Introduction: Postoperative patency and flow control are essential for success and validation of vascular graft implantation research projects. Angiography represents for many the gold standard in the follow-up after vascular surgery, but brings inherent risks: Longer anaesthesia, supplementary animal stress, possible occlusion, ischaemia, pain or limping. We studied the implementation of LVDU as an alternative to angiography in three animal species.

Methods: We appraised vascular flow and patency by LVDU, after graft implantation in different research projects in 3 rats, 5 rabbits and 25 swine at different time points: immediate, 1 and 4 weeks post-surgery. LVDU was validated by angiography immediately after surgery in 17 of the pigs and at necropsy (4 weeks post-operatively) in all the animals. During the immediate post-surgical control, bi-dimensional ultrasound with Doppler was performed using a linear Vingmed®, 10 MHz probe before the animal awakening. Sedation was no longer than 20 minutes for further examinations. Procedures and techniques were adapted for each species.

Results: Angiography confirmed ultrasonographic findings in 94% of the 17-pig cases and in 100% of the other. LVDU provides excellent image quality, allowing for reliable evaluation and follow-up of the graft and flow characteristics. Stress, inconveniences, evaluation time and supplementary risks were reduced. As a consequence of angiography, 4 pigs had ischaemic complications, 2 having to be euthanised before the end of the protocol.

Discussion: LVDU is an accessible, non-invasive technique, providing rapid, safe, repeatable and reliable results. This excellent alternative avoids angiography-related risks, therefore contributing to the animal welfare. It optimises the standards and allows an easy evaluation of surgical results.
Lecture

Magnetic Resonance Imaging of animal brain in vivo

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The tremendous success of Magnetic Resonance Imaging (MRI) in medicine is based on its ability to visualise soft tissues at high spatial resolution and with excellent sensitivity to pathophysiologic alterations. This particularly applies to studies of the central nervous system. In contrast to techniques using ionising radiation, MRI offers completely non-invasive examinations and therefore not only facilitates repeated follow-up examinations but also allows for monitoring disease progression and therapy efficacy.

The past two decades have witnessed a continuous growth of MRI studies of the central nervous system of laboratory animals. In fact, advances in imaging neuroscience of knockout and transgenic animals as well as of models of human brain disorders are expected to help bridging the gap between molecular biology and clinical applications. Moreover, the structural information obtainable by MRI has been complemented by an increasing number of techniques that attempt to characterise the functional state of the tissue rather than its mere morphologic appearance. Prominent examples include magnetic resonance angiography of the intracranial vasculature, functional assessments using a wide range of contrast media, diffusion-weighted imaging for an early demarcation of ischaemic lesions as well as for an in vivo assessment of the axonal connectivity by identifying white matter fibre tracts, and localised magnetic resonance spectroscopy which offers a neurochemical characterisation of the cellular metabolism and composition.

The purpose of this lecture is to give a brief overview of the potential of magnetic resonance studies of animal brain ranging from insects to monkeys.

Poster

The effect of training for long term restraining of rats evaluated by telemetry

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The aim of this telemetric study was to evaluate the possible stress and distress by long term restraining of conscious rats by the use of two different restraining methods, “Bollmann cage” and “Scandidact rocket”, and to evaluate the effect of training for the restraining method.

Telemetry was used to measure blood pressure and heart rate in female, Sprague Dawley rats, as these parameters are known to increase due to stress and distress.

On the first day of experimentation, blood pressure and heart rate were measured for 3 consecutive hours in the rats normal housing environment without any disturbances.

On the following 10 days of experimentation, the rats were once daily restrained for 3 consecutive hours, without any prior training, by one of the two restraining methods without changing the method of restraining during the experimentation period.

Blood pressure and heart rate data was measured as mean values of two minute intervals every 15 minutes from the start of restraining.

All data was compared to data extracted at the same time points on the first day of experimentation as an indicator of the impact of training by the repeated restraining.

The results will be presented and the importance and length of specific training for routine laboratory procedures will be discussed.

We furthermore conclude that non-invasive, telemetric obtainable recordings of blood pressure and heart rate in combination with other parameters like e.g. relevant hematological measurements and behaviour observations are very useful and reliable in the assessment of stress and distress.
Non-invasive imaging utilises specific traceable molecules to monitor spatial and temporal biological events within live animals. Since the same animal can be used throughout a study, the need to sacrifice individuals from each group at specified time points is avoided. Hence, imaging methods allow the number of animals used in a study to be reduced. As a single animal is used, individual variation is avoided, improving the reliability of experimental data. Furthermore, imaging over extended periods of time can potentially reveal mechanistic details of disease- and toxicity-related events.

Many imaging techniques obviate the need for visible clinical signs and, thus, substantially refine experimental procedures. Indeed, developments in this field may eventually allow animal studies to be replaced with clinical trials in human volunteers.

The advantages of non-invasive imaging are most poignantly highlighted by two recent approaches. The first involves the use of bioluminescent reporter systems that involve transforming mammalian cells, tumours, bacteria and viruses with genes encoding enzymes that catalyse production of bioluminescent metabolites. The genes are placed under the regulation of DNA elements that only initiate gene expression in response to a specific biological molecule or event. Thus it is possible to monitor disease progression and its response to therapeutic intervention in real-time. A second technique utilises quantum dots (nanocrystals of semi-conducting materials) which when excited by a pulse of light emit electromagnetic signals of pre-determined wavelengths. Quantum dots conjugated to biological molecules can be used as tissue- or disease-specific markers or to monitor molecular interactions.

Radio-telemetry provides an alternative means of obtaining physiological measurements from awake and freely moving small laboratory animals, without introducing stress artefacts. Until now research has mainly focussed on the responses of laboratory subjects to experimental changes but not to general laboratory handling and procedures. Traditionally, a variety of techniques have been employed to obtain physiological measurements in the laboratory. When monitoring conscious animals, it is possible to use invasive methods such as sensors, and/or electrodes. Non-invasive methods such as surface electrodes for monitoring an electrocardiogram or a tail cuff for monitoring blood pressure can also be used. Although wireless radio-telemetry technology for monitoring laboratory animals has existed for years, it has only been in the last ten years that affordable, reliable, and easy-to-use commercial products have been readily available for monitoring a variety of signals. Telemetry technologies and improved laboratory tests have allowed us to better assess the well being and physiological status of each of our research subjects. The advantages of implantable wireless telemetry transmitters include: providing a humane method for monitoring conscious, freely moving laboratory animals; eliminating stress related to the use of restraint, which can alleviate a potential source of experimental artefacts and inter-animal variability; reducing animal use by 70% in single studies, and by more than 90% in multiple studies and allowing 24 hour data collection, for days, weeks, or months, without special animal care. The application possibilities and benefits of long-term measurements of physiological parameters in mice will be presented on the basis of recent results.
Lecture

**Impact of the method of restraint on plasma corticosterone, heart rate and body temperature in laboratory mice**

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Radio-telemetry is a frequently used tool to measure physiological parameters in freely moving animals. In this study, radio-telemetry was used to assess the value of heart rate (HR) and body temperature (BT) as quantitative measures of acute stress in mice. Female C57BL/6 mice (n=9 per group) were subjected to different methods of restraint: lifting by the tail for ~5 seconds (LT), ~10 seconds of restraint by hand (RH) and 5 minutes in a plexiglas restrainer (PR).

It was found that restraint caused a tachycardia, irrespective of which method. However, during the following 90 minutes where HR recovered to baseline, HR was found to be significantly higher in the PR group compared to the LT group. RH caused an intermediate recovery to baseline of HR. These results showed a correlation with the plasma corticosterone data found in a preceding experiment, showing a gradual increase over the three methods, with LT having the lowest concentrations and PR the highest. Hyperthermia was also found after each of the three procedures, however, as BT changes are rather slow, this parameter does not seem to be a useful parameter to quantify an acute stress response.

Poster

**Faecal corticosteroid concentration vs. total faecal corticosteroids. Which measure reflects better the total amount of circulating hormone?**

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Alternative methods to the utilisation of blood and its derivatives in laboratory animals are particularly interesting, especially in the hamster due to its small size and difficulties in obtaining serial blood samples. Steroid hormone metabolites quantification in faeces, widely used in studies of free-ranging or intractable animals, shows up as a non-invasive, non-stressor, economical, animal saving technique. It allows longitudinal studies as it enables frequent sampling of the same individual that can be used as its own control. However, we remain naïve about factors that may influence the accuracy of these techniques. The aim of this study was to evaluate the relevance of cortisol faecal metabolite concentration to assess physiological stress response. Ten adult female Syrian hamsters were ovariectomised and all faeces voided by each of them collected daily during five days before and five days after surgery. Cortisol faecal metabolites were extracted and quantified by radioimmunoassay. We determined per gram faecal corticosteroid concentrations, total 24-h faecal output and total 24-h faecal corticosteroid production. Surgery affected considerably faecal output and using “per gram” vs. “total” corticosteroids yields different conclusions: while concentrations increased significantly immediately after the ovariectomy and decreased on the subsequent days, “total” excreted corticosteroids varied in a symmetrical pattern. Then, the relative, per gram measure of hormones may not reflect the total amount of circulating hormones because these measures are comparable only if the volume of material in which the hormone is contained is the same between the two groups.
Lecture

Non-invasive telemetry in comparison with invasive telemetry and its use in toxicology studies

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Conscious dog telemetry is routinely used for in vivo cardiovascular safety pharmacology studies, generally involving invasive recovery surgery for implantation of transmitters for collection of electrocardiogram (ECG) and arterial blood pressure data. Whilst the data quality is high, and dogs can be reused over a number of years, advances in non-invasive telemetry systems may allow similar data to be obtained from a less invasive procedure. These types of systems are also conducive for inclusion in toxicology studies. Here the ECG data quality surpasses that historically recorded by conventional means at “snap-shot” time-points from restrained dogs, which can lead to stress and heart rates above the normal resting values. Collecting higher quality, long-term data in toxicology studies often eradicates the requirement for separate safety pharmacology studies, thus conferring a 3Rs improvement, since an overall reduction in animals would be achieved, as well as a refined technique providing extra valued data from the same animals. Add to this the resource efficiency and the reduced compound requirements by only running one study, may save valuable time in the drug development process. The presentation will compare the advantages/disadvantages of invasive versus non-invasive telemetry, and will present data from example studies where non-invasive telemetry has added valuable data.

Lecture

Non-invasive monitoring of stress hormones in mice: A technique opening new perspectives in biomedical and animal welfare research

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In mice, the monitoring of endocrine functions is seriously constrained by the adverse effects of blood sampling. Therefore, the aim of our investigation was to establish a non-invasive technique to monitor corticosterone concentrations. In a first step, radiometabolism studies were performed revealing that Corticosterone Metabolites (CM) were predominantly excreted via the faeces and that peak excretion occurred after a lag time of about 10 h. HPLC immunograms showed that corticosterone was extensively metabolised and suggested a newly developed 5α-pregnane-3β,11β,21-triol-20-one Enzyme Immunoassay (EIA) to be suited for measuring faecal CM in mice. In a second step, the biological relevance of this EIA was investigated. Experiments comprised monitoring faecal CM after different treatments including administration of ACTH and dexamethasone, respectively. The results clearly demonstrated that pharmacological stimulation and suppression of adrenocortical activity was reflected accurately by means of CM measurements in the faeces. Furthermore, the technique proved sensitive enough to detect effects of routine laboratory procedures like blood sampling or injections. Even the naturally occurring diurnal variation of glucocorticoids could be monitored reliably. Thus, our study provided substantial information about the metabolism and excretion of corticosterone in laboratory mice. Furthermore, the developed EIA proved a powerful tool to monitor adrenocortical activity by measuring faecal CM. This non-invasive technique avoids blood sampling related stress effects and can reduce the total number of animals used for research. Since it also allows frequent sampling of individual animals over time, it contributes to implement the “3R concept” and opens new perspectives in biomedical and animal welfare research.
Biologically interesting compounds can often be labelled with radioactive isotopes of carbon (C-11, $t_{1/2}=20$ min; nitrogen (N-13, $t_{1/2}=10$ min), oxygen (O-15, $t_{1/2}=2$ min.) and fluorine (F-18, $t_{1/2}=110$ min).

These isotopes emit positrons, which in turn annihilate to two 511 keV $\gamma$ radiations, which makes it possible to observe compounds labelled with these isotopes with special Positron Emission Tomography (PET) cameras. Since a few years small animal PET cameras are being introduced in addition to the cameras for clinical human use, which were also useful for bigger animals (dogs, pigs).

Positron Emission Tomography (PET) is a quantitative functional procedure. A PET system yields the absolute tracer concentration during the acquisition time. This time dependent concentration can be converted into a quantitative description of function (like receptor binding, oxygen consumption etc. depending on the compound used) by pharmacokinetic modelling.

This makes it possible to obtain time dependent quantitative information on physiological and biochemical processes in selected organs, using a minimal amount of animals: One animal yields several time activity curves under chosen conditions.