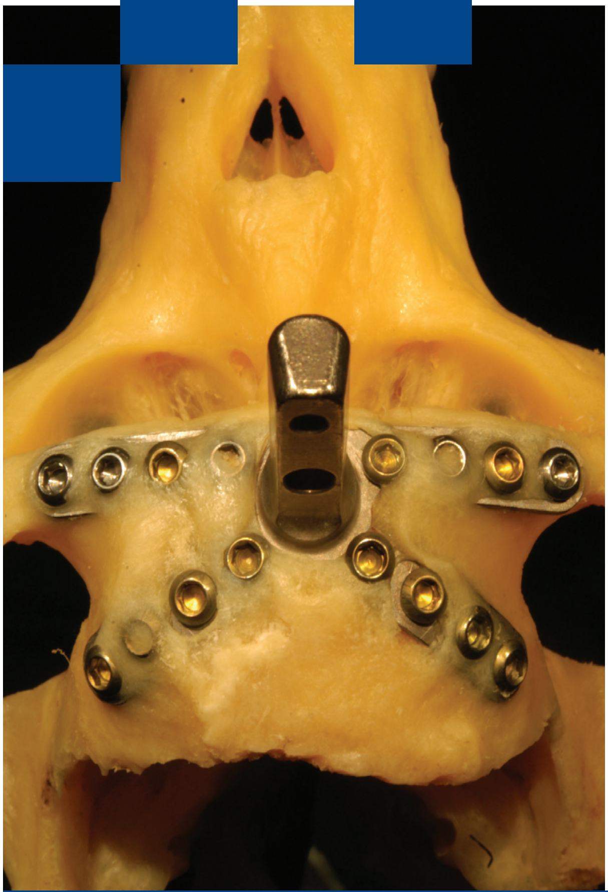
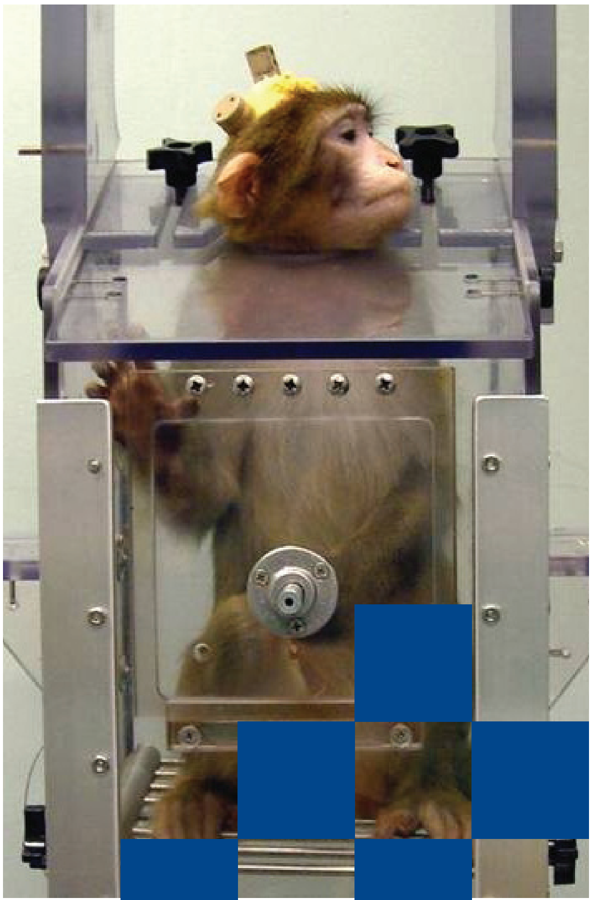




NC3Rs Primate Welfare Meeting

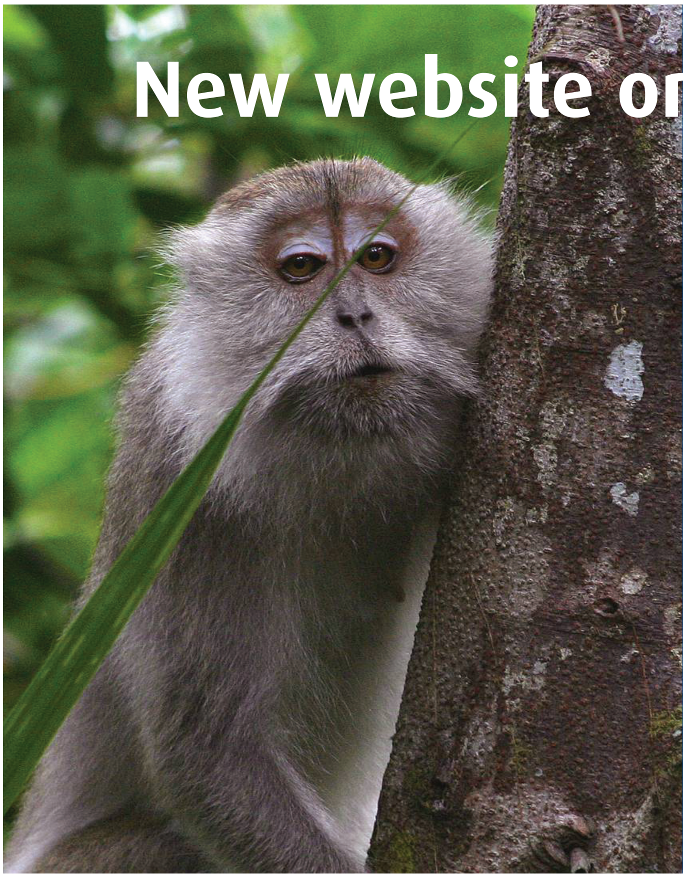
Chronic implants workshop: let's share what works

21 November 2012

Supported by
wellcome trust

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

New website on macaque welfare



The University of Stirling, funded by the NC3Rs, is developing a new website on macaque welfare to complement www.marmosetcare.com

We are seeking potential collaborators to supply images, video and sound clips for the site. Of particular interest is material from cynomolgus and rhesus macaques, including facial expressions, postures and vocalisations.

We also intend to include examples of refinement of handling and restraint, scientific and husbandry procedures, and environmental enrichment.

To contribute to the site and make it the best possible resource for understanding macaque behaviour and improving welfare, please contact Dr Lou Tasker (louisa.tasker@stir.ac.uk) or Dr Mark Prescott (mark.prescott@nc3rs.org.uk)

Welcome

Dear Colleagues,

Welcome to this workshop, the second of two held under the auspices of the 2012 NC3Rs Primate Welfare Meeting. We are pleased that this event has attracted over 110 delegates with approximately 40% from outside of the UK.

The design, maintenance and refinement of chronic implants used in primate neuroscience are topics worthy of focused discussion. This workshop builds upon a previous one held in 2008, but this time we are delighted to be working with the University of Oxford. We would like to take this opportunity to thank, in particular, Dr Caroline Bergmann, Dr Andrew Bell and Ms Maria Martinez for their input and hard work.

Together we have developed a programme to explore the pros and cons of the different implant designs and approaches currently in use, how they should be evaluated, and how the complications that can occur (such as loosening of the implant or infection) can best be prevented or addressed. We hope you will take this chance to make new contacts, including with specialists from other disciplines such as orthopaedics and dentistry, and fully participate in sharing your expertise and ideas.

Evaluation is very important to the NC3Rs, and we would ask that you contact us after this free event to tell us how attendance has enabled you to improve primate welfare and scientific outcomes, e.g. through increased awareness, data sharing or collaboration (events@nc3rs.org.uk).

The Primate Welfare Meeting is just one of several activities of the NC3Rs focused on the use of primates in research (see www.nc3rs.org.uk/primatewelfare and www.nc3rs.org.uk/primatesabpi). Where further research is needed to develop and validate new refinements, the NC3Rs is keen to support this via its research funding schemes (www.nc3rs.org.uk/fundingschemes).

Thanks to everyone for your interest and have a great day.

Dr Mark Prescott
Head of Research Management and Communications
NC3Rs

Agenda

09.30 – 10.00	**REGISTRATION and COFFEE**
10.00 – 10.10	**Welcome** *Professor Stuart Baker, Newcastle University (Chair)*
PRESENTATIONS	
10.10 – 10.30	**Development of a tissue-friendly (Tekapeek) head restraint implant** *Professor Roger Lemon, University College London*
10.30 – 10.50	**A watertight, acrylic-free titanium recording chamber** *Dr Daniel Adams, University of California San Francisco*
10.50 – 11.15	**Design and materials: the hard and soft tissue interface of dental implants in animal models** *Dr Stefan Stübinger, University of Zurich*
11.15 – 11.40	**External implants in experimental research: overview of the cascade of aseptic loosening, surgical approaches and future considerations** *Professor Brigitte von Rechenberg, University of Zurich*
11.40 – 12.00	**COFFEE and POSTER VIEWING**
12.00 – 12.15	**Guidelines for the care of non-human primates with cranial implants: a discussion of the process, deliberations and results from the Association of Primate Veterinarians** *Dr Marek A Niekrasz, University of Chicago*
12.15 – 12.30	**Individually customisable, non-invasive head immobilisation** *Dr Christopher Petkov & Ms Heather Slater, Newcastle University*
12.30 – 13.00	**Maxillofacial implants in patients: application of MR imaging and other reconstructive surgical considerations** *Mr Stephen Watt-Smith, John Radcliffe Hospital*
13.00 – 14.00	**LUNCH and POSTER VIEWING**
BREAK-OUT SESSIONS	
14.00 – 15.00	**SESSION 1** **A. Design and materials** *Dr Andrew Bell, University of Oxford & Dr Stephen Frey, McGill University* **B. Surgical technique and methods** *Dr Caroline Bergmann, University of Oxford & Professor Wolfram Schultz, University of Cambridge* **C. Wound and implant maintenance** *Professor Paul Flecknell, Newcastle University & Ms Kathy Murphy, University of Oxford*
15.00 – 15.20	**COFFEE and POSTER VIEWING**
15.20 – 16.20	**SESSION 2** – *As above*
16.20 – 17.00	**DISCUSSION and CLOSING REMARKS**
17.00 – 18.00	**NETWORKING RECEPTION**

Development of a tissue-friendly (TEKAPEEK) head restraint implant

Professor Roger Lemon
University College London, UK

I will summarise our results using the thermoplastic material Tekapeek© to construct head restraint devices for use in awake, behaving monkeys requiring optimal recording stability. The headpiece is custom-fitted to each macaque using 3D-reconstrction of the skull and brain surface from structural MRI scans carried out at 3.0T. It is securely held in place by four titanium bolt assemblies which are mounted on small discs located between dura and skull. To date we have used this material to custom-fit implants for a number of macaques, and I will describe in detail results from two of these, in which the implants remained in excellent condition for 2.8 and 2.9 years respectively. After an initial two-month post-operative 'settling-in' period, monkeys were positively reinforced to accept head restraint using the device. Thereafter we were able to carry out long-term recordings of high quality and stability. The headpiece remained in good condition throughout, with minimal need for repair. Skin margin infection was reduced compared to previous steel-based implants, and was generally well-controlled with topical antibiotics. Post-mortem examination revealed that the skull beneath the implant was strong and healthy.

Supported by an NC3Rs project grant: G0500172

A watertight, acrylic-free titanium recording chamber

Dr Daniel Adams
University of California San Francisco, USA

Neurophysiological recording in alert monkeys requires the creation of a permanent aperture in the skull for repeated insertion of microelectrodes. Polymethyl methacrylate (PMMA, acrylic or dental cement) is widely used to attach a recording chamber over the skull opening. I will describe a titanium chamber that fastens to the skull with screws, using no PMMA. The chamber is sealed to the skull using hydroxyapatite as a water-tight gasket. As the chamber base osseointegates with the skull, the hydroxyapatite is replaced eventually with bone. Rather than having a finite lifetime, such recording chambers becomes more firmly anchored the longer they are in place. By achieving a hermetic seal, many of the problems associated with PMMA-anchored chambers, such as chronic infection, bone resorption, and dural thickening can be alleviated. The resulting reduction in invasive maintenance of the implant site, and the increased duration available for recording are beneficial for both animal welfare and experimental studies.

Design and materials: the hard and soft tissue interface of dental implants in animal models

Dr Stefan Stübinger
University of Zurich, Switzerland

The orofacial system with its diversified complex composition forms a biocybernetic system in which chewing forces significantly account for remodelling processes. Thus biological, biomechanical as well as microbiological host factors play a crucial role in the development and maintenance of a stable bone-to-implant contact. Long-term success of dental implants is closely related to initial mechanical stability, osseointegration and peri-implant soft tissue integration. Thereby it is important to consider osseointegration as well as soft tissue adaptation as an ongoing biological process that reveals characteristic changes during clinical function and implant loading.

Over the last decades surgical protocols as well as endosseous implants have been continually improved, such that osseointegration into cortical and cancellous bone has been considerably refined by state-of-the-art techniques and materials. Implants can be engineered to enhance strength, interfacial stability, and load transfer by using different core materials, surface structures, and macro designs. Innovative surgical instruments like different laser systems or modulated ultrasound allow new and unprecedented ways of treating hard and soft tissue to improve the peri-implant interface. Based on a series of pre-clinical studies this overview will present current research approaches to influence the interrelation between peri-implant hard/soft tissues and implant design and surface modification.

Abstracts

External implants in experimental research: overview of the cascade of aseptic loosening, surgical approaches and future considerations

Professor Brigitte von Rechenberg
University of Zurich, Switzerland

Interfaces between implants and bone are the crucial aspect to maintain final stability for long-term use of implants or devices in the living organisms. While biocompatibility, surface structure of implants and with this wear particle issues have been extensively investigated *in vitro* and *in vivo*, very few reports are available dealing with the overall picture, or better cascade, which includes surgery, tissue interaction with the implant and the final truly existing "perpetuum mobile", once things go wrong.

Wear particles have been largely incriminated as being responsible for aseptic loosening of implants. Type, size and amount of particles were extensively tested *in vitro* and - at no real great surprise - were considered toxic for cells. However, since no surgeon really implants wear particles to begin with, the real challenging question is to figure out why the same devices are implanted without problems in some instances, whereas in another situation wear particles are formed and contribute to final failure.

In the context of aseptic loosening, initial surgery, the healing of the surrounding tissue plus the making and biomechanics of the implant itself have an intricate interplay between them. Initial surgery causes due to its invasive nature in most instances a massive trauma, which in itself causes a serious tissue reaction. This tissue reaction is dominated by inflammation, which if moderate is beneficial and mandatory for tissue regeneration. However, if this tissue reaction is excessive, for instance through excessive trauma itself (surgical technique, micro-instability), then negative aspects of inflammation take over and make the overall situation worse. There, apart from cellular reactions following trauma and ischemia, a drop of pH in the local milieu causes a change in corrosion behavior of metallic surfaces that significantly weakens the metallic surface facilitating tribocorrosion and finally wear particle formation. Once the tribo-corrosion "engine" is working, the "perpetuum mobile" is invented and nothing will stop chronic bone resorption and the formation of an active interface membrane that promotes the process of aseptic loosening and final implant failure often complicated by chronic infection. The cascade will be explained by research conducted in our own laboratory [1-3].

References

1. B. von Rechenberg et al. (2004) An animal model for interface tissue formation in cemented hip replacements. *Veterinary Surgery* 33, 495-504

2. B. von. Rechenberg et al. (2001) A reviews of aspetic loosening in total hip prosthesis. *Veterinary and Comparative Orthopaedics and Traumatology* 3, 115-124

3. B. von. Rechenberg et al. (2001) Degradation of CoCrMo hip implants - a corrosion, wear and clinical analysis. *European Cells and Materials* 2, 70-71

Guidelines for the care of non-human primates with cranial implants: a discussion of the process, deliberations and results from the Association of Primate Veterinarians

Dr Marek A Niekrasz
University of Chicago, USA

Use of non-human primates in neurobiological studies may include performing invasive cranial surgeries and implantation of chronic research devices. Success of chronic cranial implantation is a function initially of how the implant is placed and the materials used, coupled with the animal's physiology and healing responses. Cranial implantation surgery must be conducted with respect to normal host anatomy and physiology and maintaining aseptic intra-operative conditions is critical for optimal success. In procedures, it is common to stagger the placement of each attachment (e.g. head-post before the chamber) until the chamber is required to facilitate preservation of the integrity of the chamber and safeguard the health of the animal. Maintenance of operational condition is then a function of continued care of the implant and the tissues surrounding it using aseptic methods and appropriate anti-bacterial treatments. The above text aims at providing non-human primate researchers, the institutional animal care and use committee (IACUC) and veterinary staff with guidelines for conducting research involving chronic cranial implants and for assessing their routine and non-routine care. The Association of Primate Veterinarians supports the responsible use of non-human primates in neurobiological research and these guidelines are intended as general points of reference based on currently approved standards of veterinary practice. Each individual project, however, must meet specific criteria for the IACUC review and approval, verification of the investigator's skill and experience and establishing a close collaboration with the institutional veterinary staff.

Individually customisable, non-invasive head immobilisation

Dr Christopher Petkov & Ms Heather Slater
Newcastle University, UK

The development of effective non-invasive head immobilisation options for non-human primates can potentially replace the wide use of surgically implanted head posts for many neuroscientific (awake animal or recovery) procedures that require minimal head movement. Although several approaches have been proposed over the years, these continue to have limitations and have not been broadly accepted. We propose a head restraint system for macaque and marmoset monkeys that harnesses the experience of human cancer radiotherapy units, which provide effective customised non-invasive head immobilisation for both adult and child cancer patients undergoing radiotherapy treatment of cancerous head/neck tumours. We also aim to develop a version of this system that the primates can be trained to engage with for temporary head immobilisation, and to take steps to make it broadly available. This presentation will show pilot data of an animal engaging with the system and provide an overview on the planned project which has recently received support from the NC3Rs, with a proposed start date in Jan. 2013. We welcome feedback from the community as we prepare for the project start.

Supported by an NC3Rs pilot study grant: NC/K000608/1

Maxillofacial implants in patients: application of MR imaging and other reconstructive surgical considerations

Mr Stephen Watt-Smith
John Radcliffe Hospital, UK

The complexity of maxillofacial anatomy makes reconstructive surgery challenging in terms of achieving good functional and aesthetic results. Diagnostic imaging and implant technologies have evolved over many years to aid in surgical planning and enhance outcome. The choice of materials and surgical techniques has a profound impact on success.

The focus of this session will be on the surgical and imaging considerations related to implant placement. In terms of surgical management, the basic surgical principles to maximise successful outcome will be discussed. These specifically include the techniques of bone anchoring, methods of close approximation and adaptation, utilisation of types of joints, screw types, wires and glues. For soft tissue implants attention will be given to planning of pocket formation, utilisation of scar formation and contracture to the surgeons' advantage, reduction of dead space and minimising blood loss. The principles of wound closure and wound care will be discussed.

Implants may be permanent or temporary, and may consist of metal or composite materials. The key principle of adaptation, approximation and fixation are crucial for long term success. Discussion will focus on optimising approximation using the techniques of estimation, impressions and imaging. The imaging modalities of greatest benefit in such cases include plain film, CT and MRI. 3D rendering of CT and more recently MRI of bone permits the creation of anatomical models to enhance surgical planning. These new technologies and techniques will be discussed.

Poster abstracts

Poster 1

High-density scalp somatosensory evoked potentials as follow-up of functional recovery from motor cortex lesion in macaque monkeys

Anne-Dominique Gindrat[1], Charles Quairiaux[2, 3], Juliane Britz[3], Florian Lanz[1], Denis Brunet[3], Christoph M Michel[3], Eric M Rouiller[1]

[1] University of Fribourg, Switzerland

[2] University of Geneva, Switzerland

[3] Geneva University Hospital, Switzerland

The goal of this study was to develop a simple and minimally invasive method to record somatosensory evoked potentials (SSEPs) from the whole scalp surface in anaesthetised adult macaque monkeys, with the prospect of allowing repeated assessment of the cortical activity in the context of a central nervous system lesion. It is expected that SSEPs will allow us to assess post-lesion cortical reorganisation of neuronal networks and relate it to functional recovery, following a motor cortex lesion.

Experiments were conducted on four adult macaque monkeys (*Macaca fascicularis*), using a customised EEG cap containing 33 electrodes regularly distributed over the scalp while the animal was anaesthetised (2.5% sevoflurane). Electrical stimulations were delivered separately either to the median nerve or to the tibial nerve, successively on each side.

When the animals reach a behavioural plateau, they are subjected to a cortical lesion, requiring a craniotomy. Consequently, to evaluate the effect of the craniotomy itself on SSEPs, a “sham lesion” consisting in the craniotomy alone was first performed, with the bone flap put back in place. There was no SSEPs change related to the “sham lesion”. The next step is to perform a permanent unilateral lesion of the hand representation of the motor cortex.

A k-means cluster analysis of the voltage maps was applied to the SSEPs data (data-driven approach revealing a series of scalp topographies reflecting the steps in information processing). The LAURA (local autoregressive average) inverse solution algorithm with LSMAC (Locally Spherical Model with Anatomical Constraints) head model was used for source estimation of the scalp voltages.

The pre-lesional voltage topography of the SSEPs obtained after either median or tibial nerve stimulations is in line with the somatotopic organisation of the sensorimotor cortex. Post-craniotomy data are presented, as well as source localisation results.

Supported by Swiss National Science Foundation grant 310000-110005 (EMR) and NCCR Neuro

Poster 2

Surgical technique of chamber implantation for neuronal recording of the basal ganglia: which is better, ventriculography- or MR-guided?

Hirokazu Iwamuro, Ines Trigo Damas, Jose Angel Obeso Inchausti

Center for Applied Medical Research (CIMA), Spain

Electrophysiological neuronal recordings in awake macaques are essential to investigate the function of the basal ganglia, which plays a crucial role in various networks such as motor control, learning and reward representation. Since nuclei of the basal ganglia are located in a deep area in the brain and some of them are small, it is important to put a chamber on the skull in an adequate position at a proper angle for inserting a recording electrode in a target in the basal ganglia.

In two monkeys (*Macaca fascicularis*), we implanted a recording chamber which was targeted to the subthalamic nucleus (STN) by different surgical methods. One was ventriculography-guided; the target was reconstructed according to the anterior and posterior commissures on a lateral ventriculography with a sagittal atlas of *Macaca fascicularis* brain (Lanciego et al. 2011). The other was MR-guided; the coordinates of the target were calculated on a structural magnetic resonance (MR) imaging of the brain with visible markers. After implantation of the chamber by each method, electrophysiological neuronal recordings of the basal ganglia in awake state were performed and the location of the STN was identified in each monkey.

All surgical procedures were done in safety. The difference between the calculated position and the real location of the STN was 2-3mm in both monkeys.

These results suggest that the errors of chamber positioning by both methods are similar. From the viewpoint of less-invasive procedure, however, the MR-guided method is preferable.

Reference

Lanciego JL, Vazquez A. (2012) The basal ganglia and thalamus of the long-tailed macaque in stereotaxic coordinates. A template atlas based on coronal, sagittal and horizontal brain sections. Brain Structure & Function 217, 613-666.

Poster 3

New flexible arrays for chronic surface and depth recording

Chris Lewis, M Scholvinck, I Grothe, J Vezoli, E Fiedler, T Stieglitz, P Fries
Ernst Strüngmann Institute (ESI), Germany

Our lab is developing novel designs and techniques for the use of flexible multi-channel arrays for chronic recording in awake behaving primates. Our work focuses on the refinement of current techniques in order to increase the density and coverage of potential recording sites, minimize the invasiveness of implantation procedures and prolong the period of data acquisition. Towards these ends we are developing techniques along two trajectories. First, we are using dense electrocorticography (ECoG) grids, implanted subdurally, to record from large areas of cortex with high spatial resolution. This approach allows highly stable and complete coverage of large portions of cortex and is highly adaptable to target specific regions of interest. Second, we are developing multichannel shaft electrodes that can be chronically inserted through small (~400 mm) holes in the skull and used to record spiking activity and local field potentials in laminar configurations or from widely spaced sites spanning multiple areas. This enables us to alternatively sample densely from regions of special interest, while simultaneously exploring broadly across many regions. Both the ECoG grid and the shaft electrodes are made from biocompatible and extremely thin (10 mm) polyimide foil, thereby reducing the potential damage to tissue to a minimum. Further, the technology is appealing because of the flexible way in which it can be combined with other techniques (i.e. targeted acute recordings with sharp penetrating electrodes) as well as the high degree of customization available by close interaction with process engineers. In the future, we plan to increase the scale of our chronically implanted arrays, as well as further tailor our penetrating electrodes to enable high-density recording from targeted subcortical structures.

Poster 4

Cortical excitability in non-human primate quantified after permanent lesion of the primary motor cortex using chronic electromyographic implant and chronic cortical chambers

Mélanie Kaeser, Julie Savidan, Anne-Dominique Gindrat, Simon Badoud, Eric Rouiller, Eric Schmidlin
University of Fribourg, Switzerland

A lesion of the motor cortex in human patients results most of the time in intractable deficits which severity depends on the extent and the localization of the lesion. In macaque monkeys, to investigate the dynamical changes of the function of the motor cortex involved in manual dexterity after a permanent lesion, we need to assess the cortical excitability of the primary motor cortex (M1) using intracortical microstimulation at regular time points before and after a focal lesion induced by microinjections of ibotenic acid and the consequent elicited electromyographic activity (EMG) in muscles of the contralateral forelimb. To do so, we need to implant chronic intracortical microelectrodes in M1 and chronic EMG electrodes in the target muscles. The cortical chambers are made of polymer (Tecapeek) and the microelectrodes are permanently implanted through a Tecapeek grid fixed into the chronic cortical chamber over the dural surface. The chronic EMG implant is made of Teflon insulated tungsten microwires sutured over the muscular surface. The EMG activity is recorded through a connector implanted transcutaneously in the back of the animal. As the animals are also involved in a manual dexterity behavioural task (the “reach and grasp” drawer task), we implanted a total of six muscles, two in the intrinsic muscles in the hand involved in precision grip, two in the extrinsic muscles of the hand and finally two in muscles playing a more proximal role in the reach and grasp drawer task. Preliminary results show a strong correlation between recorded EMG activity and forces measured in the “reach and grasp” drawer task.

Poster 5

Using face detection to track monkeys in their home cages

Claire Witham
Newcastle University, UK

New EU legislation (2010/63/EU) requires the lifetime experience of non-human primates to be assessed. This is of particular importance to macaques used in neuroscience research, which may undergo many different experimental procedures during their lifetime. There is increasing interest in finding objective measures of monitoring cumulative stress in experimental animals; both for determining what procedures contribute to cumulative stress and for looking at cumulative stress within a single individual.

One possible measure of stress is to monitor behaviour of the monkeys in their home cages. Many primate units have CCTV cameras to monitor their animals. Using automated video analysis we could track how much time the monkeys spend in each part of their cage (e.g. foraging on the floor, sleeping etc.). There are commercial products for tracking animal movements (e.g. Ethovision, Noldus Information Technology); however these work best when the animals are housed in simple cages. Macaques are commonly housed in cages with multiple shelves at different levels and many different enrichment objects such as rubber tires, hoses and toys. Programs that just detect movement struggle to differentiate between the moving animal and other moving objects.

Face detection algorithms are commonly used in cameras to improve photos of humans and even pets. They are also commonly used in robotic vision and open source software is available for people to develop their own object detection paradigm (http://opencv.org). I have developed a face detection program that detects for each video frame whether there is a macaque face in the picture. I will present preliminary data using this program to track monkeys around their home cages and discuss ways in which we can develop this into a practical tool for monitoring behaviour.