Using normal and abnormal behaviour to assess welfare in macaques

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An assessment of the welfare of an individual animal is generally based on an evaluation of physical, physiological, neurological and immunological signs overlayed with an interpretation of the behaviours expressed or absent. The challenge this task poses is made more complex by the numerous published definitions of welfare, which can lead to different interpretations of the same information; the range of normalcy for a species or population of animal; an appropriate weighting of any abnormalities observed; and the need to minimize any personal bias of the observer. Because many of the measures of welfare entail either invasive procedures or handling/restraint of the animal, behaviour is often relied upon as the initial gauge of the welfare state of an animal.

Non-human primates in laboratory environments sometimes display abnormal patterns of behaviour. In some cases, these behaviour patterns are a symptom of other well-defined diseases or disorders (e.g., shivering and trembling during fever, hand tremors in aged macaques). In other cases, the etiology may relate to organic brain dysfunction and/or to certain environmental conditions. Abnormal behaviour in non-human primates often takes the form of stereotypic behaviour, i.e., a repetitive, frequently idiosyncratic, highly ritualized action which does not serve any apparent biological purpose. Most of these patterns are not dangerous to the monkeys displaying them and may even be adaptive under the existing environmental conditions or in the context of the physical state of the organism. Abnormal behaviour should be considered pathological if it is frequent (i.e., occupying a substantial part of the animal's time budget to the detriment of other activities), disruptive (interfering with biological functions including eating, breeding, or parental care), or intense (i.e., producing irritation or tissue damage). There are other circumstances in which behaviour may be pathological (i.e., if it occurs in an inappropriate context), but these cases may be much more difficult to identify. Thus, the frequency of these activities and the disruption they create are relevant markers for disturbances to animal welfare.

Those non-human primates that exhibit these forms of abnormal behaviour that can potentially cause harm to the animal are said to engage in "self-injurious behaviour (SIB)." Examples include hair plucking, head banging, and self-biting, which are, in fact, observed in a percentage of captive non-human primates. Unlike the stereotypic patterns described previously, SIB is potentially dangerous, sometimes causing tissue damage and increased risk of infection. It is therefore difficult to conceive of these patterns as a normal adaptation to the environment. Instead, there is likely to be some organic or psychogenic origin. Such behaviours clearly reflect compromised animal welfare. Immediate steps should be taken to identify possible causes of these behaviours and to modify the primate care and use program accordingly to mitigate their further expression and to prevent their occurrence in other members of the colony.

A variety of factors has been proposed to account for the occurrence of abnormal behaviours in monkeys. These can be divided into environmental (extrinsic) determinants and central nervous system (intrinsic) determinants. Abnormal behaviour is sometimes associated with brain damage, painful disorders such as arthritis, and brain neurotransmitter dysfunction. By far the most popular view is that abnormal behaviour emerges as a result of certain laboratory conditions associated either with early rearing practices (e.g., rearing infants without mothers) or with later housing conditions (e.g., maintaining animals in barren individual cages). Even in cases where the environment is assumed to play a major role, other factors may also be relevant. For example, although pathological behaviour is more commonly observed in animals housed in individual cages than animals housed in social groups, it is not seen in all, or even the majority, of individually housed animals. Thus, some animals may carry risk factors which make them vulnerable to this type of housing. Ultimately, the causes of psychopathology will be found in some interaction of extrinsic and intrinsic factors. And, a better understanding of these causes will result in improved laboratory primate welfare.

Non-behavioural measures of welfare in macaques

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The development of behavioural measures of welfare has had a considerable impact on the ability of those who care for in primates in captivity to assess, in the least invasive way possible, the success with which the animals are able to cope with the challenges and stress imposed by their environment. However, it is knowledge of the physiological responses to, and consequences of, stress that underpins many of the interpretations of the importance of these behavioural measures. It is also the case that many of the physiological markers of stress and poor welfare (e.g. corticosteroids) have more than one role which may confound results and has led to growing dissatisfaction with their use. There are, however, non-behavioural measures of stress that can be used to assess welfare and that do not rely on the assessment of HPA (hypothalamus-pituitary-adrenal) axis activity. This presentation will detail the use of objective scoring systems (for alopecia and physical condition) to assess the condition and general welfare status of the animal. Correlates with alopecia and the abnormal behaviour that may be responsible for its manifestation will be examined. Use of the neutrophil activation test for assessing immune response, which has been shown to reflect stress levels, will also be described.

Assessment of welfare in marmosets: Potential usefulness of anticipatory behaviour

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Behaviour and posture, unlike many physiological measures, provide an immediate, non-invasive assessment of animal welfare and clues to subjective state. It is therefore extremely important that researchers quantitatively identify these welfare measures and those working with the animals are fully trained to recognise and accurately interpret the measures in species in their care. One method to determine which behaviours are indicative of a decrease in welfare state is to administer a stressor (that is known to be unpleasant to the animal, such as removal from the social group, or restraint for non-habituated animals) and record changes in behaviour in response to this challenge. Other situations that elicit comparable responses may then be judged as similarly unpleasant. To strengthen this method, providing situations deemed to be pleasant to the animals (e.g. increased size and complexity of the enclosure) will provide comparative data on which to make the welfare assessment, and will help to distinguish between behaviours (such as agitated locomotion) that might indicate either stress or (positive) arousal. The first part of this presentation will examine behavioural welfare indicators in common marmosets (*Callithrix jacchus*) that have been exposed to an unpleasant event (capture and restraint) and a situation deemed to be pleasant (access to outside runs).

Whilst such studies are useful in elucidating which behaviours are indicators of good and poor welfare in animals, they do not directly address the question of how much an animal likes a positive event, or dislikes a negative event. This is particularly critical when there are alternative events available. For example, it is important to assess the value of environmental enrichment to ensure that it is appropriate and beneficial, or to determine which method of restraint is least stressful. Preference tests and demand functions, combined with studies of animals in the wild, have made considerable progress into understanding what animals want, and cognitive science approaches can further our understanding of emotional states. The use of anticipatory behaviour is potentially another means of addressing this animal welfare issue. It is well known that animals use environmental signals to predict events, and it has been suggested that their anticipatory behaviour between the signal and the occurrence of the forthcoming event is an indicator of their feelings towards the event. This presentation will evaluate this claim and describe a preliminary study in common marmosets.

Multidisciplinary measurement of welfare in marmosets

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There is general agreement on the desirability of identifying objective indices of welfare and understanding their biological significance. Approaches developed in our laboratory in recent research involving marmosets may have utility in this context. The work attempted to establish whether long-term adverse effects occur following exposure to either low levels of cholinesterase inhibitors or multiple vaccinations. Studies were specifically designed to be able to detect changes in key biological indices which directly reflect well-being, such as health status, sleep quality and activity patterns, ability to concentrate, muscle function and immunological competence.

This presentation will consider whether aspects of the techniques developed, many of which rely upon remote monitoring and/or home cage behavioural testing, could be incorporated into specific studies to address particular welfare questions or used as an adjunctive measure of welfare in studies with a different primary purpose. Non-invasive or minimally-invasive approaches will be emphasised.

Characterisation of the 'norm' in these circumstances is always a particular problem and suggestions will be made to facilitate sharing information across laboratories.

Welfare of macaques on food and fluid control protocols: Report on an NC3Rs Working Group

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Control of food or fluid intake is commonly used in behavioural neuroscience experiments with macaques to motivate the animals to perform extended sequences of responses on behavioural or cognitive tasks. In the UK the control typically involves strict scheduling of the time for which food or fluid is available, rather than reduction of the daily amount of food or fluid provided.

Controlled access to food or fluid can elicit physiological and behavioural responses that may compromise animal health and psychological well-being and affect the quality of scientific data being collected. Food or fluid control may further affect animal welfare if the procedures impact on the husbandry of the animals (e.g. where animals on water restriction are singly-housed). Coupled with the relatively lengthy periods of time over which behavioural neuroscience experiments are conducted, these issues make use of food and fluid control the subject of much concern. To address this, the NC3Rs convened an expert Working Group: to review and summarise current UK practice; to identify the animal welfare issues and potential and actual refinements; and to make recommendations on contemporary best practice and areas for future research.

The Working Group's report has now been submitted for publication. It recommends that researchers, in conjunction with veterinarians, animal care staff and the ERP/IACUC, address three fundamental issues. These are: the necessity for, and level of, food or fluid control; the potential animal welfare consequences of the food or fluid control; the methods for monitoring and maintaining the health and psychological well-being of the animals.

With regard to welfare assessment, it is recommended that each animal is evaluated at the start of the experiment and then regularly checked for continued good health and psychological well-being by:

- Stability in the rate of body weight gain (or body weight in fully grown animals) – comparison of body weight records against normal growth curves, and condition scoring, will help ensure the animal is receiving adequate nutrition, is not overweight or underweight, and is growing normally.
- Absence of signs of dehydration – variables that can be used to assess hydration status include food intake, urine output and specific gravity, sequential analysis of blood, moisture content of fresh faeces and assessment of skin turgor (elasticity), as well as the general appearance, demeanour, activity level, behaviour and willingness to work of the animal.

- Behavioural indicators of psychological well-being – behaviour score sheets are useful for alerting staff to such behaviours and thereby identifying animals that may be experiencing difficulties in coping with the research protocol and laboratory environment.
- Relative stability of, or incremental improvement of, performance on the experimental protocol – many factors may result in an animal failing to learn a task or to perform as expected (e.g. an animal may cease to work if it is uncomfortable in the restraint chair; performance may decline if the animal is frustrated by the reinforcement schedule).
- Periodic veterinary examinations (e.g. every 3 months) – tests of kidney function, clinical disease and physical fitness may be appropriate.

A tiered approach to the assessment of stress in cynomolgus monkeys

Anon

Currently in nonhuman primate (NHP) toxicological testing many biopharmaceuticals protocols call for the evaluation of changes in the immune cell number of both peripheral blood and lymphoid tissue (i.e. lymph nodes), in addition to other parameters normally requested. These data are relied upon to suggest changes that have occurred in the immune function. However, these are only informative when there are clear differences across control and dosed groups. Absence of such effects can present a challenge in identification of potential perturbations to the immune system.

With all this information being produced for biopharmaceutical products the reproduction of similar results across studies is essential. If there are consistent dose-response patterns and a reversal of findings with drug clearance, all this will provide credible data for the action of the test article. Individual parameter abnormalities may suggest a secondary effect other than test article administration. The battery of highly specific functional immune tests currently used with biopharmaceutical studies has produced data that have shown a great deal of inconsistency. With the restricted supply and the commendably small numbers used in NHP studies it is critical to gain a clear understanding of changes that occur in these animals during their initial period of acclimatisation prior to the start of a study. Experience shows that there is a high variability in immunological and haematological values in monkeys, and stress is sometimes cited as a reason for the inter- and intra-group variabilities.

To this end we have embarked on a 3 tier approach to the assessment of our nonhuman primates. The first stage was a short review of immunological baseline levels recorded during the acclimatisation period. This study consisted of four male and four female purpose bred cynomolgus monkeys of Mauritian origin from a 39-week preclinical toxicity study. On commencement of the study, the animals were approximately 18-21 months of age and weighed 2.2-2.8 kg (males) or 1.9-2.5 kg (females). EDTA blood samples were collected from each animal, after overnight starvation, on five occasions (Weeks 0, 6, 13, 26 and 39) via the femoral vein. At each time point, a 1 mL sample was withdrawn for standard haematology parameters (including differential white blood cell counts) and a 0.5 mL sample was taken for flow cytometry. Animals were restrained, but not anaesthetised, during the collection of samples. The absolute counts of surface antigens on lymphocytes from peripheral blood were determined by multi-colour flow cytometric analysis. A standard series of markers that are used at this facility as part of immunological assessment was used for this assessment. Samples were prepared on a TQ-Prep and the samples were then run on a Flow Cytometer. All samples were analysed on the same day as they were taken. This first study highlighted the significant variability in the absolute numbers of lymphocytes and their subsets, also the need to undertake adequate study design to minimize baseline variability. The factors controlling the degree of variability were less clear. However, in order to limit this variability a number of proposals for future toxicology studies were made.

We considered that the data demonstrated a general stability in lymphocyte counts from Week 13 onwards and that weekly assessments of the lymphocyte subset profile during the early stages of the study may reflect both stress and any early drug-related effects.

Recommendations arising from the first tier –

- In order to minimise the potential for stress-related factors, animals should be acclimatised in 'gang housed' facilities (to ensure maximum social interaction), with both environmental enrichment through "toys" and the ability to forage for eight weeks where possible.
- The acclimatisation period before starting the study should be greater than 4 weeks and during this period, animals should undergo both sham handling for dosing and dosing with saline or water. This should minimize stress from a new procedure that could otherwise impact on the lymphocyte subset profiles.
- Ideally during the acclimatisation period, at least three pre-study assessments of lymphocyte subsets and haematology should be undertaken in order to document the background variability in each monkey.

On this first tier study, four animals per sex were used as required by regulatory agencies to mimic a typical dose group used in these types of studies.

The second level of assessment is a meta-analysis of baseline data (pre-study) from over 400 individual animals. This analysis focuses on many of the factors that have been linked as stressors in NHP e.g. the type of caging. It is hoped that this will give a better understanding of the baseline data produced and what, if any factors, they are related to. Currently, the protocol used suggests that at least 3 baseline measurements be taken from the animals prior to the start of the study. If a consistent background set of data were available it may be possible to minimise this amount of pre-dosing activity. This larger set of data will hopefully give us clear links between factors that influence cynomolgus immune status.

The final tier assessment of this work is about to start. This is a detailed evaluation of the immune and biochemical status of the animals during their current 6 week acclimatisation period. This study is planned to run for at least 1 year will give insight into the changes that may take place during acclimatisation and what effect this might have on the assessment of biopharmaceutical studies. A secondary goal is to give further information about baseline measurements in these animals. A comparison will be made with the tier two dataset; again a goal is to produce robust set of data that can be used for comparisons in cynomolgus studies. The goal is to reduce the number of traumatic events such as blood sampling without compromising the integrity of safety studies and to remove stress as a source of variability in parameters that are pivotal for what may be for many biopharmaceuticals the last safety assessment before first-into-man.

Equally important to us in this research is the welfare of the animals that we must use to further human health. Whilst reducing stress improves science, it is in any event a moral requirement.

Assessing the welfare of the MPTP-treated marmoset model of Parkinson's disease

Anon

Parkinson's disease is a progressive neurodegenerative disorder causing a reduction in brain dopamine levels. It is characterised by the loss of voluntary movement, muscular rigidity, postural abnormality and tremor and affects between 1-2% of the population over the age of 50 years. The MPTP (1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine)-treated common marmoset provides a model for the study of Parkinson's disease and is highly predictive of treatment strategies in patients. Treatment over a 5 day period with MPTP induces a lesion in an area of the brain known as the substantia nigra, resulting in marked and persistent behavioural deficits including akinesia, postural instability and rigidity. To address the welfare requirements at this stage a specific care regime, including intensive hand-feeding, has been developed and this is introduced from day 2 of the procedure. Throughout the MPTP-treatment phase the animals are monitored daily for changes in bodyweight, acceptance of hand-feeding, ability to self-feed, physical condition and behaviour. This daily assessment and the care regime are maintained until body weight is stabilised and the ability to self-feed has returned (approximately 10 – 12 weeks). This continuous type of assessment allows the care regime to be adapted to the individual requirements of each animal. Changes in locomotor activity are assessed using automated testing cages, but this is not usually performed during the course of MPTP-treatment or the following 10 – 12 week period. A visual assessment of motor disability, using an established disability rating scale, is performed during the week of MPTP-treatment to determine the degree of "parkinsonism" present. In a disease model such as this, welfare assessment is crucial not only to the well-being of the animals, but also to the successful outcome of the research. The presentation aims to provide an overview of the approach to assessing the welfare of the animals during the course of the project.

Assessing and improving the welfare of SIV-infected macaques

Anon

The experimental infection of macaques with Simian Immunodeficiency Virus (SIV) is considered by many as the best virus challenge model of HIV/AIDS in man for understanding the pathogenesis of infection and identifying strategies to control the further spread of infection. Clearly the potential benefits for man from research using this NHP model could be enormous. However, it is of equal importance that, wherever possible, the welfare impact to macaques involved in this research should be minimised. We will present a review of progress from over 17 years experience of establishing simian models of AIDS and applying them for vaccine development. During this time there has been a continuing programme to refine the models and experimental protocols that will maximise the amount of scientific information that may be accrued from a study. At the same time efforts have been made to ensure that wherever possible experimental end-points are virological rather than clinical. Furthermore, regular monitoring of the health of all subjects on test ensures that unexpected events are identified at the earliest possible occasion. In parallel, there have been marked improvements to the environment in which animals challenged with SIV are housed, yet which nonetheless do not compromise the safety of animal husbandry, scientific and veterinary staff that need to work with these macaques at bio-containment level 3. Current knowledge and procedures will be described along with ideas of where future developments may lead in the next few years.

Assessing the welfare of TB-infected macaques: Refinement of end-points for a primate model of TB infection

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In order to evaluate near-to-clinic novel TB vaccines it has been necessary to establish an aerosol challenge model in two macaque species (*Macaca mulatta* and *Macaca fascicularis*). This presentation describes the establishment of a range of clinical evaluation parameters including imaging by X-ray and Magnetic Resonance Imaging (MRI) scan, to allow early intervention once infection has been confirmed and to gain maximum information from each experimental animal.

The presentation also describes the challenges associated with the use of high level containment strategies to protect staff from infection, whilst meeting the needs for housing animals in social groups and allowing rigorous observation of individual animals by trained technical staff to determine early humane end-points.

Animals were exposed to aerosols of *Mycobacterium tuberculosis* using a Henderson apparatus to generate mono-dispersed bacteria in particles of 2 to 5µm. Anaesthetised animals were exposed individually by the nose only using a mask system and plethysmography to more accurately determine the exposure time to attain the calculated dose. Doses have been calculated such that they do not overwhelm any vaccine effect but are at a minimum to cause progression to disease in unprotected animals.

Before challenge, and at 2-week intervals after challenge, chest X-rays were taken of each animal using mammography film to maximise the resolution of lung tissues. At these time-points rectal temperatures and bodyweight were measured and a blood sample was taken for immunology, bacteriology, Erythrocyte Sedimentation Rate (ESR) and haemoglobin levels.

Temperature, weight-loss, ESR, haemoglobin levels and X-rays were all successfully used to in combination to indicate progression to disease in both macaque species. This combination of factors provided the ability to apply early end-points without progression to severe disease. Other clinical signs recorded as disease progressed included rapid, shallow breathing when sedated and, in the case of rhesus, observation of cough.

The use of MRI scanning provided added value in terms of confirmation of delivery and even distribution of *M. tuberculosis* by the aerosol route. It is concluded from these results that *in vivo* MRI scanning would provide an invaluable indicator of disease progression and would give insight into the differentiation of primary and secondary TB lesions in the lungs.

These established end-point criteria are being taken forward as the main framework for assessment of vaccine efficacy in future studies.