The A P C

Animal Procedures Committee

ANIMAL PROCEDURES COMMITTEE

November 2013

REVIEW OF THE ASSESSMENT OF CUMULATIVE SEVERITY AND LIFETIME EXPERIENCE IN NON-HUMAN PRIMATES USED IN NEUROSCIENCE RESEARCH

REPORT OF THE ANIMAL PROCEDURES COMMITTEE'S PRIMATE SUBCOMMITTEE WORKING GROUP CHAIRED BY PROFESSOR JOHN PICKARD FMedSci

Review of the assessment of cumulative severity and lifetime experience in non-human primates used in neuroscience research

Contents

1. Executive summary and recommendations

The Animals (Scientific Procedures) Act (ASPA) 1986 and now Directive 2010/63/EU provide the classification of severity to be adopted when applications for licences to undertake animal research are reviewed. This severity classification has referred mainly to single procedures and includes unambiguous examples. It is now recognized that the potential lifetime experience of an animal should also be considered. However, there has been difficulty in providing a coherent set of evidence-based guidelines with illustrative examples, particularly for nonhuman primate research.

This Review by the Animal Procedures Committee's Primate Subcommittee Working Group considers how any cumulative suffering caused by multiple neuroscience research procedures over a prolonged period may be assessed in non-human primates – macaque and marmoset monkeys. It is based on wide-ranging consultations, expert meetings, a questionnaire and visits to establishments. It provides the first detailed account in this sensitive area of animal research of the nature, incidence and severity of procedures and complications, including their cumulative impact.

There was a diversity of opinion amongst contributors and members of the Working Group on the use of non-human primates in neuroscience research but, in the interest of promoting animal welfare, there was widespread engagement with the consultation process. Where possible we have reflected the range of opinions, many of which continue to be debated.

1.1 Definitions

Definitions of welfare, suffering, cumulative severity, cumulative experience and non-additive/additive potentiation effects used in this Review are as follows.

Welfare: **'Animals experience both positive (for example, reward, satisfaction) and negative (for example, pain, stress) well-being. Welfare is the state of the individual animal as regards its attempts to cope with its environment. Hence it depends on the individual's needs in relation to physical, nutritional, social and other behavioural factors and, in the case of captive animals, on the people who care for the animals or supervise such work.'**

Suffering**: 'A negative emotional state, which derives from adverse physical, physiological and psychological circumstances.'**

Cumulative severity**: 'The sum of all the events and effects that impact, adversely, positively and by way of amelioration, on the welfare of an animal over its lifetime.'**

This definition of cumulative severity includes, unlike the term suffering, both adverse and 'favourable' components. However, severity implies a net negative effect.

Cumulative experience **is a less confusing term than cumulative severity and has a similar definition: 'The sum of all the events and effects, including their quantity, intensity, duration, recovery between and memory thereof, that impact, adversely, positively and by way of amelioration, on the welfare of an animal over its lifetime.'**

It is important to consider how the effect of the first experience of a procedure impacts on the second experience of the same or a different procedure. A single procedure may have only a short-lasting impact on welfare. With sufficient time for recovery between procedures, there may be no influence on the impact of a second procedure (*non-additive*). The impact of repeated procedures may diminish (*habituation*). In contrast, if insufficient time is allowed for recovery, the residual effects of repeated procedures may add up (*additive stacking up*). Suffering from earlier events may actually increase the negative impact on welfare of subsequent events (*additive potentiation*).

This framework of definitions and scenarios is intended to render cumulative severity and lifetime experience more susceptible to objective, quantitative measurement than has been achieved hitherto.

1.2 Findings of the Review

1.2.1 Centres of non-commercial neuroscience non-human primate research in the UK.

The Review has established that non-commercial non-human primate neuroscience research in the UK is restricted to a small number of universities. All of these accept and apply the '3Rs' (Replacement, Refinement and Reduction) principles of humane experimental technique.

1.2.2 National Centre for the Replacement, Refinement and Reduction of Animals in Research.

The Review noted that the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has to date (May 2013) committed £1.4 million in grants for research to refine techniques used in primate neuroscience, and to assess objectively pain and distress. NC3Rs also leads an extensive programme of work designed to improve the welfare of laboratory primates.

1.2.3 The Questionnaire

The Review's questionnaire provides a useful first step in establishing a common methodological framework to document the various components of the lifetime experience of the animal and to facilitate systematic data collection across institutions. Following the initial completion of the survey, visits were made to the research establishments to gather further information and provide

clarification and discussion around the interpretation of the submitted data. These issues were debated at length amongst the members of the Review during the preparation of this report.

In total 17 reports were received from users in 5 UK academic institutions and 10 reports from users in EU institutions. Data were supplied by researchers, named veterinary surgeons, animal technicians and named animal care and welfare officers. Data were based on observations made on 149 macaques and 82 marmosets housed in UK facilities and 3 macaques housed in 3 EU institutions over a 10-year period. Quantitative data were extracted from records and formed the main basis for the conclusions of this Report. In addition, the respondents' subjective impressions of effects (favourable and adverse) and their severity are provided separately.

The data included in the Review did not cover every animal used but are representative of the spectrum of animal experience, based on the data collected, the interviews/discussions with investigators, veterinarians and NACWOS and the further assessment of animal use made at some centres. The data are not overtly biased to either low or high severity procedures. This substantial database has provided the quantitative data required to address the terms of reference of the Review.

1.2.4 Adverse events and cumulative experience within individual events and procedures

The Review found little evidence for adverse cumulative severity ('additive stacking up', 'additive potentiation') in the following events / procedures:

- **Weaning: in general, monkeys were not removed prematurely from their natal groups (in accordance with the Home Office requirement during the period covered by the Review).**
- **Source and condition on arrival: all monkeys used over the period of the survey had been bred in the UK. From the limited information available, there were no major issues with their condition on arrival. A policy of careful selection at source of monkeys that were appropriate for neuroscience research was being increasingly pursued by users.**
- **Housing, husbandry and care: group- or pair-housing was achieved successfully in the majority of cases, although some fight injuries occurred and there were a few compatibility issues.**
- **Non-procedural life events: apart from fight injuries, few monkeys sustained any other non-procedural life events.**
- **Anaesthesia and pain control: anaesthetic and peri-operative care had improved over the period of the survey with rapid recovery and a low level of post-operative complications (less than 1%).**
- **Surgery and maintenance of implants: the overall incidence of adverse effects resulting from surgical procedures was low, apart from the frequency of low-grade infections around**

implants (up to 23% overall, affecting 24 out of 104 macaques). For long-term implants the proportion was higher, at 39 per cent (affecting 24 out of 61). Bone infection was 6% and brain infection 1% in macaques. The incidence of seizures in studies involving lesions to the central nervous system was 9% of cases.

- **Restraint and handling: all research centres invested time in training monkeys to accept restraint, including positive reinforcement techniques. No adverse effects of restraint were reported in the majority (80%) of macaques restrained in a primate chair.**
- **Food and fluid controls, and training: food control was reported to have no adverse effects in macaques. Fluid control was also reported to be without clinical signs of adverse effects. Fluid control was individualized for each animal (macaque and marmoset) so that good performance on cognitive tasks could be maintained without compromising animal health.**
- **Behaviour: abnormal behaviours were reported in 8 per cent of macaques and 1 per cent of marmosets; pair- and group-housing reduced the incidence of neurotic behaviour.**

1.2.5 Overall cumulative severity and experience

The Review found little evidence for additive effects between procedures, whether through incomplete recovery between events (additive stacking up) or potentiation of adverse effects and suffering by earlier procedures. Some animals were reported to show diminished responses to repeated procedures (habituation), for example, in macaques, 86 per cent for restraint and handling, 71 per cent for the training chair, and 1 per cent for surgery.

Specifically, there was little evidence in the majority of nonhuman primates to suggest that *"the nature of pain, suffering, distress and lasting harm caused by (all elements of) the procedure, and its intensity, the duration, frequency and multiplicity of techniques employed"* and the *"cumulative suffering within a procedure after applying all appropriate refinement techniques"* (Directive 2010/63/EU) should have increased the severity assessment over that for single events/procedures alone.

However, there were some nonhuman primates that could not cope and were removed from study. In a small minority of cases, premature euthanasia was performed as part of the terminal phase (see section 1.2.6 below).

1.2.6 Premature killing of animals

A total of 26 cases of non-elective euthanasia were reported; 12 out of 26 (42%) of these cases were single-procedure related, whilst the remainder were attributed to disorders which were not related to the procedure, including neoplasia, acute illness and end-stage renal disease (findings confirmed at post-mortem examination). The reason for procedure-related terminations was considered to be of significant severity in 6 out of 12 (50%) cases. In such cases, 4 of the 6 animals (67%) were euthanized within 48 hours, whilst the remainder were killed within a timeframe of a few weeks once all alternative ameliorating measures had been exhausted. No nonhuman primates suffering from significant procedure-related complications were reported to have been used in further experimental studies. Although these 26 cases do not in themselves provide evidence for cumulative severity, the Report is concerned about these cases, and would expect that, if its recommendations are fully implemented, the incidence of such cases should be substantially reduced (see below).

1.2.7 Refinements

Users provided a list of advances to improve primate welfare, for example, in anaesthesia, housing, training and implants, that had made it possible to carry out improved long-term neuroscience research.

1.3 Recommendations

1.3.1 Trust and public engagement

The Review was enabled by cooperation between many of those engaged with the welfare of monkeys used in research. The welfare of the monkeys, scientific rigour and public engagement will all be served by building on the trust created. All concerned with this research must continue to work together in a spirit of openness and trust, with the emphasis on mutual education and development through listening and constructive exchange of information (Concordat on Openness on Animal Research, 2012).

1.3.2 Ethics of neuroscience research in monkeys

The Review has identified issues specific to the concept of cumulative suffering and severity. The following issues should be the subject of future ethical debate:

- **the quality of life of primates bred specifically for neuroscience research;**
- **the conflict between using a small number of subjects for longer or more subjects for a shorter period; and**

- **the weighting of the impact of the terminal phase against the overall lifetime experience of the animal when assigning severity categories.**

1.3.3 Best practice

1.3.3.1 Selection of animals, husbandry and procedures

Best practice in selection of animals, husbandry and in anaesthetic, surgical and training techniques should be encouraged by collaboration between all those engaged in neuroscience research involving non-human primates. The Review found that this process was already strongly supported by the NC3Rs and recommends further interaction through the Centre.

Every opportunity should be taken to assess individual animals for their suitability and aptitude for research procedures, and assess pairs or groups of animals for their compatibility by studying life histories and observing them at source. Animals found to be unfit or less suitable for long-term studies should be replaced rather than persisted with. Funding bodies should recognize the need for the resources for such selection and replacement.

Spare capacity for housing and staffing is highly desirable to facilitate group housing and interaction but will require the appropriate financial support.

Tasks and motivations are designed to make behavioural testing a positive experience for animals wherever possible. It is essential to have staff well versed in such techniques.

Significant adverse events, excluding issues of neglect, should be elucidated and investigated by rigorous root cause analysis conducted in a no-blame culture.

Clinicians in human and veterinary medicine should be encouraged to ensure that non-human primate neuroscience research is conducted using advanced modern anaesthetic and surgical techniques so that complications are minimized.

Opportunities should be sought to acquire, increase and share expertise to reduce the adverse consequences of head implants.

Where a centre or an investigator is conducting only a small number of procedures, advice should be sought from a specialist who is expert in the specific technique involved.

There are opportunities to advance best practice through regular interchange with networks of other neuroscientists who are engaged in non-human primate research in Europe and countries further afield.

There is also scope for further collaboration with non-human primate behavioural scientists over refining the currently available behavioural outcome measures.

There should be scope and encouragement for continuous professional development of all those involved in non-human primate neuroscience research.

1.3.3.2 Post-mortem examination and clinical chemistry

All animals used in long-term studies and all those that are killed prematurely should have a post-mortem examination performed to obtain the fullest possible diagnosis and assessment of any consequences of the neuroscience research. This information should be included in retrospective reviews of practice.

Standards established for the conduct of post-mortem examinations should specify the most appropriate person to undertake the examination and include a comprehensive description of the protocol to be used. This person should work with scientists to optimize tissue collection for both scientific and pathological analyses.

Blood samples should be taken for routine health monitoring whenever animals are under general anaesthesia for other purposes, where it will not compromise science or welfare. These should be analysed and collated along with the animals' clinical history to determine reference ranges and outliers. A mechanism should be devised to allow data to be shared and made freely available.

1.3.3.3 Importance of the management structure and a professional team approach to welfare

An institutional management culture and structure should be in place to ensure timely implementation of best practice. This should work in concert with the Animal Welfare and Ethical Review Body to optimize welfare.

The present multi-professional team approach to welfare involving the investigator(s), named veterinary surgeon, named animal care and welfare officer, animal care staff and Home Office inspector should be further encouraged to improve both welfare and science for the individual non-human primate throughout its life.

This team approach should be used to assess the severity experienced by individual animals and achieve a timely consensus over decisions about the need to terminate experiments.

The checks and balances required to avert the progression of severity of suffering should be defined within each institution.

1.3.3.4 Refinement

All investigators and staff caring for the monkeys should be involved in seeking new ways to improve the welfare of nonhuman primates. Planned refinements to improve welfare must be properly assessed to determine whether they are having the desired effect. Personnel should keep up-to-date with research on welfare assessment techniques and studies which investigate welfare in relation to positive reinforcement techniques, housing, husbandry and conduct of experiments. Where there is robust evidence for improved nonhuman primate welfare, relevant changes should be made.

The Review endorses the increasing use of CCTV to supplement monitoring of the welfare of non-human primates in their accommodation. The Review recognizes that additional resources are needed to allow detailed and timely analysis of CCTV footage to address welfare concerns and to develop more sensitive behavioural assessments than currently exist.

1.3.4 Outcome measures

Further research is required to improve objective methods to assess and quantify:

- **pain and distress in nonhuman primates;**
- **whether nonhuman primates experience long-term negative emotional states akin to anxiety and depression in humans;**
- **the impact of successive procedures;**
- **sensitive, robust surrogate biomarkers of cumulative suffering, including non-invasive magnetic resonance (MR) scanning of brain structure;**
- **the sensitivity, specificity and predictive power of welfare measures that are proposed to contribute to a practical assessment of harm–benefit analysis and to guide the appropriate timing (spacing) of future experiments.**

1.3.5 Retrospective assessment

The requirement under the new legislation (Directive 2010/63/EU) is *"to evaluate whether the objectives of the project were achieved, the harm inflicted on animals, including the numbers and species used and the severity of the procedures used and any elements that may contribute to the further implementation of the requirement of replacement, reduction and refinement"*.

Although the Working Document on a Severity Assessment Framework under the EU Directive (2012) asks only for the recording of the effects of procedural events for retrospective reporting of actual severity at the end of procedures, this Review recommends that retrospective assessment should be based on the continuous, standardized collection of data as the experiment

progresses. The information to be collected and assessed should include the following elements:

- **an overview of the animal's lifetime experience with key events and quality of the environment, including benefits of any refinements that have been developed;**
- **a log of adverse events (non-procedural, generic and intended effects of the procedure and complications) including their impact on welfare;**
- **results of post-mortem examination.**

The Review discusses the principles to be considered in choosing an appropriate method for collecting data on an ongoing basis, including the trade-off between logistic / economic feasibility and data accuracy / completeness.

1.3.6 Publication policy

All publications on non-human primate neuroscience should include the information detailed in the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines of the NC3Rs.

1.3.7 Designation of severity limits

The process of ascribing a severity classification or limit in advance of the project authorization depends upon assessing the cumulative suffering and impact on lifetime experience of the likely harm that will be informed by the retrospective reports of similar projects. The assignment of severity categories should be evidence-based. The nomenclature relating to the evaluation of severity should be clarified and criteria set to provide consistency in allocation, both prospectively and retrospectively. The Review regrets that the opportunity was not taken in Directive 2010/63/EU to extend the vocabulary that is used to describe severity limits. There is clearly a distinction to be made between Moderate, *Multiple Moderate without significant impact on welfare,* and Severe.

The Review suggests that the Animals in Science Regulation Unit Inspectorate (ASRUI) considers the following.

- **Using the term 'cumulative experience' rather than 'cumulative severity' as the more readily understood description of lifetime experience.**

- **Clarification of the phrase in the EU Directive: *"it*** [the assignment of the severity category] ***shall be based on the most severe effects likely to be experienced by an individual animal after applying all appropriate refinement techniques".***

  This phrase introduces the concept of the *probability* that a particular severity limit might be reached in contrast to the

previous use of the *worst case* to define the severity limit. The *probability* of what might happen has to be established through an iterative process based on the documented outcomes of individual investigators and institutions obtained through retrospective reporting.

- **Defining the criteria for designating categories of severity based on an overview of the animals' lifetime experience, including key events, quality of the environment and all types of adverse events, including their impact on welfare. These criteria should state unequivocal principles and provide realistic examples to distinguish 'Mild' from 'Moderate', and Moderate from 'Severe' levels of severity in both prospective and retrospective assessments. Such examples should include criteria for recovery between procedures, stacking up of the cumulative effects of successive procedures, and potentiation of adverse effects by successive procedures as reflected in objective outcome measures such as behaviour and performance.**

- **Incorporation of these factors into the prospective estimates of severity, which will be informed by data gained progressively throughout the project, including the impact of newly introduced refinements.**

- **Revisiting the issue of quantification of severity as the objective basis for the holistic assessment of severity – the Review provides an example to illustrate how, for example, the analysis of the unintended complications of procedures might be quantified for standardized scoring and reporting. Engagement should be encouraged with the type of validity, responsiveness and reliability exercise that has been widely used in clinical medicine for many years. The development of a nonhuman primate quality of life measure (NHPQOL) would complement the team approach to immediate health concerns.**

- **Under the new Directive, all those involved with nonhuman primate research will be actively involved in the retrospective assignment of severity. Researchers may well require further training once the guidelines and examples have been published by the Home Office.**

2. Terms of reference

This Review was established to consider how an assessment can be made of the cumulative severity experienced by non-human primates in neuroscience research undergoing multiple procedures over a prolonged period of time.
The Review encompassed the following.

- Consideration of the criteria by which to assess cumulative severity in non-human primates.
- Consideration of the latest research into understanding the cumulative severity experienced by animals undergoing commonly used procedures. This research may include physiological and behavioural studies.
- The implications of considering cumulative severity for future project licence applications and implications of retrospective reporting under Directive 2010/63/EU.
- Ethical considerations of cumulative severity.

The Animal Procedures Committee (APC) is an advisory non-departmental public body. Its role is to advise the Home Secretary on matters concerned with the Animals (Scientific Procedures) Act (ASPA) 1986. This relates to any experimental or scientific procedures applied to a protected animal that may have the effect of causing that animal pain, suffering, distress or lasting harm.

The APC has, over recent years, increasingly considered the lifetime experience of animals used in scientific procedures when it considers project licence applications referred to it by the Home Office (see Annex A for a description of project licences referred to the APC and Annex B for the guidance given to applicants referred to the APC; see section 8.8). This has been particularly pertinent in the case of licences involving non-human primates in neuroscience research where the animals may undergo a number of procedures over an extended period of time.

Therefore, the APC has started this Review, in conjunction with the Animals Scientific Procedures Inspectorate (ASPI, now ASRUI), to help it to assess the impact of multiple procedures administered over a period of time and the cumulative severity experienced by the animals in such procedures. Although the use of non-human primates in neuroscience research will be considered in this Review it is likely that the conclusions will have implications for assessing cumulative severity in other areas of research.

The APC notes that the timing of this Review is additionally relevant as there is an emphasis on lifetime experiences of animals together with the requirement for retrospective reporting in the new EU Directive (2010/63/EU; Annex VIII). The APC recognizes that further work is in progress by an Expert Working Group (EWG); see report of the Second Meeting of the EWG on Retrospective Severity Assessment, 3–4 May 2012. Directive 2010/63/EU requires that the assignment of the severity category shall take into account any intervention or manipulation of an animal within a defined procedure and that it shall be based on the most severe effects likely to be experienced by an individual animal after applying all refinement techniques.

Directive also highlights the need to consider the lifetime experience of animals in making decisions and points out that “procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting moderate pain, suffering or distress as well as procedures that are likely to cause severe impairment of the well-being or general condition of the animals shall be classified as Severe”.

3. Background

3.1 Non-human primate research in the UK: Weatherall and Bateson reports

The total number of non-human primates used in research worldwide is estimated at between 100,000 and 200,000 with 65 per cent involving Old World monkeys, such as macaques (Carlsson *et al*., 2004). Most (up to 85%) are used in regulatory toxicology. In addition, the most common research areas for which non-human primates are used are infectious, including HIV/AIDS (26%), neuroscience (19%), biochemistry (12%) and pharmacology/physiology (11%). The use of non-human primates in research in the UK has been comprehensively reviewed by Weatherall *et al*. (2006) and by Bateson *et al.* (2011).

Both reports established that there was a strong scientific case for research in primates benefits and listed a significant number of major contributions in biomedicine that could only have come from primate research. Both reports also agreed that primates should only be used in research programmes where there is a particular (that is case-by-case) justification and where it is judged that the likely benefits to society outweigh the likely harms inflicted on the animals that are used.

While this principle applies to the use of all animals used in research, there is a particular societal concern and uncertainty over the acceptability of using primates in research, principally because of their evolutionary proximity to human beings. This proximity has led to the view that primates may have a greater capacity for suffering than other animals because of their more developed cognitive abilities (Summerhoff, 1990).

There are concerns in general about the effects of long-distance transport, housing and care in the laboratory, which may be amenable to improvement to reduce the harms and thereby reduce concerns. It is therefore particularly necessary when examining the justification for the use of primates in research to consider the ethical issues in addition to the practical ones.

The central goal of the Weatherall report (2006) was to examine the scientific case for the use of primates in research, considering both research aimed at treatments for disease and fundamental research. The report accepted an overall case for well monitored and meticulously regulated non-human primate research, provided that it is of high quality, but also stressed the need for considerations of both scientific and welfare issues in the preparation of a cost–benefit assessment for each research proposal. The report called for work towards the refinement of research methods involving non-human primates, especially in the behavioural neurosciences and found evidence to show that, in academia, it is neuroscience research that generates the most concern about welfare. One of the recommendations was that retrospective reporting on the severity of procedures, as recommended by the Laboratory Animal Science Association (LASA) and the Animal Procedures Committee (APC) (LASA / APC, 2005), should be introduced as soon as possible.

The objective of the Bateson Review (2011) was to address one of the recommendations of the Weatherall report to undertake a thorough, retrospective

review of the quality, outputs and impacts of ten years of publicly funded UK research using non-human primates. The review encompassed all non-human primate research funded by the Biotechnology and Biological Sciences Research Council (BBSRC), the Medical Research Council (MRC), the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and Wellcome Trust and begun within the period from January 1997 to December 2006. Approximately two-thirds of the research grants reviewed by the Bateson Review Panel were in the field of neuroscience.

A bibliometric analysis of published papers arising from the research was undertaken. This supported the conclusion of the Panel that generally the research was of high or outstanding quality that was highly cited. Of the 31 neuroscience studies, around one-half were assessed as having a high welfare impact on the animals as judged by current (2011) standards. Most of these studies were also assessed as being of high scientific value. A few were also assessed as delivering, or having the potential to deliver, significant benefit. One has resulted in patents being filed, in new surgical treatments being established and in the development of new medical treatments.

In most cases, however, little direct evidence was available of actual medical benefit in the form of changes in clinical practice or new treatments. The identification and tracing of medical benefit derived from specific research projects was difficult in most cases, although this was in part because of the short time that had elapsed between the commissioning of the research and the Review. Overall, the Panel agreed that in many cases the use of non-human primates was justifiable, even in the context of current understanding of animal welfare and advances in knowledge that might now render some work on living animals unnecessary. The Panel recommended a more structured approach to knowledge and technology transfer. It was concerned about the small proportion of research programmes from which no clear scientific, medical or social benefit had emerged.

3.2 Ethics

The ethical issues posed by the use of animals in research have been extensively reviewed (Bateson *et al.,* 2011; Carlsson *et al.,* 2004; Dolan, 1999; Ferdowsian and Beck 2011; LASA / APC, 2005; Nuffield Council on Bioethics, 2005; Prescott 2010 and Regan 2004) but there has been less discussion about the ethical dilemmas posed by the accumulation of suffering over the time-course of a long project.

Ethics is concerned with doctrines concerning what it is to live 'rightly' or 'correctly'. 'Correctly' here has the meaning of a model of individual or communal life "*worthy of emulation"* by others *('Habermas'* Outhwaite 2009). All use of animals for human benefit creates a dilemma. The question whether the use of animals for human benefit is an ethical issue at all is hotly disputed. Those who think that the human use of animals is *not* an ethical issue tend to perceive animals as akin to *things*, though as naturally delicate and sensitive things that should be treated with care, just as one should treat delicate and sensitive equipment. Thus they believe that the use of animals for human benefit *does not* need special justification. People who think that the human use of animals *is* an ethical issue tend to perceive animals as more akin to *humans*: as creatures with a life of their own. Such people would regard the deliberate interference by humans with an animal's life for their own ends (for food, in research, as beasts of burden, as pets, or to kill them as vermin) as quite unlike the use for their own ends of technical equipment or machines. Hence they would insist that the human use of animals *does* need special justification (Diamond, 1991, 1996).

The Report by the Nuffield Council on Bioethics (2005) recognized that the debate around animal rights and the use of animals in research was polarized. The Nuffield Working Party could not arrive at a consensus as to *"whether one of the morally relevant features was a master property, nor whether a consequentialist, a deontological or a hybrid approach was the most appropriate framework for deciding whether or not a specific, or any, type of research was acceptable".* The Working Party was unable to agree on a single ethical stance. Instead, it presented an outline of four possible ethical positions that can be taken, which mark positions on a *continuum*.

- The *"anything goes"* view: If humans see value in research involving animals, then it requires no further ethical justification (no member of the Working Party took this position).
- The *"on balance justification"* view: In accepting research involving animals humans act with full moral justification, while accepting that every reasonable step must be taken to reduce the costs that fall on animals.
- The *"moral dilemma"* view: Most forms of research involving animals pose moral dilemmas. However humans decide to act, they act wrongly, either by neglecting human health and welfare or by harming animals.
- The *"abolitionist"* view: There is no moral justification for any harmful research on sentient animals that is not to the animals' benefit. Humans experiment on animals not because it is right but because they can.'

Whatever the ethical stance, it usually incorporates a sense of regret about animal use and hence a desire to minimize animal suffering. This is clearly embodied in codes of humane research such as the 3Rs (Russell and Burch, 1959; www.nc3rs.org.uk/3Rs The 3Rs are a widely accepted ethical framework for conducting animal experiments humanely:

- replacement – use of non-animal methods;
- refinement – methods that improve animal welfare;
- reduction – methods that reduce the number of animals used.

The ethical justification for carrying out a scientific procedure involving potential animal suffering will be a balance between the harm to the animals and the benefit to society from the knowledge gained from the work. Such 'harm–benefit' analysis is key to the ethical evaluation process (see European Directive 2010/63/EU; National Research Council, 2011). Any likely harm must be proportionate to the potential benefit of the research. The least number of animals should be used. However, the concept and practical implementation of the harm–benefit analysis is still perceived to be rather nebulous, see the American Association for Laboratory Animal Science (AALAS) – Federation of European Laboratory Animal Science Associations (FELASA) Working Group on harm–benefit analysis of animal studies, www.felasa.eu/working-groups.

Non-human primates are chosen for areas of research where it is essential to have similar anatomical, physiological and / or behavioural features to humans. However, it is precisely because of this phylogenetic proximity to humans that non-human primates are given special protection because they do show a highly developed capacity for experiencing pain, distress and anxiety and have complex behavioural and psychological needs. Higher levels of sentience are generally assumed to suggest greater capacity for suffering. Some would consider it contradictory that a species could be similar enough to humans for experimental data to be useful, yet different enough for any suffering to be morally acceptable. As a consequence, various regulatory frameworks have been recommended for the use of non-human primates in research (Smith and Boyd, 1991,Table 5.4).

Given that such checks and balances are embedded in the UK regulatory framework, the members of the Weatherall Working Party were able to agree that *"the continued use of non-human primates in research was morally required, so long as such research is directed towards significant human benefit and there are no plausibly more effective ways of pursuing such research. The alternative is to permit continued suffering to very large numbers of humans which might be alleviated or indeed removed by a careful, well monitored and meticulously regulated programme of animal research, including research with non-human primates."* Weatherall *et al.* (2006) acknowledged the blue sky nature of some non-human primate research and the issues surrounding uncertainty of the results of an experiment. If the outcome of an experiment was known for certain in advance, there would be no point in conducting the experiment. It might even be argued that such an experiment would simply be duplication and hence potentially unethical.

Bateson *et al.*'s (2011) decision model provides a very helpful overall concept of the three competing domains:

- animal suffering;
- importance of research; and
- likelihood of benefit.

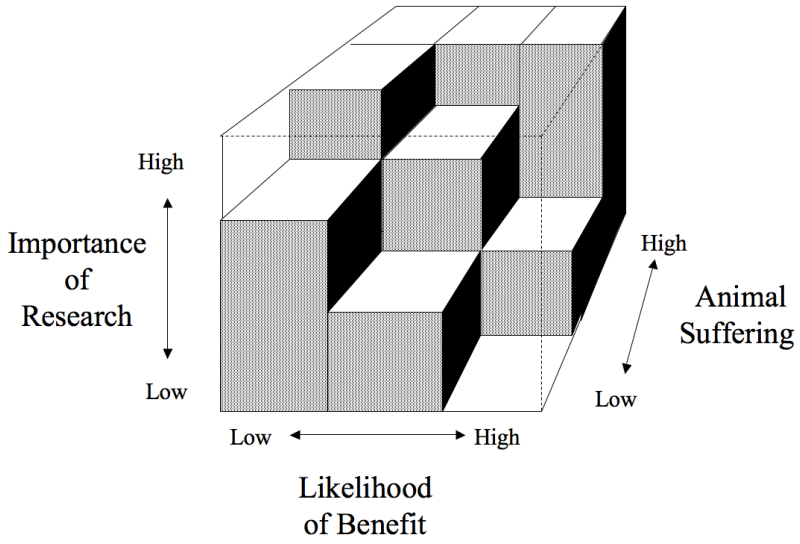


Figure 1. A cube for deciding whether a research project should proceed (clear space) or it should not (solid space) [redrawn from Bateson, 1986].

In this model three independent assessments are made:

- the total amount of suffering that the animals are likely to endure in the course of the project;
- the overall scientific importance of the project; and
- the likelihood of medical or social benefit.

The most obvious case for proceeding is when the amount of suffering is negligible, the quality of the research is high and the benefit is certain. At the other extreme, the clearest case where research should not be done is when the suffering is likely to be great, while the quality of the work and the benefit are uncertain.

There are some ethical issues that are particularly relevant to the use of non-human primates in neuroscience research:

- quality of life of purpose-bred animals;
- possession and duration of a life;
- premature killing; and
- the conflict between using a small number of animals long term or many animals short term.

For the purpose of this Review, quality of life has been defined as meaning the welfare of the individual during a period of a few days or longer.

Quality of life of a non-human primate bred specifically for research.

It is generally accepted that, when primates have to be used, they should be purpose-bred and not wild-caught. Purpose-bred animals have had no experience of life in the wild. How should the quality of their lives be judged, provided that they have been reared and maintained in an 'ethologically appropriate physical and social environment'? If careful monitoring confirms that an animal shows no distress or abnormal behaviour as the result of weaning, breeding, transport, housing, husbandry and care, handling, restraint, procedures

and any adverse effects of the procedures, is that life any less valuable than one spent in the competitive world of the natural habitat for their species?

The notions of possession and duration of a life (Harris, 1985).

There is a view that, if it is acceptable for animals to suffer in the name of scientific progress, then not only the level but also the *acceptable duration* of such suffering needs to be agreed on a consistent basis between research institutions and EU Member States. This belief reflects an implicit assumption that the longer the project, the greater the accumulation of harm. In other words, euthanasia (a good death) is better than life under these circumstances. Many neuroscience experiments require that the animal is eventually killed in order to study the brain in detail, but facilities do exist for retired non-human primates.

The Nuffield report (Nuffield Council on Bioethics, 2005) explored this issue of whether it is wrong to prematurely end an animal's life. It pointed out that, in the case of sociable animals such as primates, there were implications for other members of the group of losing a group member. They explored the issue of whether life itself is of value:

> *"It may seem that if we think that killing is wrong, then we must be committed to the view that life itself is valuable. However, this need not be the case. Some philosophers have argued that life, as such, has no value, as distinct from the experiences that happen within life. Given this view, it is entirely reasonable to treat pain, suffering and other harms within a life with great moral seriousness without attributing a similar level of concern to death. For it can be the case that there are animals that have no sense of themselves as existing in time, although they may have highly developed capacities of sensory experience. In such cases it could be argued that to the animals concerned it matters less whether they exist but more how their moment-to-moment existence is characterised. This line of thought raises the question of why we treat human life with special consideration and, in particular, why we experiment on animals precisely to find ways of prolonging the lives both of humans and animals. One possible answer, although not necessarily endorsed here, draws on two earlier points. First, most humans, and perhaps some other animals, exhibit self-consciousness and an ability to anticipate, reflect upon and fear their own death. Hence, the prospect of death usually has a significant secondary effect on the quality of lived experience. Secondly, humans, and perhaps some other animals, care about each other in the sense that the death of others is often considered a tragedy. Hence, death has special significance for highly social beings. It could therefore be argued that preserving the lives of humans and of relevant other animals should take precedence, with less regard being given to those animals that either lack self-consciousness or do not live in social groups. A simpler response is to revert to an argument implied above according to which some higher cognitive capacity generates a right to life; most humans and those animals that closely share similar features in this respect have such a right, while other animals do not. Many attempts have been made to provide a philosophical foundation for this view, although none commands wide agreement."*

The Joint Working Group on Refinement (Jennings and Prescott, 2009) explored the factors that might be explored when deciding how long to keep an animal alive which included:

- health of the animal and contingent suffering
- husbandry, care system and social environment
- regular review (say at 6 months) of the continued suitability of the animal and the continued optimised generation of scientific data.

The conflict between using a small number of animals long term or many animals short term.

It is a basic tenet of the use of any species of animals in research that the minimum number of animals should be used that is consistent with the scientific objectives based on expert statistical advice. However, should an individual be asked to suffer a lot on behalf of both man and its peers? Bateson *et al.* (2011) argued that this conflict may be overcome by the application of the principle that use of a larger number of animals may be tolerated if the welfare costs to the animals are lower. The overall index of suffering that can be fed into the decision cube shown in Figure 1 (above) is shown in Figure 2.

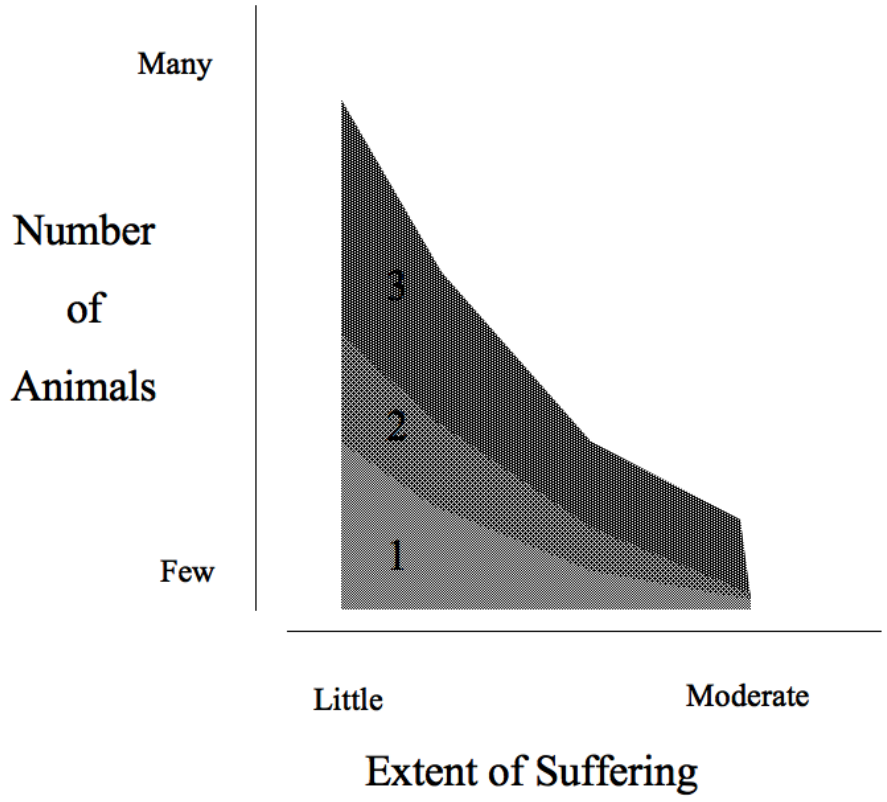


Figure 2. The number of animals used in a research programme set against the suffering that each animal is likely to endure in a proposed programme, from Bateson and Bateson unpublished, with permission.

The overall index of suffering is obtained by multiplying the index of individual suffering. The lines on the graph connect points of equal overall suffering and are agreed through consensus. The overall amount of suffering is given by the numbers that refer to the severity bands used in Figure 1 where Low = 1, Moderate = 2 and Severe = 3.

3.3 General concept of cumulative severity

The concept of cumulative severity and its effect on progression of a phenomenon is well established in nature in general and in medicine in particular. For example, sophisticated models have been developed to assess the risk of flooding with fluctuations in glacier mass, and forest fires with drought (Canadian Forest Fire Danger Rating System; Stocks *et al.,* 1989).

Repetitive insults may summate to have a major impact on health. Repetitive concussive blows to the head, none of which were sufficient to cause a severe head injury at the time, may result many years later in *Dementia Pugilistica* (Corsellis 1989; Thornton et al 2008). Repeated stressful events in humans may contribute to major depressive disorder (Eysenk *et al.*, 2006; and Young *et al.,* 2010)**.** Stressors during development can result in primates being more vulnerable or more resilient to later experiences (Parker and Maestripieri, 2011). Such events need not be major life events. Daily stressors are minor events that arise out of day-to-day living that have the potential to affect physical and psychological well-being (Almeida, 2005). Their cumulative effects may have deleterious consequences for long-term health and well-being (Lazarus, 1999; and Zautra, 2003). Some individuals are more stress-resilient than others, which may in part have a genetic basis. An investigation of this question in relation to the use of non-human primates in neuroscience research is the subject of an NC3Rs-funded research project (*see below*).

Various scales have been developed clinically to categorize the impact of multiple insults on outcome. Critical illness may be documented using a variety of scales including the APGAR score for the newborn (Apgar 1953), Glasgow Coma Scale (Teasdale and Jennett 1974) and the Acute Physiology and Chronic Health Evaluation (APACHE) score (Knaus et al 1991). The outcome following major trauma may be predicted using the Injury Severity Score (ISS; Baker et al 1974), which is an anatomically based consensus-derived global severity scoring system that classifies each injury in every body region according to its relative severity on a six-point ordinal scale.

These indices refer to snapshots that can be used for trend analysis but not in a way that is comparable to cumulative severity over the years of a non-human primate being used in a neuroscience experiment. Quality of life may be captured, tracked and quantified through both generic scales such as the SF-36 and condition-specific scales such as the EORTC-QLQC30 for cancer patients. Retinopathy of prematurity (ROP) is a common disorder of premature infants characterized by abnormal retinal vascular proliferation. While most cases regress with resumption of normal retinal development, ROP may progress to severe retinal vascular proliferation and subsequent visual compromise with or without retinal detachment. Cumulative neonatal illness severity, as measured with daily Scores for Neonatal Acute Physiology (SNAP) for the first 28 days of life, was an independent risk factor for progression from moderate to severe ROP (Richardson et al 1993, Zupancic et al 2007, Hagadorn et al 2007).

Of particular relevance to this Review is the growing clinical interest in the concept of comorbidity (de Groot et al 2003; Valderas et al 2009; Huntley et al 2012). Measures such as the Cumulative Illness Rating Scale for Geriatrics include counts of diseases with differential weighting (Linn et al 1968; Miller et al 1992)...

However, in terms of relevance to this Review, all these examples are characterized not only by clearly defined insults but also by well-defined, quantifiable outcome measures: forest fires; dementia; progressive retinopathy; depression; and death are unequivocal events. One challenge with animal experimentation is the paucity of such outcome measures. Without such measures, it will be difficult to define whether there is accumulation ('stacking up') of adverse events or whether sufficient time has been allowed to elapse for recovery between events.

3.4 Concepts of cumulative suffering, severity and lifetime experience as applied to animal experimentation, including neuroscience research.

Animals experience both positive (reward, satisfaction) and negative (pain, stress) well-being. *Welfare* may be defined as the state of the individual as regards its attempts to cope with its environment. Hence it depends on the individual animal's needs in relation to physical, nutritional, social and other behavioural factors and, in the case of captive animals, on the people who care for them or supervise such work. The ability of an individual to cope with its environment relates to its welfare, which can be good or poor (Broom, 1986; 2001; and 2008; and Broom and Johnson, 2000). In assessing welfare, it is important to examine the animal's physiological and psychological well-being in relation to its cognitive capacity and its lifetime experience.

Suffering describes the negative aspects of well-being and may be defined as: 'A negative emotional state that derives from adverse physical, physiological and psychological circumstances'.

It is usually assumed that there will be cumulative effects of disturbing procedures (for example, Bateson, 1991). The negative effect considered in this discussion is poor welfare. The magnitude of good or poor welfare can take account of both positive and negative effects. The likelihood of increasing the magnitude of poor welfare depends on the nature and intensity of each procedure and the interval between procedures. Exposure to repeated experiences can lead to a decrease in response (habituation) or to an increase (sensitization). For example, if there are several handling experiences, the first may have negative effects at the time but subsequent experiences may have no negative effect on welfare or even a positive effect as social bonds to the handler develop. On the other hand, subsequent responses could be potentiated by repeated negative experiences so that the cumulative effect is more than the sum of the individual effects (Broom and Johnson, 2000, pp 36–42; and Broom and Fraser, 2007, pp 28–29). While some repeated procedures may lead to a magnitude of poor welfare hardly greater than a single procedure, others could lead to a significant increase in poor welfare. Adverse events, particularly in early life, may lead to abnormal behaviour or may improve resilience with complementary changes in brain structure (see below).

Terminology

It would be helpful to have a term that was readily understood by professionals and lay public alike and that summarized the cumulative welfare impact of multiple procedures and lifetime experience of the animal. The term should reflect the cumulative suffering consequent on the direct effects of the procedures and the contingent suffering as a result of transport, housing, and environment, offset by any positive and ameliorating factors.

The term 'cumulative severity' has achieved some prominence amongst professionals, although its exact provenance is unclear. The terms of reference for this Review (see Section 2) refers to the concept of cumulative severity but it is important to emphasize that this term is not used in the EU Directive 2010/63 – reference is made simply to *"cumulative suffering within a procedure"* (Annex VIII).

Cumulative severity may be defined as: 'The sum of all the events and effects that impact, adversely, positively and by way of amelioration, on the welfare of an animal over its lifetime'.

A more comprehensive definition of cumulative severity might include 'a qualitative assessment that takes into account and summates (in the mathematical sense) the quantity and intensity of positive and negative welfare impacts (including expected consequences of techniques and husbandry), predictable complications (and retrospectively non-associated consequences), while also taking into account habituation, potentiation and sensitization, with a temporal element considering recovery between events and memory of them / their consequences'.

These definitions of cumulative severity include, unlike the term suffering, both adverse and 'favourable' components. However, this Review has found that the lay public has immense difficulty with the word 'severity' being used to describe anything other than adverse effects.

> *"I have never felt* severely *happy"* to quote one external referee.

In practice the members of the Review also found the term cumulative severity confusing. Cumulative severity of suffering is straightforward but excludes the possibility of positive experiences. Broom (2008) has argued that the term cumulative severity might best be replaced by *"magnitude of poor welfare after multiple events/procedures"*. This can include the net balance of negative effects and some positive effects. More concise terms include 'cumulative welfare impact' and 'cumulative experience'.

The Review commends the use of the term cumulative experience as it is the simplest, least confusing and most inclusive of the terms discussed.

Cumulative experience may be defined as 'the sum of all the events and effects, including their quantity, intensity, duration, recovery between and memory thereof, that impact, adversely, positively and by way of amelioration on the welfare of an animal over its lifetime'.

The Review's intention has been to use this term in a balanced way, not biased in favour of either negative or positive events.

<u>Assessment and 'weighting' of harm and benefit</u>

The problem comes with quantification of the summation of the interaction between positive and negative aspects of welfare in the overall assessment of the impact of a condition or treatment on an individual. Clearly, a very brief period of a certain degree of good or poor welfare is not the same as a prolonged period. However, a simple multiplicative function of maximum intensity and duration is not sufficient to capture the 'magnitude of good or poor welfare'. If there is a net intensity of good or poor welfare and this is plotted against its duration, one way of summarizing the overall assessment of welfare and so the magnitude of the good or poor welfare is the area under the curve produced. This concept was first presented by Broom (2001), and was repeated and refined in Broom (2008; and 2011, p 131).

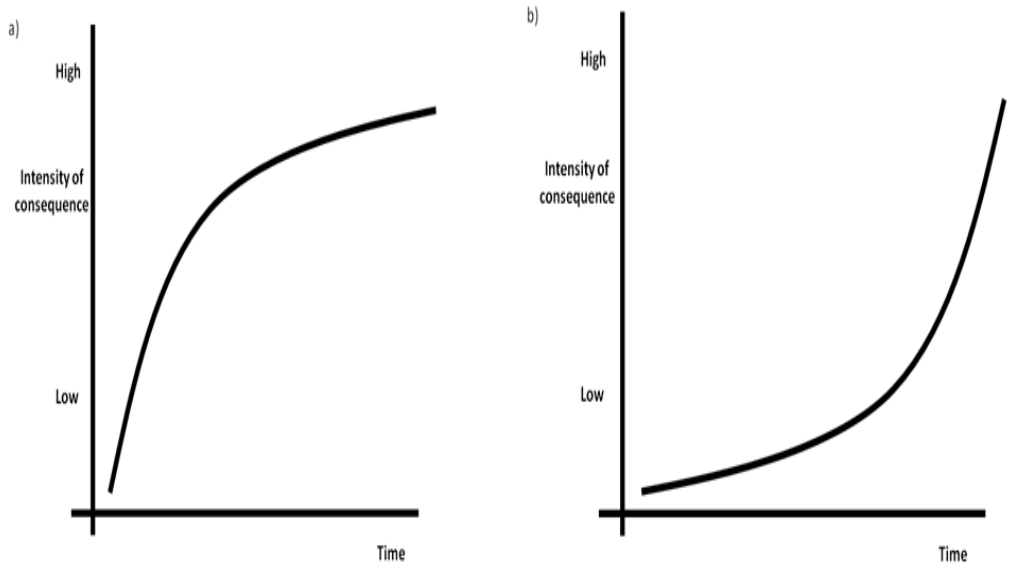


Figure 3. Broom's concept (modified after Broom 2001).

The net intensity of good or poor welfare is plotted against its duration in two examples here. The total magnitude of good or poor welfare, the area under the curve, is greater in (a) than in (b).

Cost (harm)–benefit analysis

Bateson's 'Decision Model' (1986) and the report of the Institute of Medical Ethics (Smith and Boyd 1991) have laid the groundwork for a systematic approach to the assessment of the likely cost (harm) to animals used in research. Various schemes have been developed to describe and hopefully quantify the various components that may contribute to suffering and harm (*ibid.,* chapter 7 pp 144–146.) Such schemes incorporate assessments of the welfare impact of breeding, transport, housing, husbandry and care, handling, restraint, the standards of the laboratory facilities, the skill and motivation of those handling the animal procedures and the adverse effects of the procedures. The full lifetime experience of the animals must be carefully assessed and given due weighting.

Clinical signs are commonly used to determine the degree to which an animal's physiology and mental state have deviated from normal and then use the magnitude of these perturbations for an assessment of severity (Morton, 2000). The regular collection of the relevant data requires the use of score sheets (LASA, 1990; Morton, 2000; Smith and Boyd, 1991; Smith *et al* 2006). In order to provide a practical, objective and robust overview of an individual animal's welfare, it would be ideal, but not essential, to combine a range of assessments into one usable entity. The problem is how? How should judgements about each of the relatively separate dimensions listed in schemes such as those provided by

Smith and Boyd (1991) be put together in order to arrive at an overall assessment of cost and benefit? Smith and Boyd (1991) have provided some useful case studies.

Smith et al (2006) presented score sheets to provide a battery of observable and objective measures across multiple dimensions: potential life-threatening signs, potential signs of clinical issues, atypical behaviours and laboratory assessments. They described two cases in detail in which behavioural change and laboratory assessment provided early indication of a potentially serious clinical issue in the frit case and the need for environmental enrichment in the second case. In both cases, remediable action was taken. In these cases, the use of serial observations recorded on score sheets allowed a decision to be made without overall quantification of severity. However, are there cases where overall quantification across multiple dimensions would detect subclinical cumulative severity even earlier?

Honess and Wolfensohn (2010) have produced an index of cumulative impact on welfare. Although this index does not provide any absolute measures of severity, it allows for comparison over a period of time to monitor the progress of individual animals and to monitor improvements, or deterioration, in welfare state. With further development, the authors suggest that this index could be modified and used as a tool to demonstrate the presence or absence of adaptation of an animal to a combination of procedures and environmental challenges. However, the extended welfare assessment grid has not yet been widely accepted as valid because, at present, its metrics cannot be compared with any other means of assessment. Recently (2012), the Health Protection Agency has funded work to render the extended welfare assessment grid commensurable.

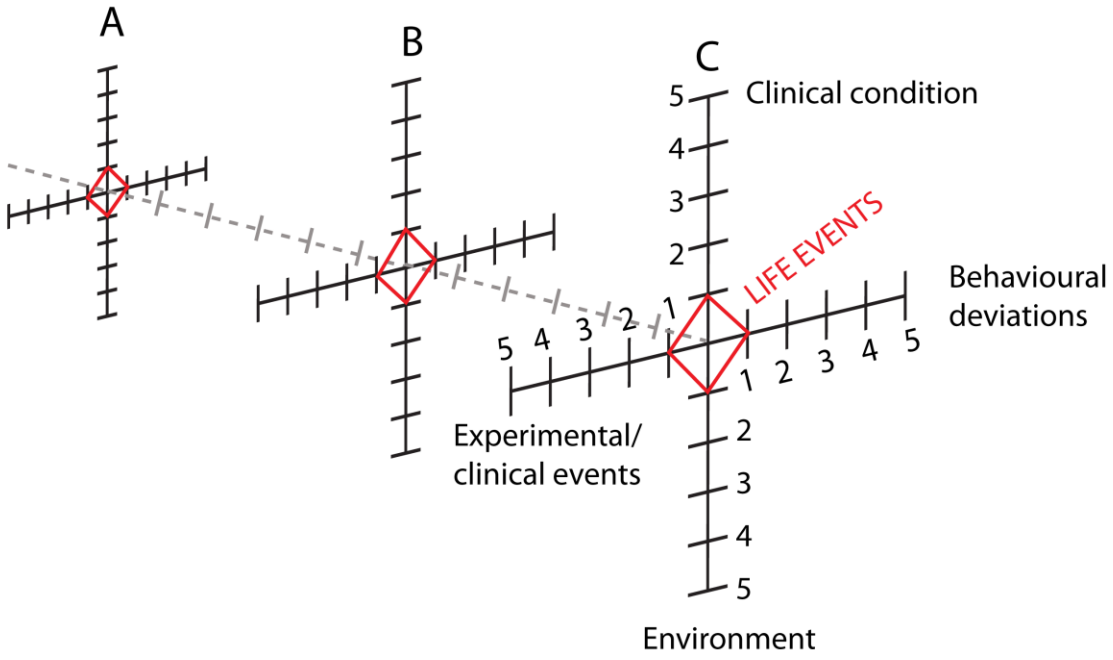


Figure 4. Extended welfare assessment grid, from Honess and Wolfensohn (2010).

Much of the work to date on quantification of cumulative severity is theoretical. Hawkins and colleagues have reviewed the practical issues involved in developing and using welfare assessment tools (2011). The problem has been the paucity of data to populate and test the

validity of these scoring systems. The quantification and tracking of alopecia is an exemplar of what needs to be achieved across a much wider range of indicators (Honess et al 2005).

Cumulative experience and neuroscience research

Neuroscience is an area of experimental research that, for reasons outlined above, poses special challenges and considerations on the use of animals, the implementation of the 3Rs and humane endpoints. The distinguishing characteristic of experimental behavioural neuroscience is that the nervous system of the animal on the study is either altered (permanently or temporarily) or measured in conscious animals.

Monkeys commonly remain on study for extended periods of time, sometimes up to several years. This may be due to the complexity of the tasks / pathways and / or advancements in technology (for example, imaging technology), which allow animals to be used in long-term longitudinal studies as their own controls (for example, collection of behavioural data before and after a brain lesion). Thus, many animals increase in 'scientific value' the longer they remain on study. When combined with the financial value of monkeys and the need for training the animals for study procedures, long-term holding is often viewed as necessary. The Animals (Scientific Procedures) Act (ASPA) 1986 states that, in animals for any procedure, the degree of severity imposed shall be the minimum consistent with the attainment of the objectives of the procedure. In practice this aspect can be challenging to implement in this area of research. It has been argued that further scientific investigation (for example, in electrophysiology) can be conducted in another animal. In this case two of the 3Rs are in conflict: refinement (that is less harm to one individual) against reduction (number of animals). The regulatory view is that refinement trumps reduction (Review of cost-benefit assessment in the use of animals in research, 2003). In the case of non-human primate neuroscience, arriving at a team consensus among the investigators, the named animal care and welfare officer (NACWO), the veterinary surgeon and the Home Office inspector (HOI) when implementing the most ethically acceptable solution for implementation of humane endpoints can be challenging.

Implementing humane endpoints may have implications beyond those of the individual animal in question. The number of animals enrolled in such studies is commonly relatively low, so terminating an animal before completing the entire protocol could invalidate the study as a whole. However, in practice this does not happen. If an animal is considered for removal from study due to the effects of cumulative severity, the decision is complicated by the ethical conflict of subjecting another animal to procedures such as surgery and training, as well as the practical implications of sourcing a suitable animal (size, age, socially compatible, transport stress) against the cumulative cost experienced by each individual animal.

Implementation of humane endpoints could be further complicated as some data analysis (such as single-cell recording, imaging data) is either done retrospectively or the entire data needs to be collected before analysis can be performed, potentially delaying an assessment of the impact of euthanizing an animal on the overall study population.

The longer an animal remains on study, the more likely it is to suffer from study-unrelated harms resulting in an increase of potential adverse effects (for example, during anaesthesia). Stressors during development can result in primates being more vulnerable or more resilient to later experiences (Parker and Maestripieri, 2011). Much work has shown that stress is 'bad for the brain' (Sapolosky 1996). Early life stresses may cause changes in parts of the brain such as the corpus callosum and hippocampus and anxious behaviour in nonhuman primates (Jackowski, Perare et al 2011; Spinelli et al 2009). However, in contrast, coping

with stress may induce hippocampal neurogenesis and buffer the deleterious effects of stress in adult monkeys (Lyons, Buckmaster et al 2010). Engaging nonhuman primates in complex tasks for fluid reward may improve their psychological well being.

With respect to depression, there are close parallels in primates and in other non-human species (Irwin, 2001) and the condition may be detected using careful behavioural observation and assessment. Some chimpanzees have been shown to develop mental health problems after many years in the laboratory setting. Social stress may increase the risk of atherosclerosis in monkeys (Kaplan et al 1983). One cause of effects that might be considered to be depression in laboratory animals is a housing condition that does not meet the needs of the animals. Repeated aversive procedures might lead to a depressed condition but efforts are usually made to detect and prevent these. It is necessary to use welfare indicators to evaluate effects and considerable knowledge of the adaptability of the individual animal is needed to predict accurately what the cumulative effects will be. This information is only now being developed.

The Working document on a severity assessment framework (2012) suggested that cumulative severity should be considered as the combination of direct suffering (the procedural details on the licence) including consideration of any clinical conditions that affect the animal (which may not be due to the procedure being carried out, for example, a bite wound) and contingent suffering (for example, housing, husbandry, transport).

As discussed above, an animal may suffer as a result of a procedure that is carried out at a certain point in time but then it may undergo another procedure or repeated procedures over and over again. It cannot be assumed that all procedures or events that cause a degree of suffering simply 'stack up' for evermore, with the result that severity would continually increase within a procedure. This would inflate the numbers of actual severity assessments deemed to be 'severe' or even 'above severe and requiring an application to invoke the safeguard clause'. Such an approach would obviously undermine the whole system of severity classification, quite possibly to the detriment of animal welfare if it meant that severity limits became meaningless and people took an 'anything goes because it's all severe' attitude.

The actual level of severity may not be higher for each individual episode – the animal may be become habituated to the procedures (such as handling or restraint). Alternatively, the animal may become hyper-sensitized as it learns to anticipate what is about to occur and becomes fearful, thus increasing the level of severity with each event. It is, however, impossible simply to add up the subsequent events to assess cumulative severity and cumulative experience. The simple quantity of techniques that cause pain, suffering, distress or lasting harm that are applied to an individual animal, their duration and an assessment of any increasing or decreasing impact must be taken into account. Predictions of cumulative severity and experience should also consider whether one significant event (for example, early maternal separation or a painful experience when a neonate) can affect how pain or distress are experienced in the long term.

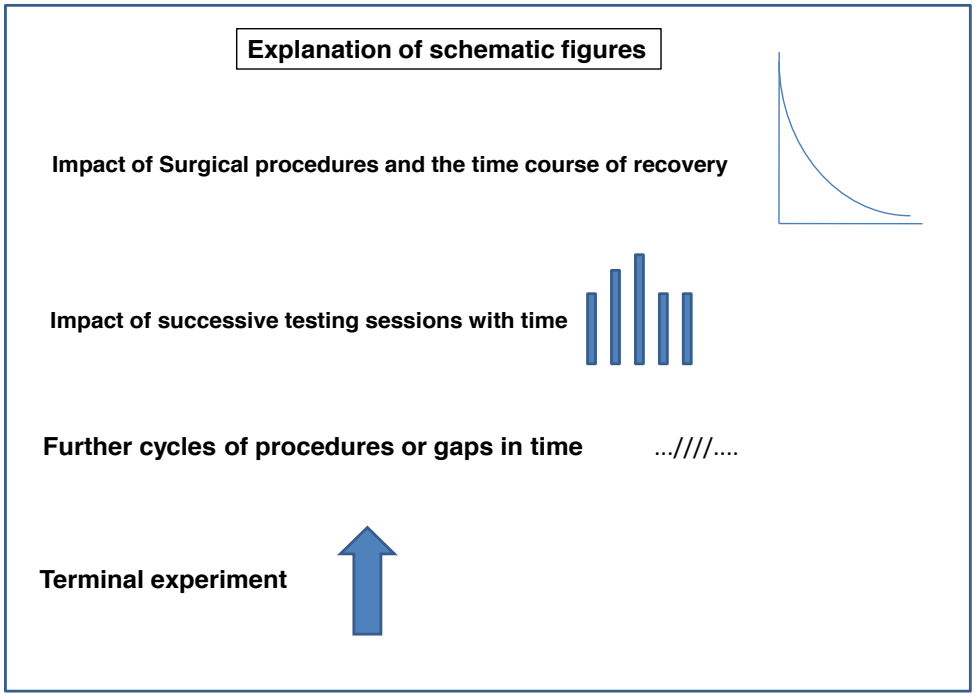
In order to clarify these issues, Figure 5 provides an overview of the differences in neuroscience experiments between:

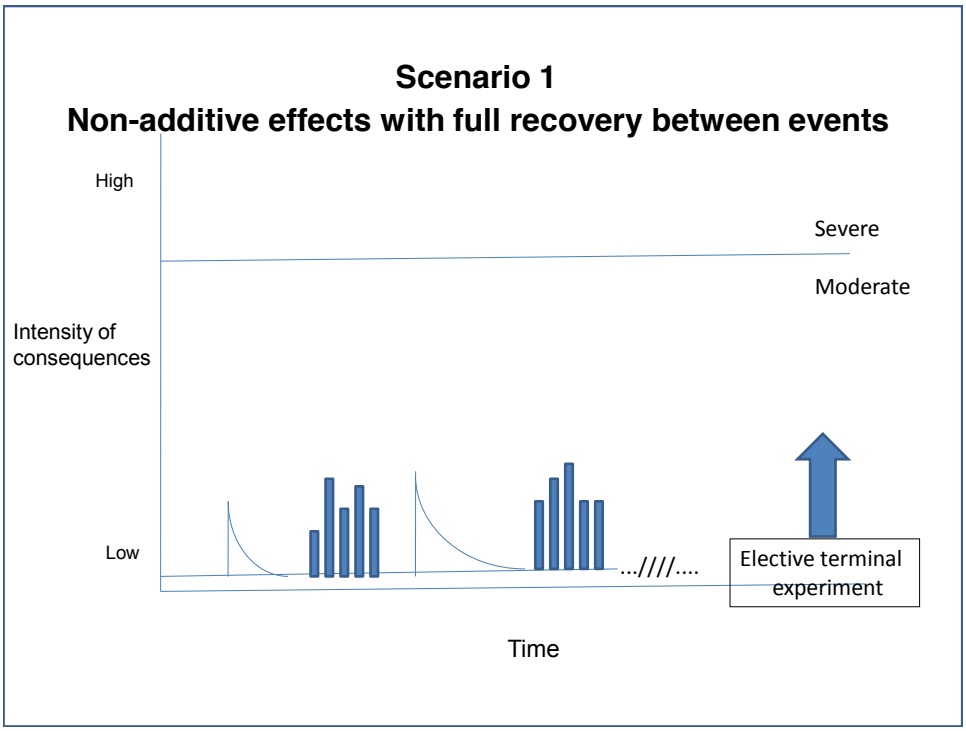
- scenario 1: Non-additive effects where there is complete recovery between events;
- scenario 2: Non-additive effects with habituation between events;
- scenario 3: Additive effects with incomplete recovery between procedures ('stacking up');

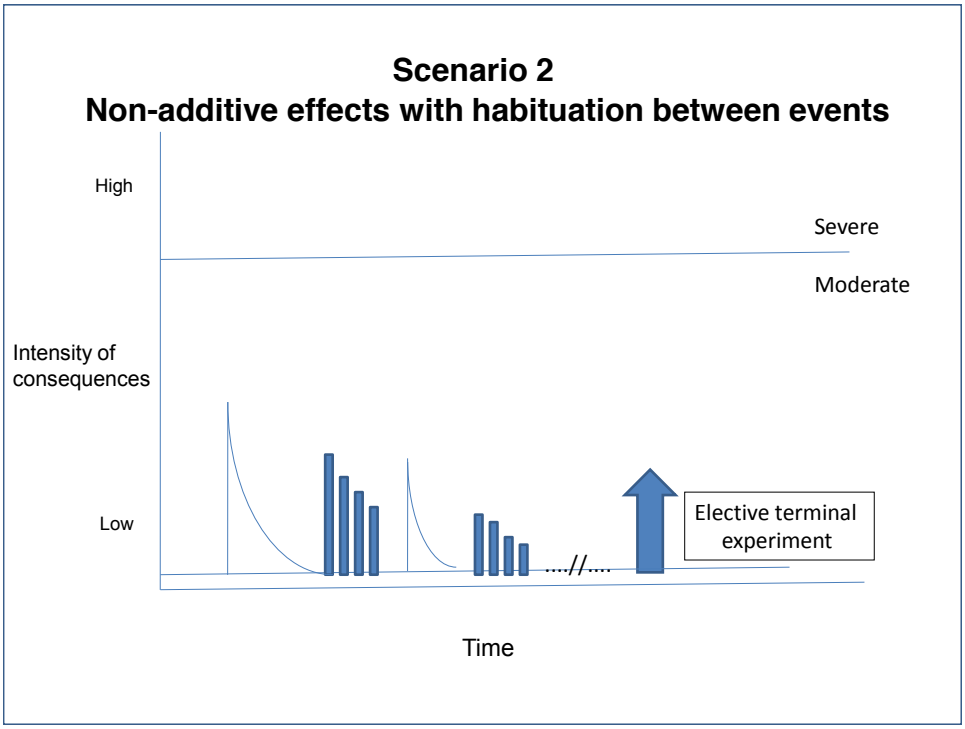
- scenario 4: Additive effects of procedures that are compounded by potentiation of suffering by earlier procedures.

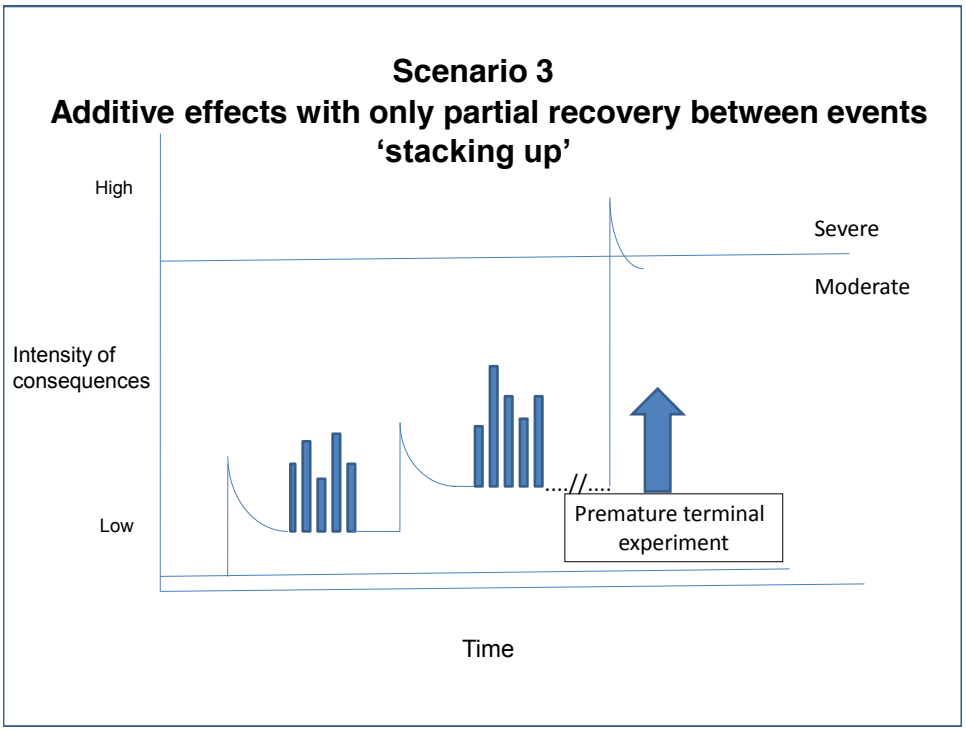
These schematic figures represent different scenarios of procedural effects on the cumulative experience of monkeys in an experimental setting. Non-additive effects do not have a lasting impact on cumulative severity (scenario 1) and, in some cases, their impact with repeated use may diminish due to habituation (scenario 2). Where fear or distress but not pain is caused, familiarity and training may reduce the effect, so that the third time an animal is exposed to a stressful situation it suffers less than the first time. The effect of stress, or lack of stress, may impact on the experience of pain and thus affect the 'background' intensity. When events occur long in the past, in humans, memories fade and impacts may reduce.

Procedures with residual effects may lead to the 'stacking up' of adverse impacts (scenario 3) that may be further potentiated by suffering from earlier events (scenario 4) and cross over a designated severity threshold equivalent to that defined in Annex VIII of 2010/63/EU [Appendix 8.9].




Scenario 1
Non-additive effects with full recovery between events
High
Severe
Moderate
Intensity of consequences
Low
..////....
Elective terminal experiment
Time


Scenario 2
Non-additive effects with habituation between events
High
Severe
Moderate
Intensity of consequences
Low
....//....
Elective terminal experiment
Time



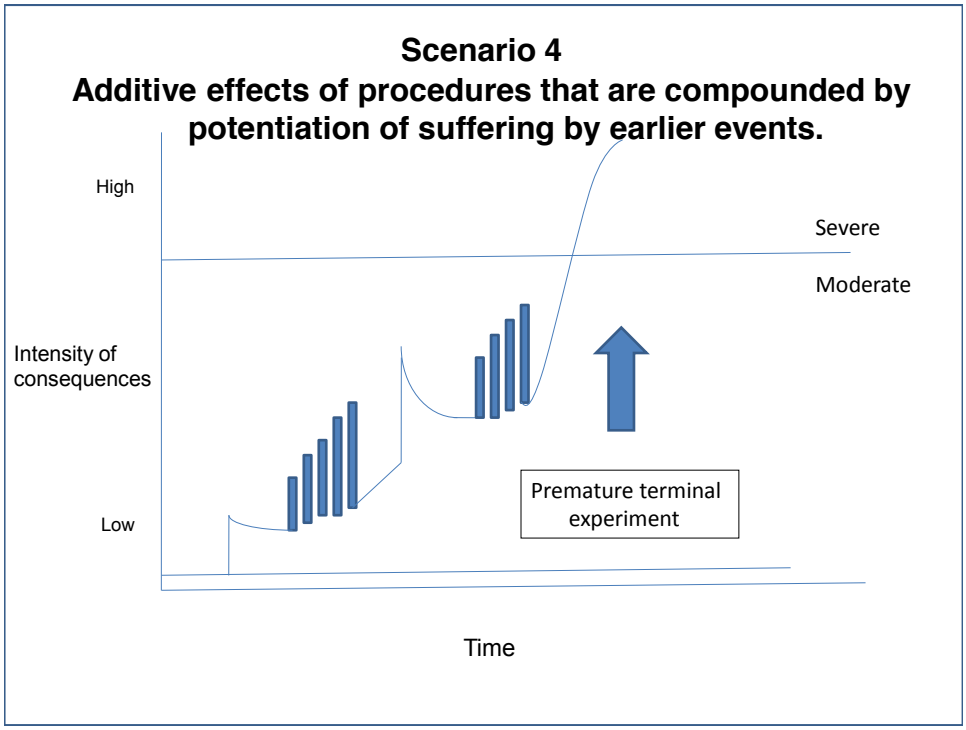


Figure 5. Schematic representations of four scenarios of procedural effects on the cumulative experience of monkeys in an experimental setting.

These scenarios create a framework for understanding the nature of cumulative severity / experience. Key questions can then be asked for each step in the lifetime experience of the individual non-human primate. Given the evidence of different personality types (e.g. bold and shy), it is reasonable to assume that different individual non-human primates will cope with adverse events in different ways and must be assessed individually.

- Was the non-human primate in good condition on arrival and suitable for a neuroscience study?
- Is the environment optimal?
- Has the non-human primate made a full recovery from a specific procedure or event? Which physiological and behavioural indicators might be used to make a judgement?
- Have all the appropriate ameliorating methods been used?
- What adverse effects (non-procedural, generic and intended effects of the procedure and complications) have occurred?
- What has been their impact on behaviour and performance?
- Is recovery from an adverse event realistic? In the context of lesions which may have a permanent effect on the brain, how far has the individual recovered and what are the criteria for deciding whether/ and when it is justifiable for him/her to continue?
- Has the threshold between 'Moderate' and 'Severe', as defined in Annex VIII of 2010/63/EU, been crossed?

3.5 Past and present systems of classification of severity of regulated procedures in the UK

In the United Kingdom, the use of animals in scientific research is regulated by the Animals (Scientific Procedures) Act (ASPA) 1986 using a three-part licensing process. A detailed account of the process is provided in Appendix 7.8.

Prior to 2013, ASPA (1986) referred to severity in two contexts.

- First, work required authorization if it was above a threshold of severity (the level at which a procedure *has the potentia*l to cause pain, suffering, distress or lasting harm). The 'threshold', the level of severity above which a technique or procedure is considered to be a 'regulated procedure', that is, requiring licence authorization, is defined as 'the skilled insertion of a hypodermic needle' or equivalent.

- Second, all procedures had to be carried out using the least severe method commensurate with achieving the scientific objectives of the study. Severity classification was used largely prospectively during the assessment and granting of project licences, but was also used to ensure that actual severity did not exceed the pre-authorized limit.

The UK used a two-tier severity classification system:

- the first tier being a severity limit applied to individual protocols;

- the second tier being the severity band of the entire project, which may contain numerous different protocols.

Both tiers used the same four-category severity classification system.

Severity limits

Unclassified: Procedures carried out entirely under general anaesthesia, from which the animal does not recover consciousness (includes the preparation of decerebrated animals).

Mild severity limit: Procedures causing only slight or transient minor adverse effects. Multiple minor procedures can lead to cumulative severity necessitating a higher severity limit.

Moderate severity limit: Effectively all procedures falling between 'Mild' and 'Substantial'. This includes most surgical procedures provided that pain and suffering are controlled by adequate anaesthesia and analgesia.

Substantial severity limit: Procedures that have the potential to cause a major departure from an animal's normal state of health or well-being; examples given are major surgical procedures, procedures involving some infectious agents, and those expected to cause significant morbidity or mortality.

The severity limit applied to a protocol was intended to reflect the worst-case scenario. Even if only one animal was expected to experience adverse effects of a higher level, then the entire protocol would carry the higher severity limit. Thus, many, or even all, of the animals used on a Substantial severity limit protocol might in reality have only experienced Moderate, or even Mild, severity.

Severity band (overall severity)

The second tier of severity classification was used to classify the 'average' severity of entire project licences. This 'severity band' was for information purposes only and had no role in

limiting the severity. It was based on the overall level of cumulative suffering to be experienced by each animal. It aimed to reflect both the typical animal experience and also the likely actual animal experience, as opposed to the worst case scenario as in a severity limit.

Severity bands in particular, but also severity limits of protocols, were used to trigger particular types of scrutiny by the UK competent authority. For example, Substantial limit protocols involving non-human primates, or Substantial severity band projects involving interference with the nervous system were automatically referred to the Animal Procedures Committee (APC) for additional assessment prior to project licences being granted.

In general, the focus of severity assessment under ASPA (1986) was on the intensity of the pain or suffering, and less so on the duration or aggregate effects of procedures.

Changes under Directive 2010/63/EU

Directive 2010/63/EU on the protection of animals used for scientific purposes came into effect on 22 September 2010 and has been transposed into the national laws of all EU Member States on 1 January 2013.

Article 8 and Paragraph 17 of the new Directive 2010/63 allow for research in non-human primates, provided that *"the purpose of the procedure cannot be achieved by the use of species other than non-human primates"*. Of relevance to this Review, research may be undertaken for a number of reasons, as defined in **Article 5**, including *"basic research"* (Article 5 (a)), and *"translational or applied research",* namely *"the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants"* (Article 5 b(i)).

The Directive makes new provisions for the classification of the severity of scientific procedures, as set out in Annex VIII of the Directive. The severity of procedures must be assessed prospectively and shall be determined by the degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure. The assignment of the severity category shall be based on the most severe effects likely to be experienced by an individual after applying all appropriate refinement techniques. Retrospectively, the severity actually experienced by each individual animal must be assessed using the same criteria at the end of the procedure.

Projects authorized in the UK from January 2013 onwards will have a severity classification applied to protocols in place of the current severity limit, and project licence bands will no longer be used.

Annex VIII is based on the system used in the UK and utilizes the same four-category classification system, but provides greater guidance on how severity is to be classified.

The severity classifications under Directive 2010/63/EU Annex VIII are as follows.

- Non-recovery: Procedures that are performed entirely under general anaesthesia from which the animal does not recover consciousness.

- Mild: Procedures on animals that are likely to cause short-term mild pain, suffering or distress, and procedures with no significant impairment of the well-being or general condition of the animals.

• Moderate: Procedures on animals that are likely to cause short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress, and procedures that are likely to cause moderate impairment of the well-being or general condition of the animals.

• Severe: Procedures on animals that are likely to cause severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress, and procedures that are likely to cause severe impairment of the well-being or general condition of the animals.

• Below threshold: Article 3 defines this as practices not likely to cause pain, suffering, distress or lasting harm. The threshold is defined as equivalent to, or higher than, that caused by the introduction of a needle according to good veterinary practice.

• Upper Limit: Article 15 (2) requires Member States to ensure that an upper limit is complied with, namely that a procedure shall not be performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated

In particular, Directive 2010/63/EU places greater emphasis on the duration of the experiment and any suffering that results from it, and of the cumulative nature of any suffering in the assessment of the severity of procedures than has been the case in the UK up to January 2013. Examples of Mild, Moderate and Severe procedures are given, but there is little guidance on how duration or any cumulative severity should be considered. Long-term neuroscience procedures involving monkeys are not included among the examples given (see also Report of the Suffering and Severity Working Group 2009).

The assignment of procedures to one of the categories of severity as part of retrospective reporting of actual severity should be based on prospectively recorded day-to-day observations of clinical signs.

Although the term 'cumulative severity' was not explicitly used in Directive 2010/63/EU, the concepts of cumulative suffering and lifetime experience are encompassed within it. Specifically the Directive requires *"…taking into account the lifetime experience of the individual animal."* (Paragraph 25), *"should have a personal history file from birth …"* (Paragraph 33) *"…to enhance the lifetime experience of the animals…"* (Paragraph 31), *"... to reduce the duration and intensity of suffering to the minimum possible…"* (Article 13.3b). It requires that the severity category assigned to the experiment shall take into account:

- the degree of pain, suffering, distress and lasting harm, and its intensity;
- the duration, frequency and multiplicity of techniques;
- the cumulative suffering within a procedure; and
- the application of all appropriate refinement techniques including methods to reduce pain, suffering and distress (Annex VIII).

3.6 The National Centre for the Replacement, Refinement and Reduction of Animals in Research

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is a scientific organization that leads the discovery, development and promotion of new ways to replace, refine and reduce the use of animals in research and testing (the 3Rs). Primarily supported by the Government, the Centre is the UK's major funder of 3Rs research. In addition to funding research, its small team of scientists works in collaboration across many sectors and scientific disciplines to advance the 3Rs.

The NC3Rs provides expertise to the major UK public funders of animal research to help to embed the 3Rs in their policies and practice. In addition to scientific peer review organized by the funders, since 2004 grant, fellowship and studentship applications to the Medical Research Council (MRC), Biotechnology and Biological Sciences Research Council (BBSRC), Wellcome Trust and Royal Society involving the use of non-human primates (or dogs, cats and equines) are also reviewed by the Centre. Its role is:

- to help to identify and address any animal welfare concerns;
- to help to ensure that any 3Rs opportunities are exploited; and
- to monitor the implementation of guidelines produced jointly with the research funders to support contemporary good practice.

As part of the Review, the NC3Rs will assess the proposed animal use and care, including the arrangements for transport, housing, anaesthesia, surgery, restraint and food / fluid control. The applicant is invited to respond to any issues raised by the NC3Rs. Its advice is then taken into account by the funding bodies during decisions about which applications to fund and when drafting the terms and conditions of grant awards. Successful applicants may have to change their practices to promote welfare and the 3Rs. Examples of impacts arising from the NC3Rs peer review are given in the Centre's evaluation framework report (www.nc3rs.org.uk/evaluationreport).

To date (May 2013) the NC3Rs has committed £1.4 million in grants for research to refine techniques used in non-human primate neuroscience – including for head restraint, electromyography recording, and training for behavioural tasks – and to develop better methods of assessing pain and distress in non-human primates (Prescott, 2012). This includes an interdisciplinary collaboration led by Professor Mellissa Bateson of Newcastle University to investigate cumulative severity in macaques used in neuroscience protocols. The aim is to develop novel psychobiomarkers of cumulative stress based on knowledge of the biological changes seen in humans with major depressive disorder. The two main approaches involve:

- measuring via qPRC changes in the length of the telomeres of chromosomes in white blood cells; and
- measuring via magnetic resonance imaging (MRI) changes in the activity and size of brain areas involved in processing of emotional information.

Awards to Dr Matthew Leach of Newcastle University and Dr Sarah-Jane Vick of the University of Stirling will be used to quantify behavioural and facial correlates of pain in macaques. Facial expressions are the gold standard for pain assessment in non-verbal humans and these projects will use a standardized research tool for the measurement of facial expression in macaques (MaqFACS) to validate facial markers of pain and develop a visual rating scale that would be both practical and sensitive enough to guide clinical

decisions about provision of pain relief. Dr Vick will also examine the influence of laboratory routines upon the behaviour of individual animals experiencing aversive events as part of ongoing research. Standardized and objective measures of pain and distress would provide an insight into the relative severity of different procedures, either in isolation or across the lifespan of a research animal, underpinning judgements about cumulative severity.

The NC3Rs also leads an extensive programme of work designed to improve the welfare of laboratory non-human primates, including annual workshops, scientific papers and online resources (www.nc3rs.org.uk/primatewelfare). Since 2010 the NC3Rs has initiated visits, attended by BBSRC and the Wellcome Trust, to UK non-human primate laboratories to monitor compliance with the NC3Rs guidelines.

4 Methodology

The Review adopted a broad approach to clarify the issues, identify any published evidence and create a questionnaire and database in order to provide an evidence-based set of recommendations.

4.1 Consultation process

The Review consulted widely, via meetings, interviews and correspondence with stakeholders, including researchers in the field, research funders and welfare organizations. Others who wished to submit written evidence to the Review were invited to do so. A detailed survey of non-human primate researchers ('User Survey') and of individual monkeys used in academic research ('Subject Survey') was carried out by means of a web-based questionnaire sent to the researchers (see Section 4.5).

It is important to note that there was a diversity of opinion amongst contributors and amongst members of the Working Party on the use of non-human primates in neuroscience research but, in the interest of promoting animal welfare, there was wide engagement with the consultation process. Throughout the consultation process, there was an emphasis on mutual trust, education and development through listening and constructive exchange of information (O'Neill 2002; Concordat on Openness on Animal Research, 2012). Where possible we have reflected the range of opinions, many of which continue to be debated.

4.1.1 Stakeholders meeting (including overview of biomarkers)

The Animal Procedures Committee (APC) / Animals Scientific Procedures Inspectorate (ASPI) organized a joint workshop at the Wellcome Trust in June 2011 to discuss the current state of knowledge with respect to the severity classification of procedures involving non-human primates. A particular goal was to establish what information was needed in order to assess cumulative suffering, severity and experience. The participants included research workers currently involved in using non-human primates in research, veterinarians, animal care technicians, Home Office inspectors and representatives from the major funding agencies. A key output of the workshop was to help to define the questions that should be asked in a proposed call for evidence, to elicit the right sort of information and identify areas where further research is required.

The main part of the workshop consisted of active participation by delegates in four breakout groups (delegates had the opportunity to input into all four):

- effects of food and fluid control and interpretation of body weight data;
- behavioural effects and interpretation of performance on tasks;
- physiological effects and markers; and
- novel ways of measuring animal welfare.

Many rich discussions resulted and have been incorporated into the relevant sections of this Review.

It was agreed that current methods of assessing welfare in non-human primates, and hence methods for assessing progressive changes in their welfare, were limited.

With regard to food and fluid control (Prescott et al 2010, 2012b), it was emphasized that the animal is not deprived of food and water, but is in an environment where the timing of the ingestion of food and fluids is controlled (see section 5.8 for details). A number of points were made that this is not necessarily placing suffering on the animal as it would not be unusual for a monkey to have irregular drinking and feeding behaviours in its home habitat (relative to, for example, safety from predators). There was a need for systematic studies of renal function and histology at post-mortem examination. Studies of blood osmolality had confirmed that monkeys regulated their day-to-day hydration levels within a narrow range of values (Yamada et al., 2010). Body weight was regarded as a crude measure of welfare.

Most participants agreed that the observation of the behaviour of individual animals was the most widely used means of assessment. However, there was very limited information as to which behavioural changes indicated suffering. Major deviations from an individual's normal behaviour, for example, development of stereotypic behaviours, were considered to be indicators of poor welfare. However it was considered that there was no reliable information available as to how these and other behavioural changes could be used to quantify suffering (or good welfare).

It was considered, for the most part, that animals do not perform well if suffering, therefore the most indicative measures/indicators would be:

- behavioural symptoms;
- less willing to be handled and a noticeable lack of willingness to do daily tasks;
- significant weight loss;
- choosing isolation rather than mixing with other animals.

However, many participants iterated that to get to this stage would be unlikely as there are many safeguards and monitoring procedures in place to pick up on such manifestations.

Similar reservations were expressed concerning physiological measures and other biomarkers of good or poor welfare. There was (2012) no experience or evidence of the usefulness of non-invasive or passive monitoring of physiological parameters, for example, heart rate, blood pressure, blood glucose. Lack of reference data was identified as an obstacle to use of biochemical markers, but if serial samples are available from individual animals this may not be an issue. There is very little useful necropsy data to indicate, retrospectively, the health of animals over the course of a study – this could easily be remedied in the future.

It was recognized that numerous factors could result in changes to measures such as cortisol and other endocrine factors, or to biochemical, cardiovascular or other physiological variables. It was suggested that, although these measures could be useful, they could change in similar ways in response to both positive and negative events. For example, the laboratory environment eliminates the many stressors that may be encountered by wild-living primates (e.g., food scarcity, predation, aggressive interactions, and parasitism; Novak et al 2013). However, the laboratory environment introduces other stressors. Anxiety may be

associated with raised levels of hair cortisol (Dettmer et al 2012). In contrast, experimentally induced alterations in early experience (e.g., nursery rearing), and the spontaneous development of behavioural pathology (e.g., self-injurious behaviour) may be associated with blunting of the stress response system (Novak et al 2013).

Changes in biomarkers that could indicate ill health could be used as part of an assessment of welfare, but there seemed to be no published data on the use of biomarkers to measure cumulative experience. Several biomarkers (for example, cortisol), when assessed in isolation, give little indication of whether an event has positive or negative effects. However, it was suggested that this could be done by measurement of the animals' affective (emotional) state. Methods of assessing emotion in animals have been developed in other species, although this area of research is still at an early stage (Mendl, 2010). An assessment of 'suffering' should use animal-centred measures that attempt to quantify, in an objective way, the effects of events on the animal's emotional state. Virtually all assessments use anthropomorphic criteria or arbitrary judgements made by human observers. These assessments can be used to develop numerical scores and this approach has been used later in this report. It is important to appreciate that this is currently the only practical approach that can be adopted. However, it must also be appreciated that such numerical scores lend a false sense of precision to the assessments made.

As discussed earlier in this report, many participants emphasized that an assessment of cumulative suffering was complex. It was generally accepted that there is currently a poor understanding of the nature of suffering in any animal species, and an even poorer understanding of the interactions between various events in an animal's lifetime experience. Although research in this area is urgently needed, participants also suggested that gathering and using currently available data would allow some assessments to be made. It was important to emphasize that these surrogate measures of suffering were, in many instances, highly subjective in their interpretation. With that proviso, assessing and documenting an animal's demeanour and behaviour, particularly in relation to its willingness to engage in the daily tasks associated with specific research projects, could be used to assess changes to its welfare. Similarly, base-line measures of physiological variables, and tracking of any changes throughout a study could be of value. A further point was made that attempts to add up these different changes to produce a metric of 'suffering' was, at present, scientifically seriously flawed.

An interesting and novel approach to the long-term impact of neuroscience research on monkeys is that funded by the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) recently (2012). It is intended to measure both changes in the length of the telomeres in the chromosomes in white blood cells and structural and functional changes in the brain of monkeys as biomarkers of stress based on human studies of depression and anxiety (www.nc3rs.org.uk/researchportfolio).

4.1.2 Animal protection organizations and other interested parties, including written submissions

The Review is very grateful for all the helpful and scholarly written responses received from Animal Defenders International (ADI) and the National Anti-Vivisection Society (NAVS), the British Union for the Abolition of Vivisection (BUAV), Four Paws, Humane Society International (HSI)and the Humane Society of the US (HSUS), People for the Ethical Treatment of Animals (PETA), Royal Society for the Prevention of Cruelty to Animals (RSPCA), the Universities Federation for Animals Welfare (UFAW), and Dr Andrew Knight of Animal Consultants International. Oral evidence was given by Ms Jessamy Korotoga (ADI/NAVS), Dr Katy Taylor and Dr Nick Palmer (BUAV), Dr Gilly Stoddart and Mr Alistair Currie (PETA), and Dr Elliott Lilley (RSPCA).

In addition to the animal protection groups, the Captive Care Working Party (CCWP) of the Primate Society of Great Britain (PSGB) and Professor John Gluck, University of New Mexico, also submitted written responses. Dr Paul Honess (CCWP, PSGB) and Professor Don Broom (University of Cambridge) gave oral evidence.

All were keen to see a focus on the benefits for the animals that should accrue from effective assessment of cumulative severity. In particular, constructive guidance on using any severity assessment to inform refinement, reduce suffering and improve welfare would be widely welcomed.

The majority of responses stressed the need for assessments of severity, including cumulative severity, to include all of the harms caused to the animals throughout their lifetimes, and not just the adverse effects of the scientific procedures involved. The RSPCA pointed out that it had been campaigning for this for some time. The RSPCA suggested some approaches to solutions, including deconstructing the animal's life experiences and recognizing the importance of considering whether the effect of one procedure will affect the response to the next. The RSPCA also outlined how harm / benefit judgements on the level of cumulative suffering and the justification for re-use may be skewed by the animal's scientific and economic value and the moral imperative to reduce primate numbers involved in research.

Respondents argued that including 'contingent suffering' arising from, for example, acquisition, transport, and housing in captivity, would lead to:

- a fairer cost–benefit assessment;
- greater attention given to refinement of all aspects of the lifetime experience; and
- improved transparency and public accountability.

Perhaps not surprisingly, some respondents called for greater clarity regarding interpretation of the terms 'cumulative suffering' and 'cumulative severity', so that there is a common understanding among all the relevant parties. Related to this, the RSPCA and PETA called for constructive guidance on how to use any future cumulative severity assessment scheme, with a process of feedback and refinement. The BUAV made suggestions as to how prospective assessment of cumulative severity could be integrated into the licensing, regulatory and statistical reporting systems.

Many recognized that assessing and classifying suffering over an animal's lifetime presents some practical problems. These included but were not limited to:

- the difficulty of predicting how the effect of one procedure will affect the animal's response to the next;

- variability between individual animals in their responses to procedures;

- the possibility that as prey species non-human primates have evolved to mask any pain they may be experiencing; and

- the complexity of summating different types of suffering.

These difficulties are compounded by the paucity of research into cumulative severity. In the words of the CCWP, PSGB: *"too little is known of the expression of accumulated experience in non-human primates, its residual effects, its welfare cost or its consequences, to be able to answer many of the questions that will arise from this review"*.

The UFAW took a more pragmatic approach, considering that *"all measures of animal welfare and decisions on severity are taken over some time period, so there is nothing intrinsically new about cumulative severity, other than the desire to explicitly include all direct and contingent harms that we may cause to the animals"*. It recognized, however, that the more one tries to include, the harder the judgements become; there is no mathematical way of integrating all positive and negative events in an animal's life, particularly when this is being done prospectively.

Most respondents recommended a multidisciplinary approach to the assessment of welfare and cumulative severity, involving both physiological and psychological aspects. Many made reference to the extended welfare assessment grid (EWAG) (Honess and Wolfensohn, 2010), and the Five Freedoms (http://www.fawc.org.uk/freedoms.htm) as possible frameworks for the assessment of cumulative severity. Professor Gluck mentioned cognitive bias and the possibility that cumulative severity may be increased in those monkeys that perceive their lives in a negative way without expectations that it will change substantially (negative cognitive bias).

Some groups listed criteria for assessing cumulative severity. For example, PETA in its focused response recommended use of well designed score sheets to record markers of welfare under the following categories (taken from the EWAG): clinical condition (physical wellbeing); behavioural deviations (psychological wellbeing); environmental conditions; and clinical/experimental events.

ADI and NAVS listed a variety of scientific, husbandry and other procedures, which can have a negative impact on the welfare of non-human primates used in research, providing many supporting references from the primatological literature. BUAV took a similar approach, citing literature reporting invasive studies with non-human primates, rodents and other animals; it also included case studies from two UK universities listing considerations that, in its view, ought to have been taken into account in the prospective assessment of severity. Dr Andrew Knight cited invasive studies using chimpanzees.

It was very helpful to have the general perspective provided by many of these detailed submissions. Unfortunately, much of the literature cited was not specific

to non-human primates in general and to the UK in particular. This is because the procedures are not necessary or allowed, the husbandry systems are different or the species is not used. For example, whilst it is the case that many breeding centres worldwide wean young macaques before the biologically normal age of around 10 to 12 months, which can affect the physiological and behavioural development of the animals and compromise their welfare in both the short and long terms, the majority of macaques used in UK universities are rhesus macaques supplied from a UK source, which now ordinarily weans at or beyond 12 months of age (Prescott *et al.*, 2012a).

During the written and oral evidence gathering it became clear that one valuable function of the Review would be to update all those interested in animal welfare on the contemporary standards applied to UK non-human primate neuroscience.

"Many primates live in small, barren cages." This is not the case in the UK, the Home Office and NC3Rs guidelines do not allow it.

"Animals with headcaps should be pair-housed." This is accepted practice in UK universities, although occasionally there have to be exceptions.

"fluid deprivation" Non-human primates on fluid control protocols are not deprived of fluid; rather their free access to fluid is scheduled in order to motivate them to work for small fluid rewards. Steps are taken to ensure that they receive sufficient daily fluid amounts, which are bounded by their normal *ad libitum* daily fluid intake at the top and the minimum amount necessary for physiological functioning at the bottom. A level somewhere in between these two bounds is mostly used and adjusted for each animal.

This Review, and the recent commitment to greater openness from the bioscience community, may help to avoid such misunderstandings in the future (O'Neill 2002; Concordat 2012).

The primatological studies cited by the HSI and HSUS were almost exclusively from US literature involving housing conditions that are not comparable with those in the UK. That said, some of the papers mentioned were of particular interest with regard to cumulative severity. For example, behavioural and psychopathologies in captive-bred primates can change in type and intensity with increasing time in captivity (Bellanca and Crockett, 2002; and Novak, 2003). The frequency of Mild procedures (for example, venepuncture, anaesthesia) and cage moves are correlated with increased behavioural pathology, as well as increased morbidity and mortality (Lutz *et al.*, 2003; Lutz *et al.,* 2007; Novak, 2003; and Vandeleest *et al.*, 2011).

HIS and HSUS suggested that the available data do not report a simple, additive dose-effect of cumulative exposures across the lifespan, but rather complex, non-additive risk profiles. In their view, a system that assumes that discrete signs of suffering arise immediately, directly and exclusively in the context of a particular procedure is inadequate for the assessment of cumulative severity. On the basis of the studies cited in their responses, increasing impacts should be anticipated and assessed. In this context, other respondents mentioned phenomena such as hyperalgesia and allodynia, although only in general terms and without specific examples from non-human primate research.

UFAW, in its response, made the point that whatever data are taken to inform decisions on welfare and severity, assessments of severity, whether cumulative

or otherwise, remain judgements and it follows, therefore, that there is merit in these judgements being taken by groups to reduce the effect of personal bias. UFAW drew attention to a non-peer reviewed article recently published in the newsletter of the Laboratory Animal Science Association (Wolfensohn and Andersen, 2012) reporting the results of surveys of attendees of professional meetings who are involved with the care and use of animals (although not necessarily non-human primates) in research, for example, named veterinary surgeons (NVSs), named animal care and welfare officers (NACWOs) Home Office liaison officers (HOLOs), Ethical Review Process (ERP) members, and others with no direct involvement in animal research. Interestingly, the surveys found that those asked scored cumulative severity as higher than would currently be assigned as a limit to the procedure by the Home Office. Unfortunately, details of information about non-human primate studies given at the meeting and the precise questions posed were not given in the article.

Ethical considerations centred around the similarity of non-human primates to humans and the potential for greater suffering in comparison with other laboratory animals on account of their complex social, behavioural and psychological needs, and the challenge of meeting these in the laboratory environment. Dr Andrew Knight made the point that animals have intrinsic value and the non-consensual termination of life at the end of the experiment raises serious moral issues, regardless of whether a humane method of euthanasia is used.

Professor Gluck and members of the CCWP, PSGB, who have direct experience of working with neuroscience non-human primates, questioned whether the apparent willingness of a monkey to separate from its cage mates, accept restraint, and work for fluid rewards for prolonged periods indicates that the work is without cost to the animal or that participation is enjoyable for the animal. Dr Honess was of the view that even in the absence of a demonstrable and quantifiable negative impact on animal welfare, any insult to the animal must warrant an entry in the ‘cost’ column of the ‘ethical ledger’.

Cumulative severity and re-use

A number of respondents, including BUAV, Four Paws and the CCWP, PSGB, were concerned that the high cost of purchase and maintenance of non-human primates could drive decisions to re-use the animals (thereby compounding the suffering of individual animals) rather than there being an indisputable scientific case for re-use.

There is an ethical decision to be made as to whether it is better to re-use one animal for longer, or, when the first use / experiment has been completed to kill it, to reduce the suffering of that one animal. Generally, the Home Office has usually advocated the use of more animals with a lower level of suffering, rather than to increase the suffering of the ‘few’.

There is never a scientific reason for re-use. One animal may be used in linked experiments, where the data from the first are required for the complete interpretation of the second and subsequent experiments. This is continuous use and is explained further in the Home Office document (http://tna.europarchive.org/20100413151426;

http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/publications/guidance/use-con-

animalsfdfd.html?view=Standard&pubID=606442). This is unavoidable, if the experiment is to be completed.

Re-use is avoidable, but may be the preferred option where the first use does not compromise scientifically the second use *and* the accumulated consequences for the animal to date and the proposed consequences of the second / subsequent uses (considered together as harms) are 'less than' the expected benefits of the uses, and do not exceed acceptable harms.

There have been strict controls over re-use (see Section 14, Animals (Scientific Procedures) Act (ASPA) 1986) such that an animal may only be re-used if:

- the Secretary of State has given permission in a project licence; and
- if an anaesthetic was given it was only for restraint; or
- if the second use is under terminal general anaesthesia only.

Changes after January 2013 retain the requirement for project licence authorization, but also require that a vet who has knowledge of the animal advises that an animal's general state of health and well-being has been fully restored. It relaxes the criteria on anaesthesia to be in line with the European Directive, so that consideration is more dependent on severity classifications of the first use and subsequent use(s). It prohibits re-use if the likely severity of the further procedure is 'Severe'. If the first use was classified retrospectively as Severe, then:

- the severity to that particular animal must be considered;
- there must be exceptional reasons why that animal should be re-used.

The consideration of severity will account for the life experience of that animal by allocation of severity retrospectively after each use, and it is expected that these restrictions will limit the harms of re-use to the individual animal to ethically acceptable levels.

4.2 Pilot literature review

The original intention was to undertake a systematic literature search. However, after discussions within the community and the major funding agencies, together with the insight gained from the extensive literature search conducted for the NC3Rs review of food and fluid control (Prescott *et al*., 2010), it was concluded that a large-scale literature review of cumulative severity should not be commissioned without a pilot feasibility study.

A pilot literature search was then undertaken based on Cochrane Principles. The PubMed database was searched with combinations of the following words / phrases: abnormal behaviour; adverse effects; cruelty; cumulative severity; deprivation; distress; electrophysiological recording; ethics; experimental; fluid control; food control; harm; husbandry; investigations; lifetime; macaques; microelectrode; motivation; neuroscience; non-human primates; pain; physiology; primates; psychology; reward; severity; stress; suffering; testing; training; welfare; and well-being. A ten-year publication date filter was added to the results and a limit set of 500 results per search. The abstract of any title that looked relevant was read and, if appropriate, the complete pdf was reviewed.

In summary, there were reports of the concept of, and difficulties surrounding, the quantification of cumulative severity but no relevant data-driven studies, apart from those of isolated components within the lifetime of a monkey.

Although not referred to as cumulative severity as such, there were some studies of factors that were predictive of increased risk of abnormal behaviour later in life such as the interaction between nursery rearing and single housing (Bellanca and Crockett 2002; Lutz et al 2003, 2007)..

The option of completing a full review was considered but, due to the paucity of the literature, it was not deemed to be time or cost efficient.

This view was reinforced by the extensive process of consultations and user submissions. At every step, respondents were encouraged to highlight the relevant literature.

4.3 Design of questionnaire and Cloud-based database: Iterative refinement

4.3.1 Background: Outline description of the experimental studies covered by the questionnaire and survey

All the studies covered by the questionnaire and survey were long-term investigations (longer than one year). Studies were mostly of two main types.

- *Neuroscience recording studies*: Techniques were used to record neural activity from the central nervous system while performing a trained behavioural task. The general objective of all these studies was to understand the brain mechanisms that underpin complex behaviour, including advanced cognitive function such as memory and decision-making. Studies of this type were carried out in macaques only. It is well established that the fundamental unit of all brain function is the single neuron, and these studies employed a variety of different recording techniques to record from single or multiple neurons. Some of these studies involved chronically implanted electrodes, and in others, electrodes were introduced in each daily recording session and removed at the end. In some but not all cases head restraint was required. In a few cases, additional monitoring of eye movements and muscle activity was carried out using other chronic implants.

- *Behavioural studies*: Both marmosets and macaques were used in these studies. The general objective here is to understand the contribution of specific brain areas to complex, cognitive behaviour. Focal brain lesions are made under general anaesthesia in trained animals and subsequently the short-term and long-term effects of these lesions on behaviour is documented and correlated with a variety of genetic, pharmacological and physiological variables.

Non-invasive magnetic resonance (MR) imaging was used extensively for both types of study.

4.3.2 Technical design and implementation

The cumulative severity retrospective questionnaire was conducted by direct user submissions online. Data submission was open between May and June 2012 (with a subsequent two-week extension) and preliminary analysis was used to inform the establishment visits where further clarification could take place.

Given the sensitive nature of submitted data, a secure Cloud-based database was established. All users were provided with a unique username and password, and web access permitted only via encrypted connection to the secure server. The database software was adapted from the ORION framework; this was developed by Obex Technologies and is used by the University of Cambridge for secure online processing of patient healthcare data. To preserve anonymity, user and establishment information was pseudonymized and identifiers were kept offline and only accessible by the APC secretariat.

Project licence holders, NVSs, NACWOs, certificate holders and other senior animal technical staff were invited to submit user and subject surveys on behalf of their institutions. Online access was also provided to animal welfare organizations in order to view the questionnaire. As the data submission period was restricted to six weeks, users were only permitted to work on one form draft at a time (a printable form was also made available to allow offline work) and editing of submitted information was disabled in order to streamline

the process. The website was reopened after the round of visits to research establishments to allow the submission of additional data.

It might be suggested that, as the Subject Survey was completed by users, there might have been under-reporting of adverse events. The visits to establishments confirmed that the NVS was either involved with the submissions or was able subsequently to identify monkeys that had posed welfare problems. The list of contingent effects was provided by the NVS independently of the users (see Section 5.4). Similarly, ASRUI was aware of monkeys where there was a history of not coping and premature withdrawal from study or euthanasia.

4.3.3 Questionnaire design and content

The questionnaire was designed to capture the events contributing to the lifetime experience of individual subjects and the overall views of users. Questionnaire content was agreed by the APC and ASRUI through a process of iterative refinement and prior testing by committee members. The initial versions of the questionnaire comprised a single document and relied on users to collate their aggregate establishment information. In order to ensure consistent analysis and permit a more detailed and quantitative approach to specific aspects of cumulative severity, a subject-specific questionnaire was developed to allow submission on a per-animal basis (including their unique establishment identifier) for central analysis by members of the committee. Qualitative aspects of a general user's experience and views were included in a separate user questionnaire. The majority of form fields were mandatory in order to ensure data completeness and explicitly to confirm important negatives (for example, complications). The scope of the subject questionnaire was restricted to rhesus macaques and marmosets used in neuroscience research. User questionnaires were also collected from other EU institutions carrying out similar research in order to inform of non-UK practice. However, only UK-based data were included in the quantitative analysis.

The subject questionnaire was divided into the following sections (see Appendix 7.5).

- General information, including subject species, key dates, and number of anaesthetics.

- Adverse effects of experimental procedures and husbandry (intended, generic and non-procedural events). Taking into account the retrospective submission of a large amount of complex data within a short timeframe, a semi-quantitative approach was chosen. Users could select whether an impact was observed for a particular procedure, and if so, were invited to submit further information in free text.

- Non-intended procedural complications, including the total incidence per animal and further free text if present, allowing detailed quantitative analysis.

- Cumulative severity, including a user assessment on whether each procedure (anaesthesia, surgery, restraint, food and fluid control, housing and husbandry, long-term implants and training) had an unchanging, diminishing or increasing cumulative effect with repeated use, and further opportunity to comment on procedure interaction. A 'N/A' option was also available if the animal was not exposed to the particular procedure or had not been in the procedure for long enough for any cumulative effects to have been measured.

  Definitions of unchanging, diminishing and increasing:

- unchanging – later procedures applied to an animal have the same welfare impact as preceding procedures of the same nature;
- diminishing – each procedure applied to an animal produces a less severe impact compared with preceding procedures of the same nature (decreasing cumulative severity, asymptotic severity, tolerance with repetition, habituation);
- increasing – each procedure applied to an animal produces a more severe impact compared with preceding procedures of the same nature (increasing cumulative severity due to, for example, hypersensitization).

- Additional information, including clinicopathological data, the need for euthanasia and user-estimated appropriate severity classification.

The user questionnaire sections included:

- general information including user country of work, experience and species used;
- supply and condition of animals on arrival, and general comments on non-procedural aspects of contingent suffering;
- methods used to record subject behaviour and signs of distress, with specific reference to individual procedures and husbandry practices;
- evolution of techniques over the past decade, with specific reference to individual procedures and husbandry practices;
- cumulative severity considerations relating to procedure frequency and motivator usage; and
- additional information, including views on severity classification, post-mortem practice and general comments.

4.3.4 Analysis of the questionnaire.

Information from both questionnaires enabled analysis and conclusions to be made in the following areas:

- dates of removal from natal groups and return dates;
- supply and condition on arrival;
- re-use;
- housing, husbandry and care;
- non-procedural life events;
- anaesthesia and pain control;
- surgery and maintenance of implants: Generic procedural, intended effects and complications;
- restraint and handling;
- food and fluid controls, and training;
- behaviour;
- refinement;
- post-mortem examination;
- lifetime experience;
- severity classification; and
- cumulative severity and suffering.

The initial analysis of the questionnaire was divided by topic, each of which was assessed independently by two members of the Working Party, including representatives from ASRUI. The two assessors then arrived at a consensus. The whole Working Party then reviewed these reports.

4.3.5 Submissions

User Survey

The total number of responses received was 27; 21 were from scientific investigators, 3 from veterinary surgeons, 1 NACWO, 1 animal technician, and 1 other. The majority (17) of the responders were based in the UK and the remaining 10 in the EU. The number of years' experience in non-human primate research for those responding was between 10 and 40 years, and involved the use of a median number of 38 animals (including marmosets; interquartile range 13 to 50).

Subject Survey

Data were returned for a total of 234 non-human primates: 152 macaques – of which 149 were rhesus (*M. mulatta*) and 3 cynomolgus (*M. fascicularis*) – and 82 common marmosets (*C. jacchus).* Responses came from five UK academic research institutions. 3 macaques were returned from 3 EU institutions. Data were returned for non-human primates for which procedures began from the year 2000 until the closing date of the Subject Survey (July 2012).

Comparison with the Home Office Annual Return of Procedures data indicated that more macaque monkeys had been started on procedures between 2002 and 2011 compared with the number of animals that were reported in the questionnaire to have started on procedures between those dates. All Annual Return subjects shown in Table 1 below had general anaesthesia with recovery, and were reported in the Return as being involved in basic research that required direct interference with the brain.

Table 1. Home Office statistical return for UK usage of macaques, 2002–2011.

	Year											
Institution	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Grand total**	**Submissions to CS website**
A	32	22	32	26	40	45	58	48	42	4	**349**	**101**
B	4	2	1	1	3	7	5	13	9	7	**52**	**27**
C						1		1	4		**6**	**0**
D	5	1		4			2	2	1	3	**18**	**12**
E			5		2	3	4		4		**18**	**9**
Non-UK												**3**
Total	**41**	**25**	**38**	**31**	**45**	**56**	**69**	**64**	**60**	**14**	**443**	**152**

The apparent discrepancy between the numbers returned in the Review's Subject Survey (152 macaques) and those reported in the Annual Return statistics (443) was addressed by direct enquiry to the institutions that took part, both at the visits to the establishments and, where necessary, by a second visit by the Chairman of the Review. It became clear that the apparent discrepancy arose for a number of reasons, including:

- animals used only in short-term procedures that were not relevant to cumulative severity (for example, a single minor procedure under anaesthesia simply for restraint, followed by a procedure under terminal anaesthesia);
- researchers who held the data had retired or moved to another institution prior to the questionnaire exercise and therefore comprehensive details were not available; and
- time constraints in filling in details of all animals used.

Additional data were supplied that greatly reduced the size of the discrepancy and further confirmed that any non-human primates omitted from the Review Survey were not selectively omitted due to them experiencing particularly Severe or Mild adverse effects. Importantly, the NVS at two institutions were able to reassure the Chairman that the great majority of the subjects with significant complications had been captured in the questionnaire. ASRUI provided an independent perspective (see Table 9).

It is clear that the data included in the Review Subject Survey, while not covering every animal used, are representative of the spectrum of animal experience and not overtly biased to either low or high severity procedures. This substantial database has provided the quantitative data required to address the terms of reference of the Review (see section 2).

5 Analysis of questionnaire and trends

5.1 Weaning dates and return dates

Introduction

The age at which young monkeys are permanently separated from their mothers, and the social environment in which they are subsequently reared, are important considerations in the provision of high quality animal models able to cope with the laboratory environment. There is substantial evidence in humans that growing up with adverse childhood experiences including social deprivation has a negative impact on brain development and increases the risk of health problems later in life (Dube et al, 2003; and Fox et al, 2010; Foresight Mental Capacity and Wellbeing Project 2008). The same phenomenon is seen in non-human primates. Permanently removing a still-sucking infant from the mother is an extremely distressing experience for both the mother and the infant. Monkeys separated prematurely from their mothers and nursery reared or socially deprived are more likely to develop anxious behaviour in response to stress, reduced exploratory behaviour, impaired cellular immune responses, illnesses in general, wounds, alopecia and higher mortality rates (Conti et al 2012; Corcoran et al 2012; Feng et al 2011; Lewis et al 2000; Prescott *et al*., 2012a; Reinhardt 2002,).

The natural (biologically normal) age of weaning is 10 to 14 months for macaque species and approximately 3 months for common marmosets. However, the young animals remain psychologically dependent on their parents for some time and continue to live in the natal group for many years or for their whole life. Commercial breeders of laboratory primates generally separate animals destined for research use from their mothers at or before the natural weaning age (so called 'early weaning'), which can have adverse welfare implications (Prescott *et al*., 2012a). Professional and regulatory bodies have set lower limits on the age at which laboratory primates can be removed from their mothers. Under the UK Home Office, this is currently (2013) eight months for macaques and marmosets; for the majority of the period covered by the Review, the guideline was six months.

The questionnaire asked for the dates of removal from the natal group, arrival at the facility, study commencement, retirement and death. Hence the age at (nutritional) weaning was generally not available whereas the age at permanent removal from the natal group was provided.

Macaques

The date of removal from their natal group related to 101 of this group on which data were submitted. It was expected that this information would correlate with weaning age. The age at removal from the natal group prior to commencing procedures varied greatly, and the range given exceeds the likely weaning range: some are possibly ex-breeding stock removed from their natal group at several years of age. All animals used on procedures commencing from 2000 onwards were approximately eight months of age or older at the time of removal from the natal group with a tendency in the latter phase of the review period to include more animals removed at between one and two years of age.

The age at the start of procedure varied between 18 months and over 9 years of age. The duration of procedures was typically between one and three years and ranged up to eight years for an animal of unknown age commencing in 1994.

Figure 6 displays the changes in age (days) at removal from the natal group (days) over time for macaques.

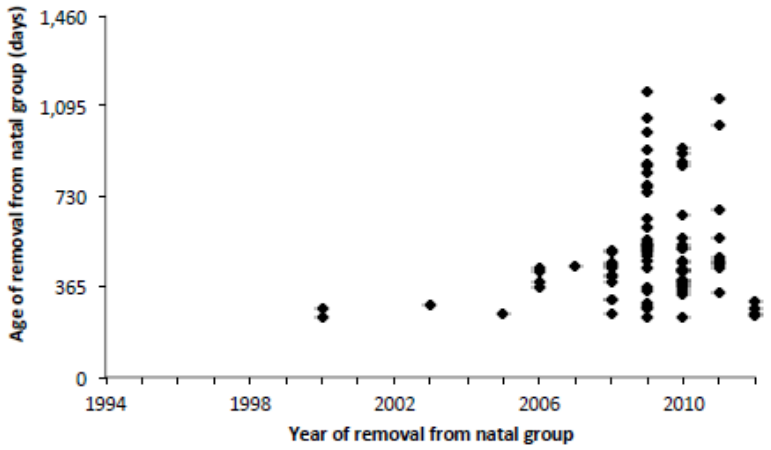


Figure 6. Changes in age (days) at removal from the natal group (days) over time for macaques.

Figure 7 displays the age (years) at the start of the procedure (years) over time.

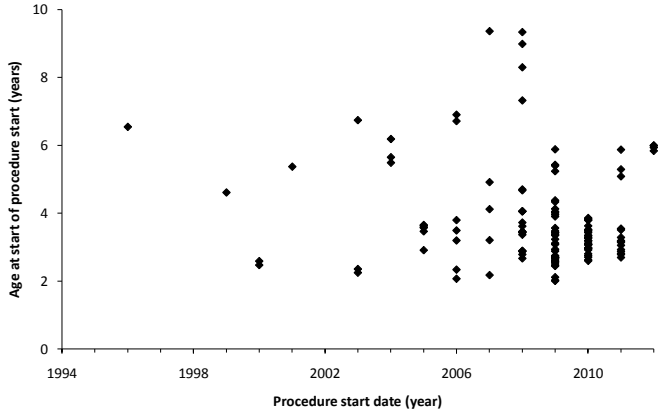


Figure 7. Age (years) at the start of the procedure (years) over time for macaques.

Figure 8 displays the duration of the procedure (years) depending on the start date for macaques. There is a recent trend towards shorter procedures.

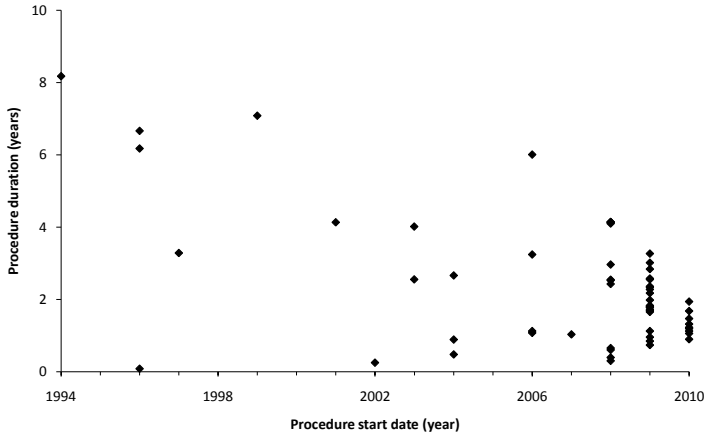


Figure 8. Duration of the procedure (years) depending on the start date for macaques.

Marmosets

Data were received on 82 marmosets; for 61 of these, details were available on date of birth and age at removal from their natal group. The average age at removal from the natal group was 655 days (with a range of 122 to 2,267 days).

The first year of starting on procedure was 2000 for those marmosets whose age at removal from the natal group was also given. The mean age at the start of the procedure varied from 258 days in 2000 (n=2) to 567 days for the 3 years 2009–11 (n=64). This increase is largely because animals were removed from their natal group much later in recent years (at more than two years of age). Animals at the younger end of the range were used in all years for which data were provided, including 2012, but virtually all animals remained in their natal group until mature.

The average duration on study, excluding some very short periods, was 370 days.

Conclusion

There was no evidence that animals used in neuroscience research were permanently removed from their mothers before the minimum age specified by the Home Office. There has been a trend towards shorter procedures for macaques.

5.2 Supply and condition on arrival.

Introduction

All monkeys used for neuroscience research over the period covered by this Review were bred in the UK. Investigators are increasingly visiting the breeding establishments to work with and select individual subjects that appear to be amenable to involvement in this type of research.

The questionnaire asked in the User Survey: *"Have animals been transferred to or from your research group / to others (e.g. previously used on immunology studies or in breeding programmes)? If so, did this involve transport between institutes or any change in social hierarchy?"*

The majority of macaques were supplied at between two and four years of age but little further substantive information was given on their condition on arrival. However, the following comments were reported in macaques:

- a female ex-breeder was deemed to be depressed / withdrawn due to intestinal neoplasia and was euthanized;
- one animal purchased that did not thrive was found to be hypoglycaemic and returned to health following modification of its diet;
- one animal was small and appeared nervous;
- one arrived with a part-missing tail.

At one designated user establishment 19 macaques had been used in immunology studies; 2 macaques had come from another department at the same establishment. It was not possible to exclude prior use from the data provided but the Animals in Science Regulation Unit Inspectorate (ASRUI) would have been aware if that was a possibility. A general comment was made that acclimatization of animals arriving from UK breeding colonies took between one and four weeks.

In addition, six marmosets were supplied from another establishment. Again, it was not possible to exclude prior use from the data provided but ASRUI would have been aware if that had occurred. Two of these were subdued and nervous on arrival.

Conclusion

All non-human primates used for neuroscience research over the period covered by this Review had been bred in the UK. From the limited information available, there were no major issues with their condition on arrival.

5.3 Housing, husbandry and care

It is generally recommended that captive primates are pair- or group-housed (Carlson 2008; Council of Europe 2006; DiVincenti et al 2011; European Directive 2010/63/EU; Honess and Marin 2006; Joint Working Group on Refinement 2009; Kelly 2007; Reinhardt et al 1995; Wolfensohn 2004).

For a non-human primate the laboratory environment is always a compromise and cannot replicate the natural environment and provide for the full range of natural behaviours. However, developments in housing and husbandry, using animals that have defined health status, have allowed units to progress away from the two-tier, single-housing on grid floors seen two decades ago. Animal management changes have resulted in less aggressive, more cooperative animals that are less stressed by capture and exhibit more natural behaviours, although continuing challenges include some social unrest within groups (Wolfensohn, 2004). Dedicated and highly trained animal careworkers ensure the physiological and psychological well being of the nonhuman primates under their care (Coleman 2011; Vitale and Pollo 2011; Joint Working Group on Refinement 2009). There is direct scientific evidence that social housing of macaques can change both the structure and connectivity of important brain regions (Sallet *et al.*, 2011).

The questionnaire asked for information on the welfare impact of housing, husbandry and care, including cleaning routines.

5.3.1 Housing

Most marmosets were kept in natal groups and then pair-housed as female / male pairs. Most macaques also appeared to be pair- or group-housed. In general it appears that group- or pair-housing was attempted and was successful in the majority of cases, but that sometimes aggression and fight injuries required separation into smaller groups, pairs or, as a last resort, single-housing. Fight injuries were reported to have the single biggest impact on welfare and these were considered to be more frequent in male groups with an increase in frequency after the age of five to six years. One user commented on a strategy of managing compatibility of macaques by initially housing in groups of four and dividing these into pairs as late as possible before physical aggression started.

<u>Effects associated with single-housing</u>

Reports for 26 macaques indicated there were no adverse effects associated with periods of single-housing. Adverse effects were reported in two animals which had led them to be single housed: one had persistently self- mutilated for three years and the other had diarrhoea, which reduced when kept in single-housing. One animal was reported to have severe behavioural pathology (stereotypy) and was withdrawn from study. In one case it was reported that pair-housing had been attempted but had provided no benefit to either of the animals. One animal was reported as *"preferring to be alone"* and showed no stereotypic behaviour. This individual was so aggressive that repeated attempts to pair-house failed due to the serious injuries inflicted on the partner.

Reports for 17 marmosets indicated that there were no adverse effects associated with periods of single-housing. Adverse effects were reported for three animals: two had come from another designated establishment as single-housed animals; attempts to pair failed. The third marmoset lost its cage mate and was reported as being subdued and quiet.

<u>Effects associated with pair-housing</u>

Reports for 76 macaques indicated that pair-housing led to no adverse effects and there were comments that pair-housing reduced the incidence of neurotic behaviour, such as

stereotypy. In one centre that had used only single-housing in the past, this amounted to some 10 per cent of animals. However, adverse effects were reported in 19 animals: 7 animals had several instances of fighting but were maintained in their pairs; 7 animals that were paired had to be separated due to fighting; there were two reports that one animal of a pair was not benefiting from the relationship and so they were separated (one was successfully re-paired); one animal managed to escape and attacked other animals, but was itself badly injured and had to be euthanized; one animal of a pair picked at wounds so had to be separated; and one was separated due to fighting but was successfully re-paired. In one case it was reported that incompatibility issues started after return from neurosurgery. In one case a subordinate male in a pair had been injured by misdirected aggression towards other males in adjoining cages. In this case moving the pair to calmer environment restored compatibility.

Reports for 75 marmosets indicated that pair-housing led to no adverse effects. One respondent believed that marmosets naturally existed as monogamous pairs in the wild, although this is not strictly accurate. There was one report of an animal fighting so it was single-housed as attempts to re-pair it were unsuccessful. On the other hand one animal that had been singly housed on arrival was successfully integrated into a pair.

Effects associated with group-housing

Reports for 57 macaques indicated that group-housing led to no adverse effects. There was a particular note for 13 animals to the effect that they were well integrated into social groups. One user reported that in order to establish stable groups of three to five adult males the space required to achieve this was three to four times the legal requirement. However, the following adverse effects were reported: 12 animals were involved in fights that required surgical repair of wounds; a further animal sustained a severe tail de-gloving injury; 39 animals had several fights which may or may not have required suturing of / surgery to wounds; one animal was ‘picked’ on – it was not clear whether this was associated with fighting; one animal had persistent (whole of time on study) diarrhoea deemed to be a stress-related effect.

In some cases animals were withdrawn from group- or pair-housing due to compatibility issues. One animal went into a terminal procedure due to unsuccessful attempts at group-housing, including moving to new peer group. Comments were made that the animal’s history should have been taken into account when assessing suitability for training.

Marmosets were initially housed in natal groups before being allocated to experimental conditions (usually in pairs). The majority of animals were reported as showing no adverse effects from either group- or pair-housing. In a few cases adverse effects were reported for animals in all three categories of housing. Reports for 78 marmosets indicated that pair-housing led to no adverse effects and in relation to 1 animal the positive impact of the natal group was noted. There were reports of fighting for three marmosets, with an additional marmoset requiring surgery.

5.3.2 Adverse effects of cleaning of housing

This appeared to have no adverse impact in almost all animals; in one animal this disrupted fluid regulation and one appeared stressed. One report indicated that changes to cleaning regimes (in this case removing the animals from the area whilst cleaning took place) had a positive effect on welfare.

5.3.3 Cumulative severity associated with housing

Table 2 summarizes the reports on the cumulative effects on individuals associated with housing.

Table 2. Cumulative effects on individuals associated with housing.

	Cumulative severity assessment			
Species	**Unchanged**	**Diminished**	**Increased**	**N/A (not housed long enough to evaluate change)**
Macaques	96 (63.2%)	48 (31.5%)	5 (3.3%)	3 (2.0%)
Marmosets	77 (94%)	4 (4.8%)	1 (1.2%)	0

Species	**Single-housed**		**Pair-housed**		**Group-housed**		**Comments**
Macaque	22		83		89		Duplication of reporting due to re-housing in different group types
Marmoset	17		75		78		
Cumulative effects	**Macaque**	**Marmoset**	**Macaque**	**Marmoset**	**Macaque**	**Marmoset**	•Number reported •(percentage for housing type)
Unchanged	15 (68.2%)	16 (94.2%)	57 (68.7%)	57 (76.0%)	51 (57.3%)	76 (97.4%)	
Increased	4 (18.2%)	0	3 (3.6%)	0	0	0	
Diminished	3 (13.6%)	1 (5.8%)	23 (27.7%)	3 (4.0%)	38(42.7%)	2 (2.6%)	

Reasons given for reporting *diminishing* severity were:

- successful re-housing with one or more cage mates (either from single-housing or, more frequently, to reduce aggressive behaviour within a larger group);
- moving to larger cages and the inclusion of swings and ropes;
- providing a play pen; and
- improving the cleaning regime.

Reasons given for *increasing* severity were generally associated with the need to separate animals from conspecifics due to aggression and fight wounds. In one case, the negative impact of removal from an incompatible pair included repeated small-scale self-inflicted wounds on the animal's legs and feet.

In some cases where severity was reported as *unchanged* there was evidence of active management of group stability by assessing the dynamic of a large group and splitting it into smaller groups to reduce aggression. One marmoset was reported to have a tendency to fight with her partner and ended up having six different partners in her lifetime. A response of *"Not applicable"* was given for three macaques and this applied to animals that either were obtained specifically for terminal procedures after receipt or were deemed unsuitable for prolonged experimental studies and were terminated (one such animal was discovered to have bilateral cataracts at the first sedation).

5.3.4 Conclusion

There was evidence of efforts being made to social-house animals in groups or pairs but that in some cases this was unsuccessful due to issues of compatibility. In at least one case it seems that more detailed knowledge or scrutiny of the animal's life history would have prevented allocation to an experiment for which it turned out to be totally unsuitable. Given that the majority of marmosets are derived from in-house breeding colonies and that macaques are derived from a single UK breeding site, it is recommended that every opportunity should be taken to assess individuals for suitability / aptitude and pairs or groups for their future compatibility by studying life histories and observing the animals at source before they are moved to the research environment. Efforts should also be made where possible to use such information to establish groups of suitable animals within the breeding unit before moving them to the experimental facility.

5.4 Non-procedural life events

These non-procedural 'life' (contingent) events are based on a named veterinary surgeon's (NVS's) records of 90 macaque monkeys in a single UK institution over the period covered by the Review (Figure 9).

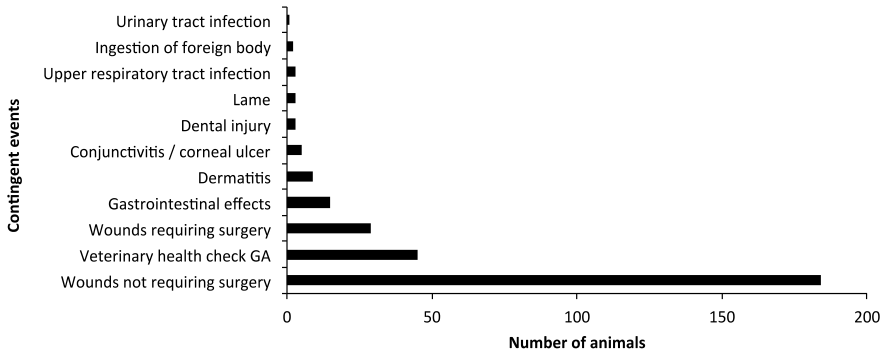


Figure 9. Total number of contingent events in 90 macaques from a single establishment over the period covered by the Review (2000 – July 2012).

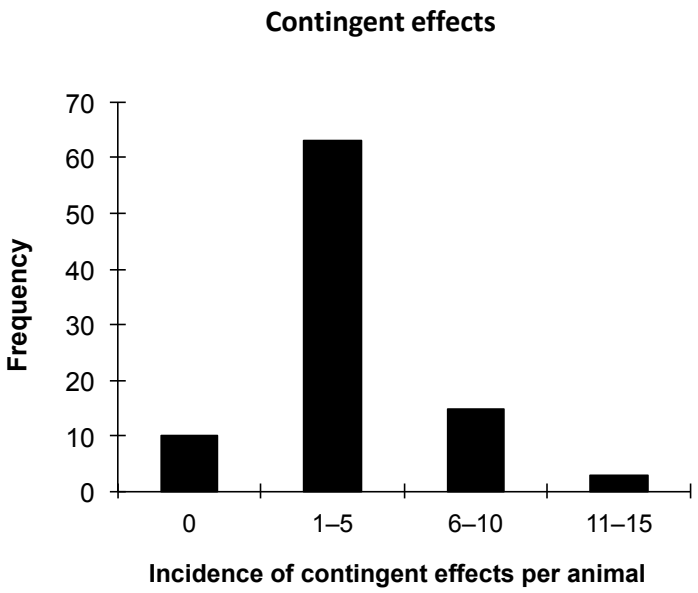


Figure 10. Incidence of contingent events per macaque from this single establishment.

Conclusion

The data show that the great majority of macaques do not experience a large number of contingent events; around 75 per cent experienced up to 5 events. The highest incidence of such events is for minor wounds (not requiring surgery) in pair- / group-housed animals and for routine health checks under general anaesthesia.

Some centres reported a lower incidence of fighting than that indicated in Figure 9. For example, one centre reported that, for a total of ten pair-housed macaques over a ten-year period, there were only two incidents of wounds inflicted during fighting that required surgery.

5.5 Anaesthesia and pain control

Introduction

It is generally accepted that modern anaesthetic techniques should always be used and by an experienced anaesthetist.

The question asked for information on the welfare impact of the anaesthetic and of post-procedure pain control.

The data provided by the questionnaire indicated that the anaesthetic practices, and the peri-operative support provided, have improved over the period covered by the survey. Most users report that anaesthesia is now (2013) most frequently maintained using inhalant agents, often with an opioid supplementation to provide a balanced anaesthetic regimen. Several respondents comment that greater use is now made of anaesthetic monitoring apparatus, and in most establishments anaesthesia was administered by a veterinarian or specialist veterinary anaesthetist, which inspectors report was not common practice historically.

A small number of anaesthetic complications and accidents were reported in the subject section of the database, including:

- five animals died or were euthanazed as a result of anaesthetic complications – in three instances, no cause was established, one was due to equipment failure or operator error, and one due to complications following endotracheal intubation.
- vomiting on recovery (four animals);
- minor changes to blood biochemistry that were resolved (one animal);
- fitting, which was resolved on treatment (one animal); and
- one non-specified reaction to anaesthesia, which was resolved.

All of these problems are similar to those reported in veterinary clinical practice (Brodbelt *et al*, 2008). The incidence of anaesthetic complications was approximately equal to 1:150 as the total number of anaesthetics given was 731.

Comments from users indicated that if best practice was adopted with anaesthesia and peri-operative care recovery could be very rapid, with animals able to move, eat and drink within an hour of recovery, and return to their cage mates within 4 to 16 hours. If such approaches are adopted, the users' assessments that the impact of anaesthesia alone was Mild seem reasonable.

Most users commented on the clinical signs they use to assess the presence of pain and some specified the analgesic regimens used to prevent or alleviate post-operative pain. The clinical signs used were similar to those listed elsewhere (for example, National Research Council of the National Academies 2009) with the addition that a change in performance on a task was often used as an indicator of pain or some other abnormality (for example, infection). The clinical signs mentioned are given in Table 3. One respondent suggested that more subtle signs of suffering might exist that will require further elucidation and validation (see also section 5.9.2, page 89).

Conclusion

Anaesthetic and peri-operative care improved over the period of the survey, with rapid recovery and a low level of complications (less than 1%).

Table 3. Clinical signs of pain or distress given by the respondents to the questionnaire

Signs that respondents say are indicators of pain or distress include

- behavioural change - negative
  - apathy
  - quiet animal
  - huddled
  - lying on ground or bottom of cage
  - no or reduced interest in treats or food
  - lack of or reduced responsiveness to humans entering room
  - not engaging with other cage mates
- behavioural change - excessive
  - excessive or unusual aggression to humans
  - agitation and excessive movements
- physical signs
  - abnormal postures - Heads down
  - shivering
  - vocalisation
  - altered gait
  - piloerection
  - weight loss
- performance change
  - failure to perform or perform tasks poorly

Possible signs that responders think indicate continuous suffering include:

- Subtle signs. Difficult to assess
- Suggested signs included
  - weight change
    - weight loss?
    - lack of weight gain over long period.
    - change in growth rate
  - increased susceptibility to infections
  - dampening of normal behaviour
  - behavioural change
    - loss of interest to handler
    - loss of interest to cage mates
  - change in ability to perform behavioural task in one animal may have been due to cumulative suffering. However, rare. Seen once only.
  - development of stereotypies and over grooming. Rare.

5.6 Surgery and maintenance of implants: Generic procedural and intended effects, and complications

Introduction

The experimental analysis of brain and behaviour that characterizes most neuroscience research involving non-human primates uses two basic types of experimental procedure. In one of these, the monkey is prepared surgically, under deep general anaesthesia, so as to allow subsequent invasive recordings from the brain while the monkey performs a trained task. This surgery requires one or more devices to be implanted in the monkey. In the second procedure, again under deep general anaesthesia, a lesion is placed in one particular part of the brain so as to allow subsequent investigation of the effect of that lesion on the monkey's behaviour and task performance.

5.6.1 Generic (expected) effects of typical recovery from planned surgical procedures

5.6.1.1 Surgical implantation of headpost or headpiece

The purpose of this implant is to allow head restraint during subsequent neurophysiological recordings where a high level of stability is required to allow reliable recordings with fine microelectrodes from single neurons while the monkey carries out its trained task. The monkey undergoes surgery under deep general anaesthesia with full aseptic conditions. Anaesthesia is carried out using appropriate anaesthetic agents and methods, including physiological monitoring, as advised by the named veterinary surgeon (NVS). Sedation prior to anaesthesia is usually carried out in the home cage, with an intramuscular injection of a drug such as ketamine. This is followed by intubation and placement of venous/arterial catheters. A number of additional drugs may be given intra-operatively to relax muscles, prevent any brain oedema, and reduce any post-surgical side effects such as vomiting. The monkey's head is held in a metal frame to allow precise positioning of the implant. The implant can be made of either biocompatible material (stainless steel, titanium, Tekapeek) or a tissue-friendly material (for example, titanium coated with hydroxyl apatite). It is often manufactured to fit the skull of the individual animal, or made by the implantation of screws and bone or dental cement used to fashion a post-holding cap. The pre-shaped implant is rigidly fixed to the skull bone by screws, bolts or other devices. The operation typically last up to six hours, during which time there is continuous monitoring of physiological condition (heart rate, blood pressure, respiratory rate and pattern, temperature, etc) and careful maintenance of normal body fluids.

The monkey is given a full programme of post-operative analgesia and antibiotic treatment. Most establishments continue to give analgesics orally for two to three days post-surgery. Typically monkeys recover rapidly and will be sitting up and moving around the recovery cage within an hour of the end of surgery, and be able to drink and eat soft foods. All animals are monitored regularly by a (PIL) holder and / or animal care staff / veterinary surgeon until the animal has returned to its pre-surgical state of health and well-being. Normal eating and drinking usually resumes the next day, and monkeys are not normally separated from their cage mates for more than one or two days. Monkeys typically re-enter the training programme within a week of surgery. There may be some small weight loss in the period immediately after surgery but this is usually made up within a few weeks. Some establishments allow a

considerable period of time (around two months) to allow the implant to stabilize before the monkey is head-restrained.

5.6.1.2 Surgical implantation of chamber or recording electrodes

In this case a surgical operation is carried out to make a craniotomy giving access to a particular brain area from which brain recordings will subsequently be taken. The dura exposed by the craniotomy is protected by the recording chamber. The craniotomy is made with a bone drill or a trephine. The chamber is usually made of biocompatible or tissue-friendly material and is secured to the skull by bone screws or other devices. The chamber is airtight and filled with sterile saline. In some establishments, the chamber is implanted but the craniotomy beneath it is not made until a subsequent minor surgery.

This surgery may be combined with the one for headpost implantation, and it may also be used for implanting arrays of electrodes permanently in different brain structures. The operation may take up to six hours. Once again recovery is rapid and the monkey will be sitting up and moving around the recovery cage within an hour of the end of surgery, and able to drink and eat soft foods. Normal eating and drinking usually resumes the next day, and monkeys are not normally separated from their cage mates for more than one or two days. Most establishments continue to give analgesics orally for two to three days post-surgery. Monkeys typically resume their training within a week of surgery. There may be some small weight loss in the period immediately after surgery but this is usually made up within a few weeks. The chamber is normally flushed out with clean saline (including 5-FU in some centres) with the head restrained every few days.

In a small number of cases a spinal chamber and / or spinal electrodes may be implanted. This surgery requires stabilization of the cervical spinal column with bone screws and bone cement. It is more invasive than the cranial implants and the operation may last up to ten hours. However recovery from anaesthesia is still rapid, and animals are usually moving around their recovery cage and eating and drinking within an hour. Full post-operative recovery is generally longer than with other operations, and more prolonged and more potent analgesic treatment may be needed for up to five days post-operatively. The animal would resume training and task performance within a week of surgery, and would not normally be separated from their cage mates for more than one or two days.

5.6.1.3 Other implants

In some establishments, other surgeries are carried out that may be additional to those listed above. These may involve implantation of physiological transducers, eye coils or implantation of surface electromyogram (EMG) electrodes, which are located subcutaneously above various hand or arm muscles. The same anaesthetic regime referred to above is used. The operation may take up to 12 hours. Recovery is normally rapid, and the monkey will be sitting up and moving around the recovery cage within an hour of the end of surgery, and able to drink and eat soft foods. Normal eating and drinking usually resumes the next day, and monkeys are not normally separated from cage mates for more than one or two days. For EMG surgeries the monkey normally wears a protective jacket for a few weeks post-surgically, to prevent it removing stitches and to promote rapid healing of skin wounds. Analgesics are given orally for at least one week and monkeys typically re-

enter the training programme within two to three weeks after surgery. There may be some small weight loss in the period immediately after surgery but this is usually made up within a few weeks.

5.6.1.4 Minor surgeries

These surgeries include those needed to repair or adjust implants, some to remove excess connective tissue in a recording chamber, or to make a small craniotomy within the recording chamber. These surgeries are carried out under short-acting general anaesthesia agents that are given by intramuscular injection, or using the same anaesthetic regimens as for more prolonged surgery. These minor operations do not usually last more than one to two hours. An anti-sedative drug may be given to reverse the action of the drugs used and speed up recovery. The monkey will be sitting up and moving around the recovery cage within 15 to 30 minutes after the end of surgery, and normal eating and drinking usually resumes the same day. Usually the monkey can re-enter the training/recording programme within one to two days.

5.6.1.5 Lesions
Macaques

Brain lesions can be performed by surgical ablation (excision / aspiration / cautery), chemical induction and / or administration of neurotoxins. The following is a specific example.

All animals undergo a detailed veterinary examination prior to surgery. Deep general anaesthesia is carried out as described above. Animals are placed in a stereotaxic or standard head holder and accurate stereotaxic placement is confirmed by X-ray. A craniotomy and durotomy is typically performed under visual guidance. Stereotactic coordinates are used to locate the exact site of lesion. The majority of animals will experience mild facial inflammation (for example, redness, swelling) due to positioning in the head holder (ventral to the eye), for up to two to three days post-operation.

The duration of the surgery varies between three to six hours, depending on the location and nature of the lesion.

For some lesions, midline retraction of the brain is required for access to sites in the centre of the brain. As a result, in the post-operative period, animals may commonly exhibit weakness in one or both limbs on the contralateral side. The gait can be altered for approximately one week and some animals can be mildly and intermittently distressed by their disability. Adapting the housing arrangements on a case by case basis is generally successful in minimizing distress.

Removal of the zygomatic arch can be required for access to some brain sites at the most lateral regions of the brain, which involves significant manipulations of the temporalis muscle. This leads to inflammation (for example, swelling) for up to 10 to 14 days post-operation. Soft and palatable foods are provided during the post-operative period and further treatment as advised by the NVS.

Neurotoxins cause localized intracranial inflammation resulting in mild cytotoxic oedema. These lesions carry the most significant adverse effects. Signs of cerebral oedema are a sleepy mental state; animals appear drowsy, lethargic and sedated. This typically lasts for between 24 and 48 hours and is not expected to extend beyond 72 hours. Immediately after surgery, animals need support to maintain a sitting position, being fed by hand and receive intravenous fluid. The mental status varies between periods of unresponsiveness (sleeping and drowsiness), apparent lack of awareness, reduced interest in their environment and lack of ability to focus on objects. Animals with bilateral lesions are commonly more severely

affected than those with unilateral lesions. If animals are more severely affected, further diagnostic tests may be performed, for example, magnetic resonance imaging (MRI) to rule out unintended pathology, such as cerebral oedema or brain haemorrhage.

Clinical signs associated with surgical ablation (excision / aspiration / cautery) result in animals being more quiet, withdrawn or slower to respond / come forward, less interested in drinking and food / treats for approximately 12 to 24 hours. Mild cerebral oedema is expected after induction of all brain lesions. Possible adverse effects of all lesions are haemorrhage / stroke (at time of surgery or following surgery) and are indistinguishable from those of cerebral oedema. Such clinical signs associated with haemorrhage are a combination of various neurological signs, such as abnormal posture, altered motor function (for example, hemi- / para-paresis; and / or plegia, ataxia; lying down on floor of cage), impaired proprioception, changes in deep pain perception, altered mental state (for example, reduced social interaction, lethargy, apathy), cranial nerve deficits (for example, problems with balance / coordination, paralysis, difficulty swallowing), transient unconsciousness and seizures.

A range of seizures (simple, complex partial or general seizures, such as absence seizures, clonic and tonic-clonic seizures) occur in a significant proportion of all animals undergoing lesion surgery. These are typically expected to occur within the first 48 hours of surgery, be single episodes (or a few in number) and transient. They usually resolve spontaneously and / or respond (that is, stop) immediately to an appropriate medication as advised by the NVS, and the animal makes a full recovery. Brain infection is unlikely and no incidents of this were reported in the lesion experiments.

All animals during the immediate post-operative recovery period (typically up to 48 hours) are observed continually (for example, direct observation, CCTV monitoring) as advised by the NVS. All animals are monitored regularly by a PIL holder and / or animal care staff / veterinary surgeon until the animal has returned to its pre-surgical state of health and well-being. Detailed records are kept, including food / fluid intake and urine / faeces output.

Occasionally an animal's appetite in the immediate post-operative period may be reduced. Regurgitation and / or vomiting occur rarely. Appropriate pre-emptive gastro-intestinal protective drugs are routinely provided, as advised by the NVS.

Typically, the animal returns to its pre-operative behaviour within 24 hours and is routinely returned to its home enclosure within 48 to 72 hours after surgery. With the exception of those with neurotoxic lesions, animals that are temporarily separated from the group remain within auditory and visual contact with their cage mates. Any surgical procedure carries the risk of disruption to the social hierarchy and can result in group instability and subsequent injuries requiring veterinary intervention. This is minimized by assessing social status prior to surgery and thereby determining the most suitable time to return an animal to its social group. However, disruption of the social hierarchy is unavoidable and the impact should be taken into consideration regarding the animal's lifetime experience. Animals with neurotoxic lesions are kept separate to ensure quiet recovery, adapting the care routine to each individual and avoiding social stress.

Marmosets

Lesions are performed to induce semi-permanent manipulations in localized brain regions in order to establish the causal involvement of specific regions in particular behaviours. The following is a specific example.

The monkey undergoes surgery under general, inhalational, anaesthetic (as described above) with full aseptic conditions. Additional medications are used both pre- and post-

operatively to prevent brain oedema and provide appropriate analgesia. The monkey's head is held in a specially designed stereotaxic frame to allow precise anatomical targeting. Small burr holes in the skull are created with a surgical micro-drill that exposes the dura over the target region. The dura is then pierced allowing infusion of the relevant neurotoxin via a fine cannula attached to a microsyringe for accurate administration. Throughout surgery there is continuous monitoring of the physiological condition (heart rate, blood pressure, oxygen saturation, respiratory rate and pattern, temperature, etc.) and careful maintenance of normal body fluids. Typically monkeys recover rapidly and will be sitting up and moving around the recovery cage within half an hour after the end of surgery, and will be able to eat and drink normally by the end of the day. If minor tremor is seen in the first 24 hours post-operation, it is controlled with oral or injected valium as required. Monkeys usually return to the home cage that evening or early the next morning. They receive three days post-operative analgesia and as a routine return to their testing regime seven days later.

5.6.2 Adverse effects and complications of surgery.

Table 4. Adverse effects and complications of surgery, including infection: Number (and proportion) of monkeys reported as showing adverse effects in relation to those that had undergone the procedure.

Species	**Macaques - *M. mulatta* N=152***			**Common marmosets - *C. jacchus* N=82**		
Adverse effects of:	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**
3a Headpost surgery	57 (37.5)	55 (96)	2 (4)	2 (2)	2 (100)	0
3b Chamber surgery	37 (24)	36 (97)	1(3)	0	0	0
3c Electrodes surgery	38 (25)	38 (100)	0 (0)	0	0	0
3d Lesion surgery	46 (30)	38 (83)	8 (17)	52 (63)	50 (96)	2 (4)
3e Other implant surgery	27 (18)	23 (85)#	4 (15)##	47 (57)	46 (98)###	1 (2)
Complications:						
1ai Skin infection (topical treatment)	104 (68)	88 (85)+	16 (15)	58 (71)	58 (100)	0
1aii Skin infection (systemic treatment)	104 (68)	80 (77)	24 (23)$	58 (71)	58 (100)	0
1aiii Bone infection	101 (66)	95 (94)	6 (6)&	56 (68)	56 (100)	0
1aiv Brain infection	101 (66)	100 (99)	1 (1)	57 (70)	57 (100)	0
1b I Partial seizures	101 (66)	95 (94)	6 (6) ~	64 (78)	62 (97)	2 (3)~~
1b ii Generalized seizures	101 (66)	92 (91)	9 (9)^	64 (78)	63 (98)	1 (2)
1c Cerebral haemorrhage	101 (66)	100 (99)	1 (1)>	63 (77)	63 (98)	1 (2)
1d Paralysis / paresis	102 (67)	98 (96)	4 (4)>>	65 (79)	62 (95)	3 (5)
1e Immobility	102 (67)	93 (91)	9 (9)<	65 (79)	65 (100)	0
1f Other neurological signs	102 (67)	100 (98)	2 (2)	65 (79)	63 (97)	2 (3)
1gi Lesions: Immediate	51 (34)	42 (82)	9 (18)	57 (70)	54 (95)	3 (5)
1gii: Lesions: Long-term	50 (33)	49 (98)	1 (2)	55 (67)	55 (100)	0 ^^

NOTES

* includes three *M.fascicularis* (cynomolgus)

\# includes eight eye coils, of which six had to be replaced one to four years later

\## includes two spinal chambers

\### includes 36 reports of *"transient discomfort from bandaging and handling in the week following abdominal implantation of blood pressure probe"*

\+ 41 out of 104 cases were treated with topical antibiotics. See section below on cranial implants

$ 40 out of 104 cases treated with systemic antibiotics; in total 100 courses / 104 non-human primates for skin infection. In 24 macaques who received at least two courses of antibiotics (range 2 – 10, median 3), the average interval between courses was 17.4 months (median 13.4 months).

& 6 out of 104 cases treated with systemic antibiotics; in total 10 courses / 101 non-human primates for bone infection

~ 12 partial seizures in 6 cases (5 in 1 case), 3 involved lesions

~~ four partial seizures in two cases

> lesion case

>> two of these were lesion cases and the paralysis was intentional

< seven lesion cases

^ 19 generalized seizures in 9 cases (7 in 1 case), 6 involved lesions

^^ there were long-term effects in 35 cases, these were intended and resulted from the lesions made.

Conclusion: Adverse effects and complications following surgery

In general, the incidence rate of adverse effects resulting from surgical procedures was reported as low. There were two exceptions to this.

- **In implanted animals a significant number of monkeys had low-level infections around the implant, which were well managed using either local or systemic antibiotics.**
- **In lesioned animals, either focal or generalized epileptic seizures were seen in a number of cases.**

A detailed analysis revealed the following points:

• A total of 528 procedures involving a general anaesthetic (GA) were carried out on 127 macaques, a median of 2 per macaque. There were 13 macaques with 10 or more GAs, the highest being 24. A total of 203 GAs were carried out on 75 marmosets, a median of 2 per marmoset. There were 5 marmosets with 5 or more GAs; the highest number was 7.

• The great majority of returns for both macaques and marmosets suggest that the incidence of unexpected adverse effects related to surgery is low (below 10%) for most categories. For example, for headpost surgery in macaques, only 2 adverse events (4%) were reported, and rates were also low for chamber (3%) and for electrode implantation (0). These incidence rates refer only to the surgical implantation: longer-term issues with headposts and chambers, including the incidence of repairs and infections are dealt with in section 5.6.3. There were 6 macaque returns that provided quantitative information, in the form of weight and task performance, on the effect of surgery for chamber implant or for cortical electrode implant and these are included below.

• In a small number of macaques (23) additional devices were implanted (eye coils, spinal chambers, EMG, transducers, etc.). There were reports of complications in 4 macaques (15%).

• There were some complications or adverse effects related to bone (6%) and brain infection (1%), and cerebral haemorrhage (1%). Paralysis (4%) and other neurological signs (2%) were anticipated in macaques with lesions.

• There was a higher incidence of comments about adverse effects of lesions in monkeys, than in those without lesions. In macaques, lesions were made in 51 cases. Adverse effects included seizures (2, 4% of cases), swollen mouth (1, 2%) leg weakness or stiffness for up to 6 days (8, 16%) temporary drowsiness (4, 8%) stomach upset (1, 2%). In marmosets, lesions were made in 52 cases. Adverse effects were seen in 14 (27%) cases, and the most frequent report (13 cases) was *"transient mild tremor and / or weakness in the first few hours post-surgery. Controlled with valium"*. In all, 21 marmosets were given valium post-operation to control tremor.

• There were reports of paralysis in 4 macaques (incidence 4%) and 3 marmosets (5%). Immobility was seen in 9 cases (all macaques, 9%), 5 were in lesion studies, which were expected to produce this effect.

• Again, lesion studies gave rise to some short-term complications in 9 macaques (18%) and 3 marmosets (5%). Long-term (unintended) complications of lesions were seen in only 1 macaque (2%) and in none of the marmosets.

• Partial seizures were unusual, there were 8 in all – 6 macaques (6%) and 2 marmosets (3%), all were lesion cases. There were 20 generalized seizures in 9 macaques (9%), 7 with lesions, and in 1 lesioned marmoset (2%).

5.6.3 Long-term issues with cranial implants

A. macaques (headposts, chambers, etc.)

Reports showed that 81 macaques (53%) had long-term implants, and adverse effects were reported in 18 of these (22%); 61 macaques had cranial implants (57 with headposts, 4 with cranial chambers or electrodes, but no headpost); and 5 macaques had additional spinal chambers).

Repairs and replacements

A reported 12 of the 61 macaques (20%) had repairs or replacement of their cranial implants. The extent of these was variable, from the addition of acrylic to firm up the attachment of loosening chambers or posts, to complete removal and replacement. Minor repairs were done under sedation with reported smooth recovery and little harm. More significant repairs required GA. There were only 5 reports of replacement of the headpost

(9%), 1 after 14 months, 2 after 2 years, and 2 after 5 years. One cortical chamber and two spinal chambers needed replacing.

In one case an x-ray was taken and suggested small fractures, which were treated with analgesia and a rest from trained tasks.

Skin infections related to the cranial implant(s)

Most of the adverse effects reported concerned infection around the implant. There were several reports concerning low-level local infection of skin margins around the headpost / chamber or other cranially implanted device (41 out of the 61 macaques, 67%). In 16 cases (26%) these were reports of adverse effects or complications. Some of these were in the five macaques with spinal chambers additional to the cranial implants. In the other cases this was not a major concern since systemic antibiotics were not needed, and the problem could be readily managed by topical application. Treatment of a wound surrounding cranial implant varies, from keeping hair short around implant, intermittent cleaning with antiseptic solution (variable regimes including using betadine), use of wound healing baby cream (Panaten crème, Flamazine), use of silver nitrate, use of analgesics, for example, meloxicam, and in some cases antibiotics (ibafloxacn, amoxycillin and cefalexin were mentioned); these may have been systemic, but topical preparation of ibafloxacin is available. Steroids were given to some macaques to suppress inflammation.

Use of systemic antibiotics to control skin infections related to cranial implants

Just under one-half (48%, 29 out of 61) macaques required one or more treatments using systemic antibiotics. In 24 cases (39%) this was because of a negative impact or complication, although in many cases the infection was reported as being well managed. The highest number of courses of antibiotic given to a macaque was 7 over a period of 30 months. In total, 109 courses were given to the 29 reported cases. The median number of courses was 3 and the median interval between courses was 13 months. Palatable preparations are available and so oral rather than injectable antibiotics are possible in most cases, but this was not specified.

In conclusion, the incidence of skin infection for the group of macaques with cranial implants was much higher than in the main group of macaques undergoing surgery. This probably reflects the open nature of the implant and the difficulty of keeping such implants clean.

B. marmosets (transducers, etc.)

Long-term implants were placed in 49 marmosets (60% of the sample) and adverse effects were reported for 3 of these (6%). These effects were related to implanted telemetry devices failing and having to be re-implanted.

5.6.4 Unintended procedural complications

The total incidence and number of unintended complications per individual monkey is illustrated in Figures 11 and 12 based on the data summarized in Table 4.

Figure 11 illustrates the total incidence of complications: Note that the questionnaire allowed data entry on the total number of episodes to allow the recording of multiple events in the same animal. The numbers shown here refer to the total incidences, not monkeys.

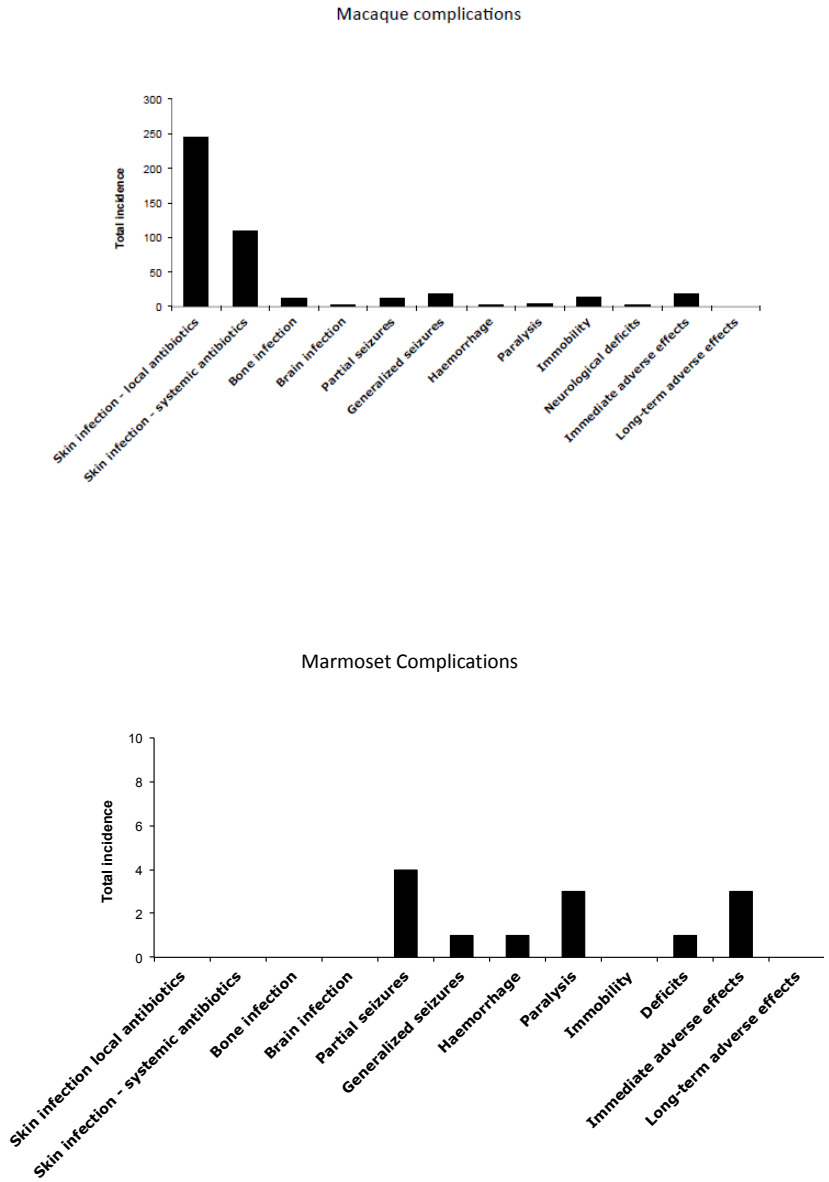
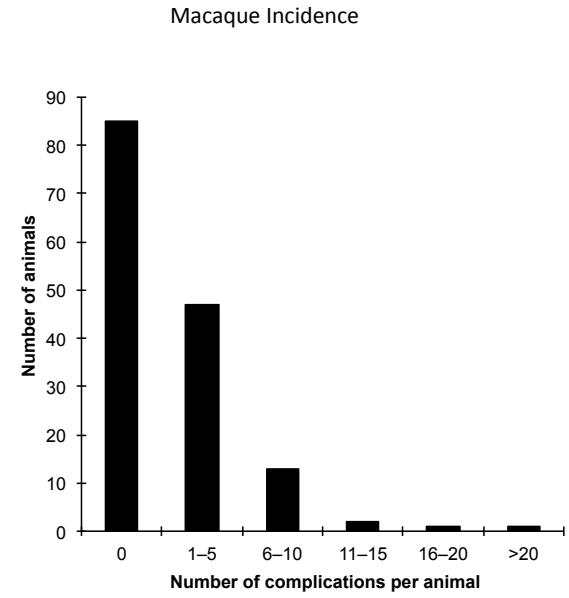


Figure 11. Total incidence of complications in macaques and marmosets.



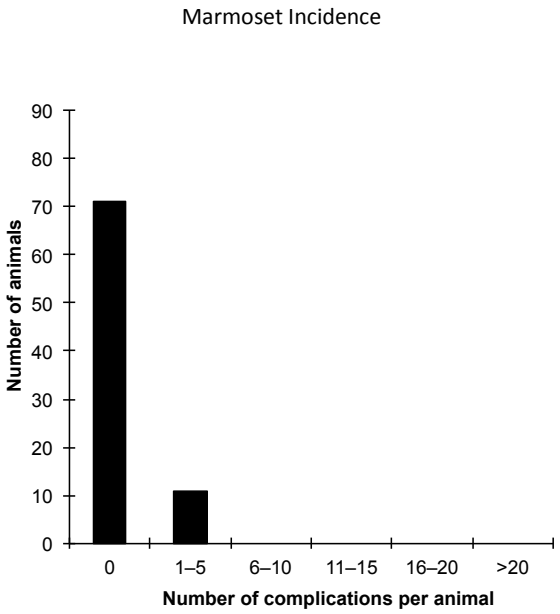


Figure 12. Number of complications per monkey.

The impact of unintended complications on user-reported severity outcome is presented later in Section 6.2 and Table 10, based on four broad categories:

- infections – superficial, systemic, meningitic and cerebral;

- seizures – partial and generalized;
- cerebral haemorrhage;
- neurological impairment – paralysis, immobility, deficits, other immediate and long-term adverse effects

Conclusion

The majority of animals (85 out of 149 macaques – 57%, and 71 out of 82 marmosets – 87%) had no reported complications. 16 out of the 149 (11%) had more than 5 complications. Of the 43% of macaques reported to have had complications, the majority were low grade skin infections, many of which were well controlled with topical antibiotics. Of the macaques requiring systemic antibiotics, the median number of courses was 3 with a median interval between of 13 months (notes to Table 4).

Intended neurological deficits as part of the experimental procedure were reported in 35 marmosets that had lesions, and these were thus excluded from the analysis.

The risk of postoperative infections was of a similar order of magnitude to human neurosurgery for comparable procedures (see section 7.2).

Significant adverse events should be investigated in a no-blame culture by rigorous 'root cause analysis' to identify and correct the factors leading to such events and thereby prevent recurrence.

5.7 Restraint and handling

Introduction

Head and body restraint are needed in non-human primate neuroscience regimes for a number of reasons.

- In animals using one hand to perform a task, it may be important to restrict movements of other body parts to avoid confounding the experimental paradigm. Similarly, in some tasks eye movements may be confounded by uncontrolled head movements.
- In some types of recording head restraint may be essential to allow stable recording from single neurons. However, restraint in primate chairs, transport boxes, head and / or neck restrainers or other devices restrict movement and may have a significant negative welfare consequence.
- Handling may also be aversive, especially if it is forced.

The Review found that all research establishments invest a lot of time in training non-human primates to accept restraint, and many use positive-reinforcement training to accomplish this (Prescott 2003).

The questionnaire asked for information on the welfare impact of the adverse effects of restraint and / or handling. Table 5 shows a breakdown of the responses received.

Table 5. Adverse effects of restraint and handling: Sample size (proportion) of subjects by question and species in relation to those to which the procedure applied.

Species	**Macaques N=148**			**Common marmosets N=82**		
Adverse effects of:	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**
Restriction in small enclosure	128 (86)	125 (98)	3 (2)	64 (78)	64 (100)	0 (0)
Restraint in primate chair with head fixation	54 (36)	43 (80)	11 (20)	1 (1)	0 (0)	1 (1)
Neck restraint	62 (42)	48 (77)	14 (23)	0 (0)	0 (0)	0 (0)
Movement of animals voluntarily into transfer cages / chairs	142 (95)	134 (94)	8 (6)	71 (87)	69 (97)	2 (3)
Use of collar and pole	22 (15)	16 (73)	6 (27)	0 (0)	0 (0)	0(0)

The majority of animals were reported to have no adverse effects or complications of restraint or handling in the context of chairs or boxes. In some establishments oral valium was used in macaques for a few days during initial training for restraint in order to reduce anxiety. Of the 54 macaques undergoing head restraint, 4 were given oral valium in the initial 2 to 4 sessions. Of 62 macaques in which neck restraint was used during training, 9 (15%) were reported in which the first 2 to 7 sessions involved oral valium, after which it was discontinued.

Several reports mentioned the non-human primate's reluctance / refusal to enter chair / move between cages and the need to motivate the animals positively to overcome this. In some cases this was explained by the relationship with cage mate, height of cage, etc. The problem was solved in some instances by positive reinforcement and change in cage mate. One non-human primate was reported as refusing to work when head restrained in the last part of study.

Neck restraint was more commonly reported as having some adverse effects. The use of collar and pole (22 reports) was usual in only one establishment where it was reported as an alternative to the use of sedation (which was used in some other establishments) at the time of neck restraint. A collar and pole was used only to position the head in the chair and not to drag the monkey out of the cage. The use of these was for a very limited time only (days) and were deemed to be necessary and in the welfare interests of the animals to reduce the stress overall during this phase of training. No long-term adverse effects were reported. The use of collar and pole in the UK is rapidly declining.

Conclusion

Restraint and handling were reported to have no adverse effects on the majority of non-human primates used, but it was stressful for some, mainly in the initial stages of training. It is important in the future to be able to establish whether there are links between different procedures – examples are given in section 5.12.

5.8 Food and fluid control, training and motivators

Introduction

Control of food or fluid intake is commonly used in neuroscience experiments with monkeys in order to motivate the animals to perform extended sequences of responses on behavioural tasks while electrophysiological recordings from the brain are made. The control may involve strict scheduling of the time for which food or fluid is available, or a reduction in the total amount of food or fluid provided per day – either way, hunger or thirst becomes a key motivator for reliable performance. Steps are taken to ensure that they receive sufficient daily fluid amounts, which are bounded by their normal *ad libitum* daily fluid intake at the top and the minimum amount necessary for physiological functioning at the bottom. A level somewhere in between these two bounds is mostly used and adjusted for each animal.

Depending on how they are implemented, controls of food or fluid can elicit physiological and behavioural responses that may compromise animal health and psychological well-being. Food or fluid control may also have an indirect impact on animal welfare if it affects husbandry. Details of the way in which food and fluid control protocols are operated in the UK, the animal welfare implications, and the available opportunities for refinement are given in Prescott *et al.* (2010; and 2012b).

The questionnaire requested information on the welfare impact of controlled access to food and water, training and motivators (rewards or punishment). Quantitative data are summarized in Table 6.

A total of 103 macaques underwent food control; none were reported to experience adverse effects or complications. In one animal, minimal impact was seen at the beginning of training when the animal was unfamiliar with the tests and had not yet learned how to obtain reinforcement. This animal quickly acclimatized to the training environment and subsequently performed tasks for food reward very well without restriction of access to food / water. Food control was not used with marmosets.

Practitioners reported that the fluid control regime used was individualized for each animal. Following is an example of such individualization of fluid control.

> *"The free water intake was determined for each animal individually, and fluid control was initially set at around 70 per cent of the free water intake. The animal's daily performance in a task that it could easily perform was then determined over three to five days. If the animal's performance was not sufficient to obtain scientifically valid data on a regular basis the fluid control was altered to a lower level (10 to 15% reduction). This was repeated until adequate performance was achieved. Thus the regime yields the minimum fluid control necessary for any given monkey. Whenever possible the minimum was increased by ten per cent and the animal's performance re-determined over five consecutive days. If the animal performed adequately with this increased minimum, it was kept at this level. Given this individualized controlled access to water, the impact on the animal's welfare was minimal or nil (even in the long term)."*

Practitioners reported that fluid control regimes could be used to maintain good performance without compromising animal health and without an impact on growth, although there were was one report of cyclical changes in weekly weight seen when using a five-day fluid control regime. Unsurprisingly, when monkeys had more fluid at the weekend, motivation to work was typically lower early in the week than later. Blood tests in macaques on long-term fluid control showed normal blood chemistry and the ability to regulate body hydration within a narrow physiological range (Yamada, Louie *et al.*, 2010)

Increased motivation was only required at the start of training and at times when task difficulty had to be increased. In such cases the animal was encouraged to learn the task by increasing motivation, such as changing / increasing rewards, temporarily tightening water control and temporarily training with an easier task. A detailed study of the effects of fluid control on the growth curves, trials worked and performance by a group of four macaques is presented in Appendix 7.6.

A total of 60 macaques underwent fluid control. None were reported to experience adverse effects or complications, although one establishment noted in response to a question about the use of motivators that *"in an initial period, one monkey might have got close to its physiological minimum"*. One non-UK establishment reported weight loss / fluctuations of *"usually not more than 10 to 15 per cent"* and *"thirst related behaviour"*. For five animals, they were unable to learn complex tasks.

Fluid control was used in 21 marmosets, and there were no reports of adverse effects. Some marmosets were used in behavioural testing, which involved presentation of a loud noise. Transient behavioural and cardiovascular alterations were observed to a 20-second stimulus that predicted an aversive 300 to 500 msec loud noise. All parameters returned to normal immediately after the noise presentation. There was no obvious long-term impact observed, determined by normal eating and drinking and social behaviour in the home cage, and stable weekly weight records. There were no reports of punishment ever being used.

Table 6. Adverse effects of food control, fluid control and training: Sample size (and proportion) of subjects reported as showing adverse effects in relation to those to which the procedure applied.

Species	**Macaques N=152**			**Common marmosets N=82**		
Adverse effects of:	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**	**Number (% of total) to which procedure applies**	**Number (% of sample) with NO adverse effects or complications**	**Number (% of sample) with adverse effects or complications**
5a Food control	103 (68)	103 (100)	0	0	0	0
5b Fluid control	60 (39)	60 (100)	0	21 (26)	21 (100)	0
8a Training in tasks	148 (97)	143 (97)	5 (3)	61 (74)	61 (100)	0
8b Use of motivators	139 (91)	138 (99)	1 (1) *	62 (76)	62 (100)	0

(*Unclear whether effects seen were adverse *("monkey gets close to physiological minimum")*

Conclusion

Food control was reported to have no adverse effect in macaques. Fluid control was individualized for each animal, both macaque and marmoset, such that it was reported to be possible to maintain good performance without compromising animal health.

5.9 Behaviour

The ability to observe behaviour in a non-human primate requires skill and training. Determining what is 'normal' and 'abnormal' for an individual is not an easy task. One research institution employed a behavioural primatologist and other individuals attended training courses at the University of Stirling, EU Prim and the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs), and on selected websites (for example, www.marmosetcare.com).

The questionnaire requested information about the routine collection of data on behaviours in the home cage with examples, references and data on:

- **the signs of a contented monkey;**
- **abnormal behaviours;**
- **signs of a monkey in pain or distress; and**
- **subtle signs that indicate a low level of continuous suffering.**

5.9.1 Behavioural signs of a contented monkey

Box 1 summarizes the general points made in the User Survey that were consistent with the behavioural signs of a contented non-human primate.

Box 1 Behavioural signs of a contented non-human primate

([n] = number of times mentioned in 27 user responses)

1. Good appetite, eating well [5], drinking well [3]
2. Good general condition of the animal, especially fur condition [4], bright eyes [4], body weight [3]
3. Interacts well with other monkeys [8], showing grooming [13], playing [6]
4. Interacts well with care staff and with experimenters [11], curiosity [4]
5. Interacts well with its home cage environment [5], forages well [7]
6. Absence of any stereotyped behaviours [7]
7. Minimum expression of aggression / fear [9], normal facial expressions [3], and vocalizations [3]
8. Absence of skin wounds, rash, hair loss due to over-grooming, diarrhoea, signs of self-injury, etc. [4]
9. Shows good cooperation with experimenters (for example, for transfers to chair, application of restraint, etc.) [7], and works with vigour on the task [2]

There was reasonable agreement amongst users about the signs of a contented monkey. Many of the above indicators are influenced by the hierarchical position of the animal, the time of day, and whether or not behaviours are recorded discretely by CCTV rather than by staff entering the housing facility.

> *"By working with the animals on a regular basis we note their typical behaviour and use this to gauge normal interactions or if adverse events (social hierarchy changes) have occurred."*
>
> *"No animal should experience ongoing/chronic distress as a result of its hierarchical status."*

Do non-human primates have any choice but to co-operate in the procedure?

Although it is clear that the cooperation of a non-human primate is an essential factor in the success or failure of a neuroscience study, it is often discussed whether they have any choice other than to cooperate. Does the monkey have any opportunity to avoid the procedure? The reality is that the work cannot be done without the cooperation of the monkey in leaving the group- or pair-housing facility for the chair / training box, allowing head or another form of restraint to be applied, and performing the task to criterion for a significant length of time / number of trials. The Review was informed that all UK project licences have clauses that specifically deal with these steps, and what to do if the monkey refuses to comply. This usually means go back, retrain and find ways to positively reinforce the monkey to cooperate. Aversive stimuli are generally not allowed on non-human primate PPLs for the purpose of training, although some negative reinforcement (for example, squeeze back) and coercion (such as pole and collar) is used in some research establishments in the initial stages.

As a result, practitioners take the view that their protocols do not in any way resemble 'learned helplessness'. This is considered to be a model of severe depression (Pryce, Azzinnari *et al* 2011) and typically involves studying the behaviour of rats subjected to inescapable electric shock (which is rated a Severe procedure under current (2013) Home Office ASPA guidelines). By contrast, in non-human primate neuroscience protocols, the monkey is being offered the chance to continue to perform a task in order to earn food or fluid rewards. It is not punished by aversive events (such as the electric shocks used in the rat model of learned helplessness). Importantly, in learned helplessness, animals do not perform because they have learnt that their actions have no consequences. One would expect them to sit passively and not to make responses. Learned helplessness stops individuals from responding and learning – it can hardly be used to explain why animals pursue a task successfully.

5.9.2 Abnormal behaviours

Abnormal behaviour has been defined as a behaviour that is *"rarely seen in wild populations and does not promote the success and the survival of the individual or its close relatives (that is, it does not increase fitness). It appears not to be goal-oriented, so that its function is not apparent"*. It *"may include elements of normal activities, but they are performed in an inappropriate fashion"* (Poole, 1988, page 4).

A list of behaviours that might indicate poor welfare is given in Table 7 (Joint Working Group on Refinement, 2009, Table 2).

This list of abnormal behaviour was not provided in the questionnaire so the responses were not as full as they might have been.

Table 7. Behaviours that may indicate poor welfare in macaques, marmosets and tamarins, taken from the Joint Working Group on Refinement (2009).

A restricted behavioural repertoire	Failure to make full use of the environment Cessation of foraging or locomotion Little curiosity towards novel objects Little or no vocalization
An abnormal time budget	Restlessness or hyperactivity (e.g. circling) Decreased activity (lethargy) General inactivity or unresponsiveness Excessive eating (hyperphagia) Psychogenic excessive water drinking (polydypsia) Increased scent marking
Inappropriate social behaviour	Increased aggression to conspecifics Excessive fear towards or withdrawal from conspecifics (e.g. hiding at the back of the enclosure, hiding within or behind enclosure furniture) Over grooming, or hair plucking of conspecifics leading to hair loss Failure to mate Killing or neglect of young Change in behaviour towards human handlers (e.g. increased aggression or withdrawal) Change in the behaviour of cage-mates towards the individual animal
Other abnormal behavioural patterns	Postural stereotypy* (e.g. saluting, floating limb, head tossing and rocking) Locomotor stereotypy (e.g. excessive pacing, weaving, circling and somersaulting) Urine drinking Consumption of faeces (coprophagy) Teeth clenching or grinding (bruxism)

[NB not all behaviours are applicable to all species. *Stereotypy: the performance of unusual motor acts, repeatedly and often invariably, which serve no apparent purpose; often indicative of an inadequate or inappropriate environment]

Of the 149 rhesus macaques, 92 per cent were reported to show normal behaviour, and 8 per cent (n=12) showed some abnormal behaviours (see Table 7). None of the three cynomolgus macaques were reported to show any abnormal behaviour, and all three were reported as having diminishing cumulative severity for restraint.

Of the 82 marmosets, only one showed any abnormal behaviour. This marmoset was described as *"nervous, subdued, and timid behaviour was seen following the move from a previous unit and thus abnormal behaviour appeared present from the beginning"*. This marmoset was described as having increasing cumulative severity for restraint.

Table 8. Descriptions of the 12 cases of abnormal behaviours in rhesus macaques and the impact on any response to the restraint involved in training and recording as an assessment of cumulative severity.

Description of abnormal behaviour	Cumulative severity of impact on restraint?
Finger licking	Diminishing
This animal showed signs of poor gastrointestinal health and vomiting was observed on several occasions. The animal performed poorly on standard behavioural tasks and so a simpler behavioural task was devised for the animal	Diminishing
Mild pacing occasionally in presence of novel humans (visitors)	Diminishing
Occasionally pressing of orbital area above eye since September 2010, no signs of harming himself, performance on task is improving with training / testing	Diminishing
This animal showed some intermittent hair pulling. The last time that this was witnessed was about five months ago and does not seem to be a problem currently	Diminishing
Self-biting on arm but no punctures, no impact	Diminishing
Some vomiting noticed. Given Ranitidine between 2 and 7 March 2011. Discontinued after animal refused oral administration. Condition did not worsen	Diminishing
Tended to hold headcap. Was a matter of welfare concern	Unchanging
In last few weeks refused to perform task whether in head-fixed or head-free conditions	Increasing
Stereotyped behaviour. Serious impact on proposed study	Increasing
This animal became withdrawn and difficult to work with	Increasing
Withdrawn and weak appetite. Prevented initiation of training	N/A

The range of abnormal behaviours listed suggests that abnormal behaviours were not perceived by respondents to be directly associated with increasing cumulative severity for restraint as only 4 out of 12 (33%) had *"increasing"* cumulative severity – all others were *"diminishing"*, except for one *"unchanging"*. In most cases the abnormal behaviour was transient and had either resolved completely or was much reduced, and in all these cases the respondent listed the cumulative severity effects as *"diminishing"* (see Table 8). In other words, adverse behaviour exhibited by a nonhuman primate did not always lead to a worse response to repeated restraint.

Conclusion

The majority of animals were reported not to exhibit abnormal behaviours; a higher proportion of rhesus macaques exhibited abnormal behaviour than marmosets. Abnormal

behaviours in macaques include locomotor stereotypies (that is, pacing), other stereotypy (that is, eye poking, finger licking), hair pulling, self-biting (no puncture), vomiting, and being withdrawn and unwilling to perform tasks. There was no clear evidence of a cumulative interaction between abnormal behaviour and the response to restraint needed for training and recording.

There is scope for the development of more accurate and sensitive behavioural outcome measures than those listed in Table 7 and guided by CCTV (see also Table 3 – 'subtle signs – difficult to assess'). Such behaviours might be used to confirm or refute whether there are subtle signs of cumulative suffering prior to premature euthanasia in nonhuman primates that cannot cope (Hawkins et al 2011). The psychological effects of fluid control regimes in nonhuman primates also merit further study.

5.10 Refinements in non-human primate neuroscience research: results from the User Survey

Users were asked to comment on any improvements or refinements in their approach to the many different aspects of non-human primate research. 'How have procedures/husbandry at establishments with which you are familiar evolved or been modified over the past decade and why? How were any improvements or impacts of changes evaluated? Please indicate if the procedures have not been used.'

The returns showed much evidence, in both the UK and the wider EU, of refinement in most areas of non-human primate research. The comments from the UK users (n=21 returns) are listed below for completeness although many have been referred to in individual sections.

The main point made by several EU and UK users is that they are actively and continuously engaged in refinement, and that it is the major advances across housing, training and implants, for example, that have made it possible to carry out the kind of long-term research that is now the subject of this Review. In addition, the accumulating experience available in the main centres of non-human primate research is highlighted; research is now conducted by highly experienced teams of (PIs) and other researchers together with named animal care and welfare officers (NACWOs), named veterinary surgeons (NVSs) and animal technologists.

Anaesthesia: In 16 out of 17 UK reports, anaesthesia was now supervised by experienced veterinary staff or by the NVS. Improvements due to use of inhalation anaesthesia were mentioned in 6 out of these 17 reports and 10 out of 17 mentioned in particular faster recovery and fewer post-operative complications.

Surgical technique: Refinements in implant or surgical techniques were reported by 11 out of the 17 users. For example, use of subcutaneous sutures for closing skin wounds has improved healing.

Restraint: Around one-half of users (8 out of 17) have moved away from use of 'collar and pole' to positive reinforcement techniques (PRTs). Others (3 out of 17) are developing new electrophysiological techniques that no longer require head restraint.

Food and fluid control / training: Several reported fluid control being tailored to individual animals. Many reports made the point that the trained task can be an enriching experience for the monkey. It is clear that that there have been no major changes over the years in the operant techniques used for training non-human primates on the task. However, it is also clear than use of PRT for all phases of the training, including restraint, is on the increase (7 out of 17 respondents). Reports also mention the use of 'automatic trainers' and pre-selection of monkeys most suitable for this type of behavioural studies. Several reports point out that training a sensitive non-human primate requires special skills, not just patience and understanding.

Housing: The User Survey highlights significant improvements in welfare due to the introduction, over the last five to ten years, of pair- or group-housing for nearly all monkeys used in long-term studies. Modern housing includes enriched exercise, play and sleeping areas, with opportunity for forage. Again users report that this has reduced stereotypical behaviours, increased expression of normal behaviour, decreased neophobia, etc. In general, UK facilities were reported to be better and larger than in some other countries carrying out similar research, such as the USA. Group- or pair-housing of monkeys with long-term implants is now the norm, and has not caused any increased rate of complications with these implants.

Implant maintenance: Reports mention the increased use of tissue-friendly materials (titanium, Tekapeek, ceramic screws, coating with bone-friendly hydroxyapatite, etc; Adams et al 2011) for implants and of various treatments to reduce inflammation and dural scarring (for example, the use of the anti-mitotic compound 5-fluorouracil) caused by implants. Overall implant stability has improved and infection rate is much lower than in the past. A decade ago implants deteriorated rapidly and monkeys had to be killed after a year or two, now (as at 2013) implants can be sustained in healthy monkeys for much longer.

5.11 Premature killing of non-human primates and post-mortem examinations

The questionnaire asked practitioners to report any cases of non-human primates having to be killed to prevent suffering. They were asked about whether a post-mortem examination was carried out and if so whether the NVS was responsible for this. They were also asked whether any clinical pathological data were collected, either during the non-human primate's lifetime or at post mortem. In particular, were there any signs of impaired renal function in life or at post mortem?

Overall, a total of 26 cases of non-elective euthanasia were reported – 12 of these (46%) were procedure-related and the remainder were attributed to age-related disorders, including neoplasia and end-stage renal disease (findings confirmed at post mortem). The reason for procedure-related terminations was considered to be of significant severity in 6 out of the 12 cases (50%). In such cases, 4 of the 6 (67%) animals were euthanized within 48 hours, whilst the remainder were killed within a timeframe of a few weeks once all alternative ameliorating measures had been exhausted. None of the animals suffering from significant procedure-related complications were reported to have been used in further experimental studies.

Macaques

The sample reported that 103 macaques were terminated or died during the sample period, while 49 were still alive and in procedure. For the great majority (89 out of the 103 cases, 86%) the macaque under study was terminated as planned at the end of the procedure. The remaining 14 macaques (14% of those killed) were killed at an earlier, unplanned stage of the procedure. In eight cases this was because of anticipated, but unwanted, consequences of the procedure and in two cases it was due to unexpected effects related to the procedure, such as long-term refusal to perform the task. In four cases it was due to unexpected effects unrelated to the procedure (neoplasia (2); acute illness (2)).

A post-mortem examination was carried out in 51 cases, 33 of which were carried out by a veterinary surgeon. The post mortems reported seven positive results, three of which was directly related to the procedure or its anticipated consequences:

- anaesthetic accidents (2 cases);
- cerebral oedema, no haemorrhage;
- pneumonia;
- intestinal neoplasia (2 cases);
- severe haemorrhagic enteritis with secondary renal involvement.

Additionally 12 animals' brains and skulls were reported to have been examined, none of which were reported to have any overt signs of injury or infection. There were two post mortems that concerned anaesthetic deaths (although it seems that there may have been one more anaesthetic-related death in which no post mortem was carried out). Only three macaques were reported to have any level of renal dysfunction, two related to anaesthesia and one due to unrelated intercurrent illness. This was despite the fact that many macaques were on long-term fluid control (see Table 6).

Marmosets

In the sample reported, 79 marmosets were terminated or died during the sample period, while 3 were still alive and in procedure. For the great majority of cases (67 out of 79, 85%) the marmoset under study was terminated as planned at the end of the procedure. A total of 12 marmosets (15% of those killed) were killed at an earlier, unplanned stage of the

procedure. In two cases this was due to complications related to the procedure (intracerebral haematoma). In the other 10 cases, it was due to unexpected effects unrelated to the procedure (acute infection (4); hepatorenal disease (2); fractured femur (1); weight loss (1); chronic cystitis (1); lymphoma (1)).

It was not reported who had performed the post-mortem examinations in the 15 marmosets, or where they were performed, but the level of detailed pathology provided suggests that all were done by a veterinary surgeon. There were 15 positive results reported from the post mortems, of which only 2 related to the procedure or its anticipated consequences:

- likely pressure consequences of abdominal implant;
- likely embolic event after abdominal surgery, causing paralysis.

Clinical pathology was available for 38 per cent of macaques and 80 per cent of marmosets. However, little data were reported. Those without overt non-associated pathology had sodium and potassium values that appeared to be within the normal range. Only four marmosets were reported to have any level of renal dysfunction, three as weight fluctuations and one as having dark urine. None of these marmosets were on fluid control protocols.

Conclusion and recommendations

Greater use could have been made of the opportunities afforded by post-mortem examinations, subject to the availability of the appropriate resources.

All animals used in long-term studies and all those that are killed prematurely should have a post mortem performed to obtain the fullest possible diagnosis and assessment of any consequences of the neuroscience research. This information should be included in the retrospective reviews of practice.

Standards established for the conduct of post-mortem examinations should specify the most appropriate person to undertake the examination and include a comprehensive description of the protocol to be used. This person should work with scientists to optimize tissue collection for both science and pathological analyses.

Blood samples should be taken when animals are under general anaesthesia for another purpose, where it will not compromise science or welfare, and analysed and collated along with clinical history to determine reference ranges and outliers. A mechanism should be devised to allow data to be shared and made freely available.

5.12 Lifetime experience.

The new EU Directive 2010/63/EU emphasizes the importance of the whole lifetime experience of a research animal; Paragraph 33 requests that non-human primates should have a personal history file from birth covering their lifetimes in order to be able to receive the care, accommodation and treatment that meet their individual needs and characteristics. Hence, the Review asked for examples of typical timelines for non-human primates in neuroscience research in order to address the impact of individual events and their mutual interaction (see Scenarios 1 to 4, Figure 5).

The questionnaire asked "*Please provide a timeline showing the life experience of this non-human primate, which records timings of key events such as start of training, first use of restraint, use of food or fluid control, and timing of major surgeries*".

Detailed timelines were not returned for the majority of animals but some timelines were reported.

An example timeline for one marmoset monkey.

"This animal was bred and housed in an enriched environment within the facility for the duration of his life. He was housed in a natal group until mature and subsequently pair-housed with a female. He received a central nervous system (CNS) lesion that caused highly selective mild cognitive and emotional impairment, which could not be seen in the home cage environment. In addition he received routine behavioural testing."

In most marmoset cases where there were unintended consequences or explanation for termination required, details were given. For example, *"…in addition, the animal received routine behavioural testing and a single blood sample. She was initially removed from the natal group due to fighting and successfully re-paired. She received monthly intramuscular injections of contraceptive for eight months. She was euthanized by the NVS due to persistent weight loss and loss of condition."*

Example timelines for macaque monkeys.

Detailed entries for timelines were provided for ten macaques. Figures 12 – 15 demonstrate the details for six of these monkeys.

In three of the cases, a surgical procedure to implant a cortical array was not obviously associated with a period of weight loss. In these cases, task performance generally fell after implant but recovered again within one to two weeks. Case 164 was the exception, with weight falling by almost 1 kg over a 2-month period after the array was implanted. During this period task performance was erratic and towards the end of it there was an epileptic seizure. This prompted immediate removal of the implant, no further seizures were observed and task performance improved, and there was a slow but steady increase in body weight.

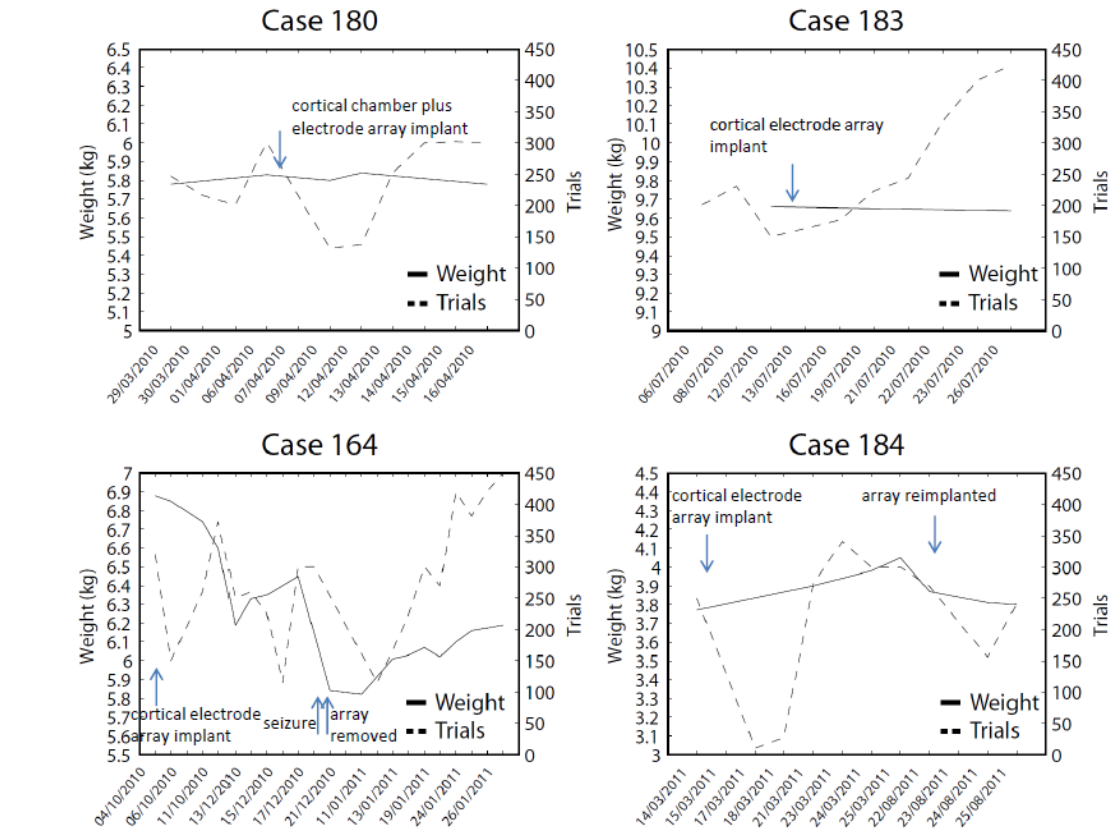


Figure 13. Examples of timelines for four macaques with implants of cortical electrode arrays.

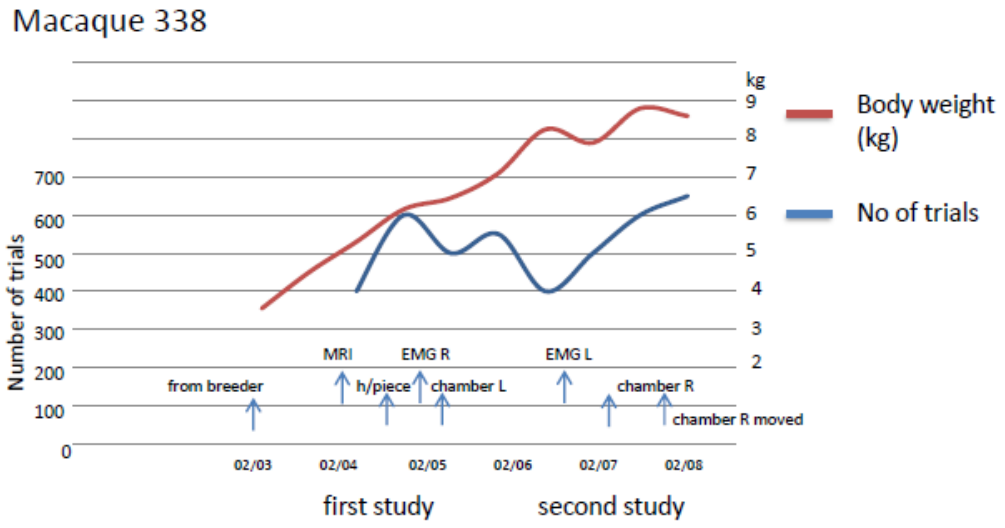


Figure 14. A typical timeline from another long-term neuroscience procedure in a macaque (case 338)

In this case, 7 procedures, each requiring general anaesthesia, were carried out over a period of 3.5 years (42 months). In Figure 14 each procedure is indicated by a short blue arrow. Note that, throughout this period, which included two main periods of neurophysiological recording (first and second studies), there was a steady increase in body weight (red line) and monthly-averaged task performance (blue line) was always above 400 trials per session.

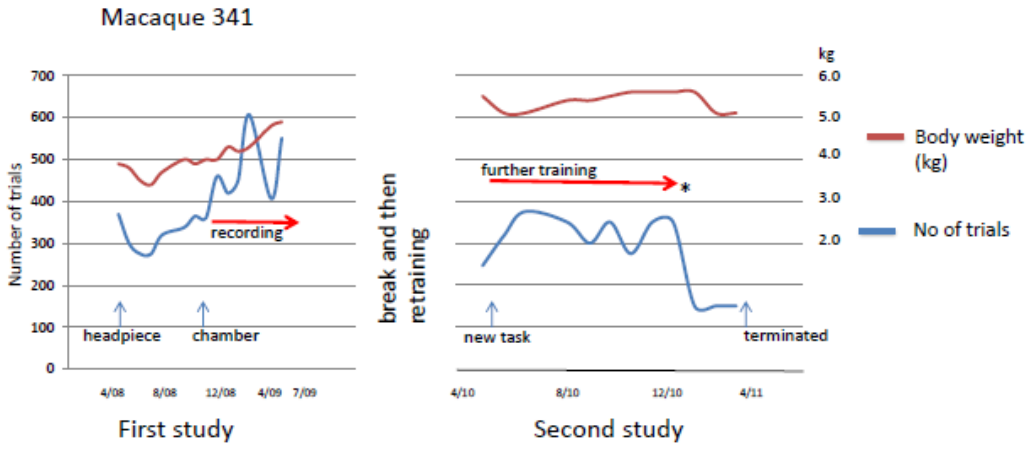


Figure 15. A single possible example of cumulative severity (macaque 341)

In this case (macaque 341) initial training was completed in April 2008 and the headpiece was implanted. During the first period of recording (first study), this monkey showed good performance on a demanding task. Once recording started, the mean number of trials per session (blue line) was always greater than 300, and there was a steady increase in body weight (red line). However, during the second study, which involved the same project but working on a different task, performance never really got up to the criterion levels needed to commence recording. Early in 2011 performance became very erratic and the monkey refused to be head restrained (marked * on Figure 15). The team decision to terminate was made at the end March 2011. There were other issues in this case, including loss of the cage mate that meant that the macaque had to be single-housed.

Conclusion

Timelines such as these can be used to assist in evaluating the impact of life events. They clearly show the sequence and type of surgeries and, if task performance is a valid surrogate for positive or neutral welfare, then they can have value in the assessment of cumulative severity. For example, in isolated examples, erratic performance on the task after surgery seems to have been a marker that there may be a problem that needed addressing (case 164 Figure 13, case 338, Figure 14). However, they need to be assessed along with veterinary records, project licence records, etc., where other relevant information is likely to be recorded. The repeated nature of procedures for that particular animal should be clear and this can then be taken into account for the classification of severity. The task performance may be used in the assessment of the duration of consequence of each surgery for the animal.

5.13 Self-reported severity assessments

User assessment

The questionnaire asked: *"In the light of the evidence you have submitted, what severity classification do you consider appropriate for the work you have described: 10 years ago and now?"*

Under the Animals (Scientific Procedures) Act (ASPA) 1986 all but very minor surgery should be classified as Moderate – see the Home Office *Guidance on the Operation of the Animals (Scientific Procedures) Act* Section 5.42 *"Moderate – … many surgical procedures (provided that suffering is controlled and minimised by effective post-operative analgesia and care)"*. This has not changed under Directive 2010/63/EU.

There were 27 user assessments; 17 from the UK and 10 from elsewhere in Europe.

Of the UK users, two considered the work could be classed as Mild, the remainder all classed it as Moderate severity, while none considered it Substantial. However, one user did consider that very long recording sessions and the use of water restriction could be classed as Substantial. Three UK veterinarians provided responses: two considered the work Moderate, one considered that electrophysiology would be considered Substantial, particularly if combined with magnetic resonance (MR) scanning and awake behaviour.

All the EU users felt that the work should be classified as Moderate or less. Some considered that the surgery aspects should be classified as Moderate for a few days afterwards and the electrophysiological recordings and functional MR scans classified as Mild.

Many expressed the strong view that the severity of the procedures used had been reduced over the last ten years through many refinements. Most considered their work with non-human primates to have been Moderate even then.

> *"Ten years ago our implants were far inferior and infections more prevalent, our anaesthetic and surgical procedures were less refined requiring longer recovery time, we used eye coils instead of infrared cameras to track eye movements. We did not readily have access to MRI* [magnetic resonance imaging] *to guide our experiments, which often meant animals had to be euthanized (to obtain histology) to verify whether we recorded data from the correct location. We did not use the variety of positive reinforcement techniques I use, we were less concerned about fluid control and monitoring growth patterns, and we had less advanced neurophysiological techniques, meaning it might take two to five times as many sessions to collect the same amount of data that I can collect with my current techniques. Without question we have made significant advancements and refinements in our research, which has reduced the severity significantly."* (UK user)

> *"Our institution has asked a particular competent and experienced primatologist, veterinarian and laboratory animal scientist for a detailed expertise on* [syn. Assessment of] *suffering based on examination not only of the procedures but also of the animals being subject to these procedures since many years. In his extensive report, he finally concluded: 'The sum of the different procedures, their combination and their duration over many years do not result in additive effects with respect to pain, suffering or damage, because even after years of participation in the*

experiment the animals show a very good health state with good general condition'." (EU user)

There was disquiet from one EU user that, despite all the refinements, there was a trend in that country to increase the level of severity classification. There was also some apparent confusion amongst EU users over how severity limits or classification of protocols has been carried out in the UK. Some respondents gave separate classifications for different parts of the same procedure, which has apparently been the custom in some EU countries.

In no cases were reasons given to explain the classification. One EU respondent referred to Annex VIII of Directive 2010/63/EU when pointing out that the surgical techniques *per se* used in neuroscience research fell within the Moderate category. The same respondent highlighted that the electrophysiological and fMRI recordings involving chair restraint and fluid control were not specifically listed in the Directive.

One user commented that he and his team *"would be deeply disturbed to have their work categorised alongside* [other Substantial] *procedures such as induction of cancer or neurodegenerative states, neuropathic pain, or application of electric shock"*.

Subject assessment

The questionnaire asked: *"In light of the evidence you have submitted, what severity classification do you consider appropriate for the work you have described on this non-human primate?"*

No specific guidance was given to the wording of the 2010/63/EU Directive.

Macaques

Data on 146 macaques were classified with respect to overall severity.

There were 30 cases (20%) classed as Mild (or in one case *"less than Moderate"*); all except two came from the same site. Those described as Mild appeared generally to be in the early stages of ongoing studies and had not had surgery, although four had undergone cranial surgery and been used on more than one project. In the view of the Working Party, these four cases should have been classified as Moderate to comply with Section 5.42 of ASPA 1986.

The remaining 116 were classed as Moderate (including *"mild form of Moderate"*). The details of life experience provided by respondents for non-human primates described as undergoing Moderate procedures were highly variable. Some provided a detailed timeline that enabled an assessment of the number and nature of general anaesthetics (GAs) and surgical procedures to be made. In other cases, the database had to be interrogated across cells to reveal many of the details of what had been done.

There was evidence of some misunderstanding of severity limits and bands. For example, one non-human primate was described as *"Moderate during agitation with chair restraint. Mild since acclimatizing to this"* but had also had a surgical implant, suggesting that the surgery was not considered in assigning severity. Another non-human primate described as *"Mild to Moderate"* had experienced one major surgery for a chamber implant and ten further craniotomies requiring anaesthesia. In one case where a detailed timeline was provided, a

Moderate severity limit was given for a non-human primate that had nine separate surgical procedures plus imaging under anaesthesia over approximately four years.

ASRUI identified four subjects that had been classified by the user as suffering overall Moderate severity (Table 9) and reassessed them according to the presented details (retrospective review). All four non-human primates were euthanized when it became clear that recovery from complications was not a realistic possibility. The ASRUI view is that all four qualified as Substantial (severe using Directive 2010/63/EU classification). However, it is not clear whether this judgement is based on the total number of GAs and procedures or on the severity of the terminal complications or both? How long should the terminal phase be to change an otherwise lifetime Moderate classification to Severe?

Table 9. Four macaques with multiple surgeries and other events and classified as Moderate.

Subject	Summary of life experience
1	Headpost and chamber implantation, total of ten GAs including *"three minor and two major",* repairs to headpost, bone growth across the chamber with osteomyelitis that could not be eradicated; abnormal behaviour holding headcap; CS maintenance increasing; PPl made decision to terminate.
2	Animal was euthanized because of implant failure before the end of the procedure; osteomyelitis proved difficult to eradicate; and the animal had housing problems. Total of five GAs.
3	Animal developed such severe behavioural problems, including stereotypy, that it had to be removed from the study, despite having been moved to another pen and having undergone surgery and repair / removal of an eye coil. Cause of stereotypy not determined. Euthanized.
4	Animal experienced loosening of spinal chamber. Total of 4 GAs and 12 sedations for head and spinal chamber and peripheral nerve and muscle electrode implants. It was euthanized due to loosening of the chamber, associated with which it lost 10 per cent of its body weight over 24 days. Previously well adapted to recording sessions that included neck restraint.

However, these four were not typical of the majority of entries. It is clear from the data returned that the overall picture was that, under a severity level assessed as 'Moderate', macaques underwent far fewer surgeries than reported for some of the macaques in Table 9: the median number of GAs for the 116 cases was 3 (and some of these were probably for MRI) and the mean value was 4 GAs per macaque.

Marmosets

Data were received for 82 marmosets from a single site in the UK, of which all except 1 were classified as either Mild or Moderate with respect to overall severity. One was described as Mild/Moderate. For one animal there was no report of surgical procedures under life experience but reference to transient tremor following lesion surgery. One animal had no report of surgery under life expectancy but reference to abdominal discomfort following implant surgery. These cases suggest that data are missing for some entries.

The 13 described as Mild did not, as far as can be determined, experience surgical procedures. Some experienced only blood samples for genotyping, some had non-invasive imaging under anaesthesia; some appeared not to have had any regulated procedures.

There were 67 marmosets classed as Moderate. Most of these experienced between one and five procedures under GA, including abdominal implant surgery, cranial surgery to create a lesion, but lesions caused only subtle or very short-term deficits, or intracerebral microdialysis and non-invasive imaging. There was no difference in the severity classification according to number of procedures.

Conclusion

In retrospect, the questions in the questionnaire should have been more explicit in terms of what was being sought in terms of overall severity. It would have been helpful to have provided the four scenarios in Figure 5 [pages 31-33] in order to illustrate the basis for consideration of overall severity. As a consequence, there appeared, within the database, to be less consideration given to the severity of the surgical procedures themselves, and more to the complications associated with surgery, etc. Hence, respondents may not have based their estimates on the severity of the entire procedure. This resulted in some cases where there had been an underestimation of severity by a minority of users, although many are experienced practitioners with detailed experience of assessing severity levels in conjunction with their Ethical Review Process (ERP) and inspector.

Follow-up visits to the establishments (see Section 6) were able to explore and clarify these issues including, for example, the time for recovery after general anaesthetic and surgery and hence the impact on overall severity.

Under the new Directive, all those involved with nonhuman primate research will be actively involved in the retrospective assignment of severity. Researchers may well require further training once the guidelines and examples have been published by the Home Office.

5.14 Cumulative severity and suffering

The questionnaire asked: *"If any of the procedures listed are undertaken repeatedly, over a long period of time (e.g. several years), do you consider the welfare impact on this non-human primate is unchanging, diminishing or increasing? Where possible, please give examples, references and/or data to support any comments."*

Definitions

Unchanging: Later procedures applied to an animal have the same welfare impact as preceding procedures of the same nature.

Diminishing: Each procedure applied to an animal produces a less severe impact compared with preceding procedures of the same nature (decreasing cumulative severity, asymptotic severity, tolerance with repetition).

Increasing: Each procedure applied to an animal produces a more severe impact compared with preceding procedures of the same nature (increasing cumulative severity due to, for example, hypersensitization).

Table 10 summarizes the responses obtained.

Table 10. Summary of responses on the welfare impact of procedures undertaken over a long period of time (several years).

Species	**Macaques – *M. mulatta**** **N=152**				**Common marmosets – *C. jacchus*** **N=82**			
Cumulative severity associated with:	Number reported	Unchanging (%)	Diminishing (%)	Increasing (%)	Number reported	Unchanging (%)	Diminishing (%)	Increasing (%)
1. Anaesthetic	120	107 (89)	12 (10)	1 (1)	67	67 (100)	0	0
2. Surgery	100	98 (98)	1 (1)	1 (1)	67	67 (100)	0	0
3. Restraint/handling	149	19 (13)	127 (86)	3 (2)	80	9 (11)	70 (88)	1 (1)
4a. Food control	97	84 (87)	12 (12)	1 (1)	0	0	0	0
4b. Fluid control	62	46 (74)	15 (24)	1 (2)	0	0	0	0
5. Housing / husbandry	149	95 (64)	48 (32)	6 (4)	81	76 (94)	4 (5)	1 (1)
6. Long-term implant maintenance	75	62 (83)	6 (8)	7 (9)	29	21 (72)	1 (3)	7 (24)
7a. Training chair	73	18 (25)	52 (71)	3 (4)	31	10 (32)	19 (61)	2 (6)
7b. Training task	138	24 (17)	112 (81)	2 (1)	77	21 (27)	56 (73)	0

*includes three *M. fascicularis* (cynomolgus).

There is no systematic evidence for increasing severity in the returns on surgery by practitioners. Practitioners do not report that increasing severity arises from repeated surgical and other procedures, and several comments specifically refute the concept.

Interaction between different procedures

The questionnaire asked: *"Which of the above procedures / husbandry* interact *and impact upon each other in a consequential unchanging, diminishing or increasing effect?"*

There were 40 different detailed text responses that were relevant to this question. Of these, 27 were of a general nature related to overall experience and were sometimes entered in the same format for each subject; 13 referred to specific subject histories. From the responses it was clear that housing, husbandry, handling, training, restraint and task all interact, but this is in general a positive interaction and, for example, a positive experience with training may help the monkey habituate to restraint. There were a few (six) negative comments; these referred to single cases.

Conclusion

There was little evidence for additive effects between procedures whether through incomplete recovery between events ('stacking') or potentiation of adverse effects and suffering by earlier procedures. Some animals showed diminished responses to repeated procedures (habituation; for example, in macaques, 86% for restraint and handling, 71% for the training chair and 1% for surgery).

Specifically, there was little evidence in the majority of monkeys to suggest that *"the nature of pain, suffering, distress and lasting harm caused by (all elements of) the procedure, and its intensity, the duration, frequency and multiplicity of techniques employed"* and the *"cumulative suffering within a procedure after applying all appropriate refinement techniques"* (Directive 2010/63/EU) led to a general increase in severity category.

However, there were some nonhuman primates (see Sections 5.11 and 5.13) that could not cope and were removed from study. In a small minority of cases, premature euthanasia was performed as part of the terminal phase.

6 Visits to establishments

Visits to the UK Research establishments undertaking academic non-human primate neuroscience work were organized a few weeks after the initial closure of the database website. The Visiting Group consisted of at least three members of the Working Party, who had no local conflict of interest, and at least two inspectors from the Animals in Science Regulation Unit Inspectorate (ASRUI). The Visiting Group met with a combination of personnel in each institution, including certificate holders, PIs and collaborating scientists, NVSs and NACWOs. The following summary was compiled from the visitors' notes.

- **Cloud – based questionnaire and database**: Many establishments had chosen to use a teamwork approach to enter subject data, where data were collected (usually by the scientist, project licence holder) and then reviewed by the NVS and animal care staff prior to entry. Not all had entered information in the user database and again a collated group response was used in some cases. Researchers complained that the format of the questionnaire did not allow the reporting of positive effects associated with procedures (for example, socialization during the experimental task). Limitations of time had restricted access to the database; the website was subsequently reopened to allow more datasets to be entered.

- **Housing**: A significant problem identified during discussions was fighting, where wounds may need to be sutured. This was a consequence of the benefit of group- / pair-housing. There was reluctance to house the animals in larger groups due to a greater extent of bullying seen in this context compared with pair-housing. However, there was some age / sex dependence in these evaluations, with compatible housing being less of a problem with the females and young animals. Planning was required where experimental termination dates were not close together, to prevent / reduce single-housing. In some cases it was necessary to keep an animal longer than scientifically necessary as a cage mate to another animal. When necessary, staff tried to pair an obvious subordinate with an older animal. When changing groups, staff used supervised playtime to introduce animals and used neighbouring cages to accustom animals to each other. Short periods in the same cage followed, which were constantly supervised and then extended to the 'sleepover', but it was clear that they needed to be monitored carefully for several weeks. Greater space and escape routes / visual barriers were likely to reduce fight injuries as animals were not forced into very close proximity. This was resource intensive. Where a unit is running close to capacity this can prove challenging for staff and the space to provide an appropriate layout, including escape routes and visual barriers, that help to reduce fight injuries.

  Conclusion: Some spare capacity for housing and staffing in this type of work is highly desirable but requires appropriate financial support.

- **Training**: It was reported that the most stressful time was early in the training phase. Positive reinforcement techniques (PRTs) were being used in some units for all phases of use. Much of this was reported to be 'led by the non-human primates themselves' – they seemed to feel that they had some control over the situation. It was reported that initiatives from the NC3Rs, such as primate welfare workshops (www.nc3rs.org.uk/event.asp?id=1825; 2012), had encouraged this development. The constraint to additional work was budgetary. There still remained some 'negative' reinforcement, for

example, for older animals that had been trained in this way, but warning was sufficient in most cases, for example, waving a broom induced the required movement and rattling cages was rarely required. It was suggested that the use of cues such as rattling a cage for a command was not necessarily negative. It was suggested that there were benefits of using a method that worked well for a particular animal through historic use, even if it was not seen as current best practice. However, most would accept that best contemporary practice should replace this for new animals.

The need to treat animals as individuals was emphasized. In some cases, playing constructively on competition between them has been used to facilitate performance. The importance of routine and giving the animal some control over the experience was explained. It was perceived that PRT is a slower approach than the 'forced' approach. The need to recognize conflict between getting things done quickly (for science and welfare) and keeping the animals in the laboratory longer was voiced. Some hoped that the later phases of training would be quicker, as the animals will have learnt more about the principles of what is required. However, no data were yet available and they may be difficult to compare. Staffing levels in different groups meant that PRT was not always possible. It was very person-dependent and needed skill and continuity. The practicalities of high staff turnover, for example, PhD students, were mentioned. Some reported that task performance and training appeared to be 'the highlight of the day' for a non-human primate, and that this was the best form of stimulation for some animals. Clearly this was task- and motivation-dependent. Additional motivation such as fluid control was required in some cases to deliver both the quantity and the quality of trials required for scientific investigation (see Appendix 8.6).

Conclusion: Tasks and motivators are designed to make behavioural testing a positive experience for animals wherever possible. It is essential to have staff well versed in such training techniques.

- **Animal selection**: It was reported that some animals do not perform well from the outset. Some of these animals are used in terminal experiments (that is, not used according to the original plan but some scientific benefits are still acquired). Careful discussions between breeding colony care staff and scientists, and in some cases by visits from the scientist to the colony, have allowed selection of animals deemed likely to succeed in behavioural studies. Over recent years, these steps were thought to have reduced the 'failure rate' of non-human primates unsuited to the procedure. As an illustration of a well-motivated subject, a monkey was seen that had already had its daily quota of fluid, but still came out of its home cage into the training cage, suggesting that monkeys do not make this transfer only when the extra motivation of anticipated fluid rewards is present.

Conclusion: Animals found to be unfit or less suitable for long-term studies should be replaced rather than persisted with. Funding bodies should recognize the potential need for increased resources for selection and replacements.

- **Anaesthesia and surgery [see also Section 5.10 on Refinements]:** The benefit of having general anaesthetics administered by experienced veterinary anaesthetists was commonly reported. Improvements in anaesthesia, post-surgical care and pain recognition were reported and

methods of diagnostics are improving, particularly imaging, which may be able to establish the extent of an infection, or give early warnings of problems. It was reported that the level of care is much higher within a UK non-human primate research institution than in many veterinary practices. There has been significant development in the field of recovery and post-operative treatment, allowing more complex techniques to be performed.

An international workshop (www.nc3rs.org.uk/downloaddoc.asp?id=1828; 2012) on refining the use of head implants was followed up by the NC3Rs convening an Expert Working Group and arranging for a consultant Maxillo-facial surgeon to observe surgical procedures in monkeys. Discussion about head implants suggested that it is probably advantageous that the implant and fixing screws should integrate with the skull bone. However, there is some reluctance to move from non-integrating methods, for example, those using bone cement. The reason given was 'why change a winning team?' It was discussed whether coating the implant (for example, with hydroxyapatite) would improve integration (Adams *et al.*, 2011) although more research in this area is needed.

There was plenty of other evidence of welfare improvements and refinements, and much active research into developing better outcome measures for pain and stress that might be associated with the different procedures (see section 3.6). Videos were shown of the methods used to allow recordings from two monkeys. The recordings involved some body restraint, but no head restraint. There was little obvious difference in the general behaviour of these two monkeys, although later it was revealed that one had been in procedure for 3 years, the other for only 18 months. However, the visiting expert behavioural primatologist was able to detect some differences.

Some monkeys were in very long-term procedures (longer than 5 years) but it was pointed out that these animals showed no signs of stress. If stress was observed, it was generally in the early part of the experiment, when associated with new housing and the initial training and surgical procedures. Researchers argued strongly against a set time limit on the duration of procedures in non-human primates, which they argue are clearly manageable by non-human primates.

Conclusion: Opportunities should be sought to acquire, increase and share expertise to reduce any consequences of head implants. There is scope for further collaboration with non-human primate behavioural scientists over refining behavioural outcome measures.

- **Severity:** The need to take a holistic approach to severity assessment was discussed. This should be on a monkey-by-monkey basis and take into account whatever quantitative and qualitative data are available. The key was knowledge of the normal behavioural pattern of a particular animal. The question was put to users: *"How long does an non-human primate have to be on a Moderate protocol before it becomes Substantial?"* ASRUI explained that it was considered that this has to be done on an individual basis. It is not directly dependent on the duration or number of surgeries but on the non-human primate's responses to them. It was considered not as a snapshot but

as a process. Impacts need to be defined, for example, for surgery there may be an impact for one week but over the lifetime of the monkey it did not appear to have a significant impact. It was reported that there is a perceptual difference between acute episodes and chronic consequences, which needs to be clarified. A distinction needs to be made between expected or predicted consequences (intended adverse events resulting from a procedure, such as a lesion) as opposed to unexpected or infrequent complications. This is routinely done in the pharmacovigilance field.

It was suggested that cumulative *well-being* should also be included in the assessment of severity. Non-human primates like routine, they learn to know what to expect. There were many observations suggesting that non-human primates may actually look forward to performing the experimental tasks. There was also discussion about whether this was just resignation on the part of the non-human primate in having to do the task, but this was not thought to be the case. The speed with which animals want to enter the chair / box are highly suggestive of willingness and not resignation.

There was reluctance to draw arbitrary decisions, for example, the number of anaesthetics / surgeries / time due to the variable responses seen. Some animals respond and recover well, but in others recurrent problems sometimes start a downward spiral. Some argued that if short-term costs are higher, then it may be better to allow a procedure to go on for longer once recovery has occurred, to give a better quality of life for longer. It was clear that there is much that is unknown about severity: Do positives outweigh negatives over the non-human primate's total life experience? If animals suffer (that much) do they forget it? Do they adapt? It should be clear what it is that is accumulating, if anything. There may be no ill effects to accumulate, once recovery from the first impacting event has occurred. It was reported that there is published evidence that repeated anaesthesia has diminishing consequences for horses and some other species. In most non-human primate studies, there will have been only one or a few Moderate events. There may then, in addition, be experience of Mild processes over a longer period, in which case, the overall life experience is different / better in later phases, especially if there is 'averaging' over the total period of the procedure. How this should be evaluated has not previously been formally determined by regulation. It was acknowledged that such a decision might be very subjective. The expected 'remaining' duration of the procedure also comes into the decision making process, where the welfare / endpoint decision is not critical, for example, if another two weeks will finalize data then euthanasia may be not be best option immediately, but in other cases that two weeks could equally take the animal beyond an acceptable endpoint, and termination should occur.

Users were asked why no single animal had been reported as Severe. A key issue here was the prospective assignment of severity limits. Some studies involving lesions were considered as more severe than most of the other work in this field, and as such would be classified as Substantial. It was pointed out that if all this non-human primate neuroscience work is classified as Substantial / Severe then there is no distinction between different types of procedure, some undoubtedly having more welfare impact than others. Users reported that a very high proportion of the work would be misrepresented according to the text in the Home Office ASPA Guidelines (Home Office,) referring to severity.

Concern was widely expressed over this severity limit issue in the academic research community using non-human primates. A minority opinion was expressed that a classification of Substantial for this work would be appropriate. Support to continue the work, even if it was reclassified, was common, always providing that the benefits justify the harms. Users wanted people to understand that non-human primate neuroscience research could be Severe, if carried out inappropriately. One of the benefits of a Moderate limit is that it is required that harms are kept within an understood lower band. At one establishment, the NVS made a very clear statement that putting non-human primate research into Severe is bad news for non-human primates, and that it would be better to use the boundary between Moderate and Severe limits as a protection for the non-human primates and as an additional cue to take action if poor welfare develops. Such a mechanism provides the NVS with legal back-up to insist on preventative action to stop a given non-human primate entering a Severe status.

The culture in EU non-human primate research might have to change if the current Federation of European Laboratory Animal Science Associations (FELASA) Table of Procedures considered to carry a Severe limit were to be changed (Annex VIII section III of Directive 2010/63/EU). In the opinion of FELASA (Guillen 2012), these examples are limited, have little descriptive power to aid assignment and relate to the procedure itself rather than to the assignment of outcome (for example, adverse effects of the procedure). For the moment, however, this Table does not include the neuroscience protocols in non-human primates that are the subject of this Review. Such protocols are also not listed in the draft guidelines for the new ASPA. There is considerable motivation in the community to maintain high welfare standards in order to avoid crossing the 'psychological barrier' between Moderate and Severe. Having such a barrier helps in the application of humane endpoints. Some felt that the practice of issuing non-human primate project licences with a Substantial/Severe limit was devaluing the efforts of researchers who are trying to improve practice to reduce harms. Others disagreed. In some cases, it was considered only as being Substantial if the procedure went wrong (that is, unexpected and unwanted adverse events) in the last week or so of the procedure.

The animal technicians that the Visiting Group met were very professional and caring. The Group was impressed by their dedication to care, which often means that technicians and/or scientists work through the night or at weekends to ensure good post-operative recovery. Technicians felt that a 'Severe' limit on the work that they are doing would completely misrepresent their work, and one said that she would feel unable to continue the work if it were all upgraded and was given a Severe limit. Researchers / NVSs stressed that their role was always to intervene in a situation where a given non-human primate might cross the line between Moderate / Severe. They agreed that a Severe limit might have the unintended consequence of longer suffering than under a Moderate limit.

Conclusion: Assignment of severity categories should be evidence based.

- **Retrospective assessment**: There was discussion about retrospective assessment, events used to classify it, and the criteria that would be used for an allocation to a different classification.

  Conclusion: The nomenclature relating to evaluation of severity should be clarified and criteria set to promote consistency in allocation prospectively and retrospectively.

7. Discussion, future trends and conclusions

7.1 Qualitative assessment of cumulative experience

Directive 2010/63/EU does not define 'cumulative suffering' and does not explicitly use the terms 'cumulative severity' or 'cumulative experience'. Reference to multiplicity and to previous use suggests that some aggregate assessment of suffering is intended and reference to training suggests that a more complex assessment is also intended.

Section 3.4 (Figure 5) provides an overview of the differences between:

- non-additive effects where there is complete recovery between events;
- non-additive effects with habituation and complete recovery between events;
- additive effects with incomplete recovery between procedures ('stacking up'); and
- additive effects of procedures that are compounded by potentiation of suffering by earlier procedures.

The concept of cumulative severity / experience must take into account:

- the simple quantity of techniques that cause pain, suffering distress or lasting harm that are applied to an individual animal;
- the intensity of impact of each of those techniques (peak);
- the potential for recovery between the techniques (return to previous 'baseline');
- an assessment of any sensitization (increasing impact) or habituation (decreasing impact) of each (effect on peak);
- potential effects, positive and negative, of ongoing environmental issues (baseline effect and potentiation);
- whether one significant unrelated life event (for example, early maternal separation or painful experience when a neonate) is likely to have affected how pain or distress are experienced in the long term (potentiation);
- the potential with prolonged inter-event intervals that memory of events is altered, possibly reduced (in Figure 5 area under curve impact *versus* time may not be an accurate assessment of long-term impact).

It is improbable that any of the models presented in graphical form (see Section 3.4) can account for all of these factors, but they should still be useful in the overall, holistic assessment of cumulative severity if their limitations are recognized and account made of other relevant factors. A holistic assessment without such quantification would not be helpful.

7.2 Quantitative assessment of cumulative experience

Many attempts have been made to quantify severity by score sheets especially when multiple consequences may occur, each of which may have different intensities (see, for example, LASA, 1990; Morton and Griffiths, 1985; Smith and Boyd, 1991; Smith, Hadzic *et al.*, 2006). These are often produced so that a definitive endpoint may be set for experiments where a single outcome measure is clearly insufficient. It is notoriously difficult to produce useful assessment matrices,

and those that have been devised often need to be modified after provisional use in an altered situation, mostly because it is discovered that the weighting given (if any) to an element does not accurately reflect the importance to the clinical condition. However, this Review's dataset allows this important issue to be revisited.

This Review has provided the first detailed account of the long-term cumulative impact of multiple neuroscience-related procedures in non-human primates using a structured and objective approach. This unique dataset is based upon quantitative data extracted from records and respondents' impressions of favourable and adverse effects and severity, together with details of the checks and balances to ameliorate suffering. The Review's questionnaire provides a useful first step in establishing a common methodological framework that facilitates systematic data collection across institutions. The Review's questionnaire has facilitated the deconstruction of the lifetime experience of a large number of different experimental primates.

In addition to defining the impact and ameliorating mechanisms for each episode in the subject's life, discrete domains explored in the questionnaire included:

- non-procedural 'life' events (contingent, non-associated consequences, for example, intercurrent infection or injury from fighting in group-housed monkeys);
- generic expected effects (typical recovery from a licensed general procedure);
- intended (for example, a lesion or disease induced as the target of the study);
- complications (for example, infection).

Due to the limitations of retrospective reporting over a ten-year time period and the short response time frame, the first three domains were captured semi-quantitatively, with users stating the presence or absence of an impact in each domain. Further qualification was included as free text, which was subsequently evaluated by the Working Party as presented in earlier sections of this report.

An example to illustrate the potential quantitative analysis of unintended complications as a basis for standardized scoring and reporting:

Unintended procedure-related complications also included event frequency thereby enabling more quantitative evaluation of this domain. A pragmatic weighted scoring system was devised to take into account both the relative magnitude and cumulative severity effect of each complication in order to explore the potential of such an approach (Table 11). A weighting value was assigned to reflect the relative severity of each complication type, and the cumulative multiplier to act as a modifier in the event of repeated complications of the same type. A cumulative multiplier equal to one denotes unchanging severity, and greater than one, increasing cumulative severity. It should, however, be noted that this approach does not account for any cumulative effects resulting from interactions between different complication categories, which would require exact event timings that were not available in the dataset. These weightings were sense-checked with clinical colleagues and non-human primate practitioners, but it is important to emphasize that they are simply illustrative at this stage.

Table 11. Weight scoring system to take account of relative magnitude and cumulative severity of a complication.

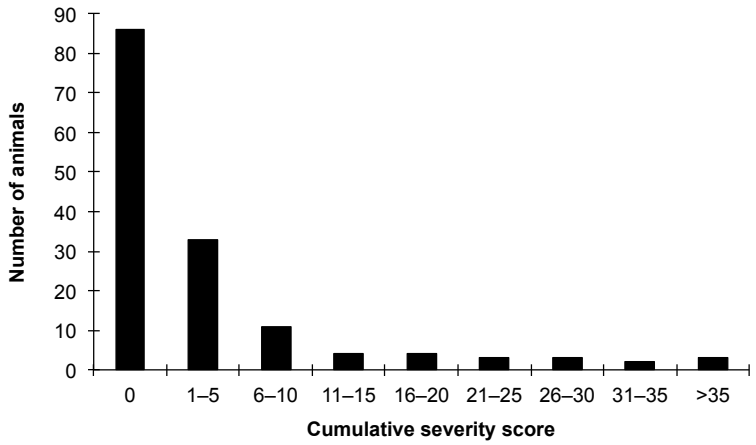
	Weighting (w)	Cumulative multiplier (c)
Infection		
Skin - local antibiotics	0.5	1
Skin - systemic antibiotics	2	1.2
Bone	5	1.4
Meningeal / Brain	8	1.5
Seizure		
Partial	1.5	1.1
Generalised	3	1.2
Haemorrhage	4	1.3
Neurological impairment		
Paralysis	5	1.3
Immobility	2.5	1.2
Deficits	5	1.2
Other immediate effects	2	1.1
Other long-term effects	7	1.3

To calculate an overall severity score (S), repeated complications of each type were modelled as a sum of a geometric series using the weighting (w), cumulative multiplier (c) and number of events (n) as parameters:

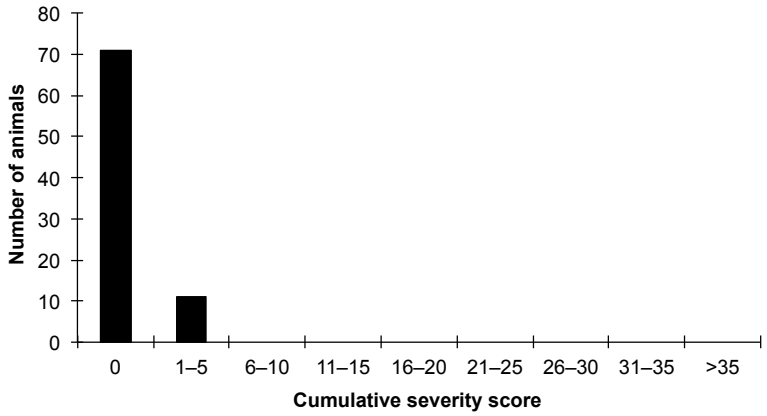
$\mathbf{S = w(1-c^n)/(1-c)}$ (except if c=1, where S=nw)

Figure 16 illustrates the severity score distribution (graphs), together with the relative contribution of each complication category to the overall scores (pie charts) for both macaque and marmoset subjects.

Macaque cumulative severity score distribution



Marmoset cumulative severity score distribution



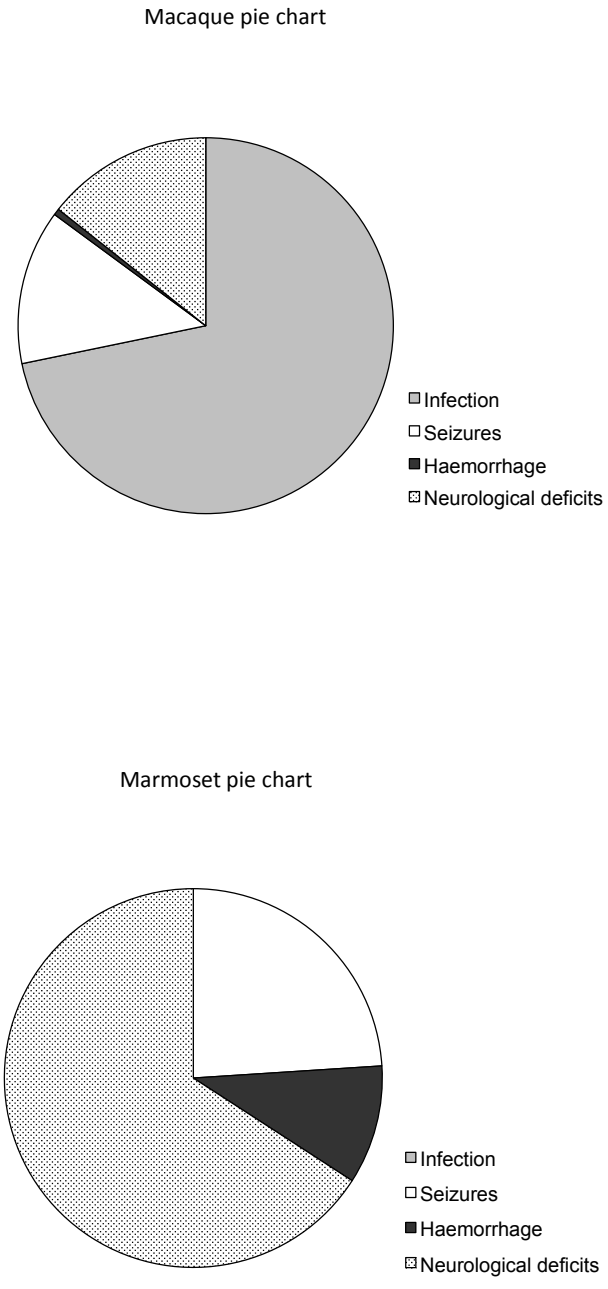


Figure 16. Severity score distribution (graphs) and relative contribution of each complication category to the overall score (pie charts) for macaques and marmosets.

Complication rates and severity scores were also correlated with the user-reported severity classification for each animal. Notably, no complications were reported in any animals with a Mild severity classification (Table 12).

Table 12. Incidence of complications and severity scores in relation to the severity classification for each macaque and marmoset.

	Complication incidence		Severity score	
	Mild	*Moderate*	*Mild*	*Moderate*
N	32	117	32	117
Mean / Median	0 / 0	3.7 / 1.0	0 / 0	6.2 / 0.5
Range	0 – 0	185	0	104

	Mild	*Moderate*	*Mild*	*Moderate*
N	14	68	14	68
Mean / Median	0 / 0	0.2 / 0	0 / 0	0.6 / 0
Range	0 – 0	0 – 3	14	0 - 5

These data are presented for illustrative purposes only to demonstrate how severity scores may be calculated. It is important to note that only complications have been included and not the procedures or contingent effects. The incidence and overall severity of unintended procedure-related complications presented in Section 5.6.4 is low, and compare favourably with surgical complication rates in veterinary and human neurosurgical practice.

Comparison of the risk of infection after nonhuman primate neuroscience procedures with veterinary and human surgery.

There are significant issues with definitions, depth and scale of surgical site infection, degree of contamination, duration of procedure, prophylactic antibiotics, age, co-morbidity and length of follow up so that any comparison can only give an order of magnitude. There is very little published data on veterinary neurosurgery practice.

Examples of risk of infection after veterinary surgery:

- minimally invasive abdominal and thoracic surgery 1.7% (dogs and cats; Mayhew, Freeman et al 2012)
- open abdominal and thoracic procedures 5.5% (dogs and cats; Mayhew, Freeman et al 2012)
- clean surgery in dogs and cats 4.7% (Brown, Conzemius *et al* 1997)
- orthopaedic surgery in dogs, cats and horses: arthroscopy 0.5%; total hip arthroplasty 2.5 to 10% (Weese 2008).

- Ventriculoperitoneal shunt for hydrocephalus in cats and dogs 8.3 % (Biel, Kramer et al 2013).

Examples of risk of infection after human neurosurgery relevant to nonhuman primate neuroscience procedures:

- Intracranial intraparenchymal pressure monitor for a few days 3.7% (Rebuck, Murry et al 2000)
- Hydrocephalus shunt requiring further surgery 4.7% with plain catheters; 3% with antibiotic impregnated catheters (Richards *et al* 2009)
- Intracranial pressure monitoring via a ventriculostomy catheter for a few days 11.3% (Rebuck, Murry et al (2000)
- Implantation of subdural electrodes to monitor epilepsy 12% (Hersh, Virk et al 2013).
- Cranioplasty (insertion of a bone or artificial plate to repair large skull defects) 12.1% (Walcott, Kwon et al 2013)
- External ventricular drainage using a plain catheter for a few days to weeks 21.4%; 12.3% with a silver impregnated catheter (Keong, Bulters et al 2012).

7.3 Considerations for establishing ongoing monitoring of cumulative experience

Overall, whilst little evidence was found for cumulative suffering within or between any of the main areas of concern, the results of the questionnaire highlight the need for more standardized methods of assessing and quantifying the impact of non-procedural, generic and intended effects resulting from non-human primate experimentation.

This review has demonstrated the feasibility of securely collecting a defined non-human primate dataset from multiple institutions for central analysis and comparison. This raises the prospect of establishing long-term retrospective reporting and assessments of a similar nature to human disease registries (Bridgewater, Keogh *et al* 2008; Richards, Seeley *et al* 2010) thus enabling both inter-establishment and temporal evaluation of cumulative experience on an ongoing basis.

Human disease registries that have proven sustainable over many years show how important it is to choose an appropriate method for collecting data on an ongoing basis, with a trade-off between logistic / economic feasibility, and data accuracy / completeness (Figure 17).

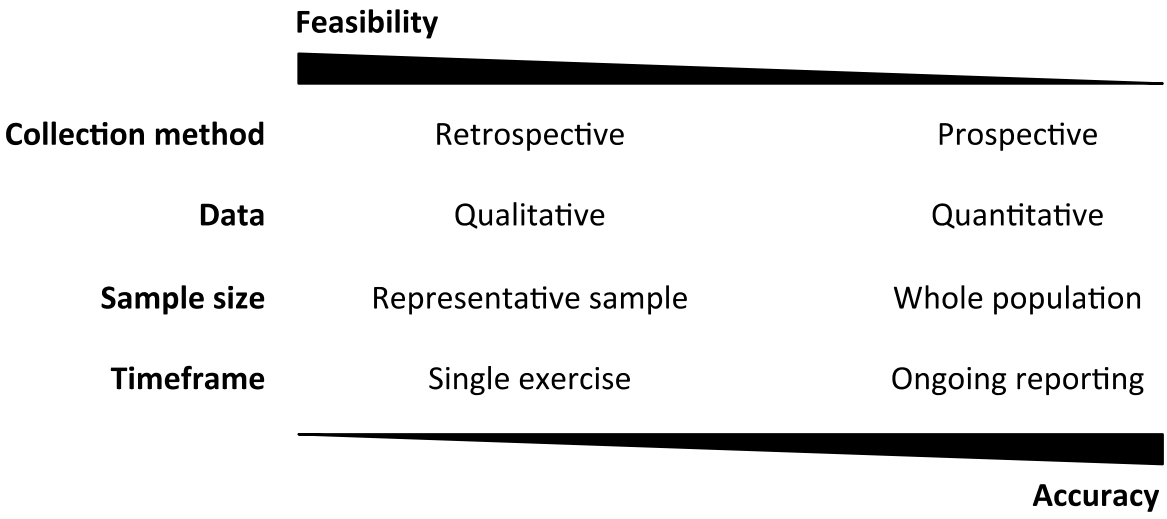


Figure 17. Trade-offs in data collection methods.

Data collection: Data items need to be consistent and easily recordable in order to ensure objective quantification that can be evaluated in an operator-independent manner. At a minimum, this would require each reported category to be assigned an ordinal variable to denote severity, which could be used for scoring computation and thus enable automated analysis. Preserving free text qualification is also important in order to enable users to enter detailed and / or contextual information that may not become apparent from a defined scoring system.

Parameter determination: Numerical values assigned for quantitative parameters within scoring systems, such as severity, weighting and cumulative effect, should be evidence-based. Whilst explicit experimental determination represents a gold standard, other approaches need to be considered when appropriate, including observational studies, correlation with clinicopathological data and expert consensus, for example, using a Delphi approach. Recognizing that each method will be associated with different degrees of confidence, establishing defined levels will be necessary in order to guide critical interpretation and future areas for improvement – this is already well established for levels of evidence used to appraise clinical evidence in medical practice in a critical way. Irrespective of the method of determination, scoring systems should be validated against other benchmark data to demonstrate their discriminatory utility.

Collection frequency: As most subjects are used over a long period of time, some form of ongoing reporting will be preferable to a single snapshot. Infrequent collective assessments are also likely to miss out on quantitative detail. Ideally, discrete events such as procedure-related effects should be reported prospectively, whilst more general welfare and non-procedural life events assessed and reported at regular intervals.

Scope: Given the relatively small numbers of subjects in diverse experimental protocols, entire population reporting will be required to obtain accurate results.

Preserving granularity: Preservation of individual data items throughout the collection process should be favoured over aggregate reporting between stages. Aside from the obvious benefits of increased accuracy, a detailed dataset enables global comparison between user-determined, physiological, clinicopathological parameters, thus accelerating the development and validation of novel outcome measures. It is also a requirement under

the Directive 2010/63/EU (Paragraph 33) to provide a personal history from birth covering the lifetime for each non-human primate.

Temporal evolution and active monitoring: Two important limitations arising from the retrospective nature of this Review are:

- the difficulty in accurately assessing lifetime evolution of cumulative severity; and
- the appreciation of the inter-dependence and interaction between different domains of cumulative severity.

Establishing ongoing reporting with standardized and quantifiable measures as described in this section creates a potentially unique opportunity for active determination of cumulative severity in real time.

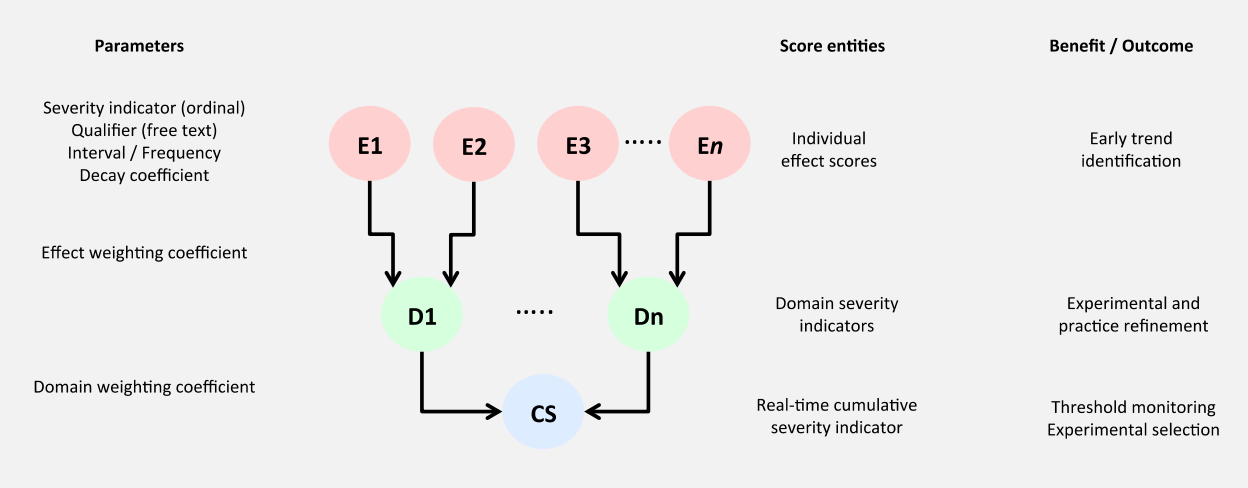


Figure 18. Conceptual model for ongoing determination of cumulative severity.

The impact of each individual effect can be quantified by an ordinal severity indicator, interval (for fixed regular assessments such as husbandry practice) or frequency (for discrete events such as complications), and a decay coefficient representing persistence over time. Individual effects can be weighted into respective domains scores which can be similarly combined to obtain a cumulative severity score at a particular point in time.

The temporal determination of severity derived from such a model can provide a quantitative approach to determine which of the scenarios depicted in Figure 5 apply for each experimental context. Furthermore, prospective determination of severity will enable monitoring for pre-determined severity thresholds on an individual subject basis in contrast to current pre-determined thresholds based on probability and prediction. Animal selection for specific experimental protocols can therefore be tailored accordingly in order to prevent and / or minimize the progression of severity at an individual level, thus addressing and informing all three potential scenarios presented in section 7.4 below.

7.4 Thresholds for designation of severity

There are various relevant scenarios including:

- single procedures that are anticipated, confirmed in retrospect and licensed to be Severe *per se*;
- single procedures that are unlikely to be Severe but, in occasional cases where they become Severe, the subject will be killed as part of a terminal experiment;
- licensed multiple procedures over a prolonged period of time during which no episode ever approaches the level of severity that would be classified as Severe if it were a stand-alone procedure.

Current (2013) regulatory procedures already deal with the first two scenarios. The Review found that the majority of non-human primates undergoing neuroscience research in the UK display little or no reported evidence of cumulative (negative) severity as defined in Figure 5, Section 3.4. None the less, some cases of cumulative severity of suffering were found for a small minority of monkeys.

There is no doubt that, for many lay people, the claim that such prolonged procedures can be carried out without cumulative (negative) severity may be difficult to believe. Too often the public is uninformed about what really goes on in primate experiments. For example,

- Animals are increasingly chosen for their suitability and aptitude for research procedures. Animals that are unsuitable are not then exposed to a prolonged training period.
- The past decade has seen major refinements that have reduced the severity and duration of individual components of a neuroscience protocol, including husbandry, anaesthetic, surgical and training technologies.

The public may be unaware of the pain and suffering caused in human patients by procedures similar to those used in monkeys. Procedures such as drilling a hole in the head to implant an intracranial pressure monitor or external ventricular drain, a small craniotomy or immobilizing the unstable neck with a halo fixation vest are fearsome procedures when first described to the public. In practice, such procedures, after completion under a general anaesthetic, are well tolerated even by children and require only simple analgesic drugs for two to three days.

It is important when ascribing a severity level to distinguish between the whole of life experience of the animal and the terminal phase of the experiment. All concerned need to ensure that, if severity increases at the end of a study, then the animal should be killed as soon as it is clear that the problem cannot be ameliorated.

It is for these reasons that the Review suggests that the Animals in Science Regulation Unit Inspectorate (ASRUI) addresses these scenarios and considers defining the criteria for designating categories of cumulative severity and experience that include unequivocal principles and examples to distinguish Mild from Moderate and Moderate from Severe levels of severity in both prospective and retrospective assessments based on the four scenarios illustrated in Figure 5.

In section 3.4, the use of score sheets that covered multiple dimensions was discussed (LASA 1990; Smith and Boyd 1991; Morton 2000; Smith *et al* 2006). These score sheets allow decisions to be made often before potentially serious problems arise (Smith and Boyd

2006). However, are there cases where overall quantification across multiple dimensions would detect subclinical cumulative severity even earlier?

Using the principles discussed in sections 3.4 and 7.3, a simple grid for repeated assessments is proposed based on generally accepted behavioural and physical welfare determinants identified through the users' comments and table 7. This welfare tool is comparable to the score sheets of Smith et al (2006) and a similar approach was proposed by Hawkins et al (2011). It is intended to provide an example of a standardized method of quantifying cumulative severity using the approach illustrated in Figure 5.

Despite the potential pitfalls of combining physiological parameters to produce a simple metric of severity discussed in 4.1.1, the validation of domain-based subjective assessments in determining human quality of life in discrete domains (e.g. physical, emotional, social) provides support to the case for developing and validating a simple ordinal welfare scale for NHPs used in biomedical research (WHOQOL Group 1995. Shaffer *et al* 2010).

The individual items within the tool are divided in to 3 domains (general welfare, abnormal behaviour and impairments) which are intended to capture the impact of intended deficits, non-procedural effects and complications, thus accounting for individual variation between animals. Procedural effects are accounted for separately, using benchmark rates of recovery which are less likely to demonstrate individual variation.

Table 13: Welfare assessment tool

General welfare	**Normal**	**Mildly affected**	**Severely affected**
Appetite			
Food intake	0	1	2
Water intake	0	1	2
Physical condition			
Fur state	0	1	2
Body weight ratio	0	1	2
Behaviour			
Peer interaction	0	1	2
Human interaction	0	1	2
Grooming	0	1	2
Cage behaviour	0	1	2
Mobility	0	1	2
Task engagement	0	1	2

Abnormal behaviour	**Absent**	**Mild / Infrequent**	**Severe / Frequent**
Restricted repertoire	0	1	2
Restlessness	0	1	2
Lethargy	0	1	2
Stereotypy	0	1	2
Urine / Faecal ingestion	0	1	2
Complication – related Impairments	**Absent**	**Mild effect**	**Severe distress**
Infection			
Non-CNS	0	2	4
CNS (bone / brain)	0	2	4
Hemiparesis			
Upper limb	0	2	4
Lower limb	0	2	4
Seizures			
Partial / generalised	0	2	4

Combining the sum of all procedural scores and current welfare score at one point in time gives an indication of current severity (potentially useful for planning the timing of successive procedures), whilst the combined area under the curve indicates cumulative severity over the animal's lifetime (Table 14):

	Overall welfare	**Procedural effects**	**Total scores**
Point assessment	Welfare Point Score (WPS) - Derived from assessment tool	Procedural point score (PPS) - $B \times D^T \times C^N$	Point severity score (PSS) - WPS + Σ(PPS)
Cumulative assessment	Welfare Cumulative Score (WCS) - Σ(WPS x Time Interval)	Procedural Cumulative Score (PCS) - $B \times C^N \times (D^T/\ln D)$	Cumulative severity score (CSS) - WCS + Σ(PCS)

B: Baseline Indicator; D: Decay Coefficient (0-1, 0 = immediate recovery, 1 = no recovery);

C: Cumulative Coefficient (<1 = decreasing cumulative severity, >1 increasing cumulative severity);

T: time from procedure (in days); N: number of preceding similar procedures

Further refinement and validation of quantitative scores could be achieved with cross-reference to known benchmarks of mild, moderate and severe documented cases once a consensus is reached on what these might be. However, benchmarks for neuroscience were not defined in Appendix VIII of the Directive 2010/63/EU.

The Review considers that a template for retrospective reporting may be helpful in this process and may allow recognition of recent refinement of practices and procedures to prevent progression of severity. The Review recommends that such a template should draw on the experience gained from development and use of the Review's questionnaire and should include the following elements:

- an overview of the animal's lifetime experience with key events and quality of the environment, including the benefits of any refinements that have been developed;
- a log of adverse events (non-procedural, generic and intended effects of the procedure and complications) including their impact on welfare;
- results of the post-mortem examination.

It would then be possible to engage with the type of validity, responsiveness and reliability exercise that has been widely used in clinical medicine (WHOQOL group 1995; Shaffer et al 2010) and advocated for application to nonhuman primate welfare by Morton (2000) and Nystrom *et al* (2001).

7.5 Vocabulary.

The Review regrets that the opportunity was not taken in Directive 2010/63/EU to modestly extend the vocabulary that is used to describe severity limits and render it less restrictive. There is clearly a distinction to be made between Moderate, *Multiple Moderate without significant impact on welfare,* and Severe.

The Review suggests that the vocabulary of the EU Directive requires clarification of the interpretation of the phrase *"the maximum expected severity likely to be experienced"*. This phrase introduces the concept of the probability that a particular severity limit might be reached based on the documented outcomes of individual investigators and institutions. This is an important distinction from the previous method of assigning severity limits based on *"the worst case scenario even if only one animal was expected to experience that level of adverse effects, whatever the number of animals used in the experiment"*. The *probability* of what might happen will have to be established through an iterative process based on the documented outcomes of individual investigators and institutions obtained through retrospective reporting.

8 Appendices

8.1 Membership of the Working Group.

Chairman: Professor John Pickard, FRCS Eng, FRCS Edin, MChir, FMedSci, is Professor of Neurosurgery and Chairman of the Wolfson Brain Imaging Centre (University of Cambridge), NHS Divisional Director for the Neurosciences at Addenbrookes Hospital and NIHR Senior Investigator / Honorary Director of the NIHR Healthcare Technology Cooperative for Brain Injury, Professorial Fellow, St Catharine's College Cambridge, Honorary Civilian Consultant Advisor in Neurosurgery to the Army; formerly President, Society of British Neurological Surgeons, Chairman, Joint Neurosciences Council, President, Academia Eurasiana Neurochirurgica, Member of Council of the Academy of Medical Sciences, Chairman, International Advisory Board for SINAPSE, Member of working parties on Brainstem Death and Vegetative State (Royal College of Physicians) and Ethical Guidelines for Research in the Mentally Incapacitated (Royal College of Psychiatrists). He was a member of the Animal Procedures Committee (2005 – 2012) and Chairman of the Primate Subcommittee. His research is dedicated to advancing the care of critically ill patients after brain injury from initial illness through recovery from coma and rehabilitation to final outcome. His early research involved non human primates (baboons) which helped to lay the scientific foundations for the creation of the Wolfson Brain Imaging Centre and thereby facilitated studies directly in critically ill patients.

Professor Hannah Buchanan-Smith, PhD, is a Professor of Psychology who heads the Behaviour and Evolution Research Group in the School of Natural Sciences at the University of Stirling. She obtained a BSc (Hons) from the University of St Andrews, and was awarded her PhD at the University of Reading. She conducts fundamental scientific research on the behaviour, ecology, evolution and welfare of mammals in a range of captive environments, with a particular focus on refinement and improving the welfare of captive primates. She is a member of the Scottish Primate Research Group, the Primate Society of Great Britain's Captive Care Working Party, and formerly was a member of the International Primatological Society's (IPS's) Captive Care Committee with shared responsibility for rewriting the *IPS International Guidelines for the acquisition, care, and breeding of non-human primates*. She became a member of the Animals Procedures Committee in 2008, sitting on its Housing and Husbandry, and Primate subcommittees.

Mr Mike Dennis, BSc (Hons) Microbiology, Professional Certificate of Management, is a scientific leader in the UK Health Protection Agency (HPA). He provides scientific input into a number of project areas involved in the development of novel vaccines and therapies against diseases that threaten human health, and is also Head of the HPA's Primate Programme. Before joining the HPA he was a microbiologist at the Institute for Animal Health where he worked on a number of infectious diseases affecting cattle and pigs. Mike has published in the fields of human and animal health research and is on the editorial board of *Laboratory Animals.* He has been a member of the Animal Procedures Committee for four years and has served on the Housing and Husbandry, Primate, and Applications subcommittees.

Professor Paul Flecknell, MA, VetMB, PhD, DipECLAM, DipLAS, DipECVA, (Hon) DipACLAM, (Hon) FRCVS, qualified from Cambridge Veterinary School in 1976. He joined Bristol Veterinary School as a feline medicine resident, then moved to the Medical Research Council (MRC) Clinical Research Centre to be the vet responsible for animal welfare in its research facilities. He completed his PhD at the University of London, and is a Diplomate of the European Colleges of Veterinary Anaesthesia and Analgesia and of Laboratory Animal Medicine. He is an honorary Diplomate of the American College of Laboratory Animal medicine and an honorary Fellow of the Royal College of Veterinary Surgeons. Recently he was awarded an honorary doctorate by the University of Ghent. He is currently (2013)

Director of the Comparative Biology Centre at the University of Newcastle and is Professor of Laboratory Animal Science in the Institute of Neuroscience. His main research interests are anaesthesia and analgesia of all species of animals and in particular the development of methods of pain assessment. He has published papers, book chapters and text books aimed at improving the welfare of both pet, farm and laboratory animals.

Dr Alexis Joannides, MA, MB, BChir, MRCS, PhD, qualified in Medicine at the University of Cambridge, completing his PhD in the use of human stem cells in experimental models of neurological disease. He is currently (2013) a Clinical Lecturer in Neurosurgery at Addenbrooke's Hospital, Cambridge, and is the project lead of the Outcome Registry Intervention and Operation Network, a national Cloud-based platform for prospectively collecting and analysing patient outcome data following neurosurgery and other neuroscience-related interventions.

Professor Roger Lemon, PhD, FMedSci, is Sobell Professor of Neurophysiology at the University College London Institute of Neurology. He is a past Secretary of the Research Defence Society and Council Member of Understanding Animal Research. He is a Council Member of the Academy of Medical Science. He is actively engaged in the public dialogue on the responsible use of animals in biomedical research, with a particular focus on the use of non-human primates. His main research interest is the control of skilled hand movements by the brain and is prompted by the need to understand why hand and finger movements are particularly affected by damage to the cortex, and its major descending pathways, for instance as a result of stroke or in spinal injury. His experiments involve the use of purpose-bred non-human primates, since these provide the best available model for the human sensorimotor system controlling the hand. He has carried out parallel studies in normal human volunteers and has sought to apply the knowledge gained from his work in monkeys to the effects of stroke on hand function in patients, to understand the process of recovery and to investigate therapies that might enhance recovery.

Dr Mark Prescott, PhD, is Head of Research Management and Communications at the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). He leads the Centre's animal welfare and peer review programmes and was a Member of the Bateson Review Panel. A primatologist by training and Honorary Research Fellow of the Scottish Primate Research Group, his current (2013) research interest is the welfare of non-human primates used in scientific procedures. He has served on the Animal Procedures Committee for almost eight years and also serves on the captive care committees of the International Primatological Society and Primate Society of Great Britain, as well as on a number of institutional ethics committees and advisory boards.

Professor Wolfram Schultz, PhD, FRS, is Wellcome Principal Research Fellow and Professor of Neuroscience in the University of Cambridge. He graduated in medicine from the University of Heidelberg, Germany and did his postdoctoral training in Germany, the USA, and Sweden. He became a Professor of Neurophysiology at the University of Fribourg, Switzerland, moving in 2001 to his current (2013) post at the University of Cambridge. During his career, he has won the 1984 Ellermann Prize of the Swiss Societies for Neurology, Neurosurgery and Neuropathology, the 1997 Theodore-Ott-Prize of the Swiss Academy of Medical Sciences, the 2002 Golden Brain Award of the Minerva Foundation (USA), and the Ipsen Prize 2005 for Neuronal Plasticity. He is a Fellow of the Royal Society. He was a Receiving Editor for the *European Journal of Neuroscience* (1998–2003), has served on the Editorial Board of the *Journal of Neurophysiology* since 1997 and is an Associate Editor of the *Proceedings of the Royal Society (B)* since 2010. He has made contributions to the understanding of the reward functions of dopamine neurons and of neurons in other parts of the brain's reward system. He has also done important work on the relationship between reward and risk information in the brain, especially in relation to learning theory and neuroeconomics.

8.2 Acknowledgements and list of contributors

The Working Group of this Review is very grateful to Professor Sir Patrick Bateson FRS, Professor Martin Bobrow CBE FMedSci FRS, Professor Donald Broom, and Professor Ray Hill FMedSci for their review of early drafts of the Report.

We acknowledge the help of the many individuals and organizations that submitted information and data related to non-human primate neuroscience research. In particular, we are very grateful to the following individuals and organisations for their significant written and oral contributions during the consultation process:

Dr Caroline Bergman, Dr Laura Boothman, Professor Donald Broom, Dr Simon Glendenning, Professor John Gluck, Dr Steve Kennerley, Dr Andrew Knight (Animal Consultants International), Professor Angela Roberts, Dr David Smith, Dr Sarah Wolfensohn, Dr Paul Honess (Captive Care Working Party of the Primate Society of Great Britain), Ms Jessamy Korotoga (Animal Defenders International and the National Anti-Vivisection Society), Dr Gilly Stoddart and Mr Alistair Currie (People for the Ethical Treatment of Animals, PETA), Dr Katy Taylor and Dr Nick Palmer (British Union for the Abolition of Vivisection), Dr Elliott Lilley (Royal Society for the Prevention of Cruelty to Animals, RSPCA), Four Paws, Humane Society International, the Humane Society of the US and the Universities Federation for Animals Welfare, Dr Penny Hawkins (Deputy Head, Research Animals Department, RSPCA; member of the Animal Procedures Committee but not of the Primate Subcommittee or its Working Party) provided helpful comments on the Report but was not asked to nor has endorsed its content or conclusions.

We also acknowledge with enormous gratitude the input from the Animals in Science Regulatory Unit Inspectors for technical assistance and operational advice, but the interpretation and conclusions are those of the Animal Procedures Committee (APC) and members of its Working Party alone.

We are very grateful to Sara Nathan OBE, Chairman of the APC, and the Secretariat of the APC for their unfailing support and to Mr Martin Ducker for conducting the pilot literature search.

We acknowledge with thanks permission to reproduce figures 1 & 2 (Professor Sir Patrick Bateson), figure 3 (Professor Donald Broom and figure 4 (Dr Sarah Wolfensohn).

Budget.

We are very grateful to all those who gave so freely of their time and resources. The following expenses were incurred: Literature review (£100); Orion database (£588 for secure hosting, security certification and backup); Stakeholders meeting (£1948).

8.3 Glossary and definitions

ADI Animal Defenders International

APC Animal Procedures Committee

ASPI Animals Scientific Procedures Inspectorate (Home Office)

ASRUI Animals in Science Regulation Unit. Inspectorate (Home Office)

BBSRC Biotechnology and Biological Sciences Research Council

BUAV British Union for the Abolition of Vivisection

CCWP Captive Care Working Party

EU Directive (2010/63/EU)

HSI Humane Society International

HSUS The Humane Society of the United States

LASA Laboratory Animal Science Association

MRC Medical Research Council

NACWO Named animal care and welfare officer

NAVS National Anti-Vivisection Society

NC3Rs National Centre for the Replacement, Refinement and Reduction of Animals in Research

NVS Named veterinary surgeon

PETA People for the Ethical Treatment of Animals

PSGB Primate Society of Great Britain

RSPCA Royal Society for the Prevention of Cruelty to Animals

UFAW Universities Federation for Animal Welfare

Definitions:

Welfare: 'Welfare is the state of the individual animal as regards its attempts to cope with its environment. Hence it depends on the individual's needs in relation to physical, nutritional, social and other behavioural factors and, in the case of captive animals, on the people who care for the animals or supervise such work.

Suffering: A negative emotional state, which derives from adverse physical, physiological and psychological circumstances.

Cumulative severity: The sum of all the events and effects that impact, adversely, positively and by way of amelioration, on the welfare of an animal over its lifetime.

Cumulative experience: The sum of all the events and effects, including their quantity, intensity, duration, recovery between and memory thereof, that impact, adversely, positively and by way of amelioration, on the welfare of an animal over its lifetime.

Non-additive, habituation, additive stacking up and additive potentiation: A single procedure may have only a short-lasting impact on welfare. With sufficient time for recovery between procedures, there may be no influence on the impact of a second procedure (*non-additive*). The impact of repeated procedures may diminish (*habituation*). In contrast, if insufficient time is allowed for recovery, the residual effects of repeated procedures may add up (*additive stacking up*). Suffering from earlier events may actually increase the negative impact on welfare of subsequent events (*additive potentiation*).

Cumulative effect:

- unchanging – later procedures applied to an animal have the same welfare impact as preceding procedures of the same nature;

- diminishing – each procedure applied to an animal produces a less severe impact compared with preceding procedures of the same nature (decreasing cumulative severity, asymptotic severity, tolerance with repetition, habituation);

- increasing – each procedure applied to an animal produces a more severe impact compared with preceding procedures of the same nature (increasing cumulative severity due to, for example, hypersensitization).

8.4 An example of the timelines for training macaques on fluid control

The following is a detailed study of the effects of fluid control on the growth curve, trials worked and performance of a group of four macaques in one establishment. The effects of the following fluid control levels, set out in the project licence (PPL), were examined (see Figures A1 to A3)

Level 0 = no control. The non-human primate has begun behavioural training, but as well as being rewarded with fluid for successful trials, it also received free access to fluid in the home cage.

Level 1 = the non-human primate was being trained on task, and received typically 200 to 500 ml of fluid (fruit juice) during testing, but also had access to a fluid supplement following return to the home cage.

Level 2 = the non-human primate was being trained on task and received most of the daily fluid requirement (approximately equal to 250 to 500 ml) during task training. However, on returning to the home cage only a small fluid supplement was provided.

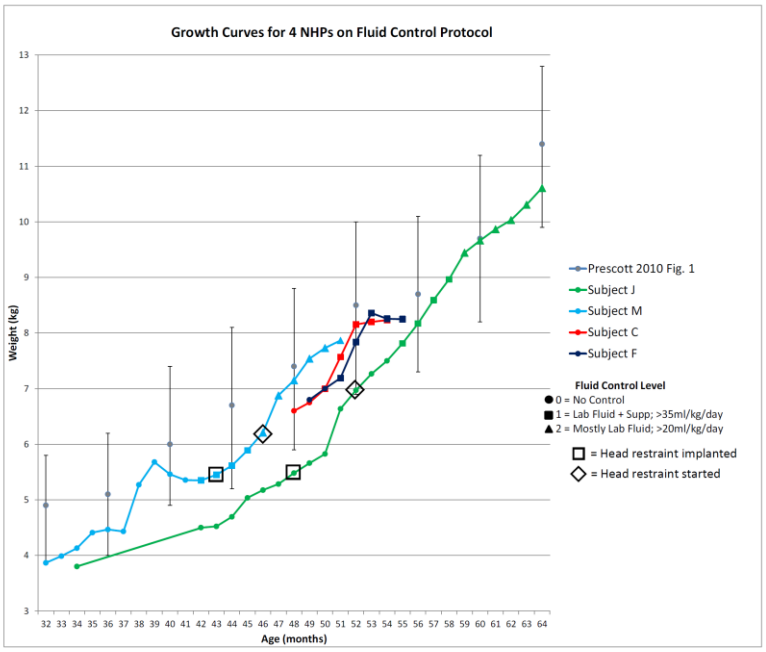


Figure A1. Growth curves of four rhesus macaques on fluid control, 2011–2012.

Figure A1 shows the growth curves of four male rhesus macaques on fluid control protocol between 2011–2012. The animals worked for different preferred juice rewards. Subject J (green) arrived aged 34 months but did not enter the protocol until 42 months. Subjects J and M received headpost implants as indicated by the black squares, and head restraint using the headpost (black diamonds) was commenced three to four months later. These animals arrived from the breeding colony at a weight below average for captive-bred male macaques (based on data from Figure 1, Prescott *et al.*, 2010). None the less, by the end of

the study all animals had achieved weights within the standard error range of normal captive-bred males. The remaining two subjects, C (red) and F (black), are in the early stages of training. Note that there is no evidence that animals on the more strict control (Level 2, triangles) exhibit stunted growth.

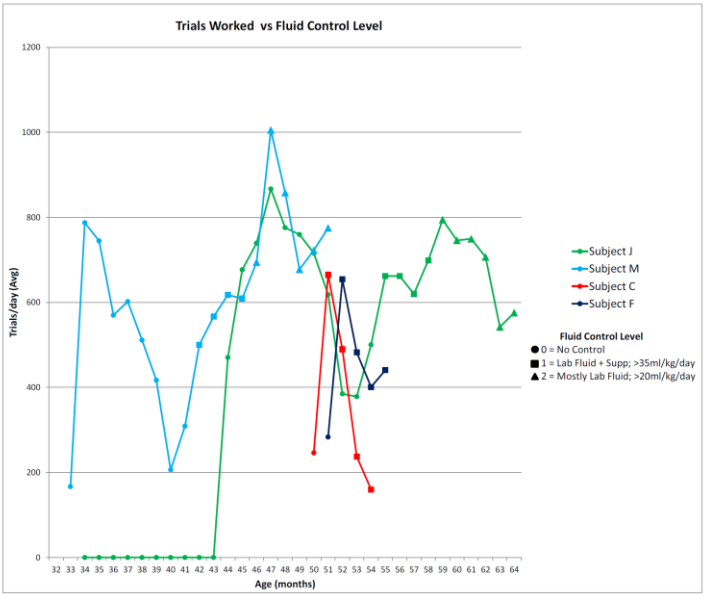


Figure A2. Trials worked per day by the four rhesus macaques and fluid control level.

Figure A2 shows the number of trials worked per day (averaged over the month) as a function of fluid control level for the same four macaques in Figure A1. The monkeys are trained to produce a large number of trials (quantity), but also to perform trials that satisfy the demands of the task (quality), carefully designed to test a scientific hypothesis (see Figure A3 for details on performance quality). 'Trials' early in the training are relatively simple (for example, move a joystick to get a juice reward); the task demands are increased progressively over the full training period until the final task design can be completed and the monkey is ready for neurophysiology to commence.

Subject M (blue) went onto Level 1 fluid control at month 42 to motivate him to work more consistently on a more advanced version of the task (see Figure A3). By month 46 he was still not consistently performing the minimum number of required trials (600) and showed signs of a declining level of performance that would not allow him to advance to the final version of the task. Given this pattern and trend in performance, following discussion with the named veterinary surgeon (NVS), he was moved onto Level 2 control at month 46. Head restraint was also introduced at month 46. From month 46 forward, he achieved the minimum performance criterion (600 trials) every month. Moreover, he continued to put on weight and has completed all training and started neurophysiological recordings. At present (2013) subject M is performing between 700 and 800 trials per day and consuming around 64 ml/kg/day (total 400 to 500 ml/day).

Subject J (green) showed good performance on an early, simple version of the task, and achieved high trial numbers. However, when the task complexity was increased, performance fell away sharply. This often happens when the quality of performance has to be improved in order to complete more demanding criteria set on the behavioural task (see Figure A3). Note that since he was moved onto Level 2 fluid control he has achieved the top level on the qualitative assessment whilst performing more than 700 trials in 4 of the 6

sessions. His task is more demanding than that of Subject M. His good performance has continued (on average 681 trials per session, data not shown), with an average fluid intake of 36 ml/kg/day (total ~350 ml per day). He continued to gain weight and has reached a performance level ready for neurophysiological recordings.

Far fewer data points were available for **Subjects C (red) and F (black)** – they have not yet been implanted with a headpost so have not yet been head restrained. They are currently (2013) on Level 1 control and are improving in performance quality (see Figure A3) although trial numbers are unstable.

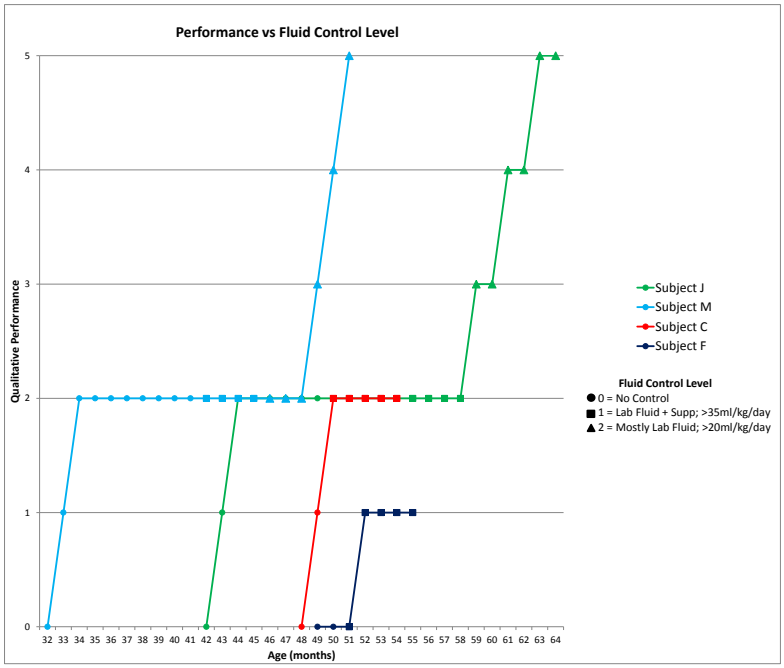


Figure A3. Performance the four rhesus macaques and fluid control.

Figure A3 shows qualitative task performance as a function of fluid control level. Since moving onto Level 2 control, Subjects J and M have both completed experimental training and commenced electrophysiological recordings in January 2013. Note that neither animal achieved the top performance until it was trained with Level 2 control (triangles). Indeed, the highest task performance (quality) level reached by any animal on Levels 0 or 1 is a score of only 2 out of 5. Note also that both Subjects C and F have not been implanted with a headpost yet. In the case of Subject F, this has slowed training as Subject F does not look at the screen long enough to understand and respond to the instructive visual stimuli.

Qualitative performance scale

0 = Not yet being trained or not yet trained to get in testing chair.
1 = Trained to get in testing chair. Moves / touches joystick for reward.
2 = May accept head restraint. Moves joystick in specific directions based on position of visual stimuli on screen. Can learn a few stimulus-outcome or action-outcome associations.
3 = Accepts head restraint, fixates / holds eyes on stimuli. Can learn and / or remember many stimulus-outcome or action-outcome associations.
4 = Can learn abstract rules and / or use strategies. Can hold and use information in working memory.
5 = Has learned final version of task, can perform sufficient number of trials per day, ready for electrophysiological recordings.

Summary

The study shows that, in four subjects on fluid control protocols, behavioural training can be achieved without compromising normal growth patterns. However, subjects that remained on Levels 0 or 1 did not progress sufficiently through the training to a point where performance quality and trial number would make it possible to obtain electrophysiological data. Monkeys on these regimes often fail to learn the task objectives (and hence are unlikely to receive a lot of rewards). They are likely to become frustrated by the task and learn that they will always receive a supplement in the home cage when they don't work on the task. Both of these outcomes are in complete opposition to the scientific objectives.

The evidence presented here suggests fluid control Levels 0 to 1 cannot be used in isolation to obtain the scientific objectives. These fluid control regimens unnecessarily prolong the duration that animals stay on the protocol and lead to excessive training (per diem) costs. Further, more relaxed fluid control protocols (Levels 0 and 1) were associated with much slower progress and additional months of training. Because of the high per diem charge for non-human primate care, slower training entailing, for example, 6 additional months of training might add as much as £20,000 to £40,000 per monkey to the cost of research, as well as holding up the overall research programme unduly.

To minimize costs, expedite and facilitate training and reduce the duration that the animal must stay on the protocol, it is advised to move to fluid control Level 2 as soon as possible. It may be possible that, once the animal is well trained on the task (and will work for many trials because of the variety and preference for juice rewards) the fluid control level can be relaxed. Note that the experience with subjects M and J is that when fully trained they are taking volumes of fluid well above the floor value of 20 ml/kg/day specified in the PPL.

In summary, there is little evidence to suggest cumulative severity during the training period. It is also worth noting that the introduction of head restraint does not impact on performance quality or quantity, rather the monkey becomes more focused on the task and earns more rewards per session.

8.5 Bibliography

Adams, D. L., Economides, J. R., Jocson, C. M., Parker, J. M. and Horton, J. C. (2011) 'A watertight acrylic-free titanium recording chamber for electrophysiology in behaving monkeys', *J. Neurophysiol.,* 2011,106, pp 1581–90.

AALAS-FELASA working group on harm-benefit analysis of animal studies. www.felasa.eu/working-groups.

Almeida DM (2005) Resilience and vulnerability to daily stressors assessed via diary methods. *Current directions in psychological science.* 14, pp 64 – 68.

Animals (Scientific Procedures) Act 1986. http://www.legislation.gov.uk/ukpga/1986/14/contents

APGAR V. (1953). A proposal for a new method of evaluation of the newborn infant. *Curr Res Anesth Analg* 32, pp 260-267.

ARRIVE Guidelines. Animal Research: Reporting *In Vivo* Experiments. www.nc3Rs.org.uk/arrivepdf.

Baker SP, O'Neill B, Haddon W, Long WB. (1974) The Injury Severity Score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 14, pp187-196.

Bateson P (1986) When to experiment on animals. *New Scientist.* 109:30-32.

Bateson, P. (1991) 'Assessment of pain in animals', *Animal Behaviour*, 42, pp 827–839.

Bateson, P., Johansen-Berg, H., Jones, D. K., Keverne, E. B., Matthews, P. M., Milner, A. D. M., Prescott, M. J., Ragan, C. I., Shattock, R., Strauss, J. and Peck, H. (2011) *Review of research using non-human primates*. Report of a panel chaired by Professor Sir Patrick Bateson FRS, London, BBSRC/MRC/NC3Rs/Wellcome Trust.

Bellanca RU, Crockett CM. (2002) Factors predicting increased incidence of abnormal behavior in male pigtailed macaques. *Am J Primatol.* 58, pp 57-69

Biel M, Kramer M et al (2013) Outcome of ventriculoperitoneal shunt implantation for treatment of congenital internal hydrocephalus in dogs and cats: 36 cases (2001 – 2009). *J Amer Vet Med Assoc* 242, pp 948-958.

Bridgewater B, Keogh B, Kinsman R et al (2008) Sixth national adult cardiac surgical database report. Dendrite Clinical Systems Ltd. Henley –on-Thames.

Brodbelt, D. C., Blissitt, K. J., Hannond, R. A., Neath, P. J., Young, L. E. et al. (2008) 'The risk of death: the confidential enquiry into perioperative small animal fatalities', *Vet. Anaesth. Analg*. 35, pp 365–373.

Broom, D. M. (1986) 'Indicators of poor welfare', *British Veterinary Journal,* 142, pp 524-526.

Broom DM (1991) Animal welfare: concepts and measurement. *J Anim Sci* 69, pp 4167-75.

Broom, D. M. (2001) 'Coping, stress and welfare'. In: *Coping with Challenge: Welfare in Animals including Humans,* Broom, D. M. (ed). Berlin: Dahlem University Press.

Broom, D. M. (2008) 'Welfare assessment and relevant ethical decisions: key concepts', *Annual Review of Biomedical Science,* 10, pp T79–T90.

Broom, D. M. (2011) 'A history of animal welfare science', *Acta Biotheoretica,* 59, pp 121–137.

Broom, D. M. and Fraser, A. F. (2007) *Domestic Animal Behaviour and Welfare,* 4th edition, p 438. Wallingford: CABI.

Broom, D. M. and Johnson, K. G. (2000) *Stress and Animal Welfare*, p 211. Dordrecht: Kluwer.

Brown DC, Conzemius MG, Shofer F, Swann H. (1997) Epidemiologic evaluation of postoperative wound infections in dogs and cats. J Am Vet Med Assoc. 210, pp1302-6.

Carlson J (2008) Safe pair housing of Macaques. Animal welfare institute, USA. www.awionline.org.

Carlsson, H. E., Schapiro, S. J., Farah, I. and Hau, J. (2004) 'Use of primates in research: A global overview', *American Journal of Primatology*, 63, pp 225-237.

Coleman K (2011) Caring for nonhuman primates in biomedical research facilities: scientific, moral and emotional considerations. *Am J Primatol.* 73, pp 220-5.

Concordat on Openness on Animal Research (2012) www.understandinganimalresearch.org.uk

Conti G, Hansman C, Heckman JJ, Novak MF, Ruggiero A, Suomi SJ. (2012) Primate evidence on the late health effects of early-life adversity. *Proc Natl Acad Sci U S A*. 109, pp 8866-71

Corcoran CA, Pierre PJ et al (2012) Longterm effects of differential early rearing in rhesus macaques: behavioural reactivity in adulthood. *Developmental Psychobiology* 54, pp 546-55.

Corsellis JA (1989). Boxing and the brain. *BMJ* 298, pp105-109.

Council of Europe (2006) Appendix A of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS No.123) Guidelines for accommodation and care of animals (Article 5 of the Convention). http://conventions.coe.int/Treaty/EN/Treaties?PDF?123-Arev.pdf.

de Groot, V., Beckerman, H., Lankhorst, G. J., Bouter, L. M. (2003) 'How to measure comorbidity. a critical review of available methods'*, J. Clin. Epidemiol.* 2003, 56 (3), pp 221–229.

Dettmer AM, Novak MA, Suomi SJ, Meyer JS (2012) Physiological and behavioural adaptation to relocation stress in differentially reared rhesus monkeys: hair cortisol as a biomarker for anxiety-related responses. *Psychoneuroendocrinology* 37, pp 191-9.

Diamond C (1991) The Realistic Spirit: Wittgenstein, Philosophy and the Mind. MIT Press, Boston.

Diamond C (1996). "Wittgenstein, Mathematics, and Ethics: Resisting the Attractions of Realism," in H. Sluga and D. Stern (eds.), *The Cambridge Companion to Wittgenstein*. Cambridge: Cambridge University Press, pp. 226–60.

DiVincenti L Jr, Wyatt JD (2011) Pair Housing of Macaques in Research Facilities: A Science-Based Review of Benefits and Risks. *J Am Assoc Lab Anim Sci.* 50, pp 856–863.

Dolan, K. (1999) *Ethics, Animals and Science*. Oxford: Blackwell Science Ltd.

Dube SR, Felitti VJ, Dong M et al (2003) The impact of adverse childhood experiences on health problems: evidence from four birth cohorts dating back to 1900. *Prev. Med.* 37, pp 268-77.

EORTC QLQ-C30. www.eortc.be/qol/.

European Directive 2010/63/EU. Available at http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm or http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

Eysenk, M., Payne S., Santos, R. (2006) Anxiety and depression: past, present and future events. *Cognition and Emotion* 20, pp 274–294.

Feng, X., L. Wang, S. Yang, D. Qin, J. Wang, C. Li, L. Lv, Y. Ma, and C. Hu (2011). Maternal separation produces lasting changes in cortisol and behavior in rhesus monkeys. *Proceedings of the National Academy of Sciences* 108, pp 14312 -14317.

Ferdowsian HR, Beck N (2011) Ethical and Scientific Considerations Regarding Animal Testing and Research *PLoS ONE* 6, e24059

Five freedoms for animals. www.fawc.org.uk/freedoms.htm

Fox S, Levitt P, Nelson CA (2010) How the timing and quality of early experience influence the development of brain architecture. *Child Dev*. 81, pp 28-40.

Foresight Mental Capacity and Wellbeing Project. 2008. The UK Government Office for Science.

Guillen J (2012) FELASA guidelines and recommendations. *J Am Assoc Lab Anim Sci* 51, pp 311-321.

Hagadorn, J. I., Richardson, D. K., Schmid, C. H. and Cole, C. H. (2007) 'Cumulative illness severity and progression from moderate to severe retinopathy of prematurity', *J. Perinatol.* 2007, 27, pp 502–509.

Harris J (1985) The value of life. An introduction to medical ethics. Routledge London.

Hawkins P, Morton DB, Burman O et al. (2011). A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working group on Refinement. *Lab Anim* 45, pp 1-13.

Hersh EH, Virk MS, Shao H, Tsiouris AJ, Bonci GA, Schwartz TH (2013) Bone flap explantation, steroid use and rates of infection in patients with epilepsy undergoing craniotomy for implantation of subdural electrodes. *J Neurosurg.* 119. Pp 48-53.

Home Office (2000) *Guidance on the Operation of the Animals (Scientific Procedures) Act*. London: Home Office.

http://www.official-documents.gov.uk/document/hc9900/hc03/0321/0321.pdf

Honess P, Gimpel J, Wolfensohn S, Mason G (2005) Alopecia scoring: the quantitative assessment of hair loss in captive macaques. *Altern Lab Anim*

Honess PE, Marin CM (2006) Behavioural and physiological aspects of stress and aggression in nonhuman primates. *Neurosci Biobehav Rev* 30, pp 413-436.

Honess, P. and Wolfensohn, S. (2010) 'A matrix for the assessment of welfare and cumulative suffering in experimental animals', *Alternatives to Laboratory Animals*, 38, pp 05–212.

Huntley, A. L., Johnson, R., Purdy, S., Valderas, J. M. and Salisbury, C. (2012) **'**Measures of Multimorbidity and Morbidity Burden for Use in Primary Care and Community Settings: A Systematic Review and Guide', *Ann. Fam. Med.*, March/April 2012, vol. 10, no. 2, pp 134–141.

Irwin, M. (2001) 'Are stress and depression related?'. In *Coping with Challenge: Welfare in Animals including Humans,* Broom, D. M. (ed) pp 271–287. Berlin: Dahlem University Press.

Jackowski, A., T. D. Perera, C. G. Abdallah, G. Garrido, C. Y. Tang, J. Martinez, S. J. Mathew, J. M. Gorman, L. A. Rosenblum, E. L. Smith, A. J. Dwork, D. C. Shungu, A. Ka_man, J. Gelernter, J. D. Coplan, and J. Kaufman (2011). Early-life stress, corpus callosum development, hippocampal volumetrics, and anxious behavior in male nonhuman primates*. Psychiatry Research: Neuroimaging* 192, pp 37-44.

Joint Working Group on Refinement (2009) *Refinements in husbandry, care and common procedures for non-human primates*, Ninth Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, Jennings, M.and Prescott, M. J. (eds). *Laboratory Animals* 43(S1), pp 1–47.

Kaplan JR, Manuck SB, Clarkson TB. (1983). Social stress and atherosclerosis in normocholesterolaemic monkeys. *Science* 220, pp 733-6.

Kelly J (2007) Implementation of permanent group housing for cynomolgus macaques on a large scale for regulatory toxicology studies AATEX 14, Special Issue, pp 107-110 Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences. http://altweb.jhsph.edu/wc6/paper107.pdf

Keong NC, Bulters DO et al (2012) The SILVER (Silver Impregnated Line Versus EVD Randomised trial): a double-blind, prospective, randomized, controlled trial of an intervention to reduce the rate of external ventricular drain infection. *Neurosurgery* 71, pp 394 – 403.

Knaus WA, Wagner DP, Draper EA et al. (1991) The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalised patients. *Chest* 100, pp1619-1636.

LASA (1990) The assessment and control of the severity of scientific procedures on laboratory animals. *Laboratory Animals* 24, pp 97-130 http://www.la.rsmjournals.com/content/24/2/97.full.pdf

LASA/APC (2005) *LASA/APC pilot study to assess the feasibility of collecting and reporting data on the severity of adverse effects caused to animals used in procedures registered under the A(SP)A 1986*. Hull: Laboratory Animal Science Association.

Lazarus RS (1999) Stress and emotion: a new synthesis. Springer, New York.

Lewis MH, Gluck JP, Petitto JM, Hensley LL, Ozer H. (2000) Early social deprivation in nonhuman primates: long-term effects on survival and cell-mediated immunity. Biol Psychiatry. 2000 Jan 15;47(2):119-26

Linn, B. S., Linn, M. W., Gurel, J. (1968) Cumulative illness rating scale. *J* Amer. Ger. Soc. 16, pp 622–626.

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, pp 1-15.

Lutz CK, Davis EB, Ruggiero AM, Suomi SJ. (2007) Early predictors of self-biting in socially-housed rhesus macaques (Macaca mulatta). Am J Primatol. **2007** May;69(5):584-90

Lyons DM, Buckmaster PS, Lee AG, Wu C, Mitra R, Duffey LM, Buckmaster CL, Her S, Patel PD, Schatzberg AF.(2010) Stress coping stimulates hippocampal neurogenesis in adult monkeys. *Proc Natl Acad Sci U S A.* 107, pp 14823-7

Mayhew PD, Freeman L, Kwan T, Brown DC (2012) Comparison of surgical site infection rates in clean and clean-contaminated wounds in dogs and cats after minimally invasive versus open surgery: 179 cases (2007 – 2008). *J Amer Vet Med Ass 240, pp 193-198.*

Mendl M, Burman OH, Paul ES (2010). An integrative and functional framework for the study of animal emotion and mood. *Proc Biol Sci.* 277, pp 2895-904.

Miller MD, Paradis CF, Houck PR et al. (1992). Rating chronic medical illness burden in geropsychiatric practice and research application of the Cumulative Illness Rating Scale. *Psychiatry Research* 41, pp 237 – 248.

Morton DB, Griffiths PH (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Vet Rec* 116, pp431-6.

Morton DB (2000) A systematic approach for establishing humane endpoints. ILAR J 41, pp 80–6.

National Centre for Replacement, Refinement and Reduction of Animals in Research *www.**nc3rs**.org.uk*

National Research Council (2011) *Guide for the Care and Use of Laboratory Animals*, 8th edition. Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council www.aaalac.org/resources/theguide.cfm.

National Research Council of the National Academies (2009) *Recognition and alleviation of Pain in Laboratory Animals*. Washington D.C: National Academies Press.

Novak M (2003) Self-Injurious Behavior in Rhesus Monkeys: New Insights into Its Etiology, Physiology, and Treatment. *American Journal of Primatology* 59, pp 3–19

Novak MA, Hamel AF, Kelly BJ, Dettmer AM, Meyer JS. (2013) Stress, the HPA axis, and nonhuman primate well-being: A review. Appl Anim Behav Sci. 143, pp135-149.

Nuffield Council on Bioethics (2005) *The Ethics of Research Involving Animals*. Nuffield Council on Bioethics, ISBN 1 904384 10 2.

Nystrom P, Schapiro SJ, Hau J (2001) Accumulated means analysis: a novel method to determine reliability of behavioural studies using continuous focal sampling. *In vivo.*15, pp 29-34.

O'Neill O (2002) A question of Trust. The BBC Reith Lectures. Cambridge University Press UK.

Outhwaite W (2009) *Habermas.* 2nd Edition. Polity Press, Cambridge UK.

Parker KJ, Maestripieri D. (2011) Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neurosciences and Biobehavioral Reviews,* 35, pp 1466–1483.

Poole, T. B. (1988) 'Normal and abnormal behavior in captive primates', *Primate Report,* 22, pp 3–11.

Prescott MJ (2003) Training nonhuman primates using positive reinforcement techniques. *J Appl Animal Welfare* 6, pp 157-161.

Prescott, M. J. (2010) 'Ethics of primate use', *Advances in Science and Research,* 5, pp 11–22.

Prescott, M. J. (2012) 'Changing the face of primate neuroscience', *BNA Bulletin*, 66, p 10.

Prescott, M. J., Brown, V. J., Flecknell, P. A., Gaffan, D., Garrod, K., Lemon, R. N., Parker, A. J., Ryder, K., Schultz, W., Scott, L., Watson, J. and Whitfield, L. (2010) 'Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research', Report of a working group of the NC3Rs. *Journal of Neuroscience Methods*, 193, pp 167–188.

Prescott, M. J., Nixon, M. E., Farningham, D. A. H., Naiken, S. and Griffiths, M.-A. (2012a) 'Laboratory macaques: when to wean?' *Applied Animal Behaviour Science*, 137, pp 194-207.

Prescott, M. J., Brown, V. J., Flecknell, P. A., Garrod, K., Lemon, R. N., Parker, A. J., Ryder, K., Schultz, W. Scott, L., Watson, J., Whitfield, L. (2012b) 'Response to Westlund's commentary: "Can conditioned reinforcers and variable-ratio schedules make food- and fluid control redundant?",' *Journal of Neuroscience Methods*, 204, pp 206–209

Pryce CR, Azzinnari D et al. (2011) Helplessness: a systematic review of theory and evidence for its relevance to understanding and treating depression. *Pharmacol Ther* 132, pp 242-67.

Rebuck JA, Murry KR, Rhoney DH, Michael DB, Coplin WM (2000) Infection related to intracranial pressure monitors in adults: analysis of risk factors and antibiotic prophylaxis. *J Neurol Neurosurg Psychiat* 69, pp 381-4.

Regan, T. (2004) *The Case for Animal Rights*. USA: University of California Press.

Report of the Suffering and Severity working group on the strengths and weaknesses of the current system of severity limits as a way of prospectively assessing suffering and severity (2009). https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/118988/suffering-severity-report.pdf

Review of cost-benefit assessment in the use of animals in research (2003). http://www.rspca.org.uk/ImageLocator/LocateAsset?asset=document&assetId=1232712104105&mode=prd

Reinhardt V (2002) Artificial weaning of old world monkeys: benefits and costs. *J Appl Welfare Sci* 5, pp151-6.

Reinhardt V, Liss C, Stevens C (1995) Social housing of previously single-caged macaques: what are the options and the risks? *Animal Welfare* 4, pp 307-328.

Richardson DK, Gray JE, McCormick MC (1993) Score for neonatal acute physiology: a physiologic severity index for neonatal intensive care. *Pediatrics* 91. pp 617-23.

Richards HK, Seeley HM, Pickard JD (2009) Efficacy of antibiotic-impregnated shunt catheters in reducing shunt infection: data from the United Kingdom Shunt Registry. *J Neurosurg Pediatr*. 4, pp 389-93

Rommeck I, Anderson K, Heagerty A, Cameron A, McCowan B (2009) Risk factors and remediation of self-injurious and self-abuse behavior in Rhesus macaques. *J Appl Animal Welfare Science* 12, pp 61-72.

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London.

Sapolsky RM. (1996). Why stress is bad for your brain. *Science* 273 pp749-760.

Sallet J, Mars RB, Noonan MP, Andersson JL, O'Reilly JX, Jbabdi S, Croxson PL, Jenkinson M, Miller KL, Rushworth MF.(2011) Social network size affects neural circuits in macaques. *Science.* 334, pp 697-700.

SF-36. www.rand.org; www.sf-36.org

Shaffer BT, Cohen MS, Bigelow DC, Ruckenstein MJ (2010). Validation of a disease-specific quality-of-life instrument for acoustic neuroma: the Penn Acoustic Neuroma quality-of-Life Scale. *Laryngoscope* 120, pp 1646 – 54.

Smith JA, Boyd KM (1991). Lives in the Balance. The ethics of using animals in biomedical research. Oxford University Press, New York.

Smith JA, Jennings M (2004) Categorizing the severity of scientific procedures on animals. www.boyd-group.demon.uk/severity_report.

Smith JJ, Hadzic V, Li XB, Liu P, Day T, Utter A, Kim B, Washington IM, Basso MA (2006) Objective measures of health and wellbeing in laboratory rhesus monkeys (*Macaca mulatta*). *J Med Primatol* 35, pp 388–96.

Sokol DK (2012) Is bioethics a bully? *BMJ* 345, p32.

Spinelli, S., S. Chefer, S. Suomi, J. D. Higley, C. Barr, and E. Stein (2009). Early-life stress induces long-term morphologic changes in primate brain. *Archives of General Psychiatry* 66, pp 658 - 665.

Stocks, B. J., Lynham, T. J., Lawson, B. D., Alexander, M. E., Van Wagner, C. E., McAlpine, R. S. and Dubé, D. E. (1989) 'The Canadian Forest Fire Danger Rating System: An Overview', *The Forestry Chronicle*, 1989, 65, pp 450–457.

Summerhoff, G. (1990) *Life, brain and consciousness*. North Holland: Amsterdam Trust.

Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness: a practical scale. *Lancet* 2 (7872) , pp 81-4.

Thornton AE, Cox DN, Whitfield K, Fouladi RT (2008) Cumulative concussion exposure in rugby players: neurocognitive and symptomatic outcomes. *J Clin Exp Neuropsychol* 30, pp *398 – 409.*

Valderas, J. M., Starfield, B., Sibbald, B., Salisbury, C. and Roland, M. (2009) **'**Defining comorbidity: implications for understanding health and health services', *Ann. Fam. Med.,* 2009, 7 (4), pp 357–363*.*

Vandeleest JJ, McCowan B, Capitanio JP (2011) Early rearing interacts with temperament and housing to influence the risk for motor stereotypy in rhesus monkeys (Macaca mulatta). *Appl Anim Behav Sci.* 2011 Jun 1;132(1-2):81-89.

Vitale A, Pollo S (2011) Introduction to the Special Section: ''The Effects of Bonds between Human and Nonhuman Primates on Primatological Research and Practice'' *American Journal of Primatology* 73, pp 211–213

Walcott BP, Kwon C-S, Sheth SA, Fehnel CR, Koffie RM, Asaad WF, Nahed BV, Coumans J-V (2013) Predictors of cranioplasty complications in stroke and trauma patients. *J Neurosurg.* 118, pp 757-762.

Weatherall, D., Goodfellow, P., Harris, J., Hinde, R., Johnson, L., Morris, R., Ross, N., Skehel, J. and Tickell, C. (2006) *The use of non-human primates in research*. London: Academy of Medical Sciences.

Weese JS (2008) A review of post-operative infections in veterinary orthopaedic surgery. *Vet Comp Orthop Traumatol* 21, pp 99 – 105.

WHOQOL GROUP (1996) The World Health Organisation quality of Life Assessment (WHOQOL): position paper from the World health organization. *Soc. Sci. Med.* 41, pp. 1403 1409,

Wolfensohn, S. (2004) 'Social housing of large primates: Methodology for refinement of husbandry and management', *Alternatives to Laboratory Animals*, 32, supplement 1, pp 149–151.

Wolfensohn S, Anderson (2012) A report on assessment of lifetime experience and cumulative severity under EU Directive 2010/63. Non-peer reviewed article, *Newsletter of the Laboratory Animal Science Association*.

Working document on a severity assessment framework (2012). ec.europa.eu/environment/chemicals/lab_animals/pdf/Consensus%20doc%20on%20severity%20assessment.pdf.

Yamada H, Louie K, Glimcher PW (2010) Controlled water intake: a method for objectively evaluating thirst and hydration state in monkeys by the measurement of blood osmolality. *J Neurosci Methods.* 191, pp 83-9.

Young E, Korszun A (2010) Sex, trauma, stress hormones and depression. *Molecular Psychiatry* 15, pp 23-8.

Zautra AJ (2003) Emotions, stress and health. Oxford University Press, New York.

Zupancic JAF, Richardson DK, Horbar JD (2007). Revalidation of the score for neonatal acute physiology in the Vermont Oxford Network. *Pediatrics* 119,pp156-163.

8.6 The process of regulation of animal experiments in the UK

In the UK the use of animals in scientific research is regulated by the Animals (Scientific Procedures) Act (ASPA) 1986 using a three-part licensing process:

- the premises where the research is undertaken is licensed, and must comply with the code of practice for the housing of the relevant species;
- all personnel carrying out procedures must hold a personal licence, and must have undergone mandatory training relevant to procedures and species involved; and
- all the work must be specifically authorized by a project licence (PPL).

PPLs are granted by the Home Office for a maximum period of five years, only if the following criteria are met:

- the work fulfils one of the *"permissible purposes"* set out in the Act;
- the benefits of the work justify the costs to animal welfare caused by the procedures, the cost–benefit assessment;
- the work cannot be achieved other than by the use of live animals; and
- it will involve the smallest number of animals of species with the lowest degree of neurophysiological sensitivity and cause the least possible pain, suffering, distress or lasting harm, commensurate with achieving the objectives of the work.

Furthermore, work involving certain species, including all non-human primates, must justify the use of those specific species.

In ensuring that applications comply with the above requirements, inspectors appointed under ASPA 1986 scrutinize each application. In some cases additional scrutiny by the Animal Procedures Committee (APC) is involved; this is the case for all PPL applications involving 'Substantial' severity protocols involving non-human primates and for all projects in overall Substantial severity band involving interference with the nervous system. In some cases further advice such as from external experts will be sought before a decision is made as to whether a licence should be granted, and on what terms.

Once the work is authorized, it is subject to ongoing scrutiny through a programme of inspection, often unannounced, to ensure compliance with all licence terms and conditions.

The process

The legal regulation of work in non-human primates in the UK is as strict as in any other country in the world. Scientific investigators wishing to carry out research in non-human primates know from the outset that they will have to satisfy a rigorous series of checks before they obtain a PPL for the work and their proposed project can begin. Providing that they continue to satisfy the PPL regulations and conditions, they do then expect to have the full protection of the law in carrying out that research. They also know that welfare provision for non-human primates in research in the UK is better than in most other research-active countries and that they will be working with teams of highly-trained professionals in UK centres of excellence for non-human primate research.

Partly because of big improvements in welfare standards, the cost of non-human primate research in the UK has risen sharply, and the UK is by far the most expensive place in the

world to carry out such research. As a result external grant funding is essential for any project involving non-human primates, and grant applications for such funding are all rigorously assessed in terms of ethical and welfare issues (see below).

Normally investigators have to pursue two parallel approaches in order to be able to carry out their research proposal. First, they have to apply for a PPL for the work, and second, they have to obtain grant funding. The approach has to be in parallel because the Home Office will want to know that the work is funded before issuing a PPL, and the funding agency will not award a research grant unless a PPL has been issued to cover the work proposed. The regulatory process for obtaining a licence is a long and very complex process, which in recent times has taken more than six months, and sometimes nine to ten months. These long timescales are partly due to Home Office processing and partly to processing at the institutional level. These timescales are, of course, significantly longer than the 40 days required under the new Directive 2010/63/EU. Clearly some streamlining will be required, but there is confidence that licence applications can still be rigorously assessed in the time allowed under the new Act.

1. **Project licence application**
   The principal investigator (PI) is advised by the Home Office inspector (HOI) to begin work on the PPL application at an early stage. An early meeting with the HOI will usually require the PI to explain the overall background to the research and the justification of using non-human primates. Thereafter, draft versions of the application will often be seen by the HOI who will probably meet the PI several times to discuss detailed protocols, for example, for food or fluid control, for surgeries, etc.

2. **Ethical Review Process**
   At this stage the draft application is subject to a full discussion under the local Ethical Review Process (ERP). This meeting usually involves the certificate holder for the designated establishment where the work is to be carried out, the named animal care and welfare officer (NACWO), the named veterinary surgeon (NVS), other PIs and animal technologists, and a lay member. The meeting may or may not be attended by the HOI. The ERP will discuss the draft PPL application and feedback to the PI on a whole range of issues within the application, ranging from the basic justification of the work proposed all the way to details on care husbandry, and use of specific drugs, dosage, etc. The ERP will be familiar with the track-record of the PI and their group, and have local knowledge of their experience, training and expertise. In addition they will be able to make a cost–benefit assessment of the work proposed and give their views on the severity limit to be discussed with the HOI.

   The PI will revise the PPL application in the light of ERP feedback and one or more further meetings with the ERP and HOI may be needed to finalize the process.

3. **Submission of project licence application**
   At this point the PPL application is submitted by the certificate holder to the Home Office. All PPLs involving non-human primates are seen by at least two HOIs. Should the local HOI advise that the work proposed is likely to extend to a Substantial severity limit, then the application will automatically pass to the APC.

4. **Attendance at the Animals Procedure Committee**
   The applicant will be asked to attend a meeting of the APC Applications Committee. They will be asked to prepare in advance responses to a number of questions related to

their application, and these can concern both scientific and welfare issues. They will also be asked to produce a typical timeline of events for the non-human primates to be used in the proposed research. The applicant is not told of the outcome of the APC recommendation at the meeting. The APC acts to advise the Secretary of State as to how to proceed with the PPL application, but no copy of their advice is given to the applicant.

5. **Further external assessment**
   Recently the Home Office has taken steps to obtain further external assessments of PPL applications from at least one expert in the research field. The assessor is asked to judge whether the work with non-human primates is justified and whether it will impact upon and advance the whole research field. Applicants are told who has been asked to assess the application and may challenge that selection if they feel that the individual selected does not have sufficient expertise in non-human primate research to assess the application.

6. **Issuing the project licence**
   If all the above steps are completed satisfactorily the PPL will be issued. There may well be additional conditions attached to the licence.

7. **Grant application**
   Although not part of the regulatory process, it is well established that all funders follow strict guidelines to assess the case for using animals in research, and for establishing that the work will be carried out to satisfy the highest possible welfare conditions. The guidelines were established after agreement between major funders and the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs), see http://www.wellcome.ac.uk/About-us/Policy/Policy-and-position-statements/WTD040129.htm.

Each application will be reviewed by a number of external expert scientists and, in the process of this peer-review, the experts will be asked not only about the quality of the proposed project but about whether or not non-human primates are essential for it.

8.6.1 Annex A. Referral of project licences to the APC.

The APC sees applications for project licences that involve:

the use of wild-caught non-human primates

the use of cats, dogs, equidae (the horse family) or non-human primates in procedures of substantial severity

a substantial severity banding (classification of suffering of an ‘average’ animal) or major animal welfare or ethical implications, involving: (a) xenotransplantation (surgical transferral from one animal to another of a different species) of whole organs or (b) chronic pain models or (c) study of the central nervous system

applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns

The APC advises the Home Secretary on such applications, offering advice on whether they should be granted and, if so, on any particular conditions they should have.

8.6.2 Annex B. Guidance to Project Licence Applicants referred to the APC Applications sub-committee

This guidance has been prepared by the APC Applications sub-committee (ASC) to help those with project licence applications referred to the APC understand and prepare for ASC review of the application. It gives some background to the review and sets out some questions commonly asked or project licence applicants.

Background

It is the duty of the APC to advise the Secretary of State (SoS) on such matters concerned with the Animals (Scientific Procedures) Act 1986 and her functions under it, as the Committee may determine or as may be referred to the Committee by the SoS.

The APC has requested, and the SoS agreed to, referral of specific categories project licence applications consideration and advice.

Since 2004, the categories of application to be referred include:

1. Any involving the proposed use of wild-caught non-human primates;
2. Any involving the proposed use of cats, dogs, equidae or non-human primates in protocols of substantial severity;
3. Any with a substantial severity banding, or major animal welfare or ethical implications, involving a) xenotransplantation of whole organs, b) chronic pain models, or c) study of the central nervous system;
4. Applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns (for example, any application involving the genetic modification of non-human primates or embryo aggregation chimaeras involving dissimilar species).

Typically, the applicant is invited to meet with members of the ASC to discuss the application in person. ASC members are scientists and non-scientists (www.apc.gov.uk/aboutapc/workgroups.htm). The ASC does not wish to create additional work for project licence applicants, but has found very helpful if applicants prepare the following in advance of the meeting:

1. A lay summary of the proposed project written so as to be readily comprehensible by a member of the general public (see Abstract section of the Project licence application form http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/publications/licences/project-licences/).
2. A schematic (e.g. graph, flow chart, GANTT chart) showing the number and scheduling (and if possible, relative severity) of all procedures involved in the project that impact on the welfare of the animals.

Preparation of these documents is, of course, voluntary, but assists the ASC to understand and explore the scientific justification for the project procedures and their costs to the animals.

Invariably, the ASC wishes to estimate the total suffering experienced by the animals on the project, during their whole life-times, and to rationalise this against the expected benefits.

Common questions asked of applicants

<u>Background, objectives and benefits</u>

What are the key objectives of the project, and the likely benefits (e.g. in terms of scientific knowledge, human or animal health, the 3Rs)?

How does the project relate to progress made under previous or current project licences?

To what extent has previous research (*in vivo/in vitro*) and existing data, literature and knowledge influenced the licence application? How has unnecessary duplication of previous work been avoided?

What is the likelihood of achieving the project objectives, and what factors are critical for success?

What are the key ethical issues?

Experimental design and the 3Rs

How was the experimental design decided, and how have each of the 3Rs been integrated into the entire plan of work?

Why is it necessary to use animals to achieve the project objectives? Why are non-animal alternatives unsuitable?

What is the justification for use of the particular animal species/model?

Was the advice of a statistician taken on minimising the number of animals to be used per experiment, and the appropriate methods for data analysis?

How else has animal use been optimised?

Scientific procedures and animal welfare

What is the justification for the particular scientific procedures to be used, and what are their effects on the animals involved?

How many animals will undergo each procedure?

How will pain, suffering, distress or lasting harm be avoided, recognised, alleviated and managed?

Will anaesthesia and analgesia be used? Has advice been taken on the most appropriate agents and regimens?

How frequently and by whom are the animals monitored before, during and after each procedure?

What are the relevant clinical signs and the humane endpoints that will be applied?

How are the animals acclimatised to, or trained to co-operate with, procedures?

What are the standards of animal accommodation, environmental enrichment and care?

Will single housing of animals be necessary?

From where will the animals be sourced?

What will happen to the animals when the work is completed?

What is the rationale for nomination of the project severity band

8.7 EU Directive 2010/63/EU: Severity categories and examples

Annex VIII Section III:

This gives examples of different types of procedure assigned to each of the severity categories, on the basis of factors related to the type of the procedure.

1. Mild:

(a) administration of anaesthesia, except for the sole purpose of killing;

(b) pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totalling less than 10% of circulating volume) and the substance is not expected to cause any detectable adverse effect;

(c) non-invasive imaging of animals, for example, magnetic resonance imaging (MRI) with appropriate sedation or anaesthesia;

(d) superficial procedures, for example, ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders;

(e) application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behaviour;

(f) administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than a mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;

(g) induction of tumours, or spontaneous tumours, that cause no detectable clinical adverse effects (for example, small, subcutaneous, non-invasive nodules);

(h) breeding of genetically altered animals, which is expected to result in a phenotype with mild effects;

(i) feeding of modified diets that do not meet all of the animals' nutritional needs, and are expected to cause mild clinical abnormality within the timescale of the study;

(j) short-term (less than 24 hours) restraint in metabolic cages;

(k) studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;

(l) models that expose animals to noxious stimuli, which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid;

(m) a combination or accumulation of the following examples may result in classification as 'Mild':

(i) assessing body composition by non-invasive measures and with minimal restraint;

(ii) monitoring electrocardiogram (ECG) with non-invasive techniques with minimal or no restraint of habituated animals;

(iii) application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour;

(iv) breeding genetically altered animals that are expected to have no clinically detectable adverse phenotype;

(v) adding inert markers in the diet to follow passage of digesta;

(vi) withdrawal of food for less than 24 hours in adult rats;

(vii) open field testing.

2. Moderate:

(a) frequent application of test substances that produce moderate clinical effects, and withdrawal of blood samples (greater than 10% of circulating volume) in a conscious animal within a few days without volume replacement;

(b) acute dose-range finding studies, chronic toxicity / carcinogenicity tests, with non-lethal endpoints;

(c) surgery under general anaesthesia and appropriate analgesia, associated with post-surgical pain, suffering or impairment of general condition. Examples include: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopaedic surgery with effective stabilization and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (for example, telemetry transmitters, minipumps);

(d) models of induction of tumours, or spontaneous tumours, that are expected to cause moderate pain or distress or moderate interference with normal behaviour;

(e) irradiation or chemotherapy with a sublethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be Mild or Moderate and would be short-lived (up to five days);

(f) breeding of genetically altered animals that are expected to result in a phenotype with moderate effects;

(g) creation of genetically altered animals through surgical procedures;

(h) use of metabolic cages involving moderate restriction of movement over a prolonged period (up to five days);

(i) studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the timescale of the study;

(j) withdrawal of food for 48 hours in adult rats;

(k) evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and that are expected to result in moderate distress.

3. Severe:

(a) toxicity testing where death is the endpoint, or fatalities are to be expected and severe pathophysiological states are induced. For example, single dose acute toxicity testing, see Organization for Economic Cooperation and Development (OECD) testing guidelines;

(b) testing of a device where failure may cause severe pain, distress or death of the animal (for example, cardiac assist devices);

(c) vaccine potency testing characterized by persistent impairment of the animal's condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering;

(d) irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease;

(e) models with induction of tumours, or with spontaneous tumours, that are expected to cause progressive lethal disease associated with long-lasting moderate pain,

distress or suffering. For example, tumours causing cachexia, invasive bone tumours, tumours resulting in metastatic spread, and tumours that are allowed to ulcerate;

(f) surgical and other interventions in animals under general anaesthesia that are expected to result in severe or persistent moderate post-operative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure;

(g) organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (for example, xenotransplantation);

(h) breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example, Huntington's disease, muscular dystrophy, chronic relapsing neuritis models;

(i) use of metabolic cages involving severe restriction of movement over a prolonged period;

(j) inescapable electric shock (for example, to produce learned helplessness);

(k) complete isolation for prolonged periods of social species, for example, dogs and non-human primates;

(l) immobilization stress to induce gastric ulcers or cardiac failure in rats;

(m) forced swim or exercise tests with exhaustion as the endpoint.

8.8 Questionnaire

Please complete all sections. Use 'N/A' if a particular section does not apply to this NHP.

General information

Subject identifier:

Subject species:

Please provide a timeline showing the life experience of this NHP, which records timings of key events such as start of training, first use of restraint, use of food or fluid control, and timing of major surgeries

Please use the date format DDMMYYYY for the relevant fields below

Date of birth:

Date of removal from natal group:

◯ Known ◯ Unknown

Date of arrival at your facility:

◯ Known ◯ Unknown

Date of study commencement:

◯ Known ◯ Unknown

Date of retirement:

◯ Known ◯ Unknown

Date of death:

◯ Known ◯ Still alive

Total number of general anaesthetics:

Further comments on life experience

Adverse effects of procedures / husbandry

Please provide information on the welfare impact of the procedures / husbandry (including adverse effects and infections) for this NHP relating to the following.

1. Anaesthetic

◯No impact ◯Impact seen ◯N/A

2. Post procedure pain control

◯No impact ◯Impact seen ◯N/A

3. Surgery and other invasive procedures

a. Headpost implantation

◯No impact ◯Impact seen ◯N/A

b. Recording chamber surgery

◯No impact ◯Impact seen ◯N/A

c. Insertion, recording from and stimulation by electrodes

◯No impact ◯Impact seen ◯N/A

d. Creating CNS lesions

◯No impact ◯Impact seen ◯N/A

e. Other implant

◯No impact ◯Impact seen ◯N/A

4. Restraint and handling

a. Restriction in a small (<100cm^3) enclosure during task performance

○No impact ○Impact seen ○N/A

b. Restraint in primate chair with head fixation

○No impact ○Impact seen ○N/A

c. Neck fixation

○No impact ○Impact seen ○N/A

d. The movement of animals voluntarily into transfer cages / chairs

○No impact ○Impact seen ○N/A

e. The use of collar and poles

○No impact ○Impact seen ○N/A

5. Food / fluid controls

a. Controlled access to food

○No impact ○Impact seen ○N/A

b. Controlled access to water

○No impact ○Impact seen ○N/A

6. Housing, husbandry and care

a. Single housing

○No impact ○Impact seen ○N/A

b. Pair housing

○No impact ○Impact seen ○N/A

c. Group housing

○No impact ○Impact seen ○N/A

d. Cleaning routines

○No impact ○Impact seen ○N/A

7. Long-term maintenance (e.g. implants)

○No impact ○Impact seen ○N/A

8. Training

a. The training of the animal to perform tasks

○No impact ○Impact seen ○N/A

b. The use of 'motivators' (e.g. rewards or punishment)

○No impact ○Impact seen ○N/A

Complications of procedures

Please provide details of any complications and non-intended effects (e.g. infection and related data) you have seen associated with the procedures detailed below, indicating the incidence in this NHP.

1. Surgery and other invasive procedures

a. Cranial infection

i. Skin only or requiring topical treatment only

◯Not seen ◯Complication seen ◯N/A

Total number of events:

Details of degree, scale, extent and duration

ii. Infection requiring systemic antibiotics

◯Not seen ◯Complication seen ◯N/A

Total number of events:

Details of degree, scale, extent and duration

iii. Infection involving bone

◯Not seen ◯Complication seen ◯N/A

Total number of events:

Details of degree, scale, extent and duration. Please also explain how this was determined

iv. Infection involving meninges and / or brain

◯Not seen ◯Complication seen ◯N/A

Total number of events:

Details of degree, scale, extent and duration. Please also explain how this was determined.

b. Seizures

i. Partial

◯Not seen ◯Complication seen ◯N/A
Total number of events:
Details of degree, scale, extent and duration

ii. Generalised

◯Not seen ◯Complication seen ◯N/A
Total number of events:
Details of degree, scale, extent and duration

c. Suspected or proven cerebral haemorrhage ◯Not seen ◯Complication seen ◯N/A
Total number of events:
Details of degree, scale, extent and duration

d. Paralysis / paresis ◯Not seen ◯Complication seen ◯N/A
Total number of events:
Details of degree, scale, extent and duration. Please also specify if unilateral / bilateral and its consequences.

e. Immobility ◯Not seen ◯Complication seen ◯N/A
Total number of events:
Describe the duration and consequences

f. Other unexpected neurological signs ◯Not seen ◯Complication seen ◯N/A
Total number of events:
Describe the signs, their consequences, the diagnosis, treatment (if any) and outcome

g. Intended and unintended adverse effects of CNS lesions

i. Immediate

◯Not seen ◯Complication seen ◯N/A
Total number of events:

Details of degree, scale, extent and duration

ii. Long term

◯Not seen ◯Complication seen ◯N/A

Total number of events:

Details of degree, scale, extent and duration

2. Over the course of the study, did the subject develop any abnormal behaviour?

◯No ◯Yes **Please specify. Did this impact on the planned study?**

Cumulative severity

If any of the procedures listed below are undertaken repeatedly, over a long period of time (e.g. several years), do you consider the welfare impact on this NHP is unchanging, diminishing or increasing? Where possible, please give examples, references and/or data to support any comments.

Definitions

Unchanging: later procedures applied to an animal have the same welfare impact as preceding procedures of the same nature.

Diminishing: each procedure applied to an animal produces a less severe impact compared with preceding procedires of the same nature (decreasing cumulative severity, asymptotic severity, tolerance with repetition)

Increasing: each procedure applied to an animal produces a more severe impact compared with preceding procedures of the same nature (increasing cumulative severity, due to e.g. hypersensitisation)

1. Anaesthetic

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

2. Surgery and other invasive procedures

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

3. Restraint and handling

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

4. Food / fluid controls

a. Controlled access to food

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

b. Controlled access to fluid

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

5. Housing, husbandry and care

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

6. Long term maintenance (e.g. implants)

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

7. Training

a. Chair

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

b. Task

◯Unchanging ◯Diminishing ◯Increasing ◯N/A

Procedure interaction

In your view, which of the above procedures / husbandry *interact* and impact upon each other in a consequential unchanging, diminishing or increasing effect? Please give examples of where repetition of a procedure may improve welfare.

Pathology

Did this NHP undergo a post-mortem? If so, what were the pathological findings?

What clinicopathological data do you have for this NHP on these studies (e.g. blood, urine parameters)?

Did this NHP show signs of impaired renal function and, if so, what?

Final outcome

Did the NHP have to be killed to prevent suffering? If so, at what stage and why?

Classification

In light of the evidence you have submitted, what severity classification do you consider appropriate for the work you have described *on this NHP?*

Please submit this questionnaire online at www.20hocs12.com

Please complete all sections - enter 'N/A' if a particular question does not apply to you.

General information

Which country do you work in?

How many years have you been working with animals in scientific procedures?

Please list all the species you use:

Approximately how many non-human primates and over what period of time are your impressions / data based upon, and what is your level of direct hands-on involvement?

Supply and condition on arrival

1. Have animals been transferred to or from your research group from / to others (e.g. previously used on immunology studies or in breeding programmes)? If so, did this involve transport between institutes, or any change in social hierarchy?

2. Considering the *non-procedural* aspects of the animal's life, do you have records from the past 10 years to indicate the level of contingent suffering from breeding practices (e.g. age of artificial weaning), transportation (method, duration), concomitant injury and disease (e.g. incidence of diarrhoea, skin lesions, woundings)? If yes, please summarise below.

Behaviour

3. Do you routinely record data on the animal's behaviour in their home cages? Please provide examples, references and data as indicated below.

If you record data, what behaviours do you record?

If you record data, what is the typical frequency and duration of your observations?

What are the signs of a 'contented' NHP?

What abnormal behaviours do you see, at what frequency, and under what circumstances?

What are the signs of a NHP in pain or distress?

Are there any subtle signs that indicate low level of *continuous* suffering?

4. Please provide details of the welfare impact of procedures / husbandry. Please provide data where available.

	What means do you use to gather data / information?	*Data / information*
Anaesthetic		
Surgery and other invasive procedures		
Restraint and handling		
Food / fluid controls		
a) Controlled access to food		
b) Controlled access for fluid		
Housing, husbandry and care		
Long term maintenance (e.g. implants)		
Training		
a) Chair		
b) Task		

Evolution of techniques used

5. How have procedures / husbandry at establishments with which you are familiar evolved or been modified over the past decade and why? How were any improvements or impacts of changes evaluated? Please indicate if the procedures have not been used.

General anaesthetic - please include who administers the anaesthetic

Surgery and other invasive procedures

Restraint and handling

Food/fluid controls, including current regime, range of fluid intake (ml/kg/day) and time of restitution of free fluid

Housing, husbandry and care

Long term maintenance e.g. implants

Training

Cumulative severity

6. Are you aware of procedures needing to be adapted over time if they are repeatedly used on a single animal, and if so, please give details to include why such adaptations are required. What safeguards are in place to prevent escalation of adverse effects to the maximum level of severity permitted?

Has there been a need to increase motivators (e.g. food/fluid control) with an increasing number of surgery/techniques performed?

Do you have a view if a sequence of 'minor' surgery / techniques is less severe on a NHP than a single major surgical procedure?

Classification

7. In the light of the evidence you have submitted, what severity classification do you consider appropriate for the work you have described: 10 years ago and now?

Pathology

8. Post-mortem

Do you, or any members of your team, carry out post-mortem examinations? Please add comments below.

◯Routinely ◯Only for specific investigations ◯Never

If you have answered yes to the question above, please indicate whether the post-mortems were full diagnostic or partial (i.e. looking for a specific finding).

Who would carry out the post-mortem?

Final comments

9. Is there anything you would further like to add on the subject of cumulative severity? This could include what further research or evidence you think would be required to assess and reduce cumulative severity.

Please submit this questionnaire online at www.20hocs12.com