to their vaccine to determine its normal molecular characteristics, and continue to monitor future batches with MAPREC while keeping production methods absolutely constant. In this way MAPREC could have been introduced as a *complete replacement* for the monkey tests with types 1 and 2 vaccines. However, today, types 1 and 2 vaccine batches are still routinely tested on monkeys.

It is very disturbing that the development of MAPREC as a full replacement method has taken more than 13 years so far. The process has been inadequately resourced, and it appears that regulators and manufacturers have been insufficiently motivated by the extensive suffering of hundreds of monkeys to act more quickly.

There is also a general preference, usually unspoken, for relying on any kind of *in vivo* test rather than on a molecular technique, albeit a sensitive and accurate one. An expert commented (in confidence) to the author that, regarding some people's preference for an NVT on monkeys rather than MAPREC, *"This is more a matter of perception rather than scientific fact, and it appeals more to the feelings than to reason. In a sense, this is some kind of high-tech sacrificial ritual."*

226 APGAW (2005). The Use of Animals in Vaccine Testing for Humans. London, UK: APGAW. See <http://www.apgaw.org> **227** MAPREC is Mutant Analysis by Polymerase chain reaction and Restriction Enzyme Cleavage - a technique which identifies and quantifies mutations which may occur during manufacture. **228** WHO Expert Committee on Biological Standardization (1999). WHO Technical Report Series 889, forty-eighth report, p13. **229** Dorsam V et al (2000). Increased safety level of serotype 3 Sabin oral poliomyelitis vaccine lots by improved seed virus, and tissue culture and virus infection conditions. Vaccine 18:2435-2443. **230** David Wood of the WHO, personal communication dated 22 March 2000. **231** Rezapkin GV et al (1998). Genetic stability of Sabin 1 strain of poliovirus: Implications for quality control of oral poliovirus vaccine. Virology 245:183-187 **232** Rezapkin GV et al (1998). Genetic stability of Sabin 1 strain of poliovirus: Implications for quality control of oral poliovirus vaccine. Virology 245:183-187. **233** Rezapkin GV et al (1999). Mutations in Sabin 2 strain of poliovirus and stability of attenuation phenotype. Virology 258:152-160 **234** WHO Expert Committee on Biological Standardization (2004). WHO Technical Report Series 924, fifty-second report, p17.

Table 7.1 Comparison between monkey neurovirulence tests, MAPREC and transgenic mice for assessing the safety of oral polio vaccine

	Animals used	Number of animals	Degree of distress	Duration of test	Cost
Neurovirulence test	monkeys	min. 80 per final bulk of vaccine	severe	3 weeks (plus 6 wks. quarantine)	high
MAPREC	none	none	none	1 week	low
Transgenic mouse	transgenic mice	min. 180 per final bulk of vaccine	moderate to severe	3 weeks	medium

In contrast, a test that uses transgenic mice has also been developed, as a 'refinement' of the monkey NVT. The mouse test, perhaps because of the 'comfort factor' that animal tests (as opposed to molecular methods) appear to offer, was fast-tracked and is already accepted and promoted by WHO, even though it causes moderate to substantial suffering to 180 mice per test[235], as shown in the Table above. The mouse test also requires microsurgical skills that are acquired by training using living animals.

While the validation and acceptance of MAPREC drags on, monkeys suffer and die in unvalidated tests for polio vaccine safety. The monkey NVT is ethically insupportable (causing substantial suffering to many monkeys); can produce unclear and borderline results; has never been proven to ensure the safety of type 1 and 2 strains; and is costly to conduct.

The solution is to ensure reliability in vaccine production, including adherence to good manufacturing practice monitored by a system of quality assurance[236]. Batch release testing would then reflect the level of consistency in production and, on good scientific grounds, could be conducted solely using MAPREC.

Regulatory tests on primates for pharmaceutical development are not required by legislation. They cause considerable suffering, cannot be relied upon to predict accurately human responses. The tests could be replaced by *in vitro*, *in silico* and human volunteer studies. The testing of oral polio vaccine for neurovirulence also causes substantial suffering to many monkeys, yet molecular methods are virtually ready to replace them. The BUAV therefore calls for the prohibition of medicines testing on primates.

235 Weisser K & Hechler U (1997). Animal Welfare Aspects in the Quality Control of Immunobiologicals. Nottingham, UK: FRAME. **236** Hendriksen CFM (2002). Refinement, Reduction, and Replacement of Animal Use for Regulatory Testing: Current Best Scientific Practices for the Evaluation of Safety and Potency of Biologicals. ILAR Journal 43 (Suppl.):S43-S48.

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CHAPTER EIGHT
Primates in fundamental research

Second only to the development and testing of human medicines, fundamental research is the largest category of experimentation on primates. Fundamental research is a broad category, ranging from curiosity- or knowledge-driven studies with no foreseen medical relevance, to basic medical research that might, in time, contribute to new ways of preventing or treating human diseases.

The scientific literature reveals the main areas of fundamental research conducted on primates throughout Europe. They include the following subjects (annotated briefly in italics with the kinds of procedures[237] endured by the monkeys used in these experiments):

- **Vision, taste, hearing and the brain (marmosets, macaques)** *Brain lesions, brain electrodes, probes in the brain, chair and head restraint, implanted eye coils*
- **Cognition and the brain (marmosets, macaques, baboons)** *Multiple neurotoxic brain lesions, cognitive damage, water deprivation*
- **Autonomic arousal and emotions (marmosets)** *Neurotoxic brain lesions, small test chamber, implanted telemetric device*
- **Functional brain anatomy (macaques)** *Injection of tracers into brain, brain electrodes and lesioning, chair and head restraint*
- **Experimental autoimmune encephalomyelitis (marmosets)** *Lesions and inflammation in the brain*
- **Schizophrenia (vervet monkeys)** *Chair and head restraint, implanted eye coils, brain lesioning, single caging for up to a year*
- **Aerospace research (macaques)** *Implanted brain electrodes and probes, replacement of skull section with plexiglass, restraint, centrifugation to 12 times normal gravity, loss of consciousness*
- **Parkinson's disease (marmosets, macaques, baboons)** *Neurotoxic brain lesions, spinal flexing, head rotation, tremor, movement disability, limb rigidity, implanted brain recording chamber, head restraint*
- **Alzheimer's disease (baboons)** *Neurotoxic brain lesions, memory damage, cognitive testing*
- **Stroke (marmosets, baboons)** *Behavioural training, blockage of cerebral artery, brain damage, behavioural tests*
- **Chronic pain (macaques)** *Single caging, severing of spinal nerves, one-sided loss of sensation, pain, self-biting and scratching for 28 days*

237 The procedures listed are taken from several different research papers. It is not intended to suggest that all monkeys experienced every procedure or every effect described, although in many cases multiple protocols were carried out on individuals.

- **Hormones and reproduction (marmosets)** *Indwelling brain cannula, drug infusion into the brain, temporary isolation, ovariectomy, hormone treatment*

- **Xenotransplantation (macaques, baboons)** *Heart or kidney removal, implantation with pig organs or cells, toxicity from immunosuppressive drugs, infections, organ inflammation and rejection, death*

- **AIDS (macaques)** *Diarrhoea, wasting, pneumonia, brain inflammation, repeat tissue biopsies*

- **Defence and bioterrorism (marmosets, macaques)** *Exposure to sarin and soman nerve gases, infection with anthrax*

8.1 'Knowledge-driven' fundamental research on primates

Given the levels of public concern about primate experiments, many scientists conducting fundamental research, if challenged, would refer to distant possibilities of their work having some medical implications. However, a reading of their published papers, particularly where the aims are stated; plus a knowledge of the journals where the work is published, give clear indications that it is fundamental in nature.

There are many examples of curiosity-driven fundamental research conducted on primates throughout Europe. They include anatomical studies of eye muscles in rhesus and cynomolgus monkeys; of the size of blood vessel channels in the skulls of 100 macaques; of the intervertebral discs from different regions of the spine of cynomolgus macaques; and of the cochlea of the ear in rhesus monkeys. Whilst this research is carried out on tissues after the animals are killed, there is no question that some of the monkeys were killed specifically for the studies.

However, more worrying by far - due to numbers of animals used and the suffering caused - are the numerous fundamental neurological studies conducted on primates each year across Europe.

8.1.1 Fundamental neurological research on primates

Primates are used in fundamental research to study cognitive, sensory, motor and other functions of the brain. Perhaps the most widely studied function is vision: invasive experiments on monkeys have been carried out in this field for more than 40 years, including at the University of Oxford[238], where it still continues[239].

In the last two years the following European laboratories have published vision research using monkeys:

- Universities of Oxford and Newcastle, England
- Catholic University of Leuven, Leuven, Belgium
- National Centre of Scientific Research, Bron, France
- Philipps University, Marburg, Germany
- Universities of Rome and Parma, Italy

238 Cowey A (1962). Visual field defects in monkeys. Nature 193:302. **239** Rolls ET et al (2005). Novel visual stimuli activate a population of neurons in the primate orbitofrontal cortex. Neurobiol. Learn. Mem. 84:111-123.

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- Utrecht University and the Ophthalmic Research Institute in Amsterdam, the Netherlands
- University of Santiago de Compostela, Spain
- University of Zurich and University Hospital of Zurich, Switzerland.

Dozens of similar experiments are conducted in the USA, Japan and China. Their purpose is to find out about the structure and function of the central nervous system as related to all aspects of vision. The main techniques are:

- electrophysiology, using intracellular or extracellular electrodes to study the activities of individual brain neurons, or groups of neurons, during various visual tasks;
- lesioning techniques to discover the function of a specific area of the brain by ablating it;
- tracer studies to follow the pathways by which information from the eyes is passed to and between different regions of the brain.

Vision research and similar studies on primates invariably cause suffering, sometimes classed as substantial. For electrophysiology, surgery typically, involves removing an area of skull to expose the brain, and cementing a metal ring over the area. To the ring is attached an electrode positioner and electrodes. Metal tubes are cemented onto the skull for restraining the monkey by the head during recording and stimulating sessions. Scleral search coils may be implanted in the eye to monitor eye movements.

Animals are sometimes deprived of food or water for many hours prior to the experiments, to motivate them to perform visual tasks. During recording or stimulating sessions, which can last for several hours a day, animals are usually conscious and restrained in chairs by the metal fixtures cemented to the skull. To avoid other animals tampering with the implants, in some laboratories monkeys are kept in solitary confinement for the duration of experiments which can last for months or years.

Some monkeys are used and re-used in similar experiments for very long periods of time. In the late 1980s, a monkey used at Oxford University in taste research had had electrode implants in the brain for five years, during which four experiments were conducted[240, 241]. At the Catholic University of Leuven in Belgium, some monkeys had been kept instrumented in single caging for two years, while being used and re-used in vision research[242].

In tract-tracing studies, monkeys are injected with tracers into the eye, or elsewhere along the visual pathways. They are later killed for post-mortem analysis. Sometimes specific areas of the brain are ablated, or fibrous tracts severed, to discover the roles of these areas in vision.

240 Scott TR et al (1986). Gustatory responses in the nucleus tractus solitarius of the alert cynomolgus monkey, J. Neurophysiol. 55:182-200. **241** Rolls ET, Yaxley S & Sienkiewicz ZJ (1990). Gustatory responses of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. J. Neurophysiol. 64:1055-1066. **242** Missal M et al (1999). Shape interactions in macaque inferior temporal neurons. J. Neurophysiol. 82:131-142.

8.1.2 Critique of primate neurological research

Although these are fundamental research studies, the ultimate species of interest is the human. Until now, ironically, more has been known about brain function in macaques than in people. As this changes with the introduction of novel, non-invasive methods, species differences are becoming apparent.

One example comes from comparative research into visual processing in the brain. This has shown that functional homologies exist between rhesus monkeys and humans at early levels of the visual hierarchy, but differences occur in the higher-order areas of the association cortex. Thus, Professor Guy Orban of the Catholic University of Leuven in Belgium, points out that[243]:

"...the intraparietal sulcus is expanded markedly in humans compared with monkeys. Functionally, the IPS appears very different in the two species at the area level."

Further species variations have been discovered in relation to the functions of the prefrontal cortex in visual processing[244]. In a comparison between monkeys and human subjects, visual stimulus-related activation of the lateral prefrontal cortex was seen in both species, but was stronger in monkeys than in humans, both in magnitude (by two- to three-fold) and in spatial extent (five-fold or more). The results indicate a difference in the level of volitional control over visual processing in humans and monkeys.

8.1.3 Replacing primates in fundamental neurological research

This kind of research has been defended on the basis that it cannot be conducted ethically in human volunteers or *in vitro*. This is no longer true.

Several newly-validated, non-invasive imaging and related techniques can now be applied safely in human volunteer studies, providing data at the level of small populations of cells and networks where, arguably, the most interesting information about brain function resides. The Nuffield Council on Bioethics has acknowledged the growing importance of human imaging studies to replace primate experiments[245]:

"Nonetheless, imaging techniques are rapidly improving and are likely to provide increasingly powerful alternatives to invasive animal research of this type."

Human studies yield highly relevant information on brain structure and function[246]. Key technologies include functional magnetic resonance imaging (fMRI), diffusion tensor MRI, magnetoencephalography (MEG) and transcranial magnetic stimulation, to name a few.

Magnetoencephalography (MEG) generates high-resolution functional maps of the human cortex, with a temporal resolution of milliseconds and spatial discrimination of around two millimetres. Early MEG research undertaken at Aston University in the 1990s showed that accurate cortical function data from humans can be obtained, and laid the

243 Orban GA, Van Essen D& Vanduffel W (2004). Comparative mapping of higher visual areas in monkeys and humans. Trends Cogn. Sci. 8:315-324. **244** Denys K et al (2004). Visual activation in prefrontal cortex is stronger in monkeys than in humans. J. Cogn. Neurosci. 16:1505-1516. **245** Nuffield Council on Bioethics (2005). The Ethics of Research Involving Animals, p. 94. London, UK: The Nuffield Council. See <http://www.nuffieldbioethics.org> **246** Langley G et al (2000). Volunteer studies replacing animals in brain research. ATLA 28:315-331.

foundations for subsequent MEG studies of human brain functional architecture[247, 248]. High-resolution cortical representation maps have been produced using MEG combined with electroencephalography, with an accuracy very close to that achieved by highly invasive microelectrode recordings in primates.

MEG has recently been used to investigate electrical changes in primary visual cortex in volunteers[249]. These changes are characterised by an increased power in the gamma (30-70 Hz) frequency range. In the past two decades, these 'gamma oscillatory' phenomena have been widely investigated, as they are thought to provide a mechanism by which the brain binds information across different cortical areas. The research has almost exclusively used macaque monkeys.

Now, scientists at Aston University have shown, using MEG with volunteers, that sensory stimuli induce gamma frequency activity that is retinotopically ordered across the visual cortex. The amplitude of the gamma oscillations is directly related to the visual stimulus contrast, providing novel information about gamma activity in the human visual system.

The work demonstrates that MEG can resolve spatial variations in local field potential activity in human volunteers - data which previously had only been available through direct electrical recording in primates. It also shows that state-of-the-art functional imaging of humans can directly replicate invasive primate studies. By the end of 2008 there are expected to be more than ten operational MEG systems in the UK.

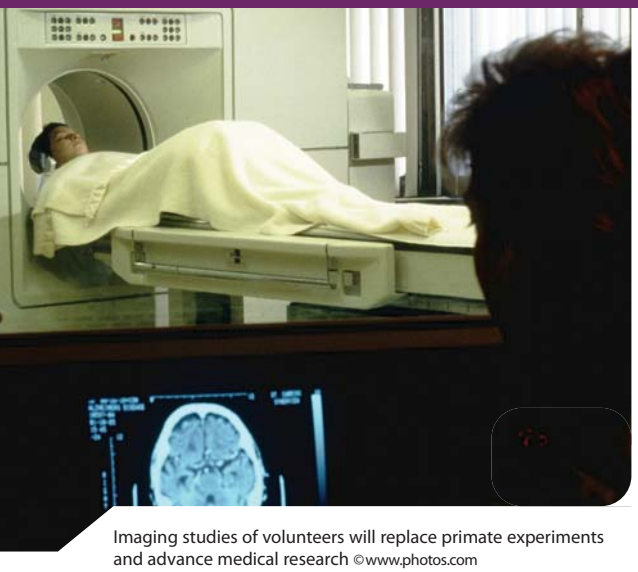
Novel applications of magnetic resonance technologies are proving a significant advance for brain lesion mapping in patients with brain damage, with wider implications for understanding human brain functions[250].

Transcranial Magnetic Stimulation (TMS) is a magnetic field technique used to create momentary, safe and reversible virtual brain 'lesions' in volunteers. It is now being used in research into visual processing in the brain, which previously had only been conducted in monkeys[251, 252]. Human studies with TMS have advantages over primate experiments[253]: TMS has transient effects, so that brain plasticity can be studied; it enables research into temporal functions and systems-level activities, which microelectrode experiments in primates do not; scientists can talk to their subjects; and species differences are eliminated.

Recent major breakthroughs now permit studies of brain tracts, and hence brain connections, in humans. Diffusion tensor imaging (DTI) is an advanced magnetic resonance technology that provides high-resolution images showing the structure and architecture of deep white matter, such as the corpus callosum. Until recently, more was known about brain connectivity in monkeys than in humans, through invasive, terminal experiments (such as electrode studies and tract-tracing). Apart from the ethical issues with such research, species differences can complicate interpretation of such data[254, 255].

DTI is already being applied to research into brain connections serving vision in the human brain. In one example, DTI and fibre tracking were used to measure the occipital lobe fibre

247 Fylan F et al (1997). Magnetoencephalographic investigation of human cortical area V1 using color stimuli. NeuroImage 6:47-57. **248** Singh KD et al (2002). Task-related changes in cortical synchronisation are spatially coincident with the hemodynamic response. NeuroImage 16:103-114. **249** Hall SD et al (2005). The missing link: analogous human and primate cortical gamma oscillations. NeuroImage 26:13-17. **250** Mort DJ et al (2003). The anatomy of visual neglect. Brain 126:1986-1997. **251** Stewart L et al (2001). The role of transcranial stimulation (TMS) in studies of vision, attention and cognition. Acta Psychologica 107:275-291. **252** Pascual-Leone A & Walsh V (2001). Fast backprojections from the motion to the primary visual area necessary for visual awareness. Science 292:510-512. **253** Walsh V et al (2002). Advances in TMS. Dr Hadwen Trust Science Review 2002. Hitchin, UK: Dr Hadwen Trust. **254** Yamamoto T et al (2004). Cerebellar activation of cortical motor regions: Comparisons across mammals. Prog. Brain Res. 143:309-317. **255** Croxson PL et al (2005). Quantitative investigation of connections of the prefrontal cortex in the human and macaque using probabilistic diffusion tractography. J. Neurosci. 25:8854-8866.



Imaging studies of volunteers will replace primate experiments and advance medical research ©www.photos.com

tracts connecting the two cerebral hemispheres of the brain[256]. These tracts are important for normal vision, and identifying them has traditionally been done in monkeys by lesioning the brain and measuring the subsequent degeneration of nerve fibres; or by the injection of tracers, both followed by post-mortem studies.

The 86/609 Directive and national legislation transposing it, such as the *Animals (Scientific Procedures) Act 1986* in Britain, requires the replacement of animal experiments where scientifically satisfactory alternatives are available. The licensing of animal experiments in Britain additionally requires a cost/benefit analysis, which should weigh the suffering of animals against the potential benefits of the research. In the case of knowledge-driven fundamental research, the likely benefits (if any) are difficult to foresee; therefore there is an argument that the suffering allowed should be proportionately reduced.

Neurological experiments on primates can cause substantial pain and distress. On this basis alone it is hard to see how the research is considered permissible. But now that there are non-invasive methods enabling safe and more relevant research to be conducted with volunteers, there is no excuse for continuing studies in primates. Britain should lead Europe in prohibiting such experiments without further delay.

8.2 Fundamental medical research on primates - Parkinson's disease

Basic medical research involves creating primate 'models' of human diseases, to study the causes, the underlying processes and how the disease progresses. It is hoped that this fundamental knowledge may contribute to the better prevention or treatment of human illnesses.

In European laboratories primates are used very widely in basic research into human Parkinson's disease (PD). This involves injecting marmosets and macaques with neurotoxins, such as 6-hydroxydopamine and MPTP, to create brain damage which, in some limited respects, is similar to that seen in Parkinson's disease[257]. It is important to realise that the Parkinson's disease syndromes found in humans have never been fully recreated in animals.

The causes and progression of PD in humans remain largely unknown[258] despite 100 years of research, mainly on rodents and primates. Patients with PD have damage to dopamine-containing cells in the substantia nigra pars compacta in the brain, as well as cell death in networks that use other neurotransmitters. The damage is linked to the typically slow and awkward movement, rigidity, resting tremor and balance disturbances of PD patients. The damaged brain cells also have microscopic inclusions called Lewy bodies,

256 Dougherty RF (2005). Functional organization of human occipital-callosal fiber tracts. PNAS 102:7350-7355. **257** Hurley MJ et al (2005). Immunoautoradiographic analysis of NMDA receptor subunits and associated postsynaptic density proteins in the brain of dyskinetic MPTP-treated common marmosets. Eur. J. Neurosci. 21:3240-3250. **258** Calne DB (2003). Parkinson's Disease over the last 100 years. In: Parkinson's Disease: Advances in Neurology 91:1-8.

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considered the pathological hallmark of PD in patients. Dopamine-replacement therapy and surgical treatments are used to relieve symptoms, but have drawbacks.

In 2001-2002, a BUAV investigator worked in a laboratory at Cambridge University where PD research was conducted on marmosets[259]. The neurotoxin 6-hydroxydopamine was injected into the marmosets' brains in order to damage dopamine-containing cells. After the neurotoxin injection, the marmosets' heads rotated over their shoulder on one side; they turned bodily in slow circles but otherwise showed little spontaneous activity; their balance and smoothness of movement was severely damaged; and they experienced tremors. Marmosets receiving bilateral injections were unable to feed or groom themselves for a period of time.

The marmosets were trained in cognitive tasks before brain lesioning and were re-tested afterwards to assess the extent and stability of brain damage. Training and testing took place over several months. The animals' food intake was restricted to motivate them to perform the tasks. Brain damage was also assessed by recording spontaneous and drug-induced body rotations (which have no counterpart in human PD) while the animals were confined in a very small perspex box, which was evidently very distressing.

Several French laboratories have published PD research using MPTP-disabled rhesus and cynomolgus monkeys. At the Victor Segalen University in Bordeaux, rhesus monkeys were used to study brain areas involved in the fine control of limb movement[260]. They were housed singly throughout the experiments. Electrode recordings were taken from regions of the brain via a steel recording chamber implanted in the skull. During recordings, the monkeys' heads were restrained. They experienced tremors, limb rigidity and freezing of movement, with *"vocalisations"*, flexing of the spine and reduced activity.

At the National Centre for Scientific Research, also in Bordeaux, cynomolgus monkeys were treated with two neurotoxins (MPTP and 3-nitroproprionic acid) to 'model' severe PD that is resistant to levodopa medication[261]. Depending on the toxin regime, symptoms included severe loss of voluntary movement, one-sided rigidity of the arm with flexing, hind limb flexing, abnormal movements and intermittent tremor. For animals whose survival in the wild depends on rapid reactions and fast, skilled movement, these disabilities would cause substantial stress. The experiment lasted for nearly a year.

Marmosets confined to perspex boxes at Cambridge University ©BUAV

259 Henderson JM et al (1998). Behavioural effects of subthalamic nucleus lesions in the hemiparkinsonian marmoset (Callithrix jacchus). Eur. J. Neurosci. 10:689-698. **260** Escola L et al (2002). Disruption of the proprioceptive mapping in the medial wall of parkinsonian monkeys. Ann. Neurol. 52:581-587. **261** Ghorayeb I et al (2002). Dystonia is predictive of subsequent altered dopaminergic responsiveness in a chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine + 3-nitropropionic acid model of striatonigral degeneration in monkeys. Neurosci. Lett. 335:34-38.

8.2.1 Critique of fundamental PD research on primates

From a scientific point of view, animal 'models' of PD should be stable over a long period of time so that the effects of new therapies can be evaluated. However, the fact is that the signs and symptoms in primates vary over time, even between individuals. Additionally, specific signs of illness in individual animals used as indicators of the severity of their condition contradict each other, making data interpretation more difficult.

There are serious underlying limitations to the primate 'models' of PD which include:

- Symptoms are caused by toxin injection and appear rapidly in primate 'models'. The causes of human PD are unknown and symptoms are slow to develop.
- Because of the artificial causation of the condition in monkeys, little can be learned of the causes and progression of the human disease.
- Compensatory mechanisms in surviving brain regions are likely to be different in lesioned monkeys compared to PD patients.
- If dosing is stopped, neurotoxin-treated primates show partial but variable recovery (and there is variation between old and new world monkeys). However, humans always show a progressive worsening of the symptoms over time.
- PD usually affects older people with co-morbidities, while the monkeys used in research are young and otherwise healthy.
- In the affected brain regions of primates, Lewy bodies are either never seen or only very infrequently. Yet in patients, these cellular inclusions are the classic hallmark of PD.
- MPTP-treated primates show limb tremor only sporadically. In PD patients tremor is marked and sustained. The cognitive patterns of impairment also differ between primates and patients[262].
- In neurotoxin-treated monkeys, specific dopamine-containing brain cells are damaged in one part of the brain. In PD, damage is more widespread and involves other neurotransmitters, in addition to dopamine.

8.2.2 Replacing primates in fundamental PD research

Functional imaging has a key role to play in human studies of Parkinson's disease, thereby avoiding problems of species differences and artificiality of the model.

Positron emission tomography (PET) imaging has been used to measure levels of dopaminergic activity in the brains of Parkinson's patients[263]. This sheds light on the pathophysiology of the condition, and permits direct study of disease progression at a biochemical level. PET imaging has revealed disturbances of brain functional interactions and cognitive information

262 Collins P et al (2000). The effect of dopamine depletion from the caudate nucleus of the common marmoset (Callithrix jacchus) on tests of prefrontal cognitive function. Behav. Neurosci. 114:3-17.
263 Leenders KL & Oertel WH (2001). Parkinson's disease: clinical signs and symptoms, neural mechanisms, positron emission tomography, and therapeutic interventions. Neural Plast. 8:99-110.

processing deficits in PD patients; and has provided direct human evidence for the role of the caudate nucleus in certain cognitive tasks in patients[264, 265]. PET studies have also shown that impaired frontal lobe function in patients with Parkinson's disease is related to low dopaminergic activity in the brain's caudate nucleus[266].

PET and SPECT[267] allow the non-invasive assessment of changes in dopamine receptor density[268]. Volunteer studies have also provided evidence that direct dopamine agonists (mimics) can inhibit the release of endogenous dopamine, possibly by the activation of presynaptic dopamine receptors[269]. Functional magnetic resonance imaging has been applied to patients to study impaired connectivity between frontal cortical regions of the brain underlying the movement disorders of PD [270].

Some researchers are studying in cell culture the role of the protein synuclein[271] (found in Lewy bodies in the brains of Parkinson's patients). Cell cultures have also been used to study oxidative stress and microglial activation as factors in Parkinson's disease. Human post-mortem studies have provided consistent evidence of biochemical damage being involved with the progression of PD[272].

Molecular epidemiology of human populations is elucidating the genetic and environmental factors which interact to cause Parkinson's disease. A molecular genetic approach has identified three genes and two or more additional genetic locations in the rarer familial forms of Parkinson's disease[273]. Preventive measures will require a knowledge of causation, and artificially induced animal 'models' are not likely to offer much information in this area.

8.3 Fundamental medical research on primates - Schizophrenia

Doctors and researchers are concerned about the slow progress in finding novel and effective treatments for the cognitive disorders experienced by people with schizophrenia[274, 275]. After fifty years of modern drug research, there has been only poor success in achieving functional improvements in patients, who experience a wide range of cognitive and perceptual symptoms.

Much schizophrenia research has traditionally been conducted on mice and rats. Increasingly it appears that primate 'models' are being developed, with the aims both of studying brain abnormalities underlying the human condition and ultimately for drug efficacy tests.

At Cambridge University marmosets have been used for many years in fundamental cognitive research into the roles of serotonin (5-HT) and dopamine circuits within, and affecting, the prefrontal cortex of the brain[276] . The main approach is to lesion dopamine- or 5-HT-containing cells in this and connecting parts of the brain by injecting targeted neurotoxins. The researchers believe that these studies have relevance to understanding cognitive problems in schizophrenic patients[277].

264 Weder B et al (2000). Disturbed functional brain interactions underlying deficient tactile object discrimination in Parkinson's disease. Hum. Brain Mapp. 11:131-145. **265** Weder BJ et al (1999). Impaired somatosensory discrimination of shape in Parkinson's disease: association with caudate nucleus dopaminergic functions. Hum. Brain Mapp. 8:1-12. **266** Bruck A et al (2001). Positron emission tomography shows that impaired frontal lobe functioning in Parkinson's disease is related to dopaminergic hypofunction in the caudate nucleus. Neurosci. Lett. 311:81-84. **267** SPECT is single photon emission computed tomography. **268** Thobois S et al (2001). Contributions of PET and SPECT to the understanding of the pathophysiology of Parkinson's disease. Neurophysiol. Clin. 31:321-340. **269** de la Fuente-Fernandez R et al (2001). Apomorphine-induced changes in synaptic dopamine levels: positron emission tomography evidence for presynaptic inhibition. J. Cerebr. Blood Flow Metab. 21:1151-1159. **270** Rowe J et al (2002). Attention to action in Parkinson's disease: impaired effective connectivity among frontal cortical regions. Brain 125:276-289. **271** Tofaris GK et al (2001). alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. FEBS Lett. 509:22-26. **272** Dawson TM & Dawson VL (2003). Molecular pathways of neurodegeneration in Parkinson's Disease. Science 302:819-822.

The experiments typically use 16-20 marmosets. The animals are water-restricted for 22 out of 24 hours, five days a week, to motivate them to learn visual discrimination tasks while shut in a box with a computer screen, in a dark room. They are then brain-damaged in the prefrontal cortex by as many as 20 stereotaxic neurotoxin injections. Outcomes include post-operative pain, temporary loss of appetite and weight reduction, loss of balance, tremors, epileptic fits (induced by some toxins) and mood and other emotional disturbances.

The marmosets are then re-tested to characterise the cognitive disabilities they develop, which include dysfunctional learning or remembering of certain tasks. The disabilities are related to the possible functions of the lesioned cells. Some marmosets undergo further brain surgery for microdialysis studies, in which a probe is inserted into the brain to monitor any recovery of the lesioned area. The whole experiment - from the start of training to post-lesion testing - can last up to four years. Astonishingly, it is the view of the British Home Office that these experiments do not cause more than moderate suffering.

Research into schizophrenia using vervet monkeys has also been conducted at the Pitié Salpêtrière Hospital in Paris[278]. Normal rapid eye movements, called saccades, are altered in people with schizophrenia, who have difficulty in switching their saccadic eye movement away from a suddenly appearing object on one side of the visual field. This is believed to be a dysfunction of attention, due to the brain's failure to re-direct the focus of attention away from such a stimulus.

The French researchers used a drug called ketamine to mimic aspects of this disorder in vervet monkeys, and they suggest this could provide a basis for further studies of schizophrenia.

The two monkeys were first surgically prepared with a head-holder anchored to the skull with screws, and a search coil implanted in each eye to measure eye movements. For a year, they were trained to perform visual tasks while held in a restraint chair with their heads fixed. The day before each training (or testing) session, the monkeys were water deprived. They were then injected with low doses of ketamine and the effects on their visual attention, as measured by their eye movements, were analysed by their performance in the same tests.

Each testing session typically took up to two hours, and the animals were tested four to five times per week for up to a year. The timing and accuracy of their saccades, and other eye movements, were affected, and they became more easily distracted. Dose-dependency differed between the individual animals. During the whole two-year experiment the monkeys were kept in individual primate cages. The source of the monkeys was not mentioned in the paper, but they are likely to have been wild-caught in the Caribbean before being transported to France (Chapter 5).

273 Shastry BS (2001). Parkinson's disease: etiology, pathogenesis and future of gene therapy. Neurosci. Res. 41:5-12. **274** Kilts CD (2001). The changing roles and targets for animal models of schizophrenia. Biol. Psychiatr. 50:845-855. **275** Hagan JJ & Jones DN (2005). Predicting drug efficacy for cognitive defects in schizophrenia. Schizophr. Bull. 31:830-853. **276** Clarke HF et al (2005). Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. J. Neurosci. 25:532-538. **277** Crofts HS et al (2001). Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cerebr. Cortex 11:1015-1026. **278** Condy C et al (2005). Ketamine-induced distractibility: an oculomotor study in monkeys. Biol. Psychiatr. 57:366-372.

CHAPTER EIGHT
Primates in fundamental research

8.3.1 Critique of fundamental schizophrenia research on primates

The experiments in Cambridge and Paris caused cognitive and visual abnormalities in monkeys that were considered to provide information about underlying deficits in people with schizophrenia. In order to provide relevant and reliable data for humans, the dysfunctions created in primates would have to sufficiently resemble a range of aspects of the human condition.

However, the primate disorders were caused by chemical methods, in different species whose functional brain anatomy, physiology and pharmacology may well differ from those of humans. The roles and functions of inter-connected brain regions, as well as the behavioural effects of the lesions in the case of the Cambridge research, would need to be similar in humans and marmosets.

In fact, species differences in the size, location and organisation of regions of the brain do occur. For example, comparative studies with eleven primate species whose brain volumes spanned more than a 50-fold range, showed that larger brains have different connections both between and within the cerebral hemispheres [279]. Clearly, marmosets and humans are at different ends of that size range.

Measuring the effects of brain lesions by assessing the behaviour of marmosets, as in the Cambridge research, has serious difficulties. Extremely complex interactions underlie functions such as thought, memory, perception and learning; and there is built-in redundancy so that, when one part of the brain is damaged, another area may take over some of that function. This organisation and re-organisation may differ, qualitatively as well as quantitatively, between species. In fact, studies of patients with specific brain damage have shown that there are also species differences in cognitive functions. Patients with lesions to the medial temporal lobe cannot perform concurrent discrimination tasks, although monkeys with this lesion can[280].

Human schizophrenia is a highly complex condition with a marked genetic component, although its precise causes are not yet known. Using other primates to study schizophrenia causes them enormous distress and suffering which cannot be justified on a scientific or ethical rationale. Moreover, clinical studies offer an alternative.

8.3.2 Replacing primates in fundamental schizophrenia research

Many areas of clinical research are already producing valuable insights into the disease. Whilst it is unethical to subject patients or healthy volunteers to invasive investigations, new technologies now offer increased scope for human studies. Clearly these offer improved relevance compared to research using other primates.

Imaging studies of patients with schizophrenia consistently show abnormalities of information processing in the prefrontal cortex of the brain. Some of these are also seen in unaffected relatives who are nevertheless genetically at risk

279 Rilling JK & Insel TR (1999). Differential expansion of neural projection systems in primate brain evolution. Neuroreport 10:1453-1459. **280** Corkin S (2002). What's new with amnesic patient HM? Nature Rev. Neurosci. 3:153-160. **281** Weinberger DR et al (2001). Prefrontal neurons and the genetics of schizophrenia. Biol. Psychiatr. 50:825-844. **282** Klei L et al (2005). Linkage analysis of a completely ascertained sample of familial schizophrenics and bipolars from Palau, Micronesia. Hum. Genet. 117:349-356. **283** Levi A et al (2005). Fine mapping of a schizophrenia susceptibility locus at chromosome 6q23: increased evidence for linkage and reduced linkage interval. Eur. J. Hum. Genet. 13:763-771. **284** Lopez-Figueroa et al (2004). Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. Biol. Psychiatr. 55:225-233.

of schizophrenia. One example is the finding of variations in a gene that may predispose people to schizophrenia. The gene codes for an enzyme that affects the metabolism of dopamine in the prefrontal cortex. People with variations in the gene perform cognitive and memory tasks differently, and functional magnetic resonance imaging confirms the effect[281]. Other genetic studies, involving linkage analysis and fine mapping of chromosomes in schizophrenic patients, their families and matched non-schizophrenic groups, provide further information[282 283].

The roles of different neurotransmitter systems in schizophrenia are being clinically researched, for example by comparing specific 5-HT receptors in key regions of the brain in healthy volunteers and patients. This confirmed alterations in 5-HT receptor sub-types in the prefrontal cortex of people with schizophrenia[284]. Diffusion tensor imaging, a new technique that traces brain connections non-invasively, has been used in volunteers to study connections between the brain regions involved in schizophrenia[285, 286].

Volunteers are also used in research which addresses the topic of the Paris experiments on vervet monkeys. Healthy volunteers, patients and patients' relatives participate in research which tracks their saccadic and smooth-pursuit eye movement, and links these to MRI-measured brain parameters[287]. Such studies have shown that errors in saccadic eye movement are a good paradigm to aid the diagnosis of schizophrenia[288]. Similar clinical research is underway to further explore the neural substrates of cognitive dysfunctions in schizophrenia, pointing to potential therapeutic approaches.

8.4 Overview of the replacement of primate research

European Directive 86/609 requires the replacement of animals in research and testing where an equivalent method, not using animals, is *"reasonably and practicably available"*. At European and national levels there have been many initiatives to progress the development of replacement, non-animal techniques, notably the European Centre for the Validation of Alternative Methods (ECVAM) and several national institutes with similar remits.

However, the mainstream scientific community appears to remain largely unaware of its responsibilities in this respect, especially in academia where much fundamental research takes place. In 1999 a survey was undertaken in Britain to assess the impact of introducing ethical review processes into research institutions[289].

The survey probed levels of awareness, among scientists involved with animal procedures, of the legislative requirement to implement the Three Rs. They were asked to respond to the statement, *"There are legal requirements for me to use alternatives in research"*. Among vets with a special responsibility for laboratory animals, 85% were aware of the requirement; but among scientists licensed to do animal experiments only 49% knew of it, while the figure for the heads of research institutions was only 46%. Incredibly, 32% of licence-holders and 43% of laboratory heads believed the statement to be false.

285 Kalus P et al ((2005). New evidence for involvement of the entorhinal region in schizophrenia: a combined MRI volumetric and DTI study. NeuroImage 24:1122-1129. **286** For a review of Diffusion Tensor Imaging (DTI) in psychiatric illness see: Taylor WD et al (2004). Diffusion tensor imaging: background, potential and utility in psychiatric research. Biol. Psychiatr. 55:201-207. **287** Schulze K et al (2005). The relationship between eye movement and brain structural abnormalities in patients with schizophrenia and their unaffected relatives. J. Psychiatr. Res. Jun 23 [Epub ahead of print]. **288** Louchart-de la Chapelle S et al (2005). A concordance of three electrophysiological measures in schizophrenia. Am. J. Psychiatr. 162:466-474. **289** Purchase IFH & Nedeva M (2001). The impact of the introduction of the ethical review process for research using animals in the UK: Attitudes to alternatives among those working with experimental animals. ATLA 29:727-744.

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More recently the results of a Europe-wide survey were published[290]. This sought to discover how widely understood the Three Rs are among animal researchers, *in vitro* scientists, regulators, animal protectionists, ethicists and others. Most of the scientific respondents were animal researchers. 71% indicated that they were very familiar with the Three Rs model, but only 25% felt that this knowledge was shared by the wider scientific community. In considering alternative methods, many respondents judged scientific quality and reliability to be very important, but fewer were moved by ethical concerns about the suffering of animals.

The authors concluded that there is a critical need for an integration between the practical and ethical aspects of the alternatives, and a more creative and successful approach in the scientific community. The BUAV and others have called for this for many years, and welcome this support of our viewpoint.

In this chapter we have discussed three examples of fundamental neurological research currently conducted on primates. We have used these as paradigms for exploring the conventional scientific justification of the experiments, the severity of suffering caused to subject animals, the question of data extrapolation to humans, and the availability of other research approaches to the same questions.

Fundamental research is inherently more difficult to justify by a cost/benefit analysis. Using primates in such research, most especially when similar studies (or others of more relevance) can be undertaken without using primates, is morally insupportable. At very best such primate experiments may provide some information that can be extrapolated to humans. At worst, the results may cause serious misconceptions that will delay a clearer understanding of human conditions.

290 Pollo S et al (2004). The '3Rs' model and the concept of alternatives in animal research: A questionnaire survey. Lab. Anim. 33:47-53.

The results from primate experiments may cause serious misconceptions that would delay understanding of human conditions.

APPENDIX ONE

Call to end the use of non-human primates in biomedical research and testing from animal protection organisations worldwide

A resolution presented at the Fifth World Congress on Alternatives and Animal Use in the Life Sciences, Berlin, August 2005

*Signed by Dr Jane Goodall plus 57 individuals and organisations from 19 different countries**

Resolution

The animal protection organisations attending the Fifth World Congress on Alternatives and Animal Use in the Life Sciences in Berlin in 2005 have united to call for an end to the use of non-human primates in biomedical research and testing. We urge governments, regulators, industry, scientists and research funders worldwide to accept the need to end primate use as a legitimate and essential goal; to make achieving this goal a high priority; and to work together to facilitate this. In particular, we believe there must be an immediate, internationally co-ordinated effort to define a strategy to bring all non-human primate experiments to an end.

Non-human primates are highly intelligent, sentient animals. They form intricate social relationships, interact with their environment in a dynamic and complex way, and engage in imaginative problem solving. It is also widely accepted that primates experience a range of negative emotions (e.g. anxiety, apprehension, fear, frustration, boredom and mental stress) as well as a range of positive emotions (e.g. interest, pleasure, happiness and excitement). In short, they are very close to humans in their biology and capabilities, and the users of non-human primates argue that this makes them ideal 'models' for research. However, this also means that primates have the capacity to suffer like humans, so there can be no question that primates can experience pain and distress.

Confining animals who would normally live in a very large and complex home range in the laboratory, must have a significant adverse effect on their welfare. At its best laboratory primate housing represents only a small fraction of their home range. The worst, still commonly used in many countries, is a small, barren metal box in which the animals can only take a few steps in any direction. Other aspects of the lifetime experience of laboratory primates also cause stress and suffering, particularly where they cannot control their environment, social grouping, or what is done to them[i]. Any pain or distress associated with experimental procedures is therefore compounded by additional adverse effects resulting from capture of wild primates, breeding practices, transport, housing, husbandry, identification, restraint, and finally, euthanasia.

For these reasons alone[ii], the use of primates in research and testing is a matter of extreme concern to the animal protection community worldwide and to the significant sector of the public who they represent. This concern has been recognised at a regulatory level with some countries making special provisions for primates in their legislation, and emphasising the need to reduce and replace primate experiments[iii].

***** Australia, Belgium, Brazil, Canada, Czech Republic, Denmark, Finland, Germany, India, Iran, Italy, Mexico, Netherlands, Norway, Poland, Portugal, Switzerland, United Kingdom and United States of America. **i** See for example: Scientific Committee on Animal Health & Welfare (2002). The Welfare of Non-Human Primates used in Research. Publ. European Commission, Health & Consumer Protection DG. **ii** Some individuals and organisations also put forward scientific arguments to question the validity of some or all experiments on primates. **iii** For example, the British Animals (Scientific Procedures) Act 1986; and Council Decision, 1989, on the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, (OJEC, 1999).

We urge governments, regulators, industry, scientists and research funders worldwide to end experiments on primates.

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