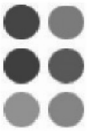

ILAF-ESLAV-ECLAM 2011
Joint Annual Meeting

WHAT'S NEW? ANIMAL MODELS, HOUSING AND TECHNIQUES

September 5th -6th, 2011, Jerusalem, Israel

Chairman of the Organizing Committee: **Dr. Rony Kalman**
Chairman of the Scientific Committee: **Prof. Alon Harmelin**

ILAF, ESLAV and ECLAM, held a joint scientific meeting entitled: "What's New? Animals Models, Housing and Techniques" The meeting was held between September 5th and 6th, 2011 in Jerusalem. It is an important achievement that for the first time ESLAV (European Society of Laboratory Animal Veterinarians) held a scientific meeting outside of Europe. This shared meeting proved to be a perfect occasion to expand professional and personal networks and exchange views in a friendly and pleasant atmosphere. The purpose of this meeting was to give those working in the field of laboratory animal science and medicine an excellent opportunity to discover and discuss the state of the art in our discipline. The scientific program included a high-level of invited speakers. Scientists from Israel, Europe and the USA gave their presentations which included unusual models, human, pig, mouse, rat, bat, birds, fish and even chameleon, each with its specific needs, enrichment, working guides and contribution. We are happy to present here the abstracts of the presentations.

Prof. Alon Harmelin
Chairman of the Scientific Committee

BRAIN IGNITIONS - THE SEARCH FOR "ATOMS" OF VISUAL PERCEPTION IN THE HUMAN BRAIN

Malach, R.

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A fundamental questions facing modern science is how mental activity emerges from the purely physical and chemical processes that occur in nerve cells. For a scientific exploration of this deep question it is critical to include the human brain as an experimental system. Several reasons make the study of the human brain imperative-first and foremost, this is the system in which mental and psychological processes can be communicated most effectively and directly to the experimenter. Second, experiments do not require long training periods so more spontaneous and natural mental phenomena can be studied. Finally, the human brain and its associated cognitive processes may be fundamentally different from other animals. For all these reasons the study of the human brain is essential. On the other hand, because of obvious ethical and methodological constraints, our ability to study the human brain is extremely limited. In my talk I will highlight recent developments in our research on the human visual system. In this work we combine non-invasive brain imaging methods with invasive recordings, conducted for clinical purpose in patients. Together these studies highlight a specific phenomenon- intense bursts of neuronal activity- as a crucial "atoms" that allow the buildup of visual images in the human brain.

LABORATORY RAT BEHAVIOUR AND WELFARE – THE IMPACT OF THE LABORATORY ENVIRONMENT

Burman, O.H.P.

Animal Behaviour, Cognition & Welfare Research Group, University of Lincoln, UK.

The laboratory environment has a major impact upon the welfare of those animals housed within it. Influential factors include the husbandry procedures that the animals experience and the size, complexity and social composition of the cage environment. But how do we go about assessing laboratory rat welfare? One of the main approaches is the use of 'welfare indicators'. Examples of behavioural indicators of welfare include: abnormal behaviour (e.g. stereotypic barchewing); injurious aggression; affiliative social behaviour; sleep behaviour; and vocalizations. By observing the relative durations and frequencies of behavioural indicators of both positive and negative welfare, we can attempt to determine the impact of particular environments on animal welfare. In this talk I will discuss the findings of a variety of research projects investigating the impact on behavioural indicators of welfare of: the timing of husbandry procedures; changes to the composition of individuals within a cage; and varying environmental complexity. The advantages and disadvantages of using behavioural indicators to assess animal welfare will be highlighted.

AN ENVIRONMENTAL ENRICHMENT MODEL FOR MICE

Sztainberg, Y., Yael Kuperman, Y. and Chen, A.

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Environmental enrichment (EE), classically defined as 'a combination of complex inanimate and social stimulation', typically consists of housing animals in large groups in relatively spacious and complex cages, with a variety of objects (e.g., nesting material, running wheels and tunnels) that facilitate enhanced sensory, cognitive, motor and social stimulation relative to standard laboratory housing conditions. Most important, an enriched environment provides the animals with opportunities to perform some of their species-specific behavioral repertoire. Since the pioneering experiments by Rosenzweig and colleagues, who introduced EE as an experimental protocol, many studies have demonstrated that environmental stimulation elicits various positive effects on the brain at the molecular, anatomical and behavioral levels. However, the extreme variability in enrichment protocols may account for some of the inconsistencies in its effects and the variance among results reported by different laboratories. In this talk, I'll describe a simple environmental enrichment strategy for the induction of a robust and replicable anxiolytic-like effect in mice and I'll present data demonstrating that the anxiolytic effect of EE is associated with alterations in the corticotropin-releasing factor receptor type 1 (CRFR1) expression levels in the limbic system.

NEURAL REPRESENTATION OF SPACE: FROM HIPPOCAMPAL PLACE CELLS TO GPS TRACKING OF BATS IN THE WILD.

Ulanovsky, N.

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The work in our lab focuses on understanding the neural basis of behavior in freely-moving, freely behaving mammals – employing the echolocating bat as a novel model system. My talk will describe our recent findings of 'place cells' in the hippocampus of crawling bats, as well as some current work in the laboratory, including: (i) the first recordings of hippocampal neural activity in freely-flying bats, using a custom neural telemetry system, which revealed that there is an elaborate 3-D spatial representation in the mammalian brain; and (ii) the first recordings of 'grid cells' in the bat's medial entorhinal cortex. I will also describe our recent studies of spatial memory and navigation of fruit bats in the wild, using micro-GPS devices, which revealed outstanding navigational abilities and provided the first evidence for a large scale map-like memory in a mammal. Finally, I will talk about a recent optimization principle that we found in how bats acquire sensory information from their environment.

NATURAL ENVIRONMENT - THE CHAMELEON

Katzir, G.

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Introduction. Because animals differ in their vision their *Umwelten* may be very different. Many mammals visually scan their surroundings in a coordinated manner, frequently with an extensive binocular overlap. Visual information is subsequently conveyed by the optic nerves, that undergo partial decussation: each eye projects to both cerebral hemispheres. Information transfer between the hemispheres is via massive connections (mainly *Corpus callosum*). However, in many vertebrates, eye movements may be independent, with little or no binocular overlap, minimal or no optic nerve decussation and minimal inter-hemispheric connections. How are the visual worlds in these vertebrates built?

Methods

We studied the common chameleon, *Chamaeleo chameleon*, as a model vertebrate to try to this question. Chameleons are slow moving arboreal lizards that forage visually for insect prey, using "sit & wait" tactics. Their highly mobile eyes perform large amplitude, independent, saccadic scanning movements. Mostly, only one eye is accommodated and attention is switched between eyes, as they scan opposite regions. Target distance is estimated by lens effort and while binocularity is used, there is no indication for stereopsis. We here present 2 patterns: response to prey and avoidance behavior. Naturally, prey detection is followed by eye convergence, loading the tongue with tension on the hyoid, initial tongue protrusion and tongue shooting.

Results and Conclusions

We show that chameleons respond to computer stimuli, allowing the determination of the effects of target motion and size on predatory behavior. In avoidance, chameleons on a vertical perch rotate their body precisely so as to minimize exposure to a threat stimulus. We show that in both eye motion and body motion there are aspects that are context dependent and aspects that are lateralized (side dependent). We discuss these issues in relation to the chameleons' perceptual world, ontogeny and well being in keeping.

USING VOCALIZATIONS AS AN INDICATOR OF WELFARE IN LABORATORY RODENTS

Burman, O.H.P.

Animal Behaviour, Cognition & Welfare Research Group, University of Lincoln, UK.

The use of vocalizations as an objective measure of welfare is becoming increasingly popular in animal welfare research because vocalizations may provide an 'honest' indication of affective state (i.e. moods and emotions) in animals. Laboratory rodents produce several different types of audible (to humans) and ultrasonic (i.e. frequencies >20kHz) vocalization. Evidence obtained from research investigating the contexts in which particular types of vocalization are emitted, and the effect of hearing those vocalizations (via 'playback' studies) on the affective state of conspecifics suggests that certain vocalizations, for example the '22kHz' ultrasonic vo-

calization in adult rats, may well indicate the affective state of the vocalising animal. Research will be introduced that investigates the potential use of vocalizations as indicators of both positive and negative affective state, and thus welfare. This research has clear implications for the assessment of laboratory rodent welfare because, if particular vocalizations can be demonstrated to influence the affective state and related behaviour of those individuals hearing the calls (e.g. cage-mates), then the measurement of these vocalisations may inform us about the welfare of not only vocalising animals, but also non-vocalising animals within auditory range.

HEAR MY SONG: OBSERVATIONS ON THE USE AND SIGNIFICANCE OF VOCALIZATION FOR COMMUNICATION IN WILD BIRDS

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From earliest writings humankind has noted and commented on birdsong and the reasons birds sing and also recognized their ability to mimic human sounds. Authors, poets and philosophers have linked bird song with feelings of wellbeing in humans and some species such as the Nightingale have been accorded almost iconic status. The calls and songs of birds however are not for our enjoyment, they serve a whole range of behavioural, territorial and defence needs crucial to the survival of each species.

Monitoring the singing of male birds during the breeding season is a quick and reliable way to map species range and population during relativity short visits, enabling large areas to be surveyed by hundreds of volunteers with the resulting data being analyzed by a small team of full time scientists. The British Trust for Ornithology is currently engaged in a project to produce a Bird Atlas to cover the UK and Ireland with the recording of singing males being a key indicator.

Mimicry is another interesting aspect of bird vocalization, the Eurasian Jay has a noisy and far-reaching screeching call and has been recorded as mimicking a range of birds including buzzard, crane, grey partridge and starling (itself an accomplished mimic). It has also been reported imitating barking dogs, human voices, whistles, motorbike horns and lawn mowers! Recent research has suggested that stress may play a role in vocal mimicry, birds learning alarm sounds when stressed and reproducing them in stressful situations.

Another amazing use of sound in birds occurs in the Honeyguide family, which communicates with a mammal species to enable it to gain access to bee grubs and bees' wax.

CHEMICAL COMMUNICATION IN HUMANS

Sobel, N.

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Charles Darwin was puzzled by the human behavior of emotional crying. This is because he identified functional antecedents for most human displays of emotion, but not for emotional crying. Put in other words, he could not explain the functional origins of this behavior. All mammals use chemical signals to communicate information, and humans are no exception. Whereas the key carrier implicated in such human signaling has been sweat, we relied on findings in mice and mole-rats to generate the hypothesis that human tears serve as a chemosignal. To test this, we obtained emotional tears, and tested for their influence. We found that although tears have no smell, they nevertheless induce a host of reactions in those that sniff them, including reductions in arousal, reductions in the hormone testosterone, and alterations of activity in the brain substrates of arousal. Taken together, we argue that the functional role of human tears is a chemosignal. In this, we have provided an answer to Darwin's open question, and also depict a potential world of ongoing sub-attentive chemosignaling between humans.

THE POWER OF MODELING HUMAN DISEASE IN NSG MICE

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The NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mouse is one of the most effective tools available for advancing cancer, infectious disease and stem cell research. These mice are unique among transplantation hosts in that they maintain the tumor-stromal microenvironment and offer superior engraftment of primary solid tumors and hematopoietic

cancers, including B-precursor acute lymphocytic leukemia and acute myeloid leukemia cells from human patients. NSG also acts as an improved platform for detecting and characterizing cancer stem cells which can initiate different human cancers. The NSG mouse can be humanized due to its ability to engraft hematopoietic stem cells (HSCs) which develop into a functional human immune system within 12 weeks. Moreover, NSG represents a viable small rodent model for the study of human-specific pathogens such as HIV and Epstein Barr virus. NSG mice were generated by crossing female NOD.CB17-*Prkdc*^{scid}/J mice with male mice bearing the X-linked B6.129S4-*Il2rg*^{tm1Wjl}/J allele. Male offspring heterozygous for the *Prkdc*^{scid} allele and hemizygous for the *Il2rg*^{tm1Wjl} allele were crossed to female NOD.CB17-*Prkdc*^{scid}/J for eight generations. Mice homozygous for *Prkdc*^{scid} and homozygous or hemizygous for *Il2rg*^{tm1Wjl} were produced by breeding heterozygous mice together. NSG mice were humanized by intracardiac or tail vein injection of human CD34+ HSCs. The NSG mouse is the most severely immunodeficient host available to date. The immunodeficiencies present in this strain affect both the adaptive and innate immune system and include a lack of mature T and B cells, loss of NK function and key interleukin signaling. NSG mice successfully passage primary human tumors without significant loss of phenotype, engraft HSCs and peripheral-blood mononuclear cells better than other immunocompromised strains and model human-specific pathogens like HIV. Taken together, these data illustrate that the NSG mouse models a myriad of human diseases and can be used for sophisticated xenotransplantation experiments.

GENETIC MODIFICATION OF PIGS – STATE OF THE ART

Wolf, E., Kessler, B., Klymiuk, N., Kurome, M., Renner, S., Wünsch, A. and Aigner, B.

Chair for Molecular Animal Breeding and Biotechnology, Department of Veterinary Sciences; and Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Munich, Germany.

Pigs can be genetically modified to recapitulate the genetic and/or functional basis of a human disease, resulting in tailored animal models for translational biomedical research. Current techniques for the genetic modification of pigs include DNA microinjection into the pronuclei of fertilized oocytes (DNA-MI), lentiviral transgenesis (LV-GT), sperm-mediated gene transfer (SMGT), and somatic cell nuclear transfer (SCNT) using transfected nuclear donor cells. Generally, the efficiency of DNA-MI is low. In the pig an average of 0.9% of the injected zygotes develop to transgenic offspring. Founder animals may be mosaic, and random integration may cause varying expression levels due to position effects or may disrupt functional endogenous sequences (insertional mutagenesis). SMGT is based on the ability of sperm to bind and internalize exogenous DNA and to transfer it into the oocyte. Although the efficiency of SMGT was discussed controversially after its first description in the mouse, continued experiments demonstrated that SMGT may work in the mouse and also in the pig. Lentiviral vectors have been shown to efficiently transduce porcine MII oocytes and zygotes. Although lentiviral vector systems can only carry <10 kilobases exogenous DNA, this is considered to be enough for transfer of expression vectors for cDNAs and small interfering RNAs. The use of SCNT facilitated the first gene targeting experiments in livestock. Since successful SCNT protocols are available for the pig, this technology is an attractive route for genetic modification of this species. It does not generate mosaics and allows for pre-selection of nuclear donor cells with regard to transgene expression or gender. SCNT from pools of transfected donor cells can be used to speed up transgenesis in the pig. The efficiency of cloning in pig is still relatively low (0.5-5% offspring per transferred SCNT embryos). The low efficiency of SCNT is attributed to failures in epigenetic reprogramming.

DISSECTING THE CENTRAL STRESS RESPONSE USING SITE-SPECIFIC GENETIC MANIPULATION IN ADULT MICE

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The biological response to stress is concerned with the maintenance of homeostasis in the presence of real or perceived challenges. This process requires numerous adaptive responses involving changes in the central nervous

and neuroendocrine systems. When a situation is perceived as stressful, the brain activates many neuronal circuits linking centers involved in sensory, motor, autonomic, neuroendocrine, cognitive, and emotional functions in order to adapt to the demand. However, the details of the pathways by which the brain translates stressful stimuli into the final, integrated biological response are presently incompletely understood. Nevertheless, it is clear that dysregulation of these physiological responses to stress can have severe psychological and physiological consequences, and there is much evidence to suggest that inappropriate regulation, disproportional intensity, or chronic and/or irreversible activation of the stress response is linked to the etiology and pathophysiology of anxiety disorders and depression.

Understanding the neurobiology of stress by focusing on the brain circuits and genes, which are associated with, or altered by, the stress response will provide important insights into the brain mechanisms by which stress affects psychological and physiological disorders. The CRF/Urocortin system is fundamental in orchestrating the organisms stress response. In addition to its hypophysiotropic action, CRF integrates the behavioral responses to stress within the central nervous system. This lecture will present an integrated multidisciplinary approch from gene to behavior using mouse genetics and animal models aim in elucidating the contribution of different members of the CRF/Urocortin family of peptides and receptors to the central stress respone. Defining the contributions of known and novel gene products to the maintenance of stress-linked homeostasis may improve our ability to design therapeutic interventions for, and thus manage, stress-related disorders.

PLURIPOTENCY AND CELLULAR REPROGRAMMING

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The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such ex vivo reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and elucidating the extent of equivalence of the in vitro derived cells to their in vivo counterparts. I will present and analyze our recent advances toward understanding these fundamental questions. I will further highlight future possibilities for utilizing epigenetic reprogramming for experimental and theoretical modeling of gene expression regulation, cell fate decisions and early mammalian development.

OPTOGENETIC INVESTIGATION OF THE EXCITATION/INHIBITION BALANCE HYPOTHESIS IN NEUROPSYCHIATRIC DISEASE

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Severe behavioral deficits in psychiatric diseases such as autism and schizophrenia have been hypothesized to arise from alterations to the cellular excitation/inhibition (E/I) balance within neural circuits. This hypothesis could unify diverse streams of pathophysiological and genetic evidence, but has not been susceptible to direct testing. Optogenetics is a technique that allows for temporally precise control of genetically-specified neuronal populations with light. Using optogenetic tools, neurons can be activated or silenced with light in precise spatio-temporal patterns. I will describe the design and application of several novel optogenetic tools that we have created to investigate the cellular E/I balance hypothesis in freely-moving mice. Using these tools, we have explored cellular and circuit-level physiology associated with E/I balance changes. We find that elevation, but not reduction, of cellular E/I balance within the mouse medial prefrontal cortex elicited profound impairment in cellular information processing, associated with specific behavioral impairments and increased high-frequency power in the 30-80 Hz range, an electrophysiological signature also reported in the clinical conditions. Consistent with the E/I balance hypothesis, compensatory elevation of inhibitory cell excitability partially rescued social deficits caused by E/I balance elevation. These results provide direct links between elevated cellular E/I balance and behavioral impairment.

GENE-BASED VACCINATION AGAINST INFLUENZA IN MULTIPLE ANIMAL MODELS

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Influenza is a highly contagious infection that affects a variety of animal populations, posing a significant threat to the economy and public health as highlighted by the recent pandemic outbreak of H1N1. Current inactivated whole-virus vaccines are effective but do not typically offer broad protection against heterologous strains and subtypes, and require inefficient egg-based production methods that limit quick response to outbreaks. Here, we present several animal models that demonstrate the high efficacy of gene-based vaccines, including those against pandemic H1N1, classical swine H1N1, highly-pathogenic avian H5N1, and equine H3N8 influenza viruses in pigs, chickens, and horses, respectively. At the NIH Vaccine Research Center, several gene-based candidate vaccines have been evaluated in mice, ferrets, and non-human primate models that have resulted in phase I clinical trials. We demonstrate that a multivalent gene-based vaccine regimen elicits protective responses against multiple strains of influenza. We also show that alternative delivery methods, such as aerosolization and needle-free injection, may enhance immune responses and increase the safety and efficiency of vaccine administration.

MAURITIAN MYNOMOLGUS MACAQUES: IMPLICATIONS OF RESTRICTED GENETIC DIVERSITY ON INFECTIOUS DISEASE RESEARCH

Wiseman, R., Karl, J., Norante, F., Burwitz, B., Greene, J., Bushmitz, M., Deiros, R.D., Rogers, J. and O'Connor, D.

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A small number of macaques were introduced to the island of Mauritius during the last millennium. Consequently, the contemporary population has extremely limited genetic diversity; there are only seven major histocompatibility complex (MHC) haplotypes and eight killer immunoglobulin receptor haplotypes. Remarkably, nearly 90% of Mauritian macaques share two MHC class IA alleles that are present on the three most common MHC haplotypes. We have determined that these MHC class IA molecules restrict 'universal' CD8+ T cell responses in simian immunodeficiency virus (SIV)-infected macaques. Ongoing studies are characterizing 'universal' CD8+ T cell responses directed against other viruses. We will discuss how monitoring these responses has improved our understanding of SIV pathogenesis and immunity. We are beginning to study variation in other Mauritian macaque loci. We speculate that genome-wide sequencing of a comparatively small number of Mauritian macaques will capture all of the genetic variation in this population. To this end, we have sequenced two complete genomes from Mauritian macaques, in addition to a single Mauritian macaque exome.

METABOLIC PHENOTYPING: THE ROLE OF CRF RECEPTOR TYPE 2 IN MEDIATING ENERGY HOMEOSTASIS

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Obesity, type 2 diabetes, and the metabolic syndrome are multifactorial diseases of considerable heterogeneity. Understanding the pathogenesis of metabolic diseases is fundamental to prevention and treatment of obesity and diabetes. Consequently, the number of genetically altered mice, which are potentially relevant to metabolic disease or glucose homeostasis alterations, continues to grow. Thorough metabolic phenotyping of these transgenic mice models requires the ability to determine whole body, tissue specific and cellular metabolic fluxes, and to delineate cellular mechanisms of signal transduction. The experimental techniques necessary for studying the impact of genetic or pharmacological manipulations on metabolic and endocrine processes are complex and require diverse specializations such as clamp studies and indirect calorimetry. Examples for these and other methodologies will be presented from research focusing on the role of corticotropin-releasing factor receptor type 2 (CRFR2) in mediating energy homeostasis.

Genetic or pharmacological manipulations of the CRFR2 and its specific ligands, Urocortin-2 (Ucn2) and Urocortin-3

(Ucn3), strongly suggest the involvement of these proteins in regulating the complex central network of energy balance. To elucidate the physiological role of CRFR2 and its specific ligands expressed by several central sites responsible for the regulation of feeding and glucose homeostasis, we used lentiviral-based system and transgenic mice models to specifically knockdown or over-express these proteins. Elucidating the metabolic and related behavioral phenotype of these mice models support an important role for central CRFR2 system in the control of food intake and energy expenditure in response to homeostatic challenge.

SAND RATS *PSAMMOMYS OBESUS* IN DIABETES RESEARCH

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The sand rat *Psammomys obesus*, also known as the fat sand rat, is a terrestrial mammal from the gerbil subfamily that is mostly found in North Africa and the Middle East. Natural habitats include sandy deserts, rocky terrains or saline-marsh areas, where the sand rats typically are found in burrows under the saltbush plants *Atriplex halimus* which are one of their most important food sources.

Sand rats have been used for biomedical research since the 1960s and are now purpose bred and commercially available from e.g. Harlan at the Hebrew University, Jerusalem, Israel in both a diabetes prone line in which more than 70% of the animals develop diabetes and a diabetes resistant line in where 90% of the animals remain normoglycemic even when fed on a high energy diet.

The sand rats are mainly used as a model for dietary induced Type 2 Diabetes as they develop the disease in a way very similar to humans if fed a normal or high-energy rodent diet due to having developed a metabolic-endocrine system adjusted to desert life on a low calorie diet.

This presentation will focus on the importance of housing sand rats under environmentally enriched species-specific conditions in order to increase animal welfare by reducing stereotypic behaviour and fulfilling their natural needs for digging and burrowing, and will scientifically prove that this can still be achieved with the same pattern of diabetes development. Furthermore, the use of sand rats in diabetes research will be scientifically discussed in relation to changes in beta cell mass and morphology and correlated changes in blood glucose and HbA1c measurements associated with the diabetes development. Advantages and disadvantages as Type 2 Diabetes model will also be presented and compared to other species being more commonly used as Type 2 Diabetes animal models.

MODELS FOR TRANSLATIONAL DIABETES RESEARCH

Wolf, E., Renner, S., Klymiuk, N., Kessler, B., Kurome, M., Wünsch, A. and Aigner, B.

Chair for Molecular Animal Breeding and Biotechnology, Department of Veterinary Sciences; and Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Munich, Germany

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from impaired insulin secretion or insulin action or a combination of both. Diabetes mellitus can be differentiated into different etiopathogenetic categories of which type 2 diabetes is most prevalent as 90-95% of all affected patients fall into this category. In type 2 diabetic patients the incretin effect is highly reduced which is mainly related to an impaired insulinotropic action of glucose-dependent insulinotropic polypeptide (GIP) while the insulinotopic action of glucagon-like peptide-1 (GLP-1) is preserved.

In order to evaluate the consequences of an impaired GIP action, we generated transgenic pigs expressing a dominant-negative GIPR ($GIPR^{dn}$) in the pancreatic islets (Renner et al., Diabetes 59:1228–1238, 2010). These pigs mimic important features of human type 2 diabetes mellitus: impaired insulinotropic action of GIP, progressive deterioration of glucose control and reduction of pancreatic beta-cell mass. The progressive nature of the phenotypic changes in $GIPR^{dn}$ transgenic pigs together with the opportunity to take serial blood samples of sufficient volume from unrestrained animals creates a unique model to screen for metabolomic footprints associated with early prediabetic stages. Clinically manifest diabetes in pig models was achieved by expression of mutant insulin (*INS*) genes in the pancreatic beta-cells. These models exhibit markedly reduced pancreatic beta-cell mass leading to reduced insulin secretion and highly elevated

fasting blood glucose levels. Transgenic pigs expressing mutant INS genes are interesting models for studying secondary lesions of diabetes mellitus, for regenerative medicine (e.g. allogeneic transplantation of porcine pancreatic islets to test their functionality before they are used for pig-to-primate xenotransplantation), and for studying consequences of maternal diabetes mellitus on the development of embryos and fetuses, and their interactions with the maternal environment.

THE GÖTTINGEN MINIPIG AS AN ANIMAL MODEL FOR DIABETES AND OBESITY

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The Göttingen minipig was developed in the early 1960s, by cross-breeding four different pig strains, at the University of Göttingen, Germany. The Göttingen minipig is especially suitable for long-term studies because of its inherent small size and ease of handling, even at full maturity.

The Göttingen minipig is very useful in many ways as a model for human physiology and pathophysiology because many of the pig's organ systems resemble those of the human. Of special relevance, in the field of developing biologics for treatment of diabetes and obesity, are the many similarities between the two species in pharmacokinetics (PK) after subcutaneous administration of compounds.

Apart from PK, it is also of paramount importance to test the pharmacodynamics (PD) and efficacy of new drugs in diseased animal models that resemble the human condition. We have therefore developed and characterized, several Göttingen minipig models, for evaluation of the efficacy of new drugs for the treatment of obesity, type 1 and type 2 diabetes and co-morbidities.

The presentation will focus on the characteristics of the different models we have developed and resemblance and non-resemblance to the human disease state. We will also show experimental data from different drug development programs where the models have been applied. Furthermore we will discuss important aspects of care, housing and handling of the different disease models.

GENETICALLY ENGINEERED PIG BEHAVIORAL AND MORPHOLOGICAL CHANGES INDUCED BY PRENATAL STRESS: FROM RATS TO HUMANS

Weinstock-Rosin, M.

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Since the 1970's, findings from retrospective studies in humans suggest that chronic stress during pregnancy may induce emotional problems, attention and learning deficits in their children. Chronic stress elevates cortisol and corticotropin releasing hormone (CRH) in the maternal circulation. Since it is impossible to control factors like genetic makeup, drug intake, maternal behavior and the nature of the postnatal environment, one cannot conclude whether the behavioral changes originate during the fetal period. However, genetic factors and the postnatal environment are more easily controlled in experimental animals. In our research we subjected pregnant Wistar rats to chronic variable forms of stress consisting of mild restraint for 30 min, placement for 5 min on an elevated platform or exposure for 15 min to a cylinder of water, each given in random order on two or three days from day 14-21 of gestation. This is the time during which the cortex and limbic system develop. The rats did not adapt to the stressors which elevated their plasma corticosterone 4-5-fold. We examined behavior and brain morphology in their offspring in adulthood. Like in humans, heightened anxiety was seen in both sexes but learning deficits were more prevalent in males. These were associated with a sex dependent reduction in hippocampal neurogenesis and dendritic morphology and alteration in CRH and its receptors in the amygdala. Anxiety, but not learning deficits results from elevated corticosterone since it was prevented by maternal adrenalectomy prior to the stress and re-instated by corticosterone administration. The data show that adrenal hormones can reach the fetal brain at a critical time during development and are able to alter its structure and function. If maternal stress occurs during the first half of gestation in humans, when the limbic system and cortex develops emotional problems and learning deficits may occur in their children.

A SWINE ANIMAL MODEL OF DIASTOLIC DYSFUNCTION: ASSESSMENT BY TWO DIMENSIONAL ECHOCARDIOGRAPHIC STRAIN IMAGING

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Background

Over 40% of heart failure is ascribed to diastolic dysfunction with significant mortality and morbidity. The treatment is empirical, limited and frequently disappointing. To enable the evaluation of new therapeutic approaches for the treatment of diastolic heart failure we have employed a model of left ventricular hypertrophy secondary to renal wrapping-induced hypertension in minipigs, in which diastolic function can be assessed noninvasively by 2D strain imaging.

Methods and results

Sixteen minipigs (ten females and six males) underwent bilateral renal wrapping for a median period of 11 weeks (5 – 22). This induced systemic hypertension and subsequent left ventricular hypertrophy. Left ventricular structure and function were characterized by echocardiography. Short axis 2D strain analysis of 6 segments at mid- ventricular and apical levels was used to evaluate systolic and diastolic mechanics. Systolic and diastolic blood pressures increased significantly (139±27 to 191±23mmHg and 83±18 to 128±21mmHg, respectively [p<0.001]). Left ventricular mass increased from 97±19gr to 142±25gr (p<0.001). 2D strain revealed a significant decrease in peak diastolic strain rate (endocardial, 2.0±0.4%/s to 1.5±0.5%/s; epicardial, 0.5±0.2%/s to 0.3±0.1%/s, [p<0.05 for both] and radial, -2.6±0.5%/s to -1.6±0.5%/s, [p<0.001]) and strain rate diastolic to systolic (E/S) ratio (endocardial, 1.43±0.2 to 1.06±0.2; epicardial, 1.32±0.32 to 1.03±0.27, [p<0.001 for both]). Early apical reverse rotation fraction decreased from 0.34±0.11 to 0.14±0.05, (p=0.001). Histology showed fibrosis of the renal capsule and hypertrophic hearts. Conclusion: This study demonstrates that diastolic dysfunction can develop experimentally in hypertensive minipigs and can be assessed non-invasively by echocardiographic 2D strain imaging.

BUILDING THE VERTEBRATE VASCULATURE: INSIGHTS FROM ZEBRAFISH

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During embryonic development, the differentiation of endothelial cells and formation of the vascular system are among the earliest events in organogenesis. Serious disruptions in the formation of the vascular network are lethal early in post-implantation, while the maintenance of vessel integrity and the control of vessel physiology have important consequence throughout embryonic and adult life. In recent years, it has become clear that many of the signals implicated in vascular development are reactivated during disease states of angiogenesis such as tissue ischemia, coronary heart disease and tumor-promoted angiogenesis. This has further reinforced the potential medical relevance of vascular development studies.

During the past years, the zebrafish has emerged as a superb model for the research of vessel formation *in vivo*. Zebrafish embryos are optically clear, providing non-invasive and high-resolution observation of the entire vascular system at every stage of embryonic development. In addition, the formation and anatomical layout of the fish vasculature are similar to that of other vertebrates, and most of the genes currently known to act as key players in embryonic vascular development are highly conserved in zebrafish.

In order to study the early stages of formation of blood and lymphatic vessels, we made use of transgenic embryos bearing robust expression of fluorescent reporters in endothelial cells. These embryos can be imaged for up to five days, without development delay or loss of viability, using long-term multiphoton microscopy. This new capability has helped elucidate many of the unique behaviors of endothelial cells in developing blood vessels *in vivo*. As most developmental processes are remarkably similar between zebrafish and humans, both in molecular aspects and function, these studies are likely to reveal conserved pathways regulating the de-

velopment and function of blood and lymphatic endothelial cells in humans.

THE NEMATODE *CAENORHABDITIS ELEGANS*: A SMALL WORM WITH SIGNIFICANT ADVANTAGES FOR THE STUDY OF TOXIC PROTEIN AGGREGATION AND AGING.

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Human neurodegenerative maladies share two common key features: a mechanistic link to the accumulation and deposition of aberrantly aggregated proteins and late onset. Typically sporadic cases onset during the patient's sixth decade of life or later while mutation-linked, familial disorders manifest during the fifth decade. This common temporal emergence pattern, among otherwise unrelated disorders, suggests that the aging process plays a key role in enabling the emergence of these maladies late in life and raises the prospect that aging manipulation could be a future neurodegeneration treatment.

To study the possible links between the aging process and toxic protein aggregation a short-lived organism whose aging process is amenable to manipulations is required. The nematode *Caenorhabditis elegans* (*C. elegans*) has substantial advantages as such model animal: wild-type worms live merely 18 days, the knockdown of nearly every gene can be achieved by feeding the nematodes with RNAi-expressing bacteria and most importantly, their aging process can be easily slowed.

C. elegans-based studies revealed that the manipulation of aging by the reduction of the Insulin/IGF signaling (IIS), a prominent aging regulatory pathway, protects the worms from neurodegeneration-linked toxic protein aggregation. This protective effect is mediated by opposing mechanisms; disaggregation and active aggregation. Recently we reported that the counter proteotoxic effect of IIS reduction is conserved from worms to mammals as IGF-1 signaling reduction protected Alzheimer's-model mice from the behavioral impairments and pathological phenotypes typical to these animals. These findings suggest that IIS reduction has a promising therapeutic potential as a future treatment for neurodegenerative disorders.

PORCINE CARDIOVASCULAR MODELS

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Swine are one of the most commonly used animal models in cardiovascular research. Their anatomic and physiologic characteristics have made them important as translational research models. Most of the models are produced either surgically or by use of interventional catheters. However, there are genetic and transgenic models which have also been available. This manuscript describes some of the most common cardiovascular models which have been utilized by our institution since 1985 with an emphasis on heart failure models. Approximately 10,000 pigs have been utilized in research and teaching protocols during that time frame. We use domestic farm pigs for acute projects, pilot procedures and short term projects. If farm pigs are utilized for more than 3-6 weeks in chronic studies then growth becomes part of the study. Consequently various miniature breeds (Gottingen, Yucatan, Hanford and Sinclair) are utilized for chronic studies and the breed is selected for each project based upon its physiologic characteristics and the goals of the study. Models which have been successfully produced include a genetic model of ventricular septal defect which has resulted in the clinical use of interventional methodologies for non surgical closure of the defect in children. A dietary produced model of atherosclerosis and menopause was produced with an atherosclerotic diet free of phytoestrogens. Surgically produced models include volume and pressure cardiac hypertrophy produced by surgical banding or arteriovenous fistulas, dilative cardiomyopathy produced by rapid epicardial pacing, pacemaker testing, vascular graft implants, heart valve replacement, aneurysm production, intravascular stent placement, fetal cardiac surgery and cardiac conduction system ablation techniques. Illustrations of the techniques will be provided during the presentation as well as the justification for the models.

MYOCARDIAL INFARCT MODELS IN RODENTS: SURGICAL ASPECTS

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Coronary disease is the most common cause of death in the US and Europe. In humans, the majority of myocardial infarcts result from thrombotic occlusion by arteriosclerotic plaques. In experimental animal models, this occlusion phenomenon can be mimicked by a ligation of one of the coronary arteries. Rodent model of acute myocardial infarction (MI) was first described in the rat in 1978. With the recent growth in utilization of genetically engineered mouse models, the opportunity to evaluate the effect of individual genes on infarct healing may become easier to achieve. Post-operative morbidity and mortality in the rodent model can be challenging and many variables are of vital importance for successful outcomes in the rodent MI model. For example, the size of the infarcted area has been extensively studied. However the impact of surgical technique and peri-operative care on success of the model has not been fully characterized. Refinement of surgical technique and peri-operative care may allow for reduction of post-operative complications. The presentation reviews improvements in animal preparation, surgical procedure, post-operative care, continuous training techniques, and quality control that may contribute to improved outcomes in MI rodent models.

GENETICALLY MODIFIED ATHEROSCLEROSIS PIG MODELS

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Atherosclerosis is the number one cause of death worldwide. By thrombosis it often leads to ischemic heart disease and ischemic stroke. Vulnerable plaques are the main cause of thrombosis and their rupture central to the disease. For obvious reasons they are difficult to study in the living human and much information about vulnerable plaques has been accumulated from post mortems. Still, there is no doubt that a good animal model would help to understand how rupture occurs and eventually to develop a treatment.

Several animal models of atherosclerosis have been developed. The question is how well they mimic the human condition. Some pig strains develop atherosclerosis spontaneously on a normal diet and are able to develop symptoms similar to humans including plaques seen to rupture and to cause sudden coronary death.

Genetically modified atherosclerosis pigs have been made at the University of Aarhus, Denmark to improve the pig model. The pigs are generated by genetic modification of differentiated cells – typically fibroblasts, followed by Hand Made Cloning and blastocyst transfer to surrogate sows.

The first successful line was a PCSK9 model. The results so far are very encouraging; the F0 generation shows moderately elevated serum cholesterol values on a normal diet. PCSK9 is expressed and decreases the LDL receptor level in hepatic cells. Furthermore LDL is selectively increased.

The second line was an ApoE knock out model which will be bred to homozygozity and subsequently tested.

The models will undoubtedly provide excellent tools for investigation of atherosclerosis and vulnerable plaques in particular - especially where a size comparable to the human being is important.

ANIMALS IN MESOPOTAMIAN ART 3500-2000 BC

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The city-states of Sumer, which evolved in the latter half of the 4th millenium BC in the south of modern day Iraq, were the first urban settlements and the first civilization with a system of writing – the cuneiform script. Artistic and textual evidence indicate that central temples kept herds of various ungulates. Depiction of animals, both wild and domesticated, is a fundamental theme of Sumerian art and reflects their important role in economic life and religion. Animals were the subject of sculptures, carved stone bowls and cylinder seals, found nowadays in a large number in public and private collections. By examining a few fragments, this talk will describe several developmental stages in the representation of animals in Sumerian and later in Akkadian art. During this period of approximately 1,500 years, the depiction of animals varied from naturalistic to stylized. On the one hand, animals, usu-

ally ungulates, were shown in peaceful scenes ("animal row"), thought to be associated with prayer for bounty and fertility. On the other hand, more violent motifs showing animals, most commonly bulls and lions, engaged in highly stylized combat ("contest scene") were popular. The style of contest scenes evolved in a well-documented manner, and has been studied by art historians and archeologists. These changes can be correlated with political and economic events of the time known from other sources.

THE USE OF THE GÖTTINGEN MINIPIG IN PRECLINICAL SAFETY TESTING

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The choice of animal species in relation to non-clinical research should always be carefully considered and justified. Pigs and minipigs offer several advantages over other species in this respect. In comparison with humans there are plenty of similarities in the anatomy of the skin, the cardiovascular system, the gastrointestinal tract, the nasal cavity and the urogenital system. Also, similarities are often found in metabolism of drugs and in physiological parameters.

Minipigs offer an advantage over pigs, due to the smaller size and thereby a markedly reduced test substance requirement. The Göttingen minipig is bred to high SPF standards, reducing background findings to a minimum. This makes this species ideal for research purposes.

For regulatory toxicity studies the minipig should therefore always be considered as a relevant test species. The minipig is fully accepted globally by regulatory authorities and considerable historical control data for the Göttingen minipig has now been generated for more than two decades.

In drug development the intended clinical route of dosing should always be applied in preclinical studies, wherever possible. All general routes of dosing (i.e. oral, dermal, subcutaneous, intramuscular and intravenous) are practically feasible in the minipig, but other routes, like intravaginal, intravesical and intranasal, is also possible. In addition to repeat dose toxicity testing, we have developed test systems for teratogenicity testing and juvenile toxicity testing in this species.

All guideline requirements for evaluation of systemic toxicity (toxicokinetics, clinical chemistry, ophthalmoscopy, electrocardiography, necropsy and histopathology) are performed on a routine basis in the Göttingen minipig. Additional sampling techniques, like bile collection, can be added. Furthermore, the Göttingen minipig is routinely used for telemetry studies.

This presentation will give the background of the Göttingen minipig and regulatory aspects will be discussed. Furthermore, practical aspects of the mentioned procedures, as well as sampling procedures, will be presented.