We can arrange a catheter course especially for you!

Integrative Wireless Monitoring of Minipigs

The Göttingen Minipig in translational neuroscience

A study measuring working memory and reference memory of pigs and minipigs using the cognitive holeboard task
First of all, I hope that you have had a nice summer and enjoyed spending time with family and friends. My colleagues and I are back from holiday, and I am pleased to see that the rising demand for minipigs did not stop during the summer. On the contrary, global demand for our Göttingen Minipigs is continuously increasing and, as we have been aware of this development in the industry, we are prepared to supply our customers with Göttingen Minipigs, a topic about which further details are available in this newsletter. With this rising demand in mind and our interest in getting involved in even more projects and further business development of Ellegaard, I have been searching for a new colleague to strengthen Ellegaard’s management team. Therefore, I am pleased to welcome Michael Lehd as our new COO, and a presentation of him is included in this newsletter. As CEO, my responsibility will focus on our licence partners, our collaboration with Göttingen University and business development in general. Michael will be responsible for the day-to-day operation of the company, as well as sales and marketing. With his background and experience, Michael will be a great asset in the process of taking Ellegaard to the next level. Based on Ellegaard’s solid foundation and our close, trusting cooperation with our customers, we continue to professionalise the development of Ellegaard to meet your demands.

A great deal of scientific knowledge and background information about the Göttingen Minipig is available and we continue to generate even more, because we appreciate how scientific information contributes to selecting the most appropriate non-rodent species for safety testing. In the future, the selection and de-selection of species for a study must be described and justified even further. Species selection should not be based on tradition and habits but science.

Actually, we just had a visitor from a large pharmaceutical company for two days who wanted to take tissue samples from some of the minipigs. This is part of the pharmaceutical company’s efforts to generate background data which will be used to select the most appropriate species. We encourage you to contact us if you would like to have the same opportunity or need any information to help decide whether the Göttingen Minipig would be the best species for your study.

Earlier this year Lars Ellegaard retired and he was very pleased with all the kind greetings he received. Lars enjoys spending more time on his fishing boat and with family and friends. Actually it has been 20 years since Lars Ellegaard founded the company “Ellegaard Göttingen Minipigs A/S” and the first Göttingen Minipigs were derived through caesarean section at Ellegaard. In 1992 the first newsletter stated: “The number of animals produced will ensure that all future demands can be met. Our aim is to produce the best minipigs in Europe.” By working together we have fulfilled Lars’s vision and I look forward to continuing the positive development of Ellegaard.

This newsletter contains several interesting articles from minipig users willing to share their knowledge and experiences. Integrative wireless monitoring, translational neuroscience and maturation of the minipig’s gastrointestinal tract in a paediatric drug disposition are the main topics of three of the articles. Furthermore, Janssen Research and Development has contributed an article about the challenges facing the company when implementing the minipig as a species. Last but not least, this newsletter includes an article from Utrecht University regarding experiments for validating a holeboard task.

Finally, I would like to encourage you to consult us if you need any information or advice. We have arranged Minipig Continuing Education sessions that will be held this autumn, and the annual meeting of the Minipig Research Forum is also an excellent opportunity to learn about other minipig users’ experiences and improve your minipig network.

Sincerely,
Jens Ellegaard, CEO
As a global developer, breeder and distributor of Göttingen Minipigs, we supply pharmaceutical companies, CRO’s and universities all over Europe with this high-quality animal model. The demand for minipigs as a non-rodent species for biomedical research is increasing and, as we have been aware of this development in the industry, we are prepared to supply existing and new customers with Göttingen Minipigs.

**Availability**

The rising tendency to use minipigs for biomedical research has compelled us to readjust our breeding continuously to enable us to meet the industry’s demands. The capacity in our two barrier units enables us to supply customers with large, uniform groups of Göttingen Minipigs. As always, the barrier facilities at Ellegaard Göttingen Minipigs guarantee that we produce and develop high-quality Göttingen Minipigs with a unique health status.

**Quality**

Although the market is changing and the demand for minipigs is increasing, one thing will never change: our high standards of health, quality, knowledge and service. We attribute great significance to the quality system as an integral part of the production process and as a prerequisite for continued growth and development. Our quality system is structured around our AAALAC accreditation with the addition of relevant elements from the ISO 9000 standard and from GLP (Good Laboratory Practice).

**Accreditation**

Minipigs have become the preferred non-rodent species for many types of studies in the pharmaceutical industry. The objective of the Rethink report was to evaluate the minipig as an alternative species in regulatory toxicity testing and the report confirms that the minipig is suitable as a non-rodent model, as are dogs and non-human primates.

Studies performed on minipigs are fully recognised by regulatory authorities worldwide and the use of minipigs is increasing.

**Values**

At Ellegaard Göttingen Minipigs A/S, we base our daily work on respecting one another and our animals. This approach makes it natural for us to focus on the Minipig welfare, an approach we incorporate into our ongoing efforts to improve the environment and conditions for our Minipigs. We cooperate throughout the company in striving to provide our customers with a product that has the best quality possible.

**Collaboration**

We want to help our customers by giving them the best opportunities to achieve the clearest scientific results. In accordance with this, we engage ourselves in projects and collaborations to investigate and evaluate background data, describe new procedures or collect other types of information about Göttingen Minipigs that can be valuable to other minipig users. You are welcome to contact us if you are interested in collaborating with us on a specific project or if you can suggest study types or areas where minipig background information is needed.

**The future**

We have a strong vision for the future: through our scientific documentation and dedicated efforts to develop and breed a well-defined animal model that contributes to achieving clear research results for our customers, we want to make the Göttingen Minipig the preferred non-rodent animal model for global biomedical research.

You are welcome to contact us for further information about Göttingen Minipigs or our company in general.

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**In August 2012 our approval from the Danish Working Environment Authority has been renewed without any remarks.**

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**NEW COO AT ELLEGAARD GÖTTINGEN MINIPIGS**

My colleagues and I would like to welcome and introduce you to Michael Lehd, our new COO, who started at Ellegaard in April. Michael holds a master’s degree in chemistry and has broad international management experience from the pharmaceutical, food and IT industries. Michael will be responsible for the day-to-day operation of the company, as well as sales and marketing.

Michael has experience in the pharmaceutical industry – but further down the line than preclinical development – notably from NNE Pharmaplan (a Novo Nordisk company) where he was a member of the executive management team. NNE Pharmaplan designs and builds production facilities for pharmaceutical companies. Michael’s previous employment includes working for a company in the food industry and most recently for EG Neoprocess, an IT company. These jobs have given him experience in working with multinational corporations and small privately-owned companies alike. During his career, he has been seconded to Germany, Russia and Japan and has learned how to do business in many other countries and cultures.

Michael’s most recent position was CEO and co-owner of EG Neoprocess where, over a three-year period of organic growth and acquisitions, he grew the company from 40 to 280 employees. One year before Michael began at Ellegaard, EG Neoprocess was sold to a private equity fund. After his vesting period, Michael searched for a new position in a small company with growth potential involved in international business. We are convinced that both Michael and Ellegaard have found the right match. Once again, please welcome Michael – I am looking forward to the exciting future for Ellegaard in rewarding collaboration with our customers.

Jens Ellegaard, CEO
**Catheter Course**

We offer one-day courses in vascular access for injections and blood samples. At this practical hands-on course you will try out different methods of catheterization and you will have time to repeat the methods and thereby increase your skills.

The specific course contents will be tailored to your exact needs and wishes and the course will be arranged to match your schedule.

Examples of techniques are central venous catheters - either percutaneous (Seldinger Method) or surgically implanted with a subcutaneous port (VAP).

Furthermore, we will introduce you to the various types of materials and kits used for vascular access and discuss their pros and cons.

After the course, we offer all the supervision you need to succeed with catheterization of minipigs.

The course instructors are our Laboratory Technician and DVM.

For further information, dates and prices, please contact us.

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**New Bedding Material**

We have tried different kinds of bedding material for minipigs, and we recently decided to use straw instead of the bedding material used previously.

We use straw for bedding material in our barriers and in the transport boxes used for transporting minipigs to our customers.

The new bedding material is wheat straw from our own farmland near the minipig barrier facilities. The wheat straw is cultivated without the use of hazardous plant growth regulators.

The wheat straw is cleared of dust at Box Straw, chopped, wrapped in a double layer of plastic bags and treated with gamma irradiation. After this procedure the straw enters the barrier units through the airlocks.

By using our own straw for bedding material, we are assured that the quality is exactly as we want it and we have also seen that the minipigs like it.

You are welcome to contact us if you have any questions or if you would like to buy some of the bedding material for your next consignment.

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**Measuring spatial in (mini)pigs using**

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**Introduction**

In recent decades, a broad range of cognitive tests has been used to investigate learning and memory in pigs (for recent reviews see: Gieling et al., 2011; Komun and Knudsen, 2011). Unfortunately, most of these tests have not been standardised, and studies attempting to replicate published findings have been performed only sporadically. We are developing and validating a spatial holeboard task for pigs that allows for a simultaneous assessment of spatial working memory (short-term memory) and reference memory (long-term memory) in pigs.

The holeboard task has been deemed suitable for testing cognition in many different species. Holeboard-type tasks are sensitive to a broad range of experimental manipulations (summarised in van der Staay et al., 2012). We expect this task to be suited for safety pharmacology and toxicological risk assessment studies and for pharmacological evaluations of putative cognition-enhancing compounds.

Holeboard-type tasks comprise of an arena in which a matrix of holes (e.g. 4 x 4 holes) can contain a food treat. Usually only a subset of these, for example 4 of the 16 holes, contains a treat. For optimal foraging, animals must learn to visit the locations with a treat only, and to avoid revisiting locations from which they collected the treat during a trial (Olton, 1987). The most efficient behaviour is to visit each location with a treat only once. To achieve this, the animal must apply a win–shift strategy and remember the places already visited to avoid revisits.

If food can only be found in a subset of potential places, two memory components can be distinguished: spatial working memory and spatial reference memory. The list of places visited is held in the working memory (WM). By contrast with WM, which holds information that is only relevant within a specific trial, reference memory (RM) holds trial-independent information, such as where food is hidden.

WM is considered a form of short-term memory, and RM a form of long-term memory (Bimonte-Nelson et al., 2003; Dudchenko, 2004; Mizuno et al., 2002). It should be stressed that WM and RM are operationally defined in spatial orientation tasks: WM is regarded as memory for trial-unique stimuli and events, whereas RM stores information which remains unchanged between trials (Prior et al., 1997). WM and RM performance is reflected by an animal’s choosing behaviour, i.e. by its visits and revisits of locations with and without a treat. WM and RM are considered operational definitions of presumably psychologically distinct mnemonic processes with different neural substrates.

Spatial WM reflects the ability of an animal to avoid revisiting holes with a treat during a trial. WM errors are revisits to holes with a treat, i.e. visits to holes which contained food, but had already been visited in the ongoing trial. Often, instead of counting WM errors, WM is defined as the number of rewarded visits divided by the number of visits to the set of holes with a treat.
Spatial RM holds information about the performance of the holeboard task, e.g. about the localisation of food and about the actions necessary to get the treat, such as dipping into a hole with food, or lifting up a ball covering a hole (Gieling et al., 2012). RM stores the general rules of a task. This information retains its relevance across many trials of an experiment and is thus trial-independent, but may be learning-task specific. RM errors are defined as the number of visits and revisits to holes that never contain a treat. Instead of counting RM errors, RM is often defined as the number of visits to the set of holes with a treat divided by the number of visits to all holes.

RM errors are defined as the number of visits and revisits to holes that never contain a treat. Instead of counting RM errors, RM is often defined as the number of visits to the set of holes with a treat divided by the number of visits to all holes.

Ratio measures of WM and RM are less biased if a pig does not find all food rewards (in which case WM and RM performance, 

Figure 1. A fully automated holeboard device for measuring spatial WM and RM in (mini)pigs. (A) Overview of the testing arena with a matrix of 4 x 4 holes; (B) Experimenter puts M&M chocolates in one of the baited holes. (C, D) Pig lifts up the ball covering a hole to gain access to the hidden M&M chocolates. For the position of the four doors, see Fig.2C,E. (photographs: Annemarie Baars).
expressed as the number of errors, may be overestimated). We routinely use the ratio measure.

**Achievement of the holeboard task by conventional pigs and Göttingen Minipigs**

We performed a series of experiments for developing and validating a holeboard task which is suited to assess the effects of experimental manipulations on spatial WM and RM in pigs.

**Animals:** Four groups of pigs were tested. Group 1 consisted of 20 conventional pigs from a study by Arts et al. (2009) which were approximately 8 weeks old when holeboard learning started. Group 2 consisted of 18 conventional pigs from a study by Gieling et al. (2012) which were approximately 9 weeks old when they were trained in the holeboard task. Group 3 comprised 8 conventional pigs and group 4 comprised 8 Göttingen minipigs from an unpublished study by Gieling, in which these two lines of pigs were compared directly. The pigs in groups 3 and 4 were approximately 13 weeks old when trained to use the holeboard.

**Device:** For testing group 1, a manually operated holeboard (8 m x 7.6 m) was used, in which buckets served as holes. The animals always entered the holeboard through the same door (see Fig. 2B; for further procedural details see: Arts et al., 2009). A semi-automatic holeboard (5.4 m X 5.4 m) – adapted to testing pigs with a body weight of up to approximately 120 kg – was used for group 2. The device was fully automated when testing groups 3 and 4. The device, manufactured by Ossendrijver BV (Achterveld, The Netherlands), is a cognitive pig holeboard (Figure 2A) consisting of a square arena with a 4 x 4 matrix of food bowls. The blue synthetic floor is slatted and the grey synthetic walls (height: 80 cm) have a steel bar across the top. The arena can be entered through any one of the guillotine doors positioned in each of the four side walls, operated from the outside. The entry door for each animal was determined individually by permutations of the numbers 1–4 (with a maximum of twice the same door in a row). By voluntarily walking down a small corridor that surrounded the arena, the animals found the open door and entered the holeboard. The device (arena and corridor) was elevated above the floor to facilitate cleaning. The entrance could be accessed by a little ramp covered with a rubber mat.

**Habituation:** Habituating piglets to the test device and testing procedure is of great importance. Pigs naturally live in groups and are not used to being alone in an unfamiliar environment. After getting them used to the experimenters (by means of movements, sounds and stroking), they were brought to the waiting area located next to the holeboard device. As a group they explored this area and the holeboard. As soon as they showed signs of relaxation, the group size was gradually decreased. Finally, the animals entered the holeboard device alone in at least 4 successive habituation sessions. During habituation, all bowls were always rewarded with M&Ms to increase search behaviour and prevent the development of place (bowl) preferences. The total habituation period lasted approximately one hour a day for 1–2 weeks.

To prevent the animals from locating rewards on the basis of smell, the food rewards (fresh M&M milk chocolates, replaced daily) were placed under the false bottom of the food bowls (Fig. 1B). To prevent the animals from locating the rewards visually, each bowl was covered with a synthetic red ball (JollyBall Dog Toy, diameter: 24 cm, weight: 400 g). The animal had to lift this ball with its snout to gain access to the reward (Fig. 1C,D). The design of the device was such that the ball fell back into place and covered the bowl, once the animal retracted its head.

Each ball contained a magnet that operated a sensor under each of the food bowls. Lifting up a ball was automatically registered as a visit. All data were gathered through an interface (LabJack) and stored on a Personal Computer (OS: Windows XP) using the

![Figure 2. Group 1: (Arts et al., 2009), Group 2: (Gieling et al., 2012), Groups 3 and 4: comparison of conventional pigs and Göttingen minipigs (submitted publication). Note that for comparison purposes, 5 block averages of 5 trials each are depicted. Panels B and C show typical configurations of holes with a treat as used for testing groups 1 (with one entry to the holeboard) and 2 (with 4 entries to the holeboard). Panel E shows a typical configuration of holes with a treat as used for testing groups 3 and 4.](image-url)
custom-made program ‘Experiment control for the University of Utrecht’ (Blinq Systems, Delft, The Netherlands). This program controlled the experiment (e.g. chose randomly per trial through which door a pig was allowed to enter the holeboard arena) and collected the data (start and end of trial, holes visited, and the time of each visit). The program also calculated WM and RM per trial. A camera was mounted above the holeboard, which recorded every trial to allow controlling the registrations by the automatic system and as backup in case of system failure.

Statistical analyses: The early learning curves for WM and RM (first 25 trials) of the four groups of (mini)pigs, measured in three different experiments, are depicted in Fig. 2. For comparison purposes, the first 25 training trials were considered. The mean of five trials each for WM and RM was calculated and subjected to an analysis of variance (ANOVA) with the factor “Groups” and the repeated measures factor “Blocks of trials”. Note that these comparisons were performed across data from three different experiments. In addition, we subjected the data of the third experiment in which conventional pigs and minipigs were presented directly to an ANOVA with the factor Groups (conventional pigs, group 3, vs Göttingen minipigs (group 4)) and the repeated measures factor Blocks of trials.

Results
All pigs were able to learn the task.

Working memory: The average WM performance of the four groups of pigs was similar (Groups: F_{3,50}=0.78, NS). The four groups of pigs slightly improved their WM performance in the course of training (Blocks of trials: F_{4,200}=13.28, p<0.0001). The rate of improvement, however, appeared to be different between groups (Groups: F_{1,23,4,3}=0.78, p=0.6713). Both groups performed at a very high level of WM from the beginning of training, and they did not improve their WM performance across the first five blocks of training [Blocks of trials: F_{4,56}=1.84, p=0.1347; Groups (3 vs. 4) by Block of trials interaction: F_{4,56}=0.59, p=0.6713]. Note that, after 79 additional training trials, WM performance in these two groups of pigs did increase (the results of the full study will be reported elsewhere).

Reference memory: The average RM performance of the four groups of pigs was different (Groups: F_{3,50}=9.65, p<0.0001). The four groups of pigs improved their RM performance in the course of training (Blocks of trials: F_{4,200}=106.43, p<0.0001), differently between groups (Groups by Block of trials interaction: F_{12,200}=4.48, p<0.0001). It appears that the pigs of group 1 (which were trained in a version of the holeboard with one entry door) improved their RM performance faster than the other groups of pigs. The version with one entry is considered less complex than the version in which four doors were used. Direct comparison of the RM performance between conventional pigs and minipigs revealed a marginal difference in the average performance level of the two pig breeds (Groups: F_{1,48}=3.38, p=0.0875). Both breeds learned the task (Blocks of trials: F_{4,48}=28.89, p<0.0001), but the conventional pigs tended to improve their RM performance slightly faster than the Göttingen minipigs (Groups by Block of trials interaction: F_{4,56}=2.42, p=0.0588).

Conclusions
We are validating the holeboard task for pigs as a cognitive task for assessing WM and RM performance in pigs. Pigs are willing to perform the task without the need to apply food deprivation. The normal food ration is distributed, but 1/3 is given in the morning and the remainder in the afternoon. Compared with published learning curves in rodents, pigs learned the task faster, and they reached a nearly error-free performance level after extended training. Göttingen minipigs appeared to learn the task slightly slower than conventional pigs, as assessed in a direct comparison (group 3 vs group 4), but they reached the same high asymptotic performance level by the end of the study (data not shown; paper submitted for publication). Commenting on a study by Manton (2010) who trained minipigs in a holeboard task for a total of 18 trials, Downes (2012) concluded that “the results of testing for learning and memory in minipigs were equivocal and ultimately disappointing”. The minimum prerequisite for testing whether putative cognition impairers or neurotoxic compounds affect learning in minipigs, however, is that the compounds are tested in a stage where the animals show clear learning under drug-free conditions. Our data demonstrate that minipigs are fully capable of learning the holeboard task, which contradicts Downes’ (2012) pessimistic view. This task is well suited for testing the spatial memory performance of Göttingen minipigs. We experienced that it is difficult to train conventional pigs which had reached a body weight of over 100 kg, in terms of both handling and motivation. These problems did not occur when testing Göttingen minipigs.

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The Göttingen minipig in translational neuroscience

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Introduction

Animal models are essential in the development of novel treatment paradigms for most diseases, and for decades rodent models of various diseases have been imperative. The translation of results and treatment options from these small animals to humans often requires the use of a large animal as an intermediate step. Non-human primates have been used for this purpose, as this species shares many similarities with human anatomy and physiology. The use of non-human primates may present both ethical and economical obstacles, which is why alternatives to this large animal model species are necessary. For the past fourteen years, the CENSE-group has worked to establish the Göttingen minipig as a research animal for neurological and neurosurgical disorders. The aim of our translational research is to examine mechanisms of action and develop new treatment paradigms of neuromodulation in a large, non-primate animal model. The Göttingen minipig has a large gyrencephalic brain (approx. 4x5x6 cm) enabling the use of conventional imaging modalities as well as operating techniques and neuromodulatory devices intended for human use. At our centre, we have the option of using CT, MRI and PET imaging in our studies, and scanning modality depends on the hypothesis and the experimental paradigm. The precision of the stereotaxic procedure allowed us to inject tracers into small brain areas to visualise the connections of the minipig brain structures, such as the putamen, the prefrontal cortex and the nucleus accumbens. Anatomical studies are, furthermore, made post mortem by immunohistological analysis, and we have inter alia characterised the hypothalamus, the pars compacta of the substantia nigra and the subthalamic nucleus in the Göttingen minipig.

Neurosurgery in the Göttingen minipig

To facilitate surgery and stereotaxic procedures, the head of the minipig is placed in a head holder that also functions as an MRI-compatible localiser box. The head is fixated in the holder by means of titanium screws in the os zygomaticum. Access to the minipig brain is achieved using standard neurosurgical techniques and instruments, and for high-precision stereotaxic placement of deep brain stimulation (DBS) electrodes and intracerebral microinjections of tracers, toxins or stem cells, a stereotaxic MRI is performed prior to surgery. Stereotaxic coordinates for the target site can be calculated by means of an implanted fiduciary marker or by external fiducials in the side plates of the head holder, the latter allowing import of the MRI into standard neuro-navigation systems to achieve a level of precision in the stereotaxic targeting equal to that in routine clinical use.

Anatomical studies in the Göttingen minipig

Figures 1 and 2 show the principle of the stereotaxic systems used by our group in the minipig. The precision of the stereotaxic procedure allowed us to inject tracers into small brain areas to visualise the connections of the minipig brain structures, such as the putamen, the prefrontal cortex and the nucleus accumbens. Anatomical studies are, furthermore, made post mortem by immunohistological analysis, and we have inter alia characterised the hypothalamus, the pars compacta of the substantia nigra and the subthalamic nucleus in the Göttingen minipig.

Parkinson’s disease in the Göttingen minipig

A Parkinson’s disease (PD) model has been established in the minipig using the toxin MPTP. We have continued to work with this model and showed a progressive nature of disease symptoms by means of a pump inserted on the back of the pig which continuously injects the toxin. The animals showed symptoms of progressively reduced motor performance and activity, and post-mortem examination showed a reduced density of dopaminergic neurons on the pars compacta of the substantia nigra. Currently, we are working to establish the rotating 6-hydroxydopamine (6-OHDA) rotating model of PD in the minipig. This model, where the selectively monoaminergic toxin 6-OHDA is injected into the nigrostriatal pathway, has been well established in rodents and primates for several decades. A unilateral lesion is made, causing asymmetry in the dopamine system, which causes the animal to rotate when stimulated with amphetamine and apomorphine, respectively. The unlesioned side of the brain serves as a normal internal control and the animals’ rotational behaviour can be used to measure the treatment effect in future studies.

Neural stem cell transplantation in the Göttingen minipig

We have tested the feasibility of neural stem cell transplantation in an MPTP model of Parkinson’s disease, grafting neural tissue from the ventral mesencephalon of 28-week-old pig-embryos and placing them in the dopamine depleted striatum of adult minipigs with PD. PET imaging revealed increased fluorodopa uptake in the transplantation sites and post-mortem examination revealed grafts surviving after 7 months. Subsequent studies have included implantation of stem cells using a newly developed intracerebral microinjection device, and...
Figure 2 shows a 6-month-old minipig fixed axially in the MRI-compatible head holder. Stereotactic coordinates for the injection of a tracer into the putamen have been calculated, the burr hole has been made and the stereotactic arch system allows for high-precision injection.

stereotaxic implantation of lentiviruses carrying an alpha-synuclein construct into the substantia nigra to overexpress alpha-synuclein locally in transfected nigral neurons.[2-13]

Deep brain stimulation in the Göttingen minipig
In order to examine the mechanisms of action of DBS for PD, we implanted a DBS system unilaterally in the chronic MPTP model of the disease. This resulted in an improvement of motor performance on the side contralateral to the stimulation, leading to rotational behaviour.[14] PET studies of the animals revealed increased blood flow around the stimulation site in the substantia nigra and increased oxygen uptake in the motor cortex, leading us to the hypothesis that DBS for PD results in a normalisation of the neural signalling in the basal ganglia system.[11]
Likewise, we have targeted the ventral hypothalamus with DBS to induce the safety as a potential treatment of obesity, as well as the subgenual area as a potential DBS target for treatment of depression. Finally we have targeted the pontine micturition centre and achieved central control of voiding in the minipig.[7;10;26] Future studies will involve DBS targeting of the limbic structure of the nucleus accumbens in an attempt to establish a model of depression.

Spinal cord stimulation in the Göttingen minipig
Spinal cord stimulation is an established clinical treatment for severe chronic pain syndromes. Its mechanisms of action, however, have yet to be clarified. The treatment involves placing an electrode in the epidural space over spinal cord segments to innervate the dermatome inflicted by chronic pain. Accordingly, we have set out to map the dermatomes of the minipig and intend to use this anatomical knowledge in addressing the mechanisms of action of the treatment by functional imaging modalities.[11]

Conclusion
The Göttingen minipig provides a unique translational platform on which to develop and test innovative therapeutic approaches, both pharmacological and surgical, and the minipig is increasingly recognised as a useful animal model for translational neuroscience.

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The studies were financially supported by the Danish Research Council, The Lundbeck Foundation, the AP Møller Foundation and the Karen Elise Jensen Foundation.

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Integrative Wireless Monitoring of Minipigs

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Abstract

The applicability of the Holst Centre wireless-sensor node technology is assessed for use on animals in light of efforts to reduce the number of animals and refine the procedures of the use of animals in biomedical (preclinical) research. The Holst Centre ECG Necklace – used on minipigs as a new non-rodent model for safety pharmacology – proved to be an accurate, valuable asset for wireless monitoring of ECG/HR and motion/positioning signals. The results obtained demonstrated the reliability of the system compared to existing technology, yet with a smaller form factor. The differences in results compared to freely moving pre-implanted minipigs (TSI telemetry) are attributable to the experimental design. The ECG sensor node sampled a clear ECG signal and calculated the heart rate in real time, which was not influenced by motion artefacts. In addition, the 3D accelerometer signals were successfully used in combination with ECG/HR and could exclude ambiguous signal changes. Its applicability to the minipig appeared very animal friendly. The pros and cons are discussed in relation to animal wellbeing and relevance for the use of animals in biomedical preclinical research. The sensor node is a stable platform for future sensor extension and integration. This warrants an integrative system for simultaneous assessment of multiple organ systems, with time contributing fully to the principles of the three Rs of animal use (Reduction, Refinement and Replacement).

1. General

1.1 Introduction

Recent advances by researchers of imec/Holst Centre comprise research platforms evidencing technological breakthroughs in the areas of wireless communication, digital signal processing, energy harvesting, sensing and read-out, leading to the realization of ultra-low power wireless body area networks (WBAN). A WBAN is used for communication among sensor nodes operating on, in or around the human body to monitor vital body parameters and movements. Ideally, multiple signals sensed from the body with numerous sensor nodes – either attached to the body surface or implanted in tissue – are transmitted to a home base station. From there, the signal may be forwarded to a hospital, clinic or elsewhere, via a wireless local area network (WLAN), cellular network or public switched telephone network. Sensor nodes in the WBAN are specifically used for electroencephalograms (EEG), electrocardiograms (ECG), electromyograms (EMG), the monitoring of skin temperature and skin conductance, and electro-oculograms (EOG). Apart from medical applications, the WBAN may also serve the user by providing lifestyle, assisted living, sports or entertainment functions. However, to the best of our knowledge, the Holst Centre wireless sensor nodes have not been applied for use on animals in biomedical research.

1.2 Wireless sensor nodes and the three Rs of animal use

The use of animals for biomedical research is a topic of intense public debate, which to some extent conflicts with the mandatory use of animals for research to protect human beings against the undesirable effects of new drugs. Legislation to protect human beings from the adverse effects of new drugs requires that animal experiments using rodents (rats, mice) and non-rodents (dogs, non-human primates) are carried out as outlined in regulatory experiment guidelines. Public interest in replacing, reducing or refining animal experimentation (the three Rs of animal use) has led to the current situation where authorities, the industry, academia and regulatory agencies work together to refine and reduce the use of animals in (preclinical) safety testing. In this context, the minipig has recently been recognized by a group of more than 40 industrial, governmental and academic experts from all over Europe as an alternative non-rodent model which can improve drug safety for humans due to the minipig’s superior predictivity and translatability for humans due to the minipig’s superior predictivity and translatability as an alternative non-rodent model which can improve drug safety for humans due to the minipig’s superior predictivity and translatability for humans due to the minipig’s superior predictivity and translatability for humans due to the minipig’s superior predictivity and translatability as an alternative non-rodent model which can improve drug safety for humans due to the minipig’s superior predictivity and translatability. We hypothesized that this contribution to the three Rs of animal use could be further improved by deploying the Holst Centre wireless sensor technology in this area of (mandatory) safety evaluation studies.

1.3 Wireless monitoring of minipigs: ECG, heart rate, acceleration

In this paper, we report on a feasibility study in which we explored – as a first initiative – an integrative application of the Holst Centre ECG necklace sensor node combined with an X, Y and Z-acceleration sensor in minipigs as test subjects. The primary focus was on 1) animal wellbeing during monitoring; 2) usefulness and quality of the signals; and, 3) relevance of the integrative simultaneous information of ECG, heart rate (HR) and acceleration (activity). For comparison, results obtained from freely moving minipigs – though pre-implanted with telemetry transponders – are reported and compared with those obtained with the Holst Centre ECG sensor node. The pros and cons of the different technologies are discussed in relation to animal wellbeing, particularly animal refinement and reduction. Finally, we speculate on the feasibility of devising an integrative animal-friendly WBAN to address multiple organ systems simultaneously. Primary focus is on safety pharmacology studies and simultaneous multimodal assessment of the cardiovascular, respiratory and nervous systems, preferably with accompanying behaviour. In the present study we focus on the minipig.

1) Part of this study appeared in the Ellegaard Newsletter no. 35, Spring of 2011
2. Methods

2.1 Holst wireless sensor nodes technology in the minipig

2.1.1 Principle of the ECG necklace sensor node

The Holst Centre ECG necklace (Figure 1A) is characterized by a low power consumption ensuring 7 days of autonomy. It contains imec’s ultra-low-power analogue readout ASIC (Application-Specific Integrated Circuit) and relies on a low-power commercial radio and microprocessor platform. The ECG algorithm used in the ECG sensor node is based on Continuous Wavelet Transform (CWT) as described by Romero et al. for its robustness to motion artefacts in ambulatory settings. Efficient and reliable R-peak detection is important for advanced ECG applications like arrhythmia monitoring. The algorithm has good performance: a sensitivity of 99.86% and positive predictivity of 99.91% when detecting R-peaks were noted by Romeo and colleagues. A wireless connection between the ECG necklace and the receiver base station transmits ECG and HR data over a range of 10 m. An optional non-volatile memory module enables continuous data logging for applications in case the receiver base station is not in the neighbourhood. This was also used in the feasibility study reported here.

The ECG sensor node is also fitted with a 3D accelerometer to monitor the orientation and movements of the individual carrying the device (Figure 1). The sensor can be extended by using other sensors over the analogue and digital interface to enable future adaptation such as monitoring of stress/emotion in (working) environments and sweat for e.g. dehydration warning. The combination of a multisensory system with advanced analysis applications creates a future-proof device for any ambulatory environment.

2.1.2 Application of the ECG necklace sensor to minipigs: ECG, HR and X, Y, Z-acceleration

The ECG sensor node detects a heartbeat if there is an R-peak to be distinguished. To test the applicability of the ECG sensor node to monitor the animal’s biomedical signals, we attached the sensor to the minipig (Figure 1B to D). Electrode patches were attached to the skin, one on the left side below the heart; the other on the right side above the heart, after shaving and cleaning the skin (Figure 1B). Sensor leads were attached to the electrodes. The sensor itself was clamped into a pre-prepared ‘pocket’ of an elastic belt attached around the pig’s midsection to maximize the freedom of movement for the minipig (Figure 1D).

Figure 1. Application of Holst Centre ECG sensor node on Göttingen Minipig.

Fig.1A: Holst Centre ECG Necklace with ECG, HR and 3D acceleration as applied to the minipig.

Fig.1B: Attachment of the sensor leads to the Meditrace ECG electrodes [cf. insertion], which are attached to the pig’s shaved and cleaned skin.

Fig.1C to Fig.1E: The Holst Centre ECG necklace was clamped in the pre-prepared ‘pocket’ of an elastic belt worn around the pig’s midsection (see 1d with insert). The orientation of the ECG necklace is recorded (cf. 1C, 1E) to enable the interpretation of acceleration changes on the X, Y and Z axes, since acceleration information is acquired simultaneously with the ECG. (Note that in Figure 1C, the ‘pocket’ on the belt has been ‘deleted’ (Photoshop) for the sake of illustration.)
The orientation of the sensor on the pig was included in the tests, allowing the translation of acceleration signals of the X, Y and Z axes in combination with the ECG and HR. The total duration of the experimental session was about 4 hours, during which the animal was continuously equipped with the Holst ECG sensor node. ECG, HR and X, Y, Z-acceleration were monitored over three periods of 30–45 minutes each. The data logged during recording were checked on a remote monitor. Changes in spatial orientation of the animal, like turning on horizontal and vertical body axes during walking or lifting of the animal – spontaneously or intentionally – were noted. Animal wellbeing was monitored by three observers; one of whom was Ellegaard’s veterinarian who is accustomed to working with minipigs on a daily basis. During the data analysis, special attention was paid to the integration of the simultaneous signals of observed behaviour, ECG, HR and acceleration (activity), in relation to animal wellbeing.

2.2 Telemetry technology for minipigs’ ECG and HR

2.2.1 Example of ECG and HR data in freely moving (pre-implanted) minipigs

The results of the Holst Centre feasibility study with the ECG necklace are discussed in relation to existing technologies for recording ECG in freely moving animals. For direct comparison, results from telemeterized pre-implanted freely moving minipigs (Table 1), are included, kindly provided by Boehringer Ingelheim Pharma GmbH & Co. KG. The relevance and potential power of the Holst Centre sensor node technology for biomedical research on animals is primarily focussed on – though not restricted to – safety pharmacology studies in minipigs.

3. Results

3.1 Results of the feasibility study using the Holst Centre wireless sensor nodes in minipigs

The continuous ECG, HR and acceleration signals were acquired and analysed with good results. A random example of the recorded ECG is shown in Figure 2A; signal morphology, i.e. the typical P, Q, R, S, T peaks and waves, is shown in Figure 2B. Subtle irregularities were observed in the ECG signal (see arrows in Figure 2A). These irregularities appear quite small and not very problematic, but could be optimized for minipig-adapting gain and filter settings. Qualitative screening of the recorded signals with the Holst Centre BAN offline Data Analysis tool indicated that the ECG trace might move up and down with changes in activity and behaviour of the animal; polarity of the ECG signal, however, appeared constant as far as this feasibility study is concerned (see Figure 3A).

As expected, the average HR (roughly 100 bpm) measured automatically by the Holst ECG sensor node (Figure 4) is similar to the HR calculated manually in the example above from RR intervals (Figure 4C). HR variations could be explained by acceleration...
Figure 3. ECG recorded with Holst ECG sensor node in a freely moving Göttingen Minipig. Fig. 3A: Random example of ECG trace obtained with Holst Centre ECG sensor node. Table 2: Summarized values for (manually) measured ECG intervals from 50 ECG complexes. Fig. 3B: Graphic representation of ECG intervals. Fig. 3C: Graphic representation of heart rate (HR) calculated from RR. Abbreviations: SD = standard deviation; ms = milliseconds; s = seconds; bpm = beats per minute.

The mean values of the different ECG intervals of the example trace of Figure 3A are shown in Table 2; these are graphically depicted in Figure 3B; HR calculated from RR intervals is shown in Figure 3C.

Figure 4. (left panel) Note the similarities between acceleration (lower panel) and HR patterns (upper panel).

Figure 5. (right panel). Fig. 5A: Changes in XYZ acceleration (arrows) during lifting of the animal (compare the sensor orientation of Fig. 1D (horizontal) and 1C (under slight change of angle). Fig. 5B: Acceleration of the minipig during moving. Note that at around 1500 seconds, the animal rotates on its own axis while standing on four hoofs, resulting particularly in changes on the x-axis (blue curve); the same behaviour and accompanying changes in the x-axis can be observed at 2200 and 3200 seconds.

3;4) These figures have previously been published (Ellegaard Newsletter no. 35, Spring 2011); and are included here for the sake of completeness.
changes (due to the animal’s activity) (see Figure 4). Such changes in orientation (X, Y, Z-position) of animal-like lifting (Figure 5A) or rotation (Figure 5B) were observed as changes in one or more of the acceleration signals leading to simultaneous HR changes (see Figure 7A, B, time: 1020–1060).

3.2 Intrinsic HR in the Göttingen Minipig (ITS telemetry)

An example of cardiovascular/ECG signals recorded in freely moving pre-implanted telemeterized minipig (ITS telemetry) is shown in Figure 6. The pre-implanted equipment allows for the simultaneous monitoring of arterial (AP) and left ventricular pressure (LVP), and also shows the maximum rate of change in left ventricular pressure (dP/dt) in addition to ECG (see Discussion).

ECG evaluation over a 24-hour period showed that the polarity of the T-wave shifted during the monitoring period without obvious reason. However, at higher HR (for example after dosing or feeding), the T-wave was positive in all animals. In summary, over the 24-hour period 45% of the T-waves were positive, 42% negative and 13% bipolar. The morphology of the P-wave was typical for each individual and never changed obviously during the experiment.

The mean values for (manually) measured ECG intervals over the entire course of 24 hours in the freely moving telemeterized Göttingen Minipig are summarized in Table 3 (means averaged over all time points, or including values at HR ≤ 80 only).

Comparison of the daylight period (1–7 hours) and the darkness period (8.3–20.3 hours) (Figure 7) showed a highly significant (p <0.0001) HR increase during the night (statistical key: paired t test, significant if p <0.05) (Figure 8B). An additional experiment

<table>
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<td>26</td>
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<td>12</td>
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</table>

Table 3: Summarized values for (manually) measured ECG intervals during 24 hours in the freely moving pre-implanted Göttingen Minipig. ECG recorded with ITS/Notocord telemetry equipment (see Figure 7). Abbreviations: HR = heart beat; bpm = beats per minute; ms = milliseconds; SD = standard deviation.
The Holst ECG sensor node measures ECG and HR and is also fitted with a 3D accelerometer to monitor the orientation and movements of the individual animal wearing the device. The sensor can be extended with other sensors by means of the analogue and digital interface to enable future adaptation such as monitoring of stress/emotion and sweat as measures of health status and well-being. The combination of a multi-sensory system with advanced analysis applications provides a scientific device for use in any ambulatory environment in the future. Therefore, we argue that application of Holst Centre wireless sensor technology on animals may substantially contribute to animal refinement and reduction, and eventually combinations of sensor nodes may even replace current testing once new biomarkers are discovered from smart combinations of different physiological outcomes.

4.2 Holst ECG necklace: an accurate and valuable asset for wireless monitoring of minipigs

The Holst Centre ECG Necklace proved to be an accurate and valuable asset for wireless monitoring of minipigs’ ECG/HR and motion/positioning signals. The results obtained demonstrated the reliability of the system compared with existing technology, yet with a smaller form factor. The ECG sensor node sampled a clear ECG signal and calculated HR in real time, which was not influenced by motion artefacts. In addition, the 3D accelerometer signals indicated the animal’s movements and orientation and were successfully used in combination with ECG/HR signals; as a matter of fact, the information on X, Y, Z acceleration helped to exclude ambiguous signal changes. Overall, ECG peaks and waves were clearly distinguishable. The total ECG trace might move up and down, however, without affecting the morphology of the ECG complexes. Moreover, changes in polarity were not observed during this feasibility study. The length of the ECG intervals could clearly be measured.

4.3 Animal wellbeing and HR: What about stress, trained handling, light/darkness, surgery, feeding?

HR values of about 110 bpm were observed in minipigs with the Holst ECG sensor node. As discussed below, relative to the HR measured in the pre-implanted telemeterized minipig (ITS telemetry), the HR measured with the Holst Centre ECG sensor node is high, but may be explained by the fact that food was available during the feasibility testing of the Holst Centre sensor node.

The HR of telemeterized pre-implanted minipigs was well monitored. The HR appeared low and remained below 100 bpm even during oral administration, suggesting that the animals were well

Figure 7. HR measured over a 24-hour monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data were summarized every 10 minutes as median values ± SD (minimum 400 sequential beats). The dotted line at 0 hours indicates oral administration of a placebo whereas the dotted line at 7 hours indicates feeding. The grey shaded area represents the dark period.

(n = 4) was performed without feeding. In this study, a mean HR during daytime of 53 bpm was followed by a HR of 51 bpm at night (Figure 8A), demonstrating that the effect depends on the postprandial condition and is not merely a diurnal effect. This supports the suggestion that the higher HR values found in the minipig of the feasibility study and measured with the Holst ECG wireless sensor node indeed originate from the fact that food was available.

Figure 8. HR measured with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Fig.8A: HR in 4 animals in daytime (mean 53 bpm) compared to night (mean 51 bpm) without feeding 7 hours after treatment; $p = 0.2101$. Fig.8B: HR in 4 animals in daytime (mean 53 bpm) compared to night (mean 64 bpm) with feeding 7 hours after treatment; $p = 0.0120$. The Holst ECG necklace provided a clear ECG signal and calculated HR in real time, which was not influenced by motion artefacts. In addition, the 3D accelerometer signals indicated the animal's movements and orientation and were successfully used in combination with ECG/HR signals; as a matter of fact, the information on X, Y, Z acceleration helped to exclude ambiguous signal changes. Overall, ECG peaks and waves were clearly distinguishable. The total ECG trace might move up and down, however, without affecting the morphology of the ECG complexes. Moreover, changes in polarity were not observed during this feasibility study. The length of the ECG intervals could clearly be measured.
trained and that the procedure of oral administration was not associated with a high level of stress. Surprisingly, however, HR markedly increased during feeding: highly significant increases in HR (up to 122 bpm) were observed which were long-lasting and never returned to the daytime level for the rest of the 24-hour monitoring period (mean of all experiments at night: 71 bpm). In the absence of feeding, it was found that the average daytime HR was 53 bpm and was followed by a similar HR of 51 bpm at night. These findings demonstrate that the effect depends upon the postprandial condition and is not merely diurnal.\(^{[11]}\)

Together, these results suggest that for minipigs, feeding per se has a higher impact on heart rate than actions like trained handling for oral administration and probably explain the differences in baseline HR measured in the latter experiment with the ITS pre-implanted telemeterized minipigs and the Holst ECG sensor node in the feasibility study. After all, in the feasibility study, food pellets and the behaviour of approaching observers were used by the observers to test animal wellbeing.

Beglinger and Becker\(^{[9]}\) reported HR of 103 (±14) bpm in singly-restricted Göttingen Minipigs (~ 20 kg). Although in this case, a stress-induced HR increase cannot be ruled out, there was no reason whatsoever to believe that the minipigs in the feasibility experiment, monitored with the Holst Centre ECG sensor node were under stress. Kano\(^{[10]}\) reported values from 72 to 76 bpm in freely moving miniature pigs weighing 17 kg (but not Göttingen Minipigs). HR in resting miniature pigs was 80 (±3.5) bpm in investigations from Kuwahara.\(^{[9]}\)

The HRs in the present study are comparable to data from freely moving, well-trained and group-housed Labrador dogs.\(^{[1]}\) At TNO we measured baseline values of HR in Beagle dogs, respectively group-housed versus solitarily housed Beagles, of 84 bpm (daylight) and 72 bpm (darkness) versus 79 bpm (daylight) and 69 bpm (darkness).\(^{[9]}\)\(^{[10]}\) Note that no differences were observed between solitarily and group-housed animals, probably because the solitarily housed animals can hear, see and smell neighbouring dogs and dogs in the animal house. Note also that HR in periods of daylight and darkness hardly differs, which is consistent with findings in telemeterized minipigs of this paper: telemeterized pre-implanted dogs in the study of Pijnappel and De Groot\(^{[9]}\) were fed during morning hours; apparently the effect of feeding on HR was temporary and recovered during the daylight period.

Together, these findings illustrate that in minipigs, feeding schedules may be important confounders when it comes to HR measures. With regard to the results obtained with the Holst ECG sensor node, it is concluded that stress levels were low but feeding probably increased the HR and, related to that, ECG intervals. So, in an animal wellbeing perspective, stress levels are clearly low for animals wearing a Holst Centre ECG sensor node.

4.4 Wireless monitoring and animal wellbeing: pros and cons of external sensor nodes and pre-implantation

Unlike pre-implanted telemeterized animals, no surgical procedure is required for the Holst sensor nodes. Otherwise, during surgery, an animal can be equipped with different sensors to address different organ systems. For example, as demonstrated here for the telemeterized minipig (TSI telemetry), a complete test battery of cardiovascular parameters is given. The disadvantage, however, is that surgery places a rather large burden on the animal when it comes to animal wellbeing, and the necessary anaesthesia, analgesia or antibiotics may change the threshold for toxicity of a drug to an unknown extent. Therefore, additional control animals may be required. A technical disadvantage of implanted telemetry transponders is the fact that the batteries of the implanted transponder may run out of energy. Although to date, transponders can be refurbished at reasonable costs, for the animal this implies that another surgical procedure is needed (with all the disadvantages for animal wellbeing and quality of research outcomes). Sometimes, the animals can still be used for other purposes where telemetry is not required, but often the animals will have to be culled and new animals will have to be implanted.

Clearly, completely non-invasive external recording is vastly superior when it comes to benefitting animal wellbeing. To date, manufacturers of telemetry equipment focus on jacketed telemetry equipment to record ECG, implying that the transponder is to be worn on the body in jacket pockets (e.g. DSI/EMKA). So far, these transponders are quite heavy, and training is needed to accustom the animal to wearing the jacket without stress. This is deemed essential for maintaining good study data. However, it takes time to train the animals, and this is a burden for the animal, although limited. In addition, time and money are involved in the training. Good-quality data of studies with ‘happy’ animals contribute to reduced variation in study results meaning that fewer individuals are needed for unambiguous study outcomes. This contributes to achieving the three Rs of animal use for increased statistical viability.

4.5 Holst wireless sensor technology and the three Rs of animal use: mouse, rat and minipig predictability

The results of the present study showed that Holst technology could add knowledge to contribute to reducing and refining the use of experimental animals. In principle – and when used up until now – Holst Centre ultra-low-power wireless sensor nodes would contribute the most to the principles of the three Rs when applied to small laboratory animals (mice, rats) as they still represent by far the largest number of laboratory animals to date. An emerging issue in biomedical research remains: the predictability of rats and mice for humans. Recently, a group of more than 40 industrial, governmental and academic experts from all over Europe reported that minipigs have many advantages for drug and chemical safety testing and could improve drug safety for humans.\(^{[11]}\) The results of the present study involving minipigs indicate that the Holst Centre wireless sensor technology could be ideal for supporting the three Rs of animal use: the minipig accepted wearing the sensor (refinement), and the signals of ECG, HR and acceleration were acquired and analysed without any problem and with very good results. Continuous, repeated and simultaneous monitoring with multiple sensor nodes addressing multiple organ systems seems to be within reach, which enables more information to be obtained from fewer animals (reduction). Otherwise, time and costs will be saved, opening up more room for further studies.

4.6. Minipig and multimodal integrative physiology platform: discovering new biomarkers

Development/application of an integrative multimodal test system is proposed to allow simultaneous animal-friendly, information-enhancing and cost-reducing assessments of physiological parameters indicative of the health status of specific organ systems and hence the overall health status of an individual. An additional challenge is simultaneous monitoring and analysis of physiological
parameters with accompanying behaviours using smart combinations of e.g. acceleration and EMG sensor nodes with ECG or EEG, to define characteristic behaviours (specific locomotion, localization or body posture) which, in turn, may be indicative of anxiety, pain, depression, etc. Hence, characteristic new biomarkers may be discovered. In the short term, this will lead to animal reduction since more information is obtained from an individual animal, also saving time and costs (e.g. for pharmaceutical industries which can abandon unpromising leads in time). In the long term, the database of toxicological information about minipigs will expand and, slowly but surely, information on rodents will not add new information to the data for non-rodent species examined with such a highly sophisticated integrative test system for simultaneous, multimodal physiological assessments. Rodent studies may become needless. In this context, it is worth remembering that the development of regulatory requirements (outlined in testing guidelines) always depends on the state of the science and state-of-the-art testing methodology. Thus, it may be possible in the future to replace the currently required tests on single endpoints in different species by a limited test battery in a single species, like the minipig, which has at least the same, but probably better, predictivity of effects and whose results can readily and reliably be translated to humans.

4.7 Sensor platform, minipig, the three Rs of animal use: future directions
To the best of our knowledge, Holst Centre ultra-low-power wireless sensor technology has only been used in biomedical research using human subjects. Once developed, the proposed system will benefit animals in the first place (reduction and reduction of animal use), society, animal rights parties, governmental and regulatory authorities and the pharmaceutical, food and chemistry industries. The system is unique in the sense that, compared to systems already on the market, it is far more animal-friendly and/or more powerful as it can address multiple organ systems, e.g. the cardiovascular, respiratory and nervous systems with accompanying behaviour, using wireless technology; simultaneously, continuously and repeatedly. Moreover, smart combinations of physiological endpoints may lead to the discovery of new biomarkers that may be very informative in the early phase of drug discovery. Moreover, continuous data logging without a computer present is possible, as is a connection to a network for ambulatory situations. The sensor node is a stable platform for future extension and integration of sensors. As indicated by Ellegaard et al.,[9] a variety of clinical signs (e.g. body posture, activity, behaviour, dehydration, etc.) may be observed in safety evaluation studies. These signs are supported by cardiovascular measurements such as HR, blood pressure, ECG, respiratory rate, EEG, and telemetry/remote monitoring. The Holst Centre sensor node is actually designed for human application to obtain ECG and HR signals, combined with activity sensors, and transmits the data over a telemetric connection. These measurements of biomedical signals were also obtained from the minipig, making the platform a versatile system for animal wellbeing. The next phases in system development could include SpO2, respiration, and galvanic skin response (GSR) in a small package and low power consumption for extended trials with minimum effort on animal behaviour. Future sensors will also measure dehydration and EEG signals, which would complete major safety pharmacology measurements. These parameters will be combined to determine HR variability, and the emotion and stress of an individual, ultimately resulting in autonomous monitoring of the individual. By translating these parameter measurements and applications for the minipig, the multi-sensory system and algorithms will also assist in fully achieving the refinement, replacement, and reduction (3 Rs) objectives highlighted in the RETHINK project.[10]

Reference list
Minipigs in Toxicological Research at Janssen: Practical Aspects


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Introduction
In recent years, minipigs have become more popular as an alternative non-rodent species for toxicity testing and are considered an acceptable alternative when the dog is not a suitable species. At Janssen the first toxicity study using minipigs was performed in 2006. Important considerations when performing toxicity testing in minipigs include the extent of experience with minipigs in toxicity studies, including background data and the level of training of animal caretakers and technicians. Improper handling can stress the animals, which can have consequences for the study. Stress can be minimized in animals that are well-socialized and acclimated to research procedures. The scope of this paper is to give a brief overview of the practical modifications that were implemented to adequately perform toxicity studies in the minipig.

Type of study
Over the past four years, studies using minipigs have been performed for several compounds at Drug Safety Sciences of Janssen, the majority of which were local tolerance studies (IM dosing, GLP and non-GLP studies). The studies were carried out using minipigs because dogs are too sensitive. Due to the many similarities between human skin and minipig skin, these types of studies using minipigs can better predict the adverse effects in humans than local tolerance studies using dogs. In addition, a number of oral gavage toleration studies were performed using both minipigs and dogs but due to the dogs’ excessive vomiting and a better TK profile for minipigs, minipigs were chosen as the non-rodent species for the First in human package including a one-month GLP study.

Practical aspects
Before the first studies were conducted in our department, the animal technicians were extensively trained by Ellegaard of Denmark. In spite of this training, some difficulties were experienced during the studies, mainly in procedures related to animal handling, housing and feeding. Recently, an additional training course was taken at Ellegaard during which it became clear that a different approach had to be taken for handling minipigs than...
for handling dogs. The training focused more on understanding minipig behaviour and how to apply this to the different procedures and techniques. After the visit, several changes were made to the housing procedures (ranking, cage enrichment, environmental conditions, feeding procedure) and the handling of the animals including experimental procedures (approaching and picking them up, putting them in a sling, blood sampling and dosing). Furthermore, theoretical and practical training was foreseen for the biotechnicians, TK biotechnicians and other employees in contact with the animals.

1.1 Housing and feeding

Minipigs at our facility are housed in dog pens which provide enough floor area and resting space. The walls of the cages are composed of Trespa® plates and glass and the floors are solid and covered with Epoxie®. In the first studies performed at our facility, minipigs were housed individually but, as they are social creatures, group-housing is preferable. In order to group-house the animals without hierarchy problems or aggression, the animals are ranked on the day of arrival based on their body weight and sibling relations. In addition, the housing conditions at the supplier Ellegaard are also taken into consideration. In recent studies, male minipigs aged 3 to 8 months on arrival were group-housed without significant problems. Some fighting was observed among older animals in the first days after arrival until the hierarchy was established. In general, however, no serious problems with aggression in male minipigs have occurred.

Minipigs are diurnal animals, meaning that the provision of 12h of light (200 lux) and 12h of dark complies with their activity pattern. The room temperature is age dependent. For animals aged 3 to 6 months, temperatures should range from 19 to 23°C, whereas for animals older than 6 months, temperatures between 21 and 25°C are suitable. Although light intensity and room temperature can easily be modified at our facility, the temperature was not originally modified for the first studies conducted at Janssen, the minipigs were fed according to their weight, similar to normal practice in dog studies. However, minipigs tend to eat everything they are given resulting in heavy animals gaining more weight than lighter animals and exacerbating the disparity. Females were routinely fed the same amount as males resulting in a marked weight difference between males and the much heavier females particularly in long-term studies.

Practice

Practically, the protocol details the date from which minipigs are fed an amount of food based on their age. In consultation with the study director, the amount of food of a certain animal can be modified if the age and weight categories no longer correspond.

1.2 Socialization, handling and experimental procedures

The following paragraph describes how procedures are currently performed and their practicalities:

**Acclimatization period**

The purpose of the acclimatization period is to allow animals to socialize and gradually become accustomed to the experimental conditions to minimize stress, not only for the animals but also for the staff. It is deemed important to create a pleasant, undisturbed atmosphere for the good conduct of the study. This period is somewhat labour intensive but can ultimately save time during the study. The acclimatization consists of touching, hand feeding and picking up the animals and training them to lie in a sling for experimental procedures. Socialization lasting approximately 3 hours per day for 3 weeks is required for a one-month GLP study. In the initial studies, less time was spent on training which resulted in more vocalization from and struggling with the animals.

**Approaching and picking up**

In the initial studies, minipigs were picked up in their cage in the same way one would pick up a dog, which resulted in a lot of vocalization from them, signifying stress. When approaching a minipig, it is important to be aware that you are dealing with a prey animal. First, a hand is offered for the animal to sniff and then food is thrown on the floor between the minipig and handler to encourage the animal to come closer. To pick them up, they are allowed to come out of their cage, a hind leg is grasped with one hand and the other hand is placed under the thorax and the pig is lifted. It is important to hold the animal loosely and to always pick them up in the same way.

### Table 1: Environmental conditions

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</tr>
<tr>
<td><strong>Room temperature</strong></td>
<td>All ages: 19–23°C</td>
<td>Age 3–6 months: 21–25°C Age &gt;6 months: 19–23°C</td>
</tr>
<tr>
<td><strong>Relative humidity</strong></td>
<td>40–70%</td>
<td>40–70%</td>
</tr>
</tbody>
</table>

Minipigs should be offered environmental enrichment in line with their natural behaviour. While 70–80% of their time is spent sleeping or lying down, the remainder is spent rooting, chewing and exploring. They are provided with bedding material and different toys for rooting and chewing which are replaced daily to maintain their interest. In addition, a stainless steel chain is affixed to their cage to allow additional chewing behaviour.

Minipigs at our facility are fed “Ssniff MPig”, and they receive fixed amounts primarily according to their age and sex and secondarily according to their body weight. Female minipigs receive less than males and animals younger than 6 months are fed twice a day. An overview of the feeding regimen is presented in Table 2. In the first studies conducted at Janssen, the minipigs were fed according to their weight, similar to normal practice in dog studies. However, minipigs tend to eat everything they are given resulting in heavy animals gaining more weight than lighter animals and exacerbating the disparity. Females were routinely fed the same amount as males resulting in a marked weight difference between males and the much heavier females particularly in long-term studies.

### Table 2: Feeding Practice

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Weight (kg)</th>
<th>Males (g)</th>
<th>Females (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–4</td>
<td>5–8.9</td>
<td>225*</td>
<td>200*</td>
</tr>
<tr>
<td>4–6</td>
<td>9–12.9</td>
<td>270*</td>
<td>240*</td>
</tr>
<tr>
<td>6–8</td>
<td>13–16.9</td>
<td>360</td>
<td>310</td>
</tr>
<tr>
<td>8–10</td>
<td>17–20.9</td>
<td>405</td>
<td>360</td>
</tr>
<tr>
<td>10–12</td>
<td>21–24.9</td>
<td>450</td>
<td>360</td>
</tr>
<tr>
<td>&gt;12</td>
<td>&gt;25</td>
<td>500 or more</td>
<td>410 or more</td>
</tr>
</tbody>
</table>

*Total amount of food/day, fed in 2 portions
Dosing
As oral dosing is a stressful procedure for the minipig, training is not considered beneficial. Ellegaard has designed a special chair on which the biotechnician sits with the minipig during the oral gavage procedure. This procedure is more labour intensive than in dogs but a level of acceptance can be achieved and the procedure takes less time and effort during a study.

Blood collection via the jugular vein
In the initial studies, blood was taken while the minipig was held steady on a biotechnician’s lap with the help of one or two other biotechnicians to take the sample. This procedure caused vocalization, struggling and stress. The current practice for young minipigs is to roll the animal onto its back into a V-trough. For older and heavier animals, blood can be collected using a sling with an opening in the sling to access the blood sampling site. This procedure does not require more time than in dogs, although an extra person may be needed to hold the minipig, particularly in younger animals.

Eye examination
Initially, animals were held in the arms to perform the eye examination although this has been modified so that now minipigs are placed in a sling. Before the start of the study, animals need to be trained for the sling, as detailed above. Putting eye drops into the eye and the eye examination itself are slightly more time consuming than in dogs because it is more difficult to correctly position the head and examine the eyes, as the pig’s eyes are more deeply set and have long eyelashes.

ECG
Other than the time required to train the animals for the sling during the pre-study period, this procedure does not require more time or personnel than in a dog study. Standard ECG leads are placed similar to those in the dog (I, II, III, aVR, aVL and aVF). For interpreting the ECG signal, the spontaneous changing of the T-wave’s polarity and a longer QT interval (than for the dog) are taken into account. The postprandial heart rate increase is surpassed by not feeding the animals during the 4 hours prior to ECG recordings.

Clinical Pathology
Haematology and clinical chemistry analysis were validated in 2007. As regards the haematological analysis, blood-sample reanalysis is required more frequently than when working with dog samples. This is probably related to the occasional difficulty of blood sampling in some minipigs, sometimes resulting in a low-quality sample (e.g. a clothed sample).

1 Device designed to roll the minipig onto its back and restrain it to collect blood.
Necropsy and pathology

Before necropsy, fasting minipigs are weighed and anaesthetised by intramuscular injection with a mixture of 1 flacon of Zoletil® 100 (250 mg tiletamine + 250 mg zolazepam, dry substance only), 12.5 ml xylazine (20 mg/ml), 2.5 ml Ketamine (100 mg/ml) and 5 ml butorphanol (10mg/ml) at an appropriate dosage volume of 0.1 ml/kg body weight. Stress should be avoided as much as possible especially to avoid malignant hyperthermia and muscle degeneration. Dogs are administered with IV heparin to prevent coagulation, but as it is more difficult to give IV injections to minipigs and they are very sensitive to stress, no anti-coagulant is given. The deeply anaesthetised minipig exsanguinates through the front or hind leg veins and arteries. Cervical veins and arteries are not cut because this further complicates the preparation of cervical tissues (thyroid, parathyroids, salivary glands). To accelerate exsanguination, a pulley is installed from which to vertically suspend the pig.

At our facility, necropsies of non-rodents (dogs, minipigs) are organized so that one animal is dissected by several people in “a chain”: the animal is passed from one technician to the next. When minipigs are to be necropsied, additional assistance is required as minipig necropsy is more laborious. The anatomy of the minipig (heavier, larger, more compact than dogs complicates the tissue dissection, where the neck region and gastrointestinal tract are particularly challenging. In the neck region, the separate thymus lobes need to be taken and the thyroid glands are not easy to localize. The parathyroid glands are embedded in the thymus during necropsy and are separated from the thymus after fixation. Yet even after careful dissection, the parathyroid glands are not always found. Salivary glands are more compact. The gastrointestinal tract is enormous compared to the dog and additional samples (compared to the dog) are taken (e.g. 4 tissue samples of the stomach versus 2 in the dog). Male genital tissue, such as testes and seminal vesicles, are very large in mature minipigs and there is a clear need for additional training of technicians and pathologists to be consistent in taking representative samples.

Bone decalcification of the distal femur takes longer due to a thicker cortex. Continuous professional training is needed to discriminate between common background lesions in the minipig from drug-related histopathological lesions. Focal accumulation of mononuclear inflammatory cells (lymphocytes, macrophages and plasma cells) in various organs (generally in perivascular and interstitial locations) is the most common histopathological background lesion noted in the minipig. These mononuclear cell infiltrates can be seen in adrenal glands, brain, kidneys, liver, parotid gland, oesophagus, stomach, etc. The interlobular fibrous connective tissue in the minipig liver is another common example of a physiological ‘pig-specific’ finding.

Apart from these anatomical variations, there are other specifics which pathologists and toxicologists should be aware of, including the sensitivity of pigs to acute heart failure due to stress and especially viral and bacterial infections. In rodents and dogs, these infections have been almost completely eradicated, but in minipigs these risks need to be kept in mind (Aujeszky’s disease, Swine Fever, Salmonella, Haemolytic E. coli, etc.), since employees can potentially come in contact with farm pigs and be a source of infection for experimental pigs. Not only should the pathology of these diseases be known and understood, but also the implications for public regulations for legally contested illnesses and the possibility of zoonoses occurring during toxicity studies.

Conclusion

The additional training both at Ellegaard and at our facility organized by the biotechnicians of the minipig expertise team led to a change in the handling approach. In the light of the important fact that pigs are more sensitive to stress, this change has obviously been beneficial. This new approach, together with modified and new techniques, led to a more optimal environment for carrying out studies with significantly less vocalization from and struggling of the animals. This is not only beneficial for the animals but also for the biotechnicians working with them – and most importantly for the study results. In addition, good knowledge of background data and histopathological background lesions is very important for the interpretation study results.

Although techniques and handling procedures have been optimized, more personnel and/or more time are still required for performing certain phases of a minipig study as compared to a dog study, not only while the animals are alive but also during necropsy. The largest difference between the two species is the high workload during acclimatization. Training and socializing minipigs require more time because minipigs are animals of prey which makes them more wary and shy, thus requiring a greater effort to gain their trust. Once this is established, minipigs can be accustomed and trained to participate in a study.

Animal caretakers who usually work with dogs can still apply their basic animal interaction skills but they should be aware that dealing with minipigs requires a different approach altogether. As biotechnicians become more accustomed to working with minipigs, they will acquire more experience, and the training and handling of the animals will flow more smoothly and the workload should be alleviated.
During prenatal and postnatal development, several morphological and physiological parameters undergo changes which can impact drug disposition. The maturation of the kidneys and the gastrointestinal (GI) system, including the liver, appears to exert the most important influence on the pharmacokinetics of most drugs.\[1\] When focusing on the gastrointestinal tract, several parameters such as length, diameter, surface area expansion factors, pH, transit time, enzymatic activity and, of course, biotransformation capacity can affect drug absorption.\[2\] In view of the current focus on paediatric drug development, detailed knowledge of these factors is a prerequisite for paediatric drug modelling, for PK/PD modelling in juvenile animals and for interpreting of juvenile toxicity studies which may be requested prior to starting clinical trials in the paediatric population.

The (mini)pig is used in (juvenile) toxicity studies and an adult animal is generally regarded as a good model for the human GI system based on morphological and physiological similarities.\[3\] However, comparative data during maturation are often lacking. In the tables below a comparison between man and pig is given for several GI parameters (limited to the stomach and small intestine in view of drug disposition) during perinatal and postnatal development. Rodents and dogs are also included as these two are commonly used as preclinical animal models. The onset of gastric acid secretion differs amongst the species. In rodents, the secretion of gastric acid only becomes prominent around weaning,\[4\] an illustration of the relative immaturity of the GI tract at birth compared to other species.

### Stomach

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Suckling</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>At 87% gestation from 7 to 3</td>
<td>Linear increase in secretion 1st week</td>
<td>6-7 fold more secretion</td>
<td>1.6-4.3</td>
</tr>
<tr>
<td>Human</td>
<td>At 60% gestation possible but minor secretion, pH below 4 (varying reports)</td>
<td>Increasing capacity (1st 4 months) [\approx 4] (varying reports)</td>
<td>By 2 y = adult (level of secretion)</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Rodent</td>
<td>At 89% gestation possible but no secretion</td>
<td>Maturation of gastric mucosa</td>
<td>By 3 weeks significant HCl secretion</td>
<td>Adult level reached by 6 wks of age</td>
</tr>
<tr>
<td>Dog</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Fasted: 5.5 (higher than human) Fed: 1.9-2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Suckling</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Pepsinogen &amp; prostaglandins appear after birth and increase, pepsinogen decreases</td>
<td>Response to diet (increases in pepsinogen &amp; prostaglandins)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Low preterm</td>
<td>Proteolytic activity shows no big age-related differences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodent</td>
<td>No to low preterm</td>
<td>No to low</td>
<td>Increase from 21 d pp</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>?</td>
<td>Peptin detectable from day 21</td>
<td>Peptin gradually increased by 63 d ~ adulthood</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Suckling</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>?</td>
<td>Biphasic</td>
<td>Biphasic</td>
<td>Biphasic</td>
</tr>
<tr>
<td>Human</td>
<td>Gastric emptying</td>
<td>2nd trimester of gestation</td>
<td>Biphasic</td>
<td>Biphasic</td>
</tr>
<tr>
<td>Rodent</td>
<td>Maturation needed of smooth muscle cells</td>
<td>Biphasic</td>
<td>Biphasic</td>
<td>Biphasic</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td>Biphasic</td>
<td>Biphasic</td>
<td>Biphasic</td>
</tr>
</tbody>
</table>
The parietal cells in the fundic gland region are responsible for the production of proteases, of which pepsin is the most well known. Again, the rodent stomach is lagging behind that of man, pig and dog. Gastric lipase (data not shown) is secreted in relatively large quantities around birth and during suckling in most species to give the secretion of pancreatic lipase some time to mature.

Data about gastric motility during gestation are scarce. In humans, gastrointestinal motility is generally believed to be present before birth. In all species, gastric emptying can be considered biphasic, i.e. a rapid phase during which liquid and small particles are emptied and a slower phase for the larger species. In suckling pigs and infants, gastric emptying is not influenced by osmolality. No data are available for dogs.

In the small intestine, the epithelium is responsible for macromolecular transport, and mature enterocytes possess microvilli coated with brush border enzymes. The unique feature of enterocytes in newborn mammals is the presence of an apical canalicular system (ACS) leading to production of large vacuoles important for colostral macromolecule uptake. After birth this ACS gradually disappears. The human intestine is generally less permeable to macromolecules compared with other species.

No big difference in peptidase or disaccharidase activities is observed between the species, except that sucrase activity is seen already before birth in humans, by contrast with pigs and rodents. Prenatal dog data are lacking.

In general, the data above indicate a similar pattern of GI maturation in (mini)pigs compared with humans, definitely more than rodents and possibly more than dogs, although we need to be cautious as maturation data for the latter species are scarce. However, several "basic" data are still lacking for the GI tract of maturing minipigs as well, such as drug metabolizing capacity and transport. In our lab, we are currently compiling data on length and diameter, surface area expansion factors and pH specifically in juvenile Göttingen minipigs and fetuses. We are also investigating biotransformation capacity to better understand and predict drug disposition in this species.

References


Small intestine

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Neonate</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>95% gestation ACS 0.2 A/M Crypts &amp; villi</td>
<td>1 type ACS disappears 2-3 d transient 0.4 A/M</td>
<td>no ACS</td>
<td>Tongue like villi</td>
</tr>
<tr>
<td>Human</td>
<td>? ACS Crypts &amp; villi</td>
<td>~ ACS</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Rodent</td>
<td>ACS Only villi</td>
<td>ACS Crypts &amp; villi</td>
<td>No ACS</td>
<td>?</td>
</tr>
<tr>
<td>Dog</td>
<td>Crypts &amp; villi</td>
<td>ACS (disappear around 14-21 d)</td>
<td>No ACS (after 21 d)</td>
<td>Stable morphology/metry at 63 d pn</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Suckling</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Increase final stage of gestation (lactase &gt; &gt;&gt; sucrose)</td>
<td>Lactase 1st week pp xx Gradual increase of others</td>
<td>Lactase maintenance level</td>
<td>Transient decline of others</td>
</tr>
<tr>
<td>Human</td>
<td>Lactase: Increase 3rd trimester: Suggest 1/2 of gestation (lactase ~&gt; sucrose)</td>
<td>Lactase xx Gradual increase of others</td>
<td>Lactase reduced to maintenance level (5 years)</td>
<td></td>
</tr>
<tr>
<td>Rodent</td>
<td>No sucrose activity</td>
<td>Lactase xx Gradual increase of others</td>
<td>Lactase maintenance level (reached at 4 wks)</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>?</td>
<td>Lactase xx (gradual decrease)</td>
<td>Gradual increase of others</td>
<td>At 21 d pn no lactase activity left Sucrose only from 63 d pn</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Preterm</th>
<th>Suckling</th>
<th>Weaning</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Increase final stage of gestation</td>
<td>Gradual decrease</td>
<td>Transient decrease to maintenance level</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>2nd trimester of gestation present, End of gestation total activity = adult</td>
<td>No changes</td>
<td>No changes</td>
<td></td>
</tr>
<tr>
<td>Rodent</td>
<td>Gradual increase</td>
<td>Steady-state</td>
<td>Decrease to maintenance level (1 month of age)</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Very low</td>
<td>Gradual increase</td>
<td>No changes</td>
<td></td>
</tr>
</tbody>
</table>

References

The 2012 meeting of the Minipig Research Forum

The annual meeting of the Minipig Research Forum will take place on 26-27 November at the Jumeirah Hotel in Frankfurt.

At this year’s MRF meeting more than 15 people will present their work and share their minipig experiences. The programme includes presentations about device testing, telemetry and syndromes etc. Two presentations will focus on the implementation of the minipig as a new species for research.

For the first time, workshops are included in this year’s programme and this will be another unique opportunity for the attendees to share experiences and discuss relevant topics.

For further information please visit the website: www.minipigresearchforum.org

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### Meeting calendar

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETS - European Teratology Society</td>
<td>2-5 September</td>
<td>Linz, Austria</td>
</tr>
<tr>
<td>BTS Autumn Meeting</td>
<td>10-11 September</td>
<td>Keele, UK</td>
</tr>
<tr>
<td>ESTP - European Congress of Toxicologic Pathology</td>
<td>11-14 September</td>
<td>Stresa, Italy</td>
</tr>
<tr>
<td>GV-SOLAS/IGTp</td>
<td>12-14 September</td>
<td>Aachen, Germany</td>
</tr>
<tr>
<td>Mip-Tec</td>
<td>25-27 September</td>
<td>Basel, Switzerland</td>
</tr>
<tr>
<td>SPS – Safety Pharmacology Society</td>
<td>1-4 October</td>
<td>Arizona, Phoenix, US</td>
</tr>
<tr>
<td>ACT – American College of Toxicology</td>
<td>4-7 November</td>
<td>Orlando, Florida, US</td>
</tr>
<tr>
<td>BSTP – British Society of Toxicologic Pathology</td>
<td>15-16 November</td>
<td>Macclesfield, UK</td>
</tr>
<tr>
<td>Minipig Research Forum Meeting</td>
<td>26-27 November</td>
<td>Frankfurt, Germany</td>
</tr>
<tr>
<td>LASA Winter Meeting</td>
<td>28-30 November</td>
<td>UK</td>
</tr>
<tr>
<td>BPS Winter Meeting</td>
<td>18-20 December</td>
<td>London, UK</td>
</tr>
</tbody>
</table>