2009 NC3Rs Primate Welfare Meeting / 9th EPV Symposium

28 October 2009

Refining scientific procedures

Agenda

	09.30 – 10.00	**REGISTRATION and COFFEE**
	10.00 – 10.10	**WELCOME and INTRODUCTION** *Professor Roger Lemon, University College London*
Neuroscience	**10.10 – 10.35**	**Procedures for animal well-being and co-operation in monkey MRI** *Professor Alex Thiele, Institute of Neuroscience, Newcastle University*
	10.35 – 11.00	**Best practice in anaesthesia and perioperative care for neuroscience macaques** *Ms Kathy Murphy, University of Oxford*
	11.00 – 11.25	**Refining the use of fluid control in neurophysiology studies with macaques** *Dr Kari Christie, California National Primate Research Center*
	11.25 – 11.45	**COFFEE**
Disease models	**11.45 – 12.10**	**Use of humane endpoints and refinements during the development of marmoset models of infectious diseases** *Dr Michelle Nelson, Defence Science and Technology Laboratory (Dstl)*
	12.10 – 12.35	**The use of imaging to refine vaccine efficacy studies** *Mr Mike Dennis, Health Protection Agency*
	12.35 – 13.00	**Refinement of primate models of Parkinson's disease (1)** *Anon* **(abstract not included)**
	13.00 – 14.00	**LUNCH**
EPV speakers	**14.00 – 14.10**	**EPV: The association of European Primate Veterinarians** *EPV Board*
	14.10 – 14.30	**Refinement of primate models of Parkinson's disease (2)** *Professor Hagai Bergman, The Hebrew University of Jerusalem*
	14.30 – 14.50	**Stem cells and biomedical research potential: production and refinement in primates** *Dr Stephanie Nichols & Dr Lynette Gierbolini, Caribbean Primate Research Center*
	14.50 – 15.10	**Refinement of non-invasive blood pressure measurement in Old and New World monkeys by the novel High Definition Oscillometry technique (HDO)** *Dr Barthel Schmelting, Covance Laboratories GmbH, Münster*
	15.10 – 15.30	**COFFEE**
Toxicology	**15.30 – 15.55**	**Using a Six Sigma approach to improve the quality of data and animal welfare: a case study on ECG recordings in cynomolgus monkeys** *Miss Janet Kelly, Covance Laboratories, Harrogate*
	15.55 – 16.20	**Macaque telemetry in large enclosures** *Dr John Finch, Charles River, Edinburgh*
	16.20 – 16.45	**Animal training: a key component of refinement in social housing and procedures for toxicology studies in macaques and marmosets** *Dr Wolfgang Müller, Covance Laboratories GmbH, Münster*
	16.45 – 17.00	**DISCUSSION and CLOSING REMARKS**
	17.00 – 18.00	**RECEPTION**

Procedures for animal well-being and co-operation in monkey MRI

Professor Alex Thiele, Institute of Neuroscience, Newcastle University

Magnetic resonance imaging (MRI) in macaques has animal welfare implications which are not present in standard electrophysiological procedures. Specifically primate MRI is faced with –

- High noise level during scanning: this can be stressful for the animal and has the possibility to result in hearing impairments. Stress can be minimized by ensuring that animals are gradually introduced to the novel environment, and scanning noise is only introduced when animals are fully accustomed. Ear protection in the form of 'mufflers' or 'ear plugs' work with some animals, but these can be stressors on their own, so an individualized approach has proven successful. These ear protectors cannot be used in auditory fMRI, although ear phones provide some protection on their own.
- Highly confined space: the space available is very restricted, in the primate chair itself (although similar to a 'normal' primate chair), but also inside the scanner. Usually this is not a major issue; gradual introduction to the environment has worked well in our laboratory.
- Special visual projection systems: animals will often view the stimuli through goggles or via mirror systems that are in very close proximity to their face, which is aversive to the animal until they are accustomed to it.
- Restriction to fluid rewards: food rewards are almost impossible to use in the scanning environment, as their delivery is a big challenge, but more importantly, chewing will result in motion induced susceptibility artefacts, which render the data useless, and cannot be adequately corrected for. This is even a problem with 'pulp food'.
- Restriction to implant materials that do not result in susceptibility artefacts: metal based implants cannot be used, as they result in susceptibility artefacts, even if the metal is non-ferrous. Thus ceramic screws are necessary, in conjunction with either dental acrylic and or plastic based implants. This is not really a problem, although dental acrylic implants can be cosmetically less pleasing.
- Motion induced susceptibility artefacts: Motion of the animal changes the magnetic field homogeneity and, under specific circumstances, it may be necessary to use motion sensors and to train animals to remain repeatedly still for e.g. 10 seconds. This can be stressful and requires additional (sometimes lengthy) training periods, but is conceptually similar to eye fixation training.

Most of the MRI-specific welfare implications can be addressed easily, and our experience shows that animals adapt well to the environment. Others can be more tricky (e.g. training animals to sit still during a trial) and may require additional training periods. Given that training and habituation can often not be done inside the scanner itself (due to prohibitive costs), a mock scanner should be available, which mimics the scanning environment as closely as possible.

Best practice in anaesthesia and perioperative care for neuroscience macaques

Ms Kathy Murphy, University of Oxford

Many of the non-human primates used for biomedical neuroscience research will at some point require anaesthesia in order to facilitate model preparation. Refinement of anaesthetic protocols and practices is essential in order to continually reduce suffering, return the animal model to its normal state as quickly as possible, and minimize any confounding factors that anaesthesia may have on the scientific data produced. Recommendations in anaesthetic practice are constantly evolving as new drugs are produced and new combinations of drugs evaluated, and so it can be difficult for researchers to ensure that they adequately refine whilst reducing variation within their studies. However, although some drug regimes are associated with particular problems that may impact on either animal welfare or the science, it is the case that preparation of the patient, monitoring of physiological parameters and appropriate intervention when problems are identified are the most important factors when refining practices.

Refining the use of fluid control in neurophysiology studies with macaques

Dr Kari Christie, California National Primate Research Center, University of California, Davis

This presentation is essentially a discussion, including case studies, regarding the use of fluid control in neurophysiology studies with macaques. First of all, behavioural and physical evaluations may facilitate selection of the appropriate study animal. Next, potential alternatives for motivating macaques to perform behavioural neuroscience tasks without fluid regulation are recommended. If water regulation is deemed necessary, the mechanical aspects of fluid regulation should be considered and determined for each animal. As a consequence, possible refinement modifications can then reduce any impact on these water regulated animals. In conclusion, an integrated approach to the monitoring and health maintenance of fluid regulated animals at the University of California, Davis and the Wisconsin National Primate Research Center will be covered. Additionally, as food for thought, the presenter will share her results on the health and stress response of animals before and during water regulation.

Use of humane endpoints and refinements during the development of marmoset models of infectious diseases

Dr Michelle Nelson, Defence Science and Technology Laboratory (Dstl)

The understanding of disease pathogenesis and the appropriate use of animal models is an important consideration when testing the efficacy of treatments such as vaccines or antibiotics. Work has been undertaken to develop the common marmoset (*Callithrix jacchus*), a New World non-human primate species, as a model of experimental tularemia and melioidosis. Both these diseases cause lethal infections in human populations; the pathogen and animals infected with the pathogens need to be handled at Containment Level 3 (CL3).

Dstl has developed the capability to work with marmosets safely at Containment Level 3, which includes the use of specially designed cages for housing and capture of the animals, as well as the use of remote monitoring (telemetry, activity and live video feed) to support the monitoring of animal welfare.

All studies are designed in consultation with statisticians to ensure the minimum numbers of animals are used to obtain valid scientific results (e.g. Dixon staircase method to estimate lethality). The maximum amount of information is gathered from each experiment including temperature and activity during the experiment and bacteriology, histology, immunology and various clinical parameters at post mortem. Data is compared to human data and other animal models to determine the relevance of the model.

Humane endpoints are determined early in the development of the models and are refined as knowledge increases. Temperature monitoring (via implants) has been key to the development of humane endpoints in our studies.

Dosing of therapies (e.g. antibiotics) has been refined by training animals to take milkshake (and subsequently antibiotic) via a syringe, offering enrichment benefits as well.

The work presented will demonstrate the use of the above examples in our studies.

This project has been funded with Federal funds from the National Institute of Allergy and Infectious Diseases (NIAID), under contract No. N01-AI-30062 through our Prime Contractor, the Health Protection Agency, Porton Down, UK.

The use of imaging to refine vaccine efficacy studies

Mr Mike Dennis, Health Protection Agency

The use of advanced imaging to quantify total lesion volume in experimentally infected animals has distinct advantages over conventional clinical and post mortem assessments in that it is quantitative, accurate, relatively simple and is a method that is easily standardised between laboratories. If facilities are available, an added advantage is that data can be collected at intervals on live animals at various stages of disease. This method is valuable at assessing in non-human primates and other model species, the efficacy of experimental vaccines and drugs intended for use in humans.

Magnetic resonance (MR) imaging could have many applications in vaccine efficacy studies and we have shown that the electronic images captured by this process from tissues *ex vivo* lend themselves to a reproducible quantification of disease using stereological techniques of image analysis. For example, if MR images of the lungs of animals could be collected from live animals throughout the study period, a comprehensive picture of disease progression over time could be built up for each study subject. Such information could be used to inform on appropriate times to terminate animals. This would minimise the risk of terminating an animal prematurely and would avoid situations where termination was predominantly based on behavioural criteria, but subsequent examination post mortem has shown levels of disease lower than predicted. Conversely, it could help to avoid cases where the clinical situation has progressed further than behavioural signs suggest. Such discrepancies could reduce the discriminatory power of a vaccine study. In-study stereology could also provide an opportunity to refine studies; if clear differences seen between control and vaccine groups could be identified, studies could be terminated early which would be beneficial both in terms of animal welfare and cost of studies. Furthermore, if serial images of the disease process can be collected this will negate the requirement for serial sacrifice and reduce the number of animals required for disease pathogenesis studies.

EPV: The association of European Primate Veterinarians

EPV Board

The non-profit network of European Primate Veterinarians (EPV) was founded in 2001 by Susanne Rensing and Thierry Decelle. The main ambition of this informal group was to exchange knowledge and information between clinical primate veterinarians, concerned with the health, care and welfare of non-human primates (NHPs).

EPV should be regarded as a platform to promote up-to-date clinical care and knowledge, as well as fellowship, among primate veterinarians. This includes veterinarians who are working in conservation with highly vulnerable and protected species as well as those working in research with purpose-bred monkeys, because all NHPs require special care and management.

In the early stages, veterinarians and postgraduates from the different European primate centres in the Netherlands, Germany and France were involved. Over the years, more and more veterinarians from universities, research institutes, industry, zoos, sanctuaries and contract research organisations joined in as well.

EPV was formally recognized as an association in 2007 when bylaws were established. At present more than 100 veterinarians from many different European nations are members.

An official annual meeting serves the purpose of educating and sharing knowledge, ideas and broad expertise. Evaluations of the need for guidelines on certain themes are handled within this scope (e.g. Guidelines for the prevention and control of tuberculosis in non-human primates: Recommendations of the European Primate Veterinary Association Working Group on Tuberculosis). Additionally, there is an update on newsworthy topics, such as politics concerning NHPs (e.g. revision of European Directive 86/609/EEC), airline transport of NHPs, the 3Rs and alternative methods.

Dissemination of expertise and sharing of information with newcomers in the field is maintained all year round via an email list of members and will be improved with our new website. For particular enquiries, a list of direct contacts and the expertise of the members is available.

EPV is open to new members and would be happy to welcome veterinarians or veterinary students engaged in professional activities related to NHPs.

http://euprimvets.u-strasbg.fr

Bushmitz M, Lecu A, Verreck F, Preussing E, Rensing S, Mätz-Rensing K (2009) Guidelines for the prevention and control of tuberculosis in non-human primates: recommendations of the European Primate Veterinary Association Working Group on Tuberculosis. Journal of Medical Primatology 38(1): 56-69.

Refinement of primate models of Parkinson's disease (2)

Professor Hagai Bergmann, The Hebrew University of Jerusalem

Parkinson's disease is one of the most common and devastating human disorders. The disease is characterized by motor deficits (poverty of movements, muscle rigidity and tremor), as well by cognitive and emotional deficits. Primate treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin reveal motor and other clinical symptoms as human patients. The responses of MPTP-treated primate to current pharmacological (e.g., dopamine replacement therapy) and surgical (e.g., deep brain stimulation) treatments of Parkinson's disease, and the pathological changes in their brain (dopamine depletion in the basal ganglia) are very similar to those of the human patients. That is the reason why MPTP-treated monkeys are commonly used in studies seeking better understanding of the basal ganglia and improved therapeutic methods for Parkinson's disease.

MPTP can be administrated to primates either systemically or through carotid or local brain injections in acute or chronic ways. The systemic MPTP primate model offers the most similar model of the human conditions; however the severe clinical symptoms of the treated monkeys demand intensive care that should include frequent follow ups (e.g. special attention should be given to the animal's general condition, weight loss and pressure ulcers), multiple nasogastric feeding sessions daily and frequent position changes.

In my talk, I will review the anatomy and physiology of the basal ganglia and dopamine system, and why dopamine depletion in the basal ganglia is leading to the clinical symptoms of Parkinson's disease. I will discuss the advantages and disadvantages of the different primate MPTP models, as well as their ethical use. Finally, I will present current refinement strategies for MPTP-treated Parkinsonian monkeys.

Stem cells and biomedical research: production and refinement in primates

Dr Stephanie Nichols & Dr Lynette Gierbolini, Caribbean Primate Research Center, University of Puerto Rico

Embryonic stem (ES) cells hold great promise in the treatment of degenerative diseases such as diabetes, Parkinson's, Alzheimer's, neural degeneration and cardiomyopathies. These cell lines offer a unique opportunity for drug discovery and testing as well as the development of replacement therapies in disease treatment because of their intrinsic potential to be directed into different cell types (e.g., neural, cardiac and blood cells). The most appropriate model for clinical applications of ES cells in therapeutic medicine is the non-human primate. Macaques are the most highly utilized and well-described non-human primates in biological research and provide a faithful model for preclinical research. By using ES cell lines derived from macaque embryos in this capacity, it is possible to replace or greatly reduce the numbers of animals required for biomedical research by providing a renewable *in vitro* resource for investigation. In addition, use of ES cell lines provides a much more consistent experimental model and presents some practical and ethical advantages for ES cell research, complementing studies with human ES cells. The majority of existing non-human primate ES cell lines have been obtained from embryos created *in vitro* and required considerable interaction with study animals. We will present a protocol to obtain *in vivo* produced embryos and ES cell lines with minimal animal disturbance. This can be accomplished through observation of the menstrual cycle (via sex skin coloration and urine analysis of sex hormone profiles) in combination with natural mating to fertile males. Cell lines obtained from the embryos of these matings are inherently high quality and provide a "gold standard" for ES cell investigations. With the macaque as our model, we have a unique opportunity to make a big impact in this promising field of research and invite other primate centers to investigate the potential of an ES cell resource in addressing human disease.

Refinement of non-invasive blood pressure measurement in Old and New World monkeys by the novel High Definition Oscillometry technique (HDO)

Dr Barthel Schmelting, Covance Laboratories GmbH, Münster

Nonhuman primates are being used increasingly as animal models during preclinical toxicology and safety assessment. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) demands in its guideline that the effects of a test substance on the cardiovascular system should be assessed appropriately.

Most non-invasive blood pressure methods fail due to technical limits. Currently, approaches for accurate blood pressure determination rely predominantly on invasive techniques, like indwelling arterial catheters or implanted cardiovascular telemetry. These approaches impose variable degrees of invasiveness which often impedes their routine use in toxicological studies and veterinary examinations.

The novel high definition oscillometry (HDO) technique was evaluated as a potential non-invasive approach for accurate blood pressure recordings in cynomolgus monkeys and common marmosets. HDO enables visible, real time analysis of each measurement on a screen by the use of blood pressure amplitude scans with up to 16.000 Hz within 10-15 sec. HDO was used in both species in repeated measurements in order to reveal the degree of precision and accuracy. In telemetry implanted cynomolgus monkeys, telemetry and HDO were able to detect cardiovascular changes simultaneously. The minimal intra-individual standard deviation in consecutive measurements confirmed the high precision of the HDO technique. In marmosets with high heart rates (>400 beats per minute), no limitations for HDO measurements were encountered.

Our data on cynomolgus monkeys and common marmosets gained by HDO provide a clear advantage over standard oscillometry in terms of precision and accuracy. The vigilance status and temperament of the animals during measurement is pivotal, as stress-related mean arterial pressure (MAP) increases, often shown in non-trained animals, may mask potential drug-related effects. Using precise and sensitive HDO, stress-associated MAP increases are avoided, which is crucial when evaluating drug related haemodynamic changes.

Using a Six Sigma approach to improve the quality of data and animal welfare: a case study on ECG recordings in cynomolgus monkeys

Miss Janet Kelly, Covance Laboratories, Harrogate

Cynomolgus macaques are an important safety model for predicting the adverse effects of new medicines before clinical testing in humans. The regulatory authorities require collection of electrocardiogram (ECG) data for the detection of cardiac arrhythmias. Pharmaceutical companies are striving to improve the development process to identify as soon as possible the risk of new drugs to cardiac parameters, so that they can make appropriate decisions on the future development of the drug.

Covance adopted a novel approach to refining ECG data recording in the cynomolgus monkey for regulatory toxicology, utilizing a Six Sigma approach. Process improvement tools such as Six Sigma have been widely implemented in business management strategies and are usually associated with efficiency gains or cost reduction. In this instance, Six Sigma tools were used to enable the introduction of a high quality digital platform for the acquisition and real-time analysis of electrocardiogram data from macaques. This innovative approach provided a systematic and directional stepwise rationale for improvement in data quality including refinements to restraint procedures during data capture.

We present data that show significant improvements to the sensitivity and reliability of macaque ECG measures during preclinical testing. In addition behavioural measures were used to evaluate changes to restraint and show benefits for animal welfare. This process has been successful in improving validity of this important animal model for pharmaceutical development and strongly demonstrates the link the between good welfare and good science.

Macaque telemetry in large enclosures

Dr John Finch, Charles River, Edinburgh

Regulatory safety pharmacology studies require detailed evaluation of test item effects on blood pressure, electrocardiography (ECG) and body temperature. Robust, reliable continuous measurements are obtained from unrestrained animals with an implanted physiological transmitter. Rodents are not ideal for this work because of their small size, and so studies are typically conducted in dogs. Occasionally dogs are not appropriate and non-human primates are the only relevant test species. We are developing a system in monkeys using advanced technology, in order to allow physiological measurements in animals housed in social groups in large environmentally-enriched enclosures. The presentation shows how we decided the most appropriate apparatus/study design to capture robust data to assess the potential cardiovascular effects of new medicines, while complying with the highest standards of animal care and housing, which exceed the requirements of the Council of Europe Convention (ETS 123). Meeting these two requirements requires the commitment of considerable care, planning and funding.

Animal training: a key component of refinement in social housing and procedures for toxicology studies in macaques and marmosets

Dr Wolfgang Müller, Covance Laboratories GmbH, Münster

In a facility where pharmacological and toxicological investigations in macaques and marmosets are performed, the housing for the monkeys and the way in which the scientific procedures are performed must complement each other, to allow test substance administration, blood sample collection and monitoring of physiological parameters, as single events but also repeatedly over weeks and months. This requires competent staff and appropriately trained monkeys that are self confident and cooperative and, as a consequence, unstressed and safe to handle. We have performed reproduction toxicity studies with pregnant females for many years and manual handling of the monkeys has always been our routine.

The monkeys are housed in social groups in cages that have been optimized over generations of prototypes to provide a stimulating environment for the occupants and to support our caretakers in handling the animals. Macaques are trained in steps by positive reinforcement training (PRT) to move to an elevated location in the back of the cage and stay there so that a caretaker can enter the cage and easily capture the animal for procedures. Since the monkeys have a vertical flight reaction, they perceive this elevated location as the safest place in the cage. Thus the training makes use of their natural behaviour and has also influenced the cage design. The training is initiated prior to a study and continues throughout the study. The trained method of capture represents a clear refinement in comparison to involuntary restraint techniques.

In our facility, animal caretakers and study technicians are organized in separate groups with different tasks. The caretakers perform or support all the animal-related procedures and give the rewards to the animals afterwards. They are therefore the "good guys" for the animals which supports our PRT efforts. Training the monkeys helps to reduce animal stress and shifts their interaction with the caretakers towards collaboration and is therefore a key component of refinement.

In summary, our approach to monkey housing is a three pillar concept which includes: 1) optimized cage systems for macaques and marmosets; 2) training of staff and monkeys; and 3) systematic selection of compatible cage mates. Both the cage design and training consider the natural behavioural patterns of the monkeys. Social housing is possible only with compatible cage mates. All three pillars are equally important and indispensable.