Pain of low and middle grade is difficult to detect in laboratory mice because mice are prey animals that intend to protect themselves from their predators by hiding signs of weakness, injury and pain. Therefore, telemetry was used to exclude the influence of the investigators presence with the aim, to identify indicators of low to middle-grade post-operative pain. Male mice bearing telemetric transmitters were subjected to vasectomy, either without pain therapy or with the application of two different analgesic regimens. Postoperatively, all the animals exhibited no overt signs of pain. The telemetrically measured locomotor activity behaviour remained stable, which confirms the intended stage of low/middle-grade pain and the absence of intolerable pain. Body core temperature showed negligible increase, suggesting, that post-surgical inflammation was of no influence.

The only group of animals showing significant changes of the hearts actions (heart rate, heart rate variability), that point to pain and sympathetic activation were the animals with no analgesic treatment. For these animals also the food intake was significantly diminished and body weight was slightly reduced.

Both analgesic regimens were able to prevent any changing of heart actions and also the post-operative food consumption and body weight were unchanged when animals received pain therapy.

The results show, that i.) the method was able to identify signs of low to middle grade pain in mice by telemetry, which could not be clearly detected otherwise in this species ii.) analgesic regimens acted successfully in relief of low to middle-grade post-operative pain in mice.

Poster
Assessment of post-operative pain in laboratory mice by telemetry
Margarete Arras, Andreas Rettich, Hans P. KAESERMANN, Paolo Cinelli and Kurt Buerki
University of Zurich, Institute of Laboratory Animal Science, Zurich, Switzerland

Pain of low and middle grade is difficult to detect in laboratory mice because mice are prey animals that intend to protect themselves from their predators by hiding signs of weakness, injury and pain. Therefore, telemetry was used to exclude the influence of the investigators presence with the aim, to identify indicators of low to middle-grade post-operative pain. Male mice bearing telemetric transmitters were subjected to vasectomy, either without pain therapy or with the application of two different analgesic regimens. Postoperatively, all the animals exhibited no overt signs of pain. The telemetrically measured locomotor activity behaviour remained stable, which confirms the intended stage of low/middle-grade pain and the absence of intolerable pain. Body core temperature showed negligible increase, suggesting, that post-surgical inflammation was of no influence.
Poster

Major animal stressors overlooked in the laboratory environment

Jonathan Balcombe, Chad Sandusky and Neal Barnard
Physicians Committee for Responsible Medicine, Research and Toxicology, Washington, DC, USA

Most objections to animal experimentation concern the experiments themselves. This paper addresses two aspects of laboratory animal welfare separate from the experiments: 1) housing conditions, and 2) routine procedures. Ninety published studies were reviewed to assess the effects of standard laboratory housing conditions on the behaviour of rodents, particularly mice and rats. Preference studies show that mice and rats value opportunities to take cover, build nests, explore, forage, and gain social contact, behavioural needs that are often thwarted by institutional laboratory housing systems. We also reviewed eighty additional studies to assess the potential stress associated with three routine laboratory procedures: Handling, blood collection, and gavage (force-feeding). Pronounced and significant changes in stress indicators (e.g. concentrations of corticosterone, heart rate, blood pressure) occurred for all three procedures, indicating fear, stress, and/or distress. These literature reviews depict a life where chronic lack of stimulation is exacerbated by regular stressful episodes. Resulting physiological (e.g. stunted brain development) and behavioural symptoms (e.g. stereotypies) compromise both scientific and ethical integrity. Providing animals with naturalistic living environments where they can engage in strongly motivated behaviours, while not obviating humane concerns, is feasible and ethically desirable.

Lecture

Cardiac and vascular responses to repeated restraint in mice

Robyn Billing and Rosemarie Einstein
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This study was conducted in mice to examine changes in body weight and heart rate (HR, using telemetry) in response to restraint stress. Animals were weighed (controls) or weighed and then restrained for 1 hour each day for 14 days. Following humane killing, the vas deferens and tail artery were mounted in organ baths for concentration-response curves to noradrenaline (NA) and electric field stimulation (EFS). There was a significant difference between the two groups in body weight gain; restrained animals showed no significant weight gain; control animals gained ~3 g over 14 days. HR increased significantly during restraint on all days and remained elevated during the entire restraint period. There was also a reversal of the light-dark rhythm, with higher HR during the light phase and lower HR values during the dark phase. A degree of habituation was observed, with these effects on HR being somewhat reduced by the end of the experimental period. In tissues from stressed animals, there was an increase in the maximum response of vas deferens to NA and concentration-response curves in tail arteries were significantly shifted to the left, indicating increased sensitivity of the tissues to NA. The EC50 in tissues from restrained animals was 2x10^-8M and in tissues from control animals was 1x10^-7M. Contractions in response to EFS (1 Hz and 10 Hz) in tail artery segments from stressed animals were also significantly increased. Thus, despite some apparent habituation of the heart rate response to restraint, underlying changes in sympathetically innervated tissues persisted.
Lecture

Workshop of international experts held on the definition, recognition, assessment and alleviation of animal distress in the laboratory

Kathleen Conlee¹ and L. A. King²

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²Linacre College, Oxford University, Oxford, UK

Distress in animal research can be difficult to address and is often overlooked, despite legislative and regulatory mandates in the U.S. to minimise animal distress and pain. Consequently, on February 11-14, 2004, The Humane Society of the United States, as part of its Pain and Distress Campaign, held a workshop of international experts to discuss the state of knowledge on animal distress and whether it is possible to define and measure distress in operational terms for application to animal research. Although the U.S. Department of Agriculture has proposed to define distress, current US regulations do not define the term. The seventeen participants represented the fields of animal welfare, applied ethology, veterinary medicine, physiology, ethics, and animal protection. Workshop discussion included topics such as what an operational definition of distress should encompass; concepts of distress and suffering; causes, prevention, and measurement of distress; and incorporation of distress into regulation. The lack of definition of distress hinders progress toward a comprehensive consideration of laboratory animal distress in the U.S., which has consequences for both animal welfare and quality of science. The participants agreed that creating a meaningful and practical definition is a challenge, but this can be addressed by crafting a general description of what might constitute animal distress, supported by a set of specific examples. An executive summary produced from the workshop includes the description of distress agreed upon by the participants, as well as additional information and conclusions drawn from the workshop discussions.

Poster

Responses in mice to restraint of cage-mates

Andrew Gilmore, Robyn Billing and Rosemarie Einstein*

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Performing stressful procedures in view of cage mates may cause stress in observer animals. However, it is not known if stressful procedures in close proximity to, but not in view of cage mates is stressful for the (observer) cage mates. Radiotelemetry and post mortem in vitro studies of the vas deferens were used to determine the effects of stress on observers. Heart rate (HR), body temperature (T) and locomotor activity were recorded for 1 hour following weighing of a cage mate, or 1 hour during restraint of a cage mate and the hour following return of the restrained mouse to the cage. This procedure was repeated for 15 days. HR, T and activity were increased in observers of both restraint and weighing of cage mates. Analysis of the AUC showed that HR and T in observers were significantly higher during restraint of a cage mate than after weighing of a cage mate. When mice were returned to the cage after weighing or restraint, HR and T were significantly higher in the cage mates of restrained animals. Results from previous studies have shown that chronic stress causes the vas deferens to become hypersensitive to noradrenaline. In this study, vas deferens from observers of restraint had a significantly increased responsiveness to noradrenaline. These results indicate that stressful procedures should be conducted in isolation from other mice. Furthermore, they show that monitoring stress using a single parameter may not give an accurate indication of the stress experienced by experimental animals.
Halothane and ketamine are two commonly used anaesthetics. There have been numerous studies to determine the effect these anaesthetics have on hormone levels. For instance, ketamine increases prolactin and cortisol levels in rhesus monkeys, but has not been found to affect testosterone in cynomolgus monkeys. In rats, no effect was observed on thyroxine, triiodothyronine, oxytocin or LH pulses. Halothane was also observed not to have any effect on testosterone, LH, and FSH in the rat, but did increase ACTH-like and corticosterone-like immunoreactivities up to 24 hours after exposure. A previous study in this laboratory found that testosterone levels in rat plasma were increased up to 24 hours after ketamine anaesthesia. This study investigates the effect of ketamine and halothane on male rat plasma testosterone and LHRH and will discuss the validity of using anaesthesia in experimental conditions when hormones are to be measured.

**Poster**

**The effect of anaesthesia on plasma hormones in the norway rat**

*Elaine M. Gould and Christian J. Cook*

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Introduction: The beagle is the most used dog strain in preclinical pharmacology. However, there are no systematic studies on the influence of repeated inhalation anaesthesia on body functions, general health, and on the impact on pharmacological compounds.

Methods: 2 groups of 3 female Beagle dogs each were investigated in a cross-over study with two anaesthesia (premedication: xylazine and Polamivet®, inhalation anaesthesia: isoflurane) regimens: 5 times vs. 2 times in 8 weeks. After a wash-out phase of 3 months, the groups were reversed. The general clinical examination parameters, blood pressure, and fitness were recorded 1 day before and 24 hours, 7, and 14 days after anaesthesia.

Results: The results show that the anaesthesia interval and frequency do not have a biologically relevant impact on the investigated parameters in general, except the ALP-8-week-interval. Only, 24 hours after each anaesthesia some aberrations were observed (weight loss, rise of body temperature, higher activity of liver enzymes, loss of body-fitness).

Discussion: The interval of 2 weeks of the tested anaesthesia regime offers sufficient safety for dogs and also for the interpretation of pharmacological results. The dogs showed no lasting effects on health, and the well-being of the animals was only temporarily impaired.
The mouse can replace larger laboratory animal species for the study of diseases and treatments. Although rodent models are widely used to study physiological mechanisms and treatment of pain, most clinical dosing of analgesics for laboratory mice is based on “best guess” extrapolations. There is lack of evidence to indicate the extent to which mice suffer from post-operative pain and for adequate treatment. If mice do suffer significant morbidity from pain, the physiologic effects of this may have an important impact on the research model being studied, as well as welfare implications. The authors have developed a model to test the hypothesis that pain or other morbid conditions related to surgery might impair mobility and food or water intake, and that effective analgesic doses, timed appropriately, might act to attenuate behavioural abnormalities in C57Bl6 female mice. We will present a scientific evaluation of mice in a surgical pain setting, evidence that analgesics can improve weight loss and behavioural abnormalities following surgery, and contextual information from the rodent basic science literature that suggests strategies for surgical pain alleviation in mice.

**Lecture**

**Evidence based surgical analgesia in the laboratory mouse**

*Alicia Karas¹, Richard Karas² and Mark Aronovitz²*

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Mitral valve grafts were implanted in six sheep (50-66 kg BW) during cardiopulmonary bypass via a left-sided thoracotomy. After IM premedication (midazolam at 0.6 mg/kg, ketamine at 4 mg/kg), anaesthesia was induced IV (midazolam at 0.1 mg/kg, propofol at 4 mg/kg) and first maintained IV for surgical preparation (propofol at 10 mg/kg/hr). Oxygen was insufflated (5 L/min) via a cannula in the endotracheal tube. In the operating room, anaesthesia was maintained with isoflurane (end-tidal 1.2-1.4%) in oxygen, plus fentanyl (5 µg/kg bolus, 2-10 µg/kg/hr) and lidocaine (2 mg/kg bolus, 1.8 mg/kg/hr) IV. Intercostal nerve blocks (3rd to 8th intercostals spaces) were performed with lidocaine/bupivacaine (1/1 mg/kg) before the first incision. All sheep were heparinised (100 UI/kg) but not at all times antagonised (protamine). Ephedrine, dobutamine and norepinephrine were administered to effect to maintain arterial blood pressure. Respiratory support was provided and adjusted according to arterial blood gas values. Intrapleural bupivacaine was administered during closure of the thorax. Postoperatively, regular and frequent evaluations, using composite pain scores were performed and analgesic therapy adjusted accordingly (Intercostal nerve blocks, carprofen at 4 mg/kg SID, dexamethasone at 1 mg/kg BID, fentanyl infusion at 2-5 µg/kg/hr or buprenorphine at 15 µg/kg QID). In view of their peri-operative cardiovascular stability, their calm recoveries and their low pain scores, it would appear that all sheep received adequate analgesia. One sheep showed considerable respiratory depression and prolonged recovery. This resolved after the administration of naloxone.

In view of their peri-operative cardiovascular stability, their calm recoveries and their low pain scores, it would appear that all sheep received adequate analgesia. One sheep showed considerable respiratory depression and prolonged recovery. This resolved after the administration of naloxone.

The anaesthetic and analgesic protocol used were suitable for cardiac surgery in these sheep. The use of pain scores allowed for satisfactory analgesic therapy in these animals.
Lecture

**Controlled release of analgesics for rodents**

*Timothy Mandrell¹, Atul Shukla², Quanmin Chen² and Shipeng Yu²*

¹University of Tennessee Health Science Center, Department of Comparative Medicine, Memphis, Tennessee, USA; ²University of Tennessee Health Science Center, Department of Pharmaceutical Sciences, Memphis, Tennessee, USA

Analgesics are administered to laboratory animals to relieve pain due to surgical procedures. Opioids are the analgesic of choice for moderate to severe pain. Morphine, the prototypic opioid analgesic, is widely used in animals and provides excellent analgesia. Sustained analgesia requires repeated parenteral administration, an inherently stressful procedure. The objective of this study is to develop a controlled release gel formulation of morphine that can maintain analgesia in mice for 3 to 5 days following a single administration.

In vitro tests were conducted on 12 formulations of morphine in biodegradable polymer gel and five formulations were selected for in vivo pharmacodynamic testing. Eight experimental groups of 6 mice were subjected to tail flick analgesia testing. The groups were: morphine gel formulations A2r, A3, A3r, A5 and A7; blank gel control; negative control (no injection); and positive control (morphine suspension). The baseline tail flick latency was determined for each mouse, gel was injected subcutaneously, and the analgesic effect measured. Analgesic effect was similar in formulations A2r, A3, A3r and A5, lasting for 12 to 24 hours. The analgesic effect for formulation A7 lasted for 120 hours and was significantly higher (p<0.01) than the untreated control group. Analgesia from morphine suspension lasted 28 hours. There was no analgesic effect from the blank gel. Biodegradable gels with morphine can provide analgesia to mice for 5 days following a single subcutaneous injection. This methodology can enhance the treatment of rodents subjected to surgery or other painful procedures by eliminating repeat dosing and providing sustained delivery of drug.

Poster

**The combined use of epidural analgesia and fentanyl patch for relief of post-operative pain**

*Thomas Martin and Christina Winnicker*

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The ethical use of animals includes the minimisation of discomfort, distress, and pain. Pain cannot always be evaluated easily in animals and one must assume that animals experience pain in a manner similar to humans, and therefore pain management appropriate to the species, the procedure, and the circumstances must be provided. One procedure which requires careful post-operative pain management is thoracic surgery (sternotomy and thoracotomy). There are many options available for management of post-operative pain after thoracic surgery including intermittent and/or continuous delivery of analgesia via intravenous, oral or intramuscular routes. However, these protocols require continued intermittent handling of the patient at a time of greatest patient sensitivity. Analgesia for 48 hours can be provided with minimal handling of animals post-surgically by the combined use of fentanyl patches and epidural anaesthesia.

This paper discusses a refinement of analgesia through the use of fentanyl patches combined with epidural analgesia (morphine, fentanyl or buprenorphine), for control of post-operative pain in dogs and pigs after thoracic surgery.
Lecture

**Are 20 kHz ultrasonic vocalisations a reliable indicator of post-operative pain in rats?**

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Our objective was to determine whether ultrasonic vocalisations (USVs) can be used as an objective measure of post-operative pain in rats. 40 male CD-IGS rats underwent either anaesthesia or anaesthesia plus surgical implantation of a cardiovascular telemetry device into the abdomen for an unrelated study. Within each treatment group, animals were given Parecoxib (i.v.) at 1 mg/kg, 5 mg/kg or 20 mg/kg pre-operatively, to produce a graded response. Pre-operatively and at hours 1, 2, and 4 post-operatively we recorded the number of 20 kHz USVs produced by isolated animals during a 10 min period. At hour 1 we also recorded the frequency of five behaviours (twitch, writhe, stagger/fall, back arch, belly press) that have previously been effective for pain assessment following abdominal surgeries, and produced a composite score. No animals produced USVs during the pre-operative recording. Post-operatively, 27% of control animals and 22% of surgical animals produced USVs during one of the three recording periods, and these USVs were not associated with the composite pain score. Overall, the composite pain scores were higher for the surgical group than the control group (Mann-Whitney test, U22, 18 = 320, p<0.001). However, the different levels of analgesic did not produce a dose-dependent response, possibly due to analgesic efficacy. Differences in USVs pre- and post-operatively suggests that 20 kHz USVs may be indicative of distress, but the lack of association with other pain scores demonstrates that they are not indicative of post-operative pain under these testing conditions.

Poster

**Investigation for objective indicator of anaesthetic depth in laboratory animals: Visual evoked potentials for ketamine anaesthesia in mice**

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In many research laboratories, various anaesthetics are used for anaesthetising mice. Whereas the animals can be reliably maintained for several hours to be anaesthetised with additional doses of these anaesthetics, the anaesthetic level is roughly monitored by somatic reflex, respiratory rate and heart rate. In the present study, we performed intermittent recording of visual evoked potentials as a reliable indicator for the depth of anaesthesia to make sure the level obtained by conventional ketamine-based anaesthetics, which are commonly used for laboratory mice.

Adult mice were anaesthetised with ketamine (80 mg/kg) and xylazine (20 mg/kg) and a monopolar stainless wire electrode was put on visual cortex. When the withdrawal reflex appeared, additional anaesthesia of ketamine only or a mixture of ketamine and xylazine were added. After this addition of anaesthetics, averaged VEP in response to stimuli of flashing LED light and the withdrawal reflex were monitored every ten minutes until the withdrawal reflex appeared again. In each mouse, the amplitude of VEPs was normalised relative to the VEP that was recorded just after the appearance of the withdrawal reflex.

The withdrawal reflex of the mice with ketamine and xylazine appeared later than that with ketamine alone. The relative amplitude of the mice with ketamine and xylazine was significantly lower than that of the mice with ketamine alone.

This result showed that the lower amplitude of VEP could reflect deeper anaesthetic stage and this method could be used as a reliable indicator for the depth of anaesthesia to reduce animal suffering.
Wherever animals are used in research, minimising pain and distress is as important and objective as achieving the experimental results. This is important for good welfare and for good science. In recent years, much attention has been focused on recognising and controlling the adverse effects of scientific procedures on animals, and also on the need to improve the environment in which laboratory animals spend their lives.

Significant and immediate improvements to animal husbandry and scientific procedures can be made in a number of ways. The Joint Working Group on Refinement was convened by the BV A(AWF) / FRAME / RSPCA and UFAW to ensure up-to-date information on good practice is available. The JWGR has a broad range of membership with representatives from science and industry, veterinary and animal welfare. The group has produced a series of reports setting out good practice for the following:

- Removal of blood from laboratory mammals and birds
- Refinements in rabbit husbandry
- Refining rodent husbandry: the mouse
- Refining procedures for the administration of substances
- Laboratory birds: refinements in husbandry and procedures
- Refinement and reduction in production of genetically modified mice
- Refinements in telemetry procedures
- Husbandry refinements for rats, mice, dogs and non-human primates used in telemetry procedures
- Refining dog husbandry and care

The poster to be presented at the 5th World Congress (on behalf of the organisations that form the JWGR) will provide further details including references and highlighting the availability for many of these reports to be downloaded for free from the "Laboratory Animals" website: www.lal.org.uk.

Refinement in animal experiments means to diminish pain, suffering and harm. The use of analgesics in experiments is applied refinement and an essential part of good veterinary care for laboratory animals.

Profound knowledge is needed to rule out the most effective pain management protocol by use of general anaesthesia, regional anaesthesia and systemic analgesics. This has to take account the side effects of used drugs to physiological parameters, behaviour and pathophysiological patterns. Recommendations for pain treatment in common experimental procedures help researchers to find out the most suitable medication for their specific experimental design in the respective animal species. Analgesic treatment should be the standard in all not insignificant painful experimental procedures. It should be pointed out that less stressful and painful protocols lead to better and more valid results.

Indeed the use of analgesics is not recommended in all experiments because effects and side-effects of the drug may interfere with the expected results.
Poster

Rehabilitation of laboratory New Zealand rabbits

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Introduction: Modern legislations on animal experimentation almost always include the credo of the Three Rs, which most often takes care of the “well being” of the experimental animal but does not always protect the “life” of the animal. The concept of the 4th R – rehabilitation of laboratory animals is a befitting continuum of Three Rs credo. In this paper we present a case study on the rehabilitation of 181 rabbits used in experiments.

Methods: Direct observation of these rabbits, and veterinary intervention were used to assess the condition of the rabbits.

Results: It has been observed that in rehabilitating these animals often succumb to spinal chord damage even if they have not been experimented on, due to the small dimensions of the cages that they are restrained, in laboratories, allowing them no freedom of movement.

Discussion: Rehabilitation of rabbits has to be done remembering that rabbits are delicate and sensitive animals and that cannot be directly rehabilitated in a natural environment with many incitements as they tend to be over active and this could be fatal. The paper discusses the finer nuances in their successful rehabilitation, physical environment, stabling options, degree and scaling of freedom of movement, social interaction and essential veterinary care. Ethological and behavioural studies on rehabilitated animals besides being a source of direct information to help refine and better understand the problems encountered in rehabilitation will open new views in learning and understanding laboratory animal care and use.

Poster

Back to basics: Human influence in animal experiments

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Over time our knowledge and perception with regards to laboratory animal science has changed considerably. Advances particularly in molecular sciences have lead to the development of sensitive animal models, but there are basic issues that still need to be addressed. The influence of environmental variables on test outcome in particular is one area where knowledge is lacking. Although many physical variables have been investigated, one variable that has received little attention is the human factor in animal experiments. Yet, humans play a vital part in all animal research, and differ both between labs and within.

This paper reviews a large amount of information on how direct and indirect contact with humans alters an animal’s physiology and behaviour. Crucially, it will give examples of how this impacts on test results of commonly used tests. For instance, studies carried out at the Central Science Laboratory have shown that experimenter identity was a highly significant variable in a standard anxiety test, despite the fact that all experimenters followed identical procedures.

The paper will finish by discussing ways of reducing this influence. Being aware of human factors in animal research is one step towards more consistent test results.
Introduction: From our point of view monitoring of anaesthetic depth during major surgical interventions in large laboratory animals is essential, especially when NMB’s are being used. EEG is principally suitable for monitoring anaesthetic depth but much experience in interpreting results or specially designed computer-based systems are necessary. The system used in our study was validated with measurement of oxygen consumption, another parameter for intra-operative stress.

Methods: 6 german landrace pigs undergoing thoracic surgery were monitored. Anaesthetic agents (Propofol/Fentanyl/Pancuronium) were administered total-intravenously. Pigs were ventilated mechanically. EEG monitoring was performed using BIS Monitor 2000 XP (AspectMS, Leiden, Netherlands). Target parameters were Bispectral Index (BIS – a number between 0, representing isoelectric EEG, and 100, representing wide-awakening) and suppression ratio (SR – ranges from 0-100, represents the percentage of isoelectric EEG in the past 63 seconds). Further parameters were: Oxygen consumption, blood pressure, ECG.

Results: The electrode fitted with the proportions of the pig-forehead. Stable measurements were possible over a period of many hours. According to measurement of oxygen consumption reliable anaesthetic depth could be expected while BIS values were lower than 60 and SR was higher than 10%.

Discussion: Thus, the situation seems to be comparable to human patients for whom the system is well evaluated. Intra-operative stress can nearly be excluded while BIS is lower than 60 and SR higher is than 10%.