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Göttingen Minipigs
MAGAZINE

ELLEGAARD
GÖTTINGEN MINIPIGS

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Dear reader

For the first time, the Minipig Research Forum 2022 was held at our breeding and research site in Dalmose, Denmark, with more than 100 participants from around the world.

It was a true pleasure for us to be the host and learn from all the interesting presentations given over the course of the three days.

Since January, we have seen a significant increase in the demand for Göttingen Minipigs and we are doing our utmost to meet market demand.

Although production has been scaled up, we still encourage our clients to place their orders well in advance.

We are humble that so many of you have chosen Göttingen Minipigs and also very pleased that you come to our premises in Dalmose to perform your scientific research. Recently, we had the pleasure of welcoming University of Antwerp, who came to Dalmose to do their research in juvenile studies.

In this edition of Göttingen Minipigs magazine, you can among others read about the expansion of our office facilities and the renewal of our outdoor surroundings at the head office.

This benefits both employees, visitors, and not least the biodiversity.

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Expansion at the Head Office

By Ellegaard Göttingen Minipigs A/S

In 2021, we doubled our office facilities in Dalmose, Denmark. Today, we have several meeting rooms and a huge conference room, which can serve both the employees and the many visitors who every year find their way to the breeding and research site in Dalmose, Denmark.

When you arrive in the morning at Ellegaard Göttingen Minipigs' head office in Dalmose, Denmark, two things are noteworthy; the silence and the beautiful surroundings.

Göttingen Minipigs are being bred in the 3 barriers, B2, B3 and B5, and with more than 50 employees and several visitors every year, the premises were suddenly too small. A couple of years ago, it was decided to expand the office facilities, and now the offices include a large conference room, meeting rooms, and several offices in a modern style.

Many opportunities at our facilities

A smell of straw being burnt means that autumn is getting closer and that the big straw-fired boiler is warming up all 3 barriers and the offices. The high-ceilinged conference room have windows to all sides for the employees to enjoy their lunch and the nature outside at the same time.

The conference room is not only suitable for the employees but also for hosting conferences and workshops. A maximum of 100 persons can be seated in the conference room and in May we introduced the conference room for a large number of participants during the MRF 2022 with about 100 seated participants.

Expansion is not new to us, as we in 2016 launched our research barrier that allows access to a state-of-the-art Göttingen Minipigs housing facility including a surgical suite. Our research barrier provides the optimal facilities for education and training sessions and offers advanced services to facilitate your research.

At Ellegaard Göttingen Minipigs, we welcome you in both our research area or for a participation in a workshop or conference surrounded by the pastoral idyll.

Image 1 + 2: Offices and conference room

Biodiversity

Not only the office facilities have undergone a transformation. The outdoor area has been renewed and benefits biodiversity. In 2022, we have planted more than 700 plants. Of these, about 25 are different species.

In particular, we are proud of our atrium. In the middle of it, a steel bed filled with water has been installed. This benefits the birds and insects during summer and when the wheather is good the employees can enjoy their lunch in the atrium.

UN Global Goals

The outdoor plan for biodiversity was finalized at internal UN Global Goals meetings and resulted in a multi-annual plan for the outdoor environment in cooperation with a landscape architect.

For the foreseeable future, we will expand our surroundings even further and consider both the biodiversity and experience value for the employees and visitors.

This makes sense to us and to future generations.

MORE INFORMATION

Would you like to know more about our facilities?
Please contact: Peter Vestbjerg, Head of International Sales, pve@minipigs.dk, T +45 5818 5818

Image 3 + 4: Biodiversity in our atrium

Emesis in minipigs and relevance in nonclinical safety testing

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Emesis (the act of vomiting) and nausea (the sensation of an urge to vomit) are amongst the most common side effects of marketed drugs for human use (Percie du Sert 2012).

Introduction

Emesis (the act of vomiting) and nausea (the sensation of an urge to vomit) are amongst the most common side effects of marketed drugs for human use (Percie du Sert 2012).

Emesis is the forceful oral expulsion of solid and/or liquid gastric contents and, is associated with contraction of the abdominal and chest wall musculature. This is an important physiological defense mechanism against intoxication. Numerous natural and synthetic chemicals can induce emesis and are thus exploited therapeutically as emetics in cases of acute poisoning in animals and humans. On the other side, anti-emetics are used to prevent or reduce drug-induced emesis such as from the widely applied chemotherapy agent cisplatin (Horn 2008; Aung and Soo 2016).

There are important species differences regarding emesis. The ferret is the most recognised model for assessing emesis (Holmes et al 2009). Several commonly used laboratory animal species such as rat, mouse, and rabbit lack a vomiting response (Horn 2008, 2013), where structural differences in the esophagus and diaphragm are considered responsible. Nonrodent species commonly used in nonclinical toxicology studies such as the dog, minipig, and nonhuman primates, i.e. marmoset and macaque monkey can vomit, but these species significantly differ to each other in their sensitivity to respond to an emetic stimulus. In addition, emesis is modifiable and can be conditioned in a number of species, including human. Such differences in emesis sensitivity can be important aspects for nonclinical safety testing. For example, dose-limiting emesis of a drug candidate may prevent the meaningful use of the dog in toxicology testing. After oral dosing, emesis can be a main contributor to highly variable plasma exposure, which may result in challenges to correlate effects to dose and respective exposure levels. Emesis can also lead to secondary clinical symptoms (e.g., abnormal body position and decreased activity), body weight loss, and anatomic side effects such as histopathological changes in the upper gastrointestinal tract, limiting the potential of a particular species to predict human relevant toxicities.

The Beagle dog has been used over decades as the primary nonrodent species in safety testing. However, Beagles are particularly sensitive for the induction of emesis. Even under therapeutic use of the sedative $\alpha 2$-agonist xylazine vomiting can occur in 10%-20% of treated dogs (Lumb and Jones 2007). The minipig, another nonrodent species increasingly used in toxicology, is largely resistant to emesis, although the perception that minipigs do not vomit is mistaken. Nevertheless, the fact that the minipig is significantly less prone to emesis adds to the many other physiological and biological advantages in regulatory safety testing compared to the dog. For example, minipigs tolerate NSAIDs much better and show less / less severe gastrointestinal lesions than dogs; minipigs largely tolerate vitamin D-analogues whilst the dog is hypersensitive; minipigs show less arteriopathy and other cardiovascular lesions, when dogs are often overly sensitive (McAnulty et al. 2012, Schmitt et al. 2015).

There are excellent reviews on the absence of emesis in rodents (Horn et al. 2013) and on emesis in dogs (Elwood et al, 2010). This mini review highlights emesis in minipigs and its relevance for the use of the minipig as a nonrodent species in nonclinical safety testing, mainly as opposed to the dog.

Common causes for minipigs to vomit

There are multiple causes leading to emesis in many species including the pig/minipig. For a high-level overview on emesis in minipigs see for example: https://thepigsite.com/articles/whats-wrong-with-my-pigs-vomiting. Interestingly, pregnancy-induced nausea and vomiting seen in humans during the first trimester (also called morning sickness) is not known for any other species. This unique feature is considered to be related to a particularly broad diet in humans compared to other mammals, and a developmental mechanism that helps the mother avoid substances which could be dangerous to the developing embryo (Flaxman and Sherman 2008).

It is important to reflect and triage the various possible causes for emesis in the minipig to enable conclusions to be drawn on potential test article-induced emesis in nonclinical safety studies. In the following, the main causes for emesis in the minipig/pig are discussed.

Pathogens, diseases, and physiologic conditions

Emesis is a prominent clinical sign of several infections and parasites in pigs/minipigs, including hemagglutinating encephalomyelitis, porcine epidemic diarrhea, colibacillosis (E. coli diarrhea), transmissible gastroenteritis, and swine fever (https://vetmed.iastate.edu/vdpam/FSVD/swine/index-diseases). In contrast, experimental animals such as minipigs are generally purpose-bred, grown and kept under stringent and well-controlled housing conditions. Colonies are regularly health monitored and tested for the absence of a wide range of pathogens according to FELASA guidelines (Berset Convenor et al. 2020).

Organ based diseases such as liver or kidney disease, tumors in the gastrointestinal tract, liver or pancreas, hypoglycaemic shock, constipation and intestinal obstruction as well as some central nervous system or psychiatric conditions such as post-operative pain are also known causes of vomiting in the minipig. Consequently, although rather a rare event, minipigs must be deprived of food for up to 12 hours before anesthesia to prevent vomiting (Alstrup 2000).

Motion-induced emesis has an early evolutionary origin and is present in many animal species including the minipig. Therefore, pigs/minipigs are generally not fed before and during transportation (Bradshaw et al. 1996, Randall and Bradshaw 1998).

Toxicity

Various drugs, in addition to intentionally emetic-inducing drugs (e.g. metoclopramide), can lead to emesis in the minipig. Examples include sedatives and analgesics (e.g., propofol), CNS active drugs (e.g., the dopamine-receptor agonist pramipexole), NSAIDs (e.g., meloxicam), opioids (e.g., apomorphine), and chemotherapeutic medications (e.g. cisplatin). Also nutritional components (e.g., deficiency or excess of vitamin D) or contaminations (e.g. the mycotoxin deoxynivalenol) can lead to emesis in the pig/minipig.

Irradiation

Like in many other species, including the human, whole and partial body irradiation was shown to induce emesis also in the minipig (Kaur et al 2018).

Emesis animal models in Research and Development

Anti-emetic drugs such as NK1 receptor antagonists, which block differing types of emesis (e.g., induced by drugs, motion or vagal stimulation) strongly indicate the presence of a common pathway for emesis (Andrews and Rudd 2004).

Animal emesis models exist in a number of species including ferret, cat, dog, and pig/minipig (Florczyk et al 1982; Andrews and Rudd 2004; Holmes et al 2009; Szelenyi et al 1994; Shim et al. 2014, Kaur et al. 2008). Ferret and dog were historically considered as the 'gold standard', likely due to the ease to induce emesis in these species, with a particular sensitivity to conditioning in dogs (Holmes et al 2009). Species differences in the emetic sensitivity to the dopamine receptor agonist apomorphine highlight the high predictive value of both dog and pig for humans (Holmes et al 2009).

Szelenyi et al. (1994) report that the sensitivity to emetogenic anticancer drugs and their susceptibility to currently known antiemetic drugs are comparable in the domestic pig and cancer patients. Retrospective data analysis of anti-cancer drugs revealed that the dog is better than the monkey in predicting vomiting in humans (Schein et al., 1970). Emesis is also used as a key indicator in a neonatal swine model for peanut allergy (Helm et al., 2002). Overall, no animal species is a universal predictor of emetic liability in human and the choice of species should be an informed decision based on the type of compound investigated and the type of emetic mechanism activated (Percie du Sert et al. 2012).

While these animal emesis models have shown utility, the value and use of regulatory safety testing in general to predict the human susceptibility to emesis can be questioned given the high percentage of drugs with emesis as side effect, sometimes limiting their therapeutic value. Dependent on the incidence and severity in toxicity studies emesis can sometimes be considered an adverse effect or indicating target organ toxicity in the CNS and/or the gastrointestinal tract. Overall, there is a good correlation for the occurrence of vomiting in preclinical studies and gastrointestinal adverse drug reactions in clinical studies (Tamaki et al 2013). The minipig in particular may provide relevant results for radiation-induced GI injury due to similar physiological parameters as humans, including transit time and pH, as well as being less prone to emesis than canines (Singh et al. 2016).

The biological basis of species differences for emesis

The exact biological reason why rodents fail to produce either retching or vomiting is unclear. While there are certain anatomical constraints (e.g., reduced muscularity of the diaphragm and long esophagus) the absent brainstem neurological component (i.e., lack of key neural circuits) has been suggested as the most likely reason (Horn et al. 2013).

From an evolutionary viewpoint, dogs are predators with a natural tendency to vomit while pigs are prey animals. Thus, the high sensitivity of dogs to emesis is related to their feeding behavior as carnivores. The preference of some dog breeds, including the Beagle, to consume large meals very rapidly reflects the competitive feeding behavior of its ancestor, the wolf. Also, dogs prefer a diet rich in fat in the attempt to maximize caloric intake whenever food is available, although large amounts of fat-rich diet can lead to digestive problems. As a result, dogs often overeat when offered excess diet and vomit in particular too much fat-rich food consumed too fast (Bradshaw 2006, WALTHAM Centre for Pet Nutrition 2012). Similarly, oral administration of large amounts or volumes of almost any test article can cause a dog to vomit (Gad 2017). A review on emesis in dogs is provided in Elwood et al. 2010. In contrast, the omnivore pig will eat almost anything while foraging for food. Therefore, diet used for minipigs generally has a high fibre content and is specifically designed to ensure that restricted feeding provides the animals with sufficient nutrients (Ellegaard et al. 2010). Overall, its biology and natural behavior makes the minipig much less prone to emesis than the dog. The minipig is consequently a preferred nonrodent species for nonclinical safety testing whenever large amounts of test item

formulation need to be dosed, particularly by the oral route.

Nausea is a distinct unpleasant human sensation and thus it has been argued that animal species may not display a similar subjective experience. Yet, other gastrointestinal effects observed in animals such as hypersalivation, loss of appetite, diarrhea and vomiting can be used as indication, preferably together, to predict nausea (Elwood et al. 2010, Parker and Limebeer 2006; Parkinson 2012).

Examples comparing drug-induced emesis in regulatory nonrodent species

Minipigs and cynomolgus monkeys showed comparable emesis induction after oral gavage of a positive allosteric modulator of the metabotropic glutamate receptor 5 (mGlu5), RG7342. In a subsequent human clinical trial, tolerability of RG7342 was indeed shown to be limited by adverse events including nausea and vomiting (Sturm et al. 2018). These results demonstrate the clinical relevance of emesis findings in both minipig and monkey to predict nausea and vomiting in humans. Toxicity studies in the dog were not performed for this project.

A negative allosteric modulator of mGlu5, basimglurant, showed emesis in Beagle dogs and cynomolgus monkeys (internal data). Nausea and vomiting was amongst the observed adverse effects in humans (Quiroz et al. 2016). Toxicity studies in the minipig were not performed for this project.

Rupniak et al. (2019) reported that the Neurokinin-2 receptor (NK2R) agonist LMN-NKA caused less emesis in minipigs than in dogs after intravenous or subcutaneous administration, concluding the minipig may be a better species for nonclinical safety testing.

Emesis seen after oral administration can be followed up and thus confirmed with intravenous dosing to reach high peak levels often associated with CNS mediated emesis.

Selection of the nonrodent species for compounds with emetogenic potential

To support the development of human pharmaceuticals, toxicity studies in a rodent and a nonrodent species are generally required. The selection of an appropriate species for nonclinical safety testing should primarily consider scientific, practical and ethical factors (Prior et al. 2020). However, comparative pharmacokinetic studies in several nonrodent species are not necessarily performed to assist in the selection of the best suitable species. This is also a reason why the dog is still the routine species in many projects. Nonhuman primates such as the cynomolgus monkey should only be used if unequivocally necessary and once all other possible avenues of potential alternatives have been explored (Schmitt et al. 2015). This species selection paradigm particularly applies to small molecule testing.

Minipigs are generally less prone to emesis than dogs, although both species tend to eat their vomit and, therefore, when not carefully and continuously monitored emesis can be overlooked. Yet, overall the minipig may often allow higher dose-levels to be tested in nonclinical safety studies, thus exploring the full range of toxicity of compounds with emetogenic potential. An additional advantage of such lower sensitivity for emesis is related to reduced issues with variable and/or undefined exposures associated with vomiting shortly after oral administration of a compound. Therefore, emesis can be a limiting factor in the selection of a nonrodent species in toxicology. Overall, the minipig offers several advantages over the dog in nonclinical safety testing, particularly with regards to the gastrointestinal system due to its greater anatomical similarity to humans and a lower sensitivity for emesis (Bode et al, 2010).

Conclusion

Similar to humans and many other species the minipig is able to vomit, yet much less sensitive than the dog. In general, minipigs can be considered human-relevant model for emesis. Based on the totality of factors to be considered for species selection, the minipig is a favorable nonrodent for the safety testing of small molecules with emetogenic potential.

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The effect of GMP housing and handling on the outcome of Peripheral Neuritis Trauma (PNT) model for neuropathic pain

A preliminary report of an ongoing study

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Abstract
The Peripheral Neuritis Trauma (PNT) model is a model for chronic neuropathic pain. Briefly, pain is induced by ligating the lateral part of the sciatic nerve with silk threads presoaked in CFA. Animal behavior is evaluated using several methodologies, including response to mechanical stimulation, behavior scoring of social behavior, and locomotor activity.

The model was running in two different vivariums: one is a standard swine facility for research, and the other is a new, state-of-the-art facility, specifically purpose-built for swine behavior studies.

The standard facility houses pigs in a corridor of rectangular pens on both sides, which are separated by opaque walls. Pigs are housed in groups of two or three, and the animals and pens are washed each morning with water. The animals are fed twice daily, and enrichments includes a choice of materials and balls. A well-trained caretaker enters the pens once daily for handling. While all tests are carried out in the pig´s home pen, locomotor activity is evaluated by gently walking the pigs to the open field arena, (sized 1.2mX2.4m). Supportive medications such as antibiotics are delivered by IM injections.

The new facility is built in a U-shape with square pens separated by bars allowing the pigs to interact with one another visually and vocally. The open side of the U-shape has a gate leading to a shared, middle space in which the pigs can enter and play. Pigs may interact freely with pigs from other pens. The pigs are held on sawdust that is replaced daily with no washing and are fed twice daily. Pens are opened twice daily for the animals to play with the caretaker (rope, balls, “feed-me”, and other tasks). All medication is administered orally after training.

Pigs are also taught to climb on to the balance weighing scale.
This paper reports the preliminary results of an on-going study that looks at the effect of the different housing and handling conditions on the outcome of a PNT model.

Introduction
Across species, it is an established fact that behavior assays depend on housing environmental conditions such as light/dark cycles, temperature and humidity, noise, bedding, enrichment, and handling. In farm pigs, poor housing in barren conditions can cause damaging behaviors such as tail and ear biting. These factors lead to chronic stress and alteration of the pig’s immunity, and can affect pain behavior significantly.

Pain by definition, is a subjective experience. This makes pain evaluation in humans challenging and almost impossible to do by another person. In humans, the assessment of pain mostly relies on verbal reporting, while in animals, the assessment of pain relies on animal withdrawal from a stimulus and/or behavioral scoring. Both approaches are highly dependent, among other things, on the animal’s stressful or stress-free environment.

In this report, we examine the effect of Standard Housing Conditions (SHC) and Specifically Designed Housing Conditions (SDHC) focused on behavioral assessment on the pig outcome following PNT induction of chronic pain.

Parametes for comparison	Standard housing condition (SHC)	Specifically Designed Housing Conditions (SDHC)
Facility characterization		
Pen size (holds 2-3 animals)	1X2.4m	1.8X1.8m
Type of walls	Smooth without a connection between pens	Bars- allowing connection between pens
Pens organization in the housing room	Corridor with pens at 2 sides	U share with a shared area 
Air condition and humidity	Controlled temperature, 15 air replacement per hour, humidity control	Controlled temperature, 15 air replacement per hour, humidity control
Light/dark cycle	12 hours/12 hours	12 hours/12 hours
Open field arena	Rectangle 2.4X4.7 meters (combination of pens together) Within a regular corridor	Square 4X4 meters Separate specifically built.
Floor cover	No cover	Sawdust cover
Water	ad libitum	ad libitum
Feeding	Twice daily	Twice daily
Handling protocol		
Number of people handling 60 pigs	5-6	2-3
Washing procedure	The animals and the pens are washed daily	The sawdust is replaced daily, with no washing at all during the study
Presence of caretaker	Twice daily	Twice daily
Enrichment program	Toys within each pen. Normally, each pen hold one type of toy	Twice daily the pens are open and the animals are free to walk into the shared space. The shared space included different toys such as “Feed me”, balls, ropes. During that time a caretaker is initiating different tasks for the pigs.
Supporting Medication (pain killers and antibiotics)	Mostly by injections	Mostly oral
Anesthesia procedure using isoflurane mask	A trained caretaker is lifting the pigs and placing the mask on their face	The pigs are trained for accepting the mask without lifting them.

Above, Methods; Animals and Housing. Female Göttingen Minipigs at age of 4 months were used in this study. The animals were housed and handled in 2 facilities: a SHC Facility for research in pigs and a new SDHC facility, specifically designed for evaluating animal behavior. The table above shows the characterization of both facilities.

Methods

Anaesthesia and PNT surgery

An anaesthetic facemask (Stephan Akzent Color, Germany) was placed on the pig's mouth and nose, as described previously. Each animal was anaesthetised with a 3% isoflurane/100% oxygen mixture. While connected to the anaesthesia, the pig was shaved and swabbed with 70% ethanol, and then carried to the operation room. The pig was placed in the sternal position on the operating table. The area of the incision was swabbed with antiseptic liquid polidine solution (Polysept solution, Rekah Pharmaceutical Industry Ltd., Israel) and the non-operated areas were covered with sterile sheets. During anaesthesia, blood oxygen saturation was monitored (Spacelab Medical, USA) .

An incision of 8-10 cm was made through the skin and fascia on the left side of the lower back, towards the caudal end, and approximately 1.5 cm lateral and parallel to the spine line of the pig. The muscles were then retracted and the entire sciatic nerve exposed. Peripheral Neuritis Trauma (PNT) was induced by three 3-0 silk threads (Assut-UK), each 3 cm in length, which were immersed in complete Freund's adjuvant (CFA) (1 mg/ml) overnight. Following sciatic nerve exposure, the pre-soaked threads were used to create 3 loose ligations (1-2 mm apart) surrounding the lateral half of the sciatic nerve bundle.

Behaviour Tests

Approaching test to assess acclimation

The normal behavior of the pigs upon the entry of the researcher entering their housing pen is to initially move away from the intruder and then approach them. The more familiar the pigs are with the person and the more comfortable they feel, the less time it takes them to approach. The latency to approach the researcher is recorded in seconds. In this test the researcher does not approach the animals, but simply enters their cages. The approach time test can serve as a tool to assess acclimation.

von Frey testing

Mechanical sensitivity was assessed using von Frey (vF) filaments (Touch Test (Von Frey) Sensory Evaluator Kit, model 58011, Stoelting Co., Wood Dale, IL, USA). The tests were performed in the pigs' home pen. Filaments ranging from a minimum of 0.07 g to a maximum of 15 g were used. The filaments were applied for a period of few seconds on the dorsal area of the animals' foot. Each filament was applied three times with a 5-10 second interval between applications using the up-down method[iii], If withdrawal was not achieved, a thicker filament was applied. If withdrawal was achieved, a thinner filament was applied. By alternating the filaments, the force required to achieve a withdrawal reaction was determined. This procedure was carried out at the time points of 1 day pre-surgery (baseline), and at days 14 and 21 post-surgery.

Open field test

The open field apparatus size was either 2.5m wide by 4.8 meters long (smaller open field), or 4 meters wide by 4 meters long (new open field). The walls of the small open-field were smooth and 1.6m high. The new open field was a separate room. The animals were introduced to the open field for 5 minutes. The walking pattern of the animals was recorded using a CCTV camera connected to the AnyMaze data acquisition software. After each open field session, the total walking distance was presented, as well as the walking pattern.

Results

Approach time as assessment of acclimation

The first reaction of the animal when entering their pen is to move away from the intruder followed by slowly approaching the intruder. The time for the animals to approach the intruder was measured in the two facilities during the acclimation period. The data collected in both facilities show that the time for the animals to habituate to the researcher's entry was shorter in the SDF than in the SHF. It shows that the approaching time 4 days post animals' arrival to the SHF was reduced by nearly 60% vs. the first day of their housing. In comparison, 4 days of housing in the new SDF facility resulted in faster acclimation as expressed by the reduction in nearly 100% in the approaching time (p<0.001 vs. traditional facility).

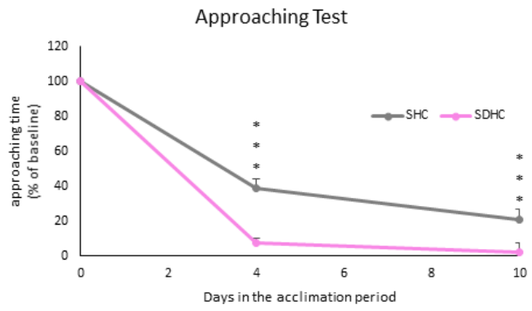


Figure 1:
The time in seconds that it took for the animals to approach the researcher entering their pen. Animals housed in the SDHC facility acclimated faster as was expressed by the faster reduction in the approaching time on study day 4 and 10.
***p<0.001 vs. SDHC

Withdrawal force

The withdrawal response to mechanical stimulation was assessed using the von Frey methodology. The withdrawal force at baseline was not different between animals housed in the SHC faclity and those housed using the behavior-specific design SDHC housing approach. In both cases, the highest withdrawal force ranged between 10g to 15g.

Fourteen days following PNT operation, the withdrawal force recorded was reduced significantly in both groups. A lower mean group withdrawal force was expressed in the animals housed in the SHC facility (2.09 ±0.29 vs. 0.84±0.15g p<0.01). Twenty-one days post PNT, the withdrawal force of the animals housed in the SHC facility was increased (1.92±0.39g; p<0.05 vs the withdrawal force measured on day 14.). No change was observed in the withdrawal force of the animals housed in the SDHC facility. Two weeks later, on study day 35, the withdrawal force of the animals housed in the SDHC facility was unchanged.

The withdrawal force of the animals housed in the SDC facility was significantly lower (Figure 2).

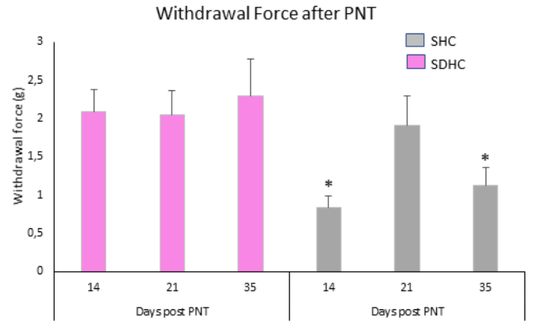


Figure 2:
Whithdrawl force (g) post PNT. The animals housed in the SDHC facility showed more stable results and also expressed higher withdrawal force vs. the animals housed in the SHC facility.
*p<0.05 vs. SDHC

Open field assay

Previously, we have shown that following PNT operation, the walking pattern detected in the open field arena was altered in castrated young male domestic pigs[v].

In this study, we are comparing the effect of the open field size and the housing environment on the walking pattern of adult female GMP. The data show that at baseline- before PNT operation- animals introduced to the small open field walked less than the animals introduced to the large open field.

Walking pattern

Fourteen days post PNT operation, the animals that were introduced to the old open field tended not to enter the central zone and preferred to walk next to the open field arena walls. This phenomenon was observed also 21 days post PNT but to a lesser extent.

The animals that were introduced to the large open field arena, showed similar behavior at baseline, meaning the animals covered the entire area without a specific preference. Fourteen days post PNT the animals tended to stay closer to the entry door. Three weeks post PNT, this tendency strengthened and some of the animals developed what appears to be a repetitive behavior, meaning they went back and forth to the entrance door. This phenomenon was not observed in the small open field arena (figure 3 page 14).

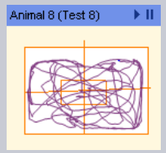
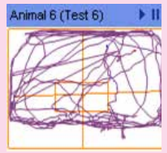
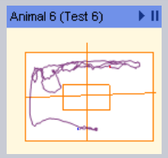
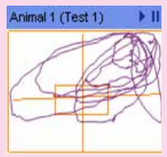
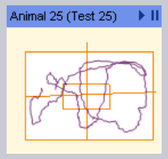
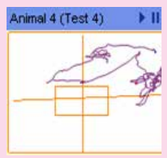
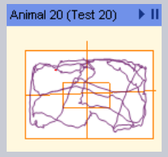
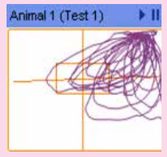
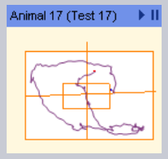
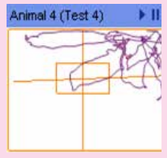
Open field walking pattern in the SHC facility	Open field walking pattern in the SDHC facility	Observations and explanations
Baseline		
A: Animal 8 	B: Animal 6 	The animals walked freely and covered the entire open field arena without specific location preference regardless of the size of the open-field and housing facility.
D14 post PNT		
C: Animal 6 	D: Animal 1 	The walking pattern in the SHC facility open field (C and E): the animals walked less and avoided the central zone. The walking pattern in the SDHC facility open field (D and F): the animals walked the same distance with a clear preference for the entrance door area.
E: Animal 25 	F: Animal 4 	
D21 post PNT		
G: Animal 20 	H: Animal 1 	The walking pattern in the SHC facility open field (G and I): the animals walked the same distance as they did at baseline without a specific preference. The walking pattern in the SDHC facility open field (H and J): the animals walked the same distance as they did at baseline, but almost exclusively focused on the door area. The walking pattern of animal #1 (H) may suggest a repetitive behavior.
I: Animal 17 	J: Animal 4 	

Figure 3: Walking pattern in the open field. A-B baseline pattern recording. C-F walking pattern 14 days post PNT. G-J walking pattern 21 days post PNT.

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Walking distance

At baseline, before the injury, the animals that were exposed to the larger open-field arena in the SDHC facility walked significantly more than the animals exposed to the smaller open-field arena in the SHC facility (figure 4) (56.33±21.13 meters vs. 73.57±35.72).

Following PNT the animals exposed to the smaller open field arena, at the SHC facility, showed a transient decrease in locomotor activity. Animals exposed to the large open field arena showed no change in the locomotor activity and no reduction in walking distance.

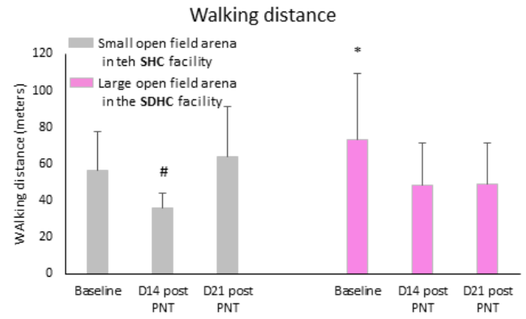

Figure 4: Walking distance during 5 minutes of exposure to the open field. In gray- small open field in the SHC facility; in pink- large open field in the SDHC facility.
*p<0.05 vs. small open field arena in the SHC facility
#p<0.05 vs. baseline at the small open field.

Discussions

In the last six months, we have been operating from a new animal facility that was specifically designed for behavior assessment of large swine studies (60 animals at all times). The move created an opportunity to re-validate specific pain models and investigate an improved facility design. In this study, we report on an ongoing re-validation of the chronic pain model in GMP. Although this is an interim report, some differences have already been observed and can be discussed.

Acclimation

The acclimation period is very critical in behavior studies, if it is carried out mainly at the animal's housing space involving the presence of investigators. The normal behavior of pigs is to move away from a person entering their pen, followed by their approach to the investigator. The latency time for the pigs to approach the intruder reflects their calm or distressed behavior. Under distressed conditions, pigs do not tend to approach the intruder. In this study, we show that the habituation of the pigs to a repeat intruder in their home pen was more rapid in the SDHC facility than in the SHC facility. This was shown by the relatively shorter approach time measured in the SDHC facility compared to that of the SHC facility. This also suggests that the pigs were more calmer and far less stressed, which shows that the SDHC facility potentially offers a better solution for behaviour assays in pigs.

Mechanical Allodynia testing

This study shows that following PNT operation all animals developed a robust and significant decrease in the withdrawal force regardless of the housing environment. However, 14 and 35 days following PNT operation, a clear difference was found between the withdrawal force of the animals housed in the SDHC facility and the animals housed in the SHC facility. Animals in the SDHC facility had higher sensitivity compared to the animals housed in the SHC facility. This difference was not noticed at 21 days post PNT operation as the mean group withdrawal force on the animals housed in the SCH facility was slightly increased. The relatively higher withdrawal force obtained in animals housed in the SDHC facility is possibly due to increased animal mobility fact that the animals were able to move more. In humans, it is shown that moderate regular exercise decreases excitability and improves inhibition in both the central nervous system (brainstem inhibitory/facilitatory sites) and the immune system. In rodents, it was shown that moderate daily exercise results in a slight increase in withdrawal threshold 3 weeks after spared nerve injury model . The SDHC facility provides pigs more opportunities for physical activity during their housing period. They walk more, can freely access the shared space, and are intrigued by different tasks.

Another explanation for the slight increase in the withdrawal force 3 and 5 weeks after PNT is that the animals were calmer. It is possible that the animals housed in the SHC facility were more sensitive to mechanical stimulation post PNT due to exposure to daily persisting stress, which resulted from daily washing. Persistent stress from daily washing can increase sensitivity to pain and pain behavior. The results obtained from the SDHC facility may have resulted from the stress-free conditions experienced by the animals. Further observations and studies are being carried out to better understand this point.

Locomotor activity

The animals were introduced to a small open field (SHC) or a large open field (SDHC). The animals that were introduced to the large open field (SDHC facility) walked more before the PNT operation. In both cases, the animals covered the entire open field arena without a specific location preference or a specific pattern. This is in line with other studies run on domestic pigs, suggesting that the behavior of the pigs is to cover the entire space when first introduced to the arena. The longer walking distances of animals housed in the SDCH open field facility may be ascribed to more opportunities for activity at their home-pen; these animals are able to be more active. Post PNT, the animals housed in the SHC facility had a transient decrease in walking distance while no change was observed in animals housed in the SDHC open-field facility.

An interesting difference was found in the walking pattern after PNT operation between the animals housed in the SHC facility and those housed in SDHC facility. The animals housed in the SHC facility tended to walk in circles, avoiding the central zone. This behavior suggests distress, and is in line with what we have previously reported in young castrated domestic pigs. This might further support the cross-talk between light chronic stress and chronic pain that is expressed in the animals housed

in the SHC facility. Animals housed in the SDHC facility showed no change in the distance but a dramatic change in the walking pattern. They focused almost exclusively near the entrance door, suggesting a very fast learning behavior that was not affected by the painful condition. After one time, the animals learned that the open door allows them to freely walk back to their home pen, a safe and comfort zone. Stress has a complex effect on learning and memory, some papers suggest that acute stress can enhance learning and memory while others show that it interfers with learning and memory. However, most researchers believe that chronic stress can impair the learning and memory process.

Economic aspects

The housing of 14-21 pigs in one room with 2-3 animals per pen require 15 minutes handling per day, at least twice daily. This means that it can take 3.5 hours per day (15 minutes per pen, 2 animals twice daily) for 14 animals. Handling the pigs using the shared space approach requires 0.5 hour to 1 hour depending on the program of handling a reduction of up to 70% in handling time. Changing the sawdust while the pigs are playing in the shared space takes about 0.5 hours for 2 people per room. The savings in the water cost and waste per day is about 80% since there is no daily pen washing.

The new SDHC facility allows housing, handling, and medicating of 50-60 pigs at any given time, which can be achieved with a staff of only 2 Full Time Employee (FTE) positions. Similar studies that run in an SHC facility typically require 5- 6 FTE to fulfill the same mission.

The time saving for a full-screen veterinarian check-up

Traditional housing requires the veterinarian to stand in front of or inside of each pen every morning to check the animals, observe whether animals are standing or walking, and look for abnormalities. This requires about 10-30 minutes per pen. In a room with 7 pens, the morning checkup can take 1-3 hours. The new pig housing approach allows the veterinarian to enter the shared space. All the animals will approach the pen's gate upon the veterinarian's entry. This allows an immediate screen of all the animals as well as an immediate observation of any abnormalities. This approach allows the veterinarian to screen the pigs and focus on the abnormalities within minutes, which is a more efficient use of time.

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Training minipigs to participate in research procedures

By Stine Drent Larsen and Cathrine Juel Bundgaard, Animal Caretakers at the Animal Unit, Novo Nordisk A/S, Denmark

Training minipigs to participate in research procedures is both more safe, effective, and gentle for the minipig and for the people working with them. It enhances the minipig welfare because the minipigs are being stimulated.

Laboratory animals are subjected to different husbandry and/or research procedures. This could be small and easy procedures, like moving the animal from one place to another or more complex procedures that are not possible to do without either forcing the animal using fixation or anaesthesia or by training the animals to freely participate in the procedure.

The daily husbandry procedures should be recognized as an important part of the animals' lives which means that it is as important to refine these procedures as the research procedures. Forcing animals against their will and without them knowing what is expected of them will often lead to confrontations between handler and animal and stress for both parties.

Clicker training

At Novo Nordisk we are working on letting the minipigs be able to predict and control its environment. This is done with a combination of habituation, socialization and actively training the minipigs. We train the minipigs using a method based on positive reinforcement training also called clicker training. Clicker training is a positive method of minipig training, where the trainer reinforces (rewards) a correct behaviour and ignores unwanted behaviour.

Clicker training is a very simple setup that can be used for all animal species. Most often the animals are trained with food and treats that they really like and therefore are willing to work to obtain. When using clicker training, the animals will initially learn to associate the click-sound with a reinforcer (bridging) and once this connection is established in the animal, the click-sound is used as a positive reinforcer to precisely signal to the animal when it is performing the desired behaviour and get the desired reinforcer.

A minipig that is clicker trained will participate in husbandry as well as research procedures because it knows that it will get a reinforcer afterwards. The minipig participates because it chooses to. It can also choose not to participate and success is therefore depended on a cooperation between minipig and human and that requires trust and connection. This makes it a very powerful training tool.

Training minipigs to participate in research procedures is both more safe, effective and gentle for the minipig and for the people working with them. It enhances the minipig welfare because the minipigs are being stimulated. Most minipigs find it fun to train. Negative stress is reduced, which is a refinement for the minipig and it is also beneficial for the scientific results since stress hormones can be a scientific confounder. Reduced stress levels and calmer minipigs in the experimental situation is also desirable to avoid mistakes, have more precise measurements (e.g. precise dosing), and to decrease the variability between each experiment. The time used on the experimental procedures is reduced and we can do experiments that we would otherwise not have been able to do, as the animal and the animal caretaker cooperate on the task. To train the minipigs and to handle well-trained minipigs add to job satisfaction for the animal caretakers and others working with minipigs. A positive relation – or bond – is built between the trainer and the minipigs.

New ways of training research animals

For many years, the animal caretakers at Novo Nordisk have been training dogs used in research. In 2017, an animal caretaker finished a certified dog trainer education. He was then appointed responsible training coordinator and put his experience and knowledge in system, which was then passed on to colleagues. At this time, we started experimenting with the use of clicker training for minipigs and the results were surprisingly good. In 2017, it was official decided from management that all research animals at Novo Nordisk should be trained for husbandry and research procedures as far as possible.

Several animal caretakers were sent on courses in positive reinforcement training of animals, which had a specific focus on research animals. It was new procedures for everyone and a lot was learned while doing it. We developed new ways of training the animals and we developed a system to register training performance. In the beginning, not everybody was equally excited about this new way of working with the animals, but slowly as the trainer's knowledge and experience grew and the results started to speak for themselves, people were convinced that it is the right way to work with animals.

Reproduceable training system

When training research animals, we face challenges that are different from training companion or zoo animals. The animals have a limited time to learn the behavior since they are to participate in procedures shortly after arrival in the facility. Another challenge is that the animals will be trained by different trainers on different days. This caused us to develop a reproduceable training system and a reporting system that is easy to use and understand so that colleagues relatively easy can collaborate on the training of a group of animals. When working with minipigs, we are also favored by the fact that minipigs are very willing to train and they are curious and preceptive.

Today, most animal technicians at Novo Nordisk are responsible for training specific animals, from arrival to the end of the study. This means that they together with the scientist plan what should be trained, how and when, and they secure that colleagues can take over when they cannot train themselves. We also have a person who is functions as a training coordinator and is the overall responsible for training the animals.

No need for anaesthesia

Training minipigs have given us further advantages. We have been able to do some very complicated studies that would otherwise have been difficult or impossible to perform. An example of this is a study we are doing where a device given orally can be followed as it passes from the ventricle to the lower intestines without anaesthetizing the animals. The minipig is trained to walk up on a platform, where it will stand completely still (freeze) while an x-ray image is taken. The minipig leaves the platform and the next minipig walks up to have its x-ray taken. The minipig will then enter the platform again after five minutes. In this way, we can follow the device as it moves through the minipig, while the minipig is cooperating and having fun.

If the minipigs were not trained for the procedure, they would have had to be anaesthetized for half an hour during several days, which would have affected the research results to an extent that it could not have been done. It would also have been stressful for the minipigs and very time and manpower consuming. It takes us approximately 8 hours distributed over 3-4 weeks to train 8 minipigs for this procedure.

Images: Show the minipig following a long stick onto a raised platform. Once it enters the platform, it freezes, the x-ray is taken and the minipig gets a reinforcer and walks down on the other side of the platform and the next minipig can enter the platform.








Images above: A minipig is being trained to follow a target stick onto a scale (a wooden stick with some green vetflex in the end). It is rewarded with limonade in a bottle. It takes about 5 minutes a day for 5 days for a new minipig to learn to follow a target stick. Step 1: The minipig learns that a click sound means that it is doing something right (it gets a reward). Step 2: The minipig learns to touch the target stick and gets a reward. Minipigs are natually curious and when it sees the stick, it will explore by touching it. Step 3: the target stick is moved and the minipig must follow it and then get the reward.

Repeated behaviour

Another example of a marked refinement in a study setup was a setup where we had to do measurements from the expiration air from the minipig. The normal way of taking these samples is to fixate the minipig and force a mask with an expiration bag over mouth and nose. The minipig is not easily fixated and will make a lot of noise scream during the procedure. Both minipigs and personnel are stressed and drained from energy afterwards.

We therefore decided to train the minipigs to voluntarily put their mouths and snout into the mask and then blow into the expiration bag. When we started training the minipigs, we only trained them to put their mouth and snout into the bag, hoping that we would be able to capture enough expiration air. We did not know that we would be able to train the minipigs to make a blow into the bag. But they quickly offered that behavior by themselves and all we had to do was to reinforce the behavior and then they repeated it. On study day, everything was calm in the stable while the samples were collected and the researcher got all their results with success.

Training minipigs to participate in husbandry and research procedures is rewarding both for the minipigs and animal technicians. It is a major refinement because it almost eliminates fixation and negative stress in the daily routines and during procedures.

It is our experience that it does not take long to train simple procedures and, for minipigs and dogs at least, it does not have to be the same person training the animal if there is a clear plan for the training and progress and shortcomings are communicated between the people training the minipigs. If the minipigs are in short term studies (few weeks), they will not be trained as much as minipigs that are here for a longer period, but we only use target training, luring and treats to move the minipig around and onto scales and strive to have as force free an environment as possible.

Luckily, to help us on our journey, our vendors of both farm pigs and Göttingen Minipigs are now starting the habituation and socialization from when the pigs are born.

This is a substantial contribution to our training and the pigs we receive are more curious and less anxious when we get them, and training can therefore begin 1-2 days after arrival.

We hope to be able to inspire others to train their research animals and are always open for questions and advice.

Images: A minipig is being trained to put mouth and snout into the mask and give it a blow.

It is rewarded with A38 mixed with lemonade light in a bottle.

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Study Insight

Development of a minipig model for excessive arterial bleeding as tool for Proof of Concept in TL-101

ABOUT STUDY INSIGHTS: Göttingen Minipigs are increasingly selected for all aspects of pharmaceutical research and are fully recognized as a reliable and established animal model by all regulatory authorities worldwide.

Insight provided by:
Charlotte Videbæk, CMO & Co-Founder of Tissue-Link ApS, Denmark

Background
Ellegaard Göttingen Minipigs was chosen as key partner for model development by Tissue-Link for establishment of a pre-clinical pig model in excessive bleeding to support the development of our innovative bio surgical devices.

This section aims at providing an insight into the wide use of Göttingen Minipigs within biological research. If you know of an interesting study, you are welcome to reach out.

Unmet medical need
Excessive hemorrhage is the leading cause of preventable civilian- and combat related deaths. In addition, excessive hemorrhage increases mortality, extend intensive care unit treatments and increases societal costs for patients surviving severe bleeding. Early control is the most effective strategy for treating hemorrhage and therefore a key target for new development of a safe and effective therapy.

Solution
Tissue-Link is developing hemostatic products encompassing a construct of the human protein, tissue factor, as first-in-class. Tissue factor initiates and amplifies clot development whereby bleeding is stopped effectively including bleeding from large arteries which is high pressure bleedings. TL-101 is our first non-resorbable dressing, which is able to control excessive life-threatening bleedings. Tissue-Link's methodology circumvents the risk previously known by use of tissue factor as a treatment option through an innovative linking of tissue-factor to a dressing.

Research challenge
To validate the hemostatic efficacy of TL-101 Tissue-Link, a robust and relevant bleeding model in a relevant species is needed. The coagulation among species is different and compared to humans most animals are hypercoagulable and therefore models, in smaller animals such as rats, can be difficult to evaluate both in relation to safety as well as efficacy.

A femoral artery lesion model in regular swine has been used by e.g. the US army for efficacy evaluation (Kheirabadi et al. 2011). In this model the femoral artery is damaged by a 6 mm arteriotomy followed by free bleeding 30 seconds, followed by typical 3 minutes compression with the examined device. With the most used hemostat in the US army QuikClot (not indicated for arterial bleedings), the survival rate and hemostatic success is around 30% in this model.

We wanted to develop a model with smaller animals keeping the significance of species selection intact and therefore initiated a collaboration with Ellegaard Göttingen Minipigs by use of Göttingen Minipigs. Due to anatomic differences, the use of the femoral artery was difficult in minipigs as it is located rather deep in the femoral region and would require complicated surgery. Therefore, Kirsten Rosemary Jacobsen and a team from Ellegaard Göttingen Minipigs suggested to explore the use of the carotid artery instead as the bleeding site as an alternative.

Method
The experiment was approved by the Danish Animal Experiments Inspectorate (license number: 2020-15-0201-00569) and all animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals in an AAALAC international approved barrier facility. A study outline as seen in image 2 was developed and the first study took place in 2020.

Coagulation status of the animals were evaluated by thromboelastographic parameters (TEG). The minipigs received a minute dose of 25iu/kg heparin to humanize their coagulation status. Anesthesia was induced by Zoletil 50 (tiletamin 125mg, zolazepam 125mg) + 6.25 ml xylazine (20 mg/ml) + 1.25 ml Ketamine (100 mg/ml) + 2.5 ml butorphanol (10 mg/m), dose 1 ml/10 kg IM. Once anesthetized, animals were intubated and prepared for surgery lege artis. Anesthesia was maintained by isoflurane inhalation, 0-2%, to effect and the following parameters are evaluated and maintained within normal range: FiO2, MV, RR, EtCO2, HAR, SpO2, IBP and body temperature. IV access was obtained by a midline catheter either in the ear vein or v. femoralis. A pressure catheter was placed in the femoral artery, and ringers Acetate was provided at various rates to maintain a mean arterial pressure (MAP) above 65mmHg.

Image 1:
Photo from actual study

For the carotid artery lesion skin, subcutaneous tissue were dissected and the left carotid artery was identified. The vessel was cleaned from connecting tissue and non-invasive holding sutures were placed underneath the artery to allow to be lifted more dorsal for ease of precise puncture. A few drops of lidocaine (20mg/ml lidocainhydrochlorid monohydrat) were added a few minutes prior to puncture to allow for maximum vasodilation. A vascular puncture was made with a 14G syringe in a 45-degree angle and twisted with a visual significant lesion as a result. After the lesion was confirmed visually, the artery was lowered to its normal position and upon releasing the artery 30 sec of free bleeding.

Thereafter, the test device was compressed at the lesion for 5 minutes. After compression the device was removed and the cavity checked for hemostasis. We studied TL-101, placebo, and the active comparator QuikClot. Placebo and TL-101 were blinded but QuikClot was visually and material wise different from the other devices.

Experience with model development and value for Tissue-Link
Our experience with the model development together with Ellegaard Göttingen Minipigs has been very positive. We have achieved convincing preclinical results in a new model, a relevant species and an arterial bleeding model. We can now build our further device development on this convincing model. Ellegaard Göttingen Minipigs has been very supportive, knowledgeable, and flexible in their support. We have also enjoyed the opportunity for a true collaboration, where we were allowed to be participants – It has truly been teamwork.

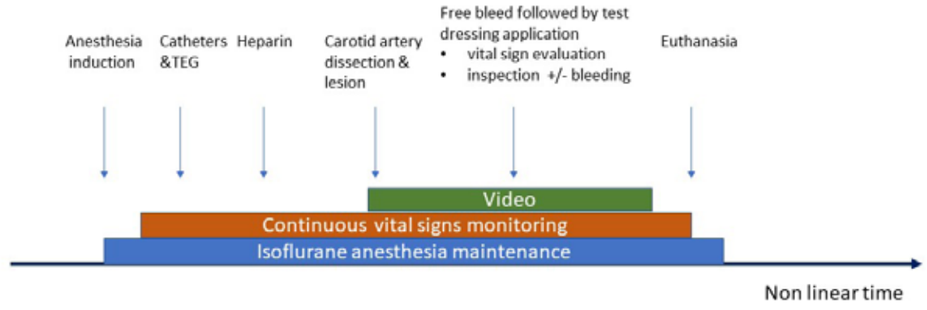

Image 2:
Study outline for carotid artery bleeding model

The 14th Minipig Research Forum took place in Dalmose, Denmark

MRF 2022 offers minipig users from all over the world the unique opportunity to meet, discuss and share knowledge and experiences on minipig care, handling and use within various areas of biomedical research. The 3-day scientific conference is packed with scientific lectures, breakout sessions and not least great networking with minipig users from all over the world!

The MRF 2022 was facilitated by and hosted primarily at the breeding and research site of Ellegaard Göttingen Minipigs located approx. 1½ hours outside the city of Copenhagen in the beautiful Danish countryside.

Twentythree knowledgeable speakers addressed their specific areas of interest and shared recent scientific results.

The scientific program of MRF 2022 was centered around five topics:

- **NUTRITION AND METABOLISM**
- **JUVENILE AND REPRODUCTIVE TOXICOLOGY**
- **PHARMACOLOGICAL MODELS**
- **IMMUNE SYSTEM**
- **PAIN - MODELS AND MANAGEMENT**

The scientific program further included two breakout sessions:

- **DOSING AND SAMPLING**
- **TRAINING AND MONITORING WELFARE**

The conference was kicked off by a welcome lecture entitled 'Developing clinically relevant models of self-poisoning in Göttingen Minipigs at the Edinburgh Large Animal Facility' presented by Eddie Clutton and Michael Eddleston, University of Edinburgh, Scotland.

According to a MRF tradition, everyone was invited to a get-together evening with food and drinks on the first evening of the MRF. This year it was held at the Vilcon Conference & Hotel.

In the afternoon of the second day, after having enjoyed two scientific sessions and a guided tour around the site of Ellegaard Göttingen Minipigs, the MRF 2022 continued at the historical Borreby Castle, situated only 15 minutes from Dalmose. The attendees had signed up to join one of the two breakout sessions which took place in the quite astonishing settings and with lively debating and sharing of knowledge among the participants and speakers. Before dinner which included Danish seasonal food, a keynote talk 'Exploring the safety of a novel anti-SARS-CoV-2/antiviral inhalation treatment in Göttingen Minipigs' was presented by Elin Lisby Kastbjerg Jørgensen, University of Copenhagen, Denmark.

Mark your calendar. The 15th Minipig Research Forum will take place from 10 to 12 May 2023. Location will be announced soon.

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Scientific Program at the MRF 2022

mrf research forum

SESSION 1: NUTRITION AND METABOLISM

Feeding of the trial animal – bear in mind possible effects of the ration	Ludwig Maximilian University of Munich, Germany
Metabolomics-based phenotyping on minipigs ranging from healthy to morbid obesity	INRAE-Université Clermont-Auvergne , France
Göttingen Minipigs as a model for NASH	Novo Nordisk, Denmark
Microbiota in metabolic Göttingen Minipigs	Novo Nordisk, Denmark

SESSION 2: JUVENILE AND REPRODUCTIVE TOXICOLOGY

Regulatory toxicity studies in juvenile animals – an industry perspective with a focus on minipigs	F. Hofmann-La Roche, Switzerland
What's good to know when considering the minipig for juvenile toxicology studies	Charles River Lyon, France
Embryo-fetal development studies in Göttingen Minipigs - technical feasibility and special considerations	Scantox, Denmark
The juvenile Göttingen Minipig: role of organ development in view of drug and food safety in neonates, infants and toddlers.	Universiteit Antwerpen, Belgium
Safety assessment of food additives in juvenile Göttingen Minipigs	Scantox, Denmark

SESSION 3: PHARMACOLOGICAL MODELS

Going beyond occlusion-reperfusion models for myocardial infarction in Göttingen Minipigs	KU Leuven, Belgium
Assessing cardiovascular control mechanisms in pharmacodynamic studies – species considerations and experiences using telemetry in Göttingen Minipigs	Bayer, Germany
Learnings on immunosuppression in Göttingen Minipigs	AstraZeneca, Sweden
Göttingen Minipigs - A new model for gut microbiome studies	University of Copenhagen, Denmark

SESSION 4: IMMUNE SYSTEM

Göttingen Minipigs - an experimental model for immune modulatory drug testing	University of Veterinary Medicine Vienna, Austria
Göttingen Minipigs as a model for skin immunology	Charles River Lyon, France

SESSION 5: PAIN - MODELS AND MANAGEMENT

Overview of anesthesia and analgesia in Göttingen Minipigs	University of Edinburgh, Scotland
Analgesia and antinociception: pros (and cons) in animal experimentation	Unibern, Switzerland
Machine vision for facial recognition in pigs: animal welfare applications	Scotland's Rural College, Scotland
The effect of GMP housing and handling on the outcome of PNT model for neuropathic pain	MD Biosciences, Israel

Follow MRF on LinkedIn

The Minipig Research Forum group on LinkedIn is an informative and useful platform where minipig users connect and interact, ask questions and share experiences. Apply for the MRF LinkedIn group membership at www.linkedin.com

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New publications on Göttingen Minipigs

Ellegaard Göttingen Minipigs gives high priority to collaborative projects that aim to better characterize and validate Göttingen Minipigs as a translational animal model and which facilitate and refine the use of Göttingen Minipigs in research projects and safety testing. Do you have an idea for such a collaborative project? Please contact ellegaard@minipigs.dk.

Stefan Eirefelt, Martin Stahlhut, Naila Svitacheva, et al.
Characterization of a novel non-steroidal glucocorticoid receptor agonist optimized for topical treatment
Scientific Reports, volume 12, Article number: 1501 (2022)
Doi: 10.1038/s41598-022-05471
www.nature.com/articles/s41598-022-05471-w

Noah Pearson, Gregory M Boiczyk, Vivek Bhaskar Kote, et al.
A Strain Rate-Dependent Constitutive Model for Göttingen Minipig Cerebral Arteries
J Biomech Eng. Aug 2022, 144(8): 081007
Doi: 10.1115/1.4053796
https://pubmed.ncbi.nlm.nih.gov/35147172/

Maria Meyhoff-Madsen, Esben Østrup, Merete Fredholm, and Susanna Cirera.
Investigating the effect of obesity on adipose-derived stem cells (ASCs) using Göttingen Minipigs
Department of Veterinary and Animal Sciences, Denmark,.
Feb 12 2022
Doi: 10.1101/2022.02.11.477943
www.biorxiv.org/content/10.1101/2022.02.11.477943v1

Kyung Oh Jung, Ashok Joseph Theruvath, Hossein Nejadnik, et al.
Mechanoporation enables rapid and efficient radiolabeling of stem cells for PET imaging
Scientific Reports - Article number: 2955 (2022)
Doi: 10.1038/s41598-022-06938-6
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Marta Liliana Musskopf, Amanda Finger Stadler, Ulf ME Wikesjö, et al.
The Minipig intraoral dental implant model: A systematic review and meta-analysis
Doi: 10.1371/ journal.pone.0264475
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Sabrina Halecker, Ludwig Krabben, Yannick Kristiansen, et al.
Rare isolation of human-tropic recombinant porcine endogenous retroviruses PERV-A/C from Göttingen Minipigs
Doi: 10.1186/s12985-022-01742-0
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Acta Cir. Bras. 37 (01) • 2022
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Michelle Fischer Carlsen, Berit Østergaard Christoffersen, Rikke Lindgaard, et al.
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Volume 115, May–June 2022, 107168
Doi: 10.1016/j.vascn.2022.107168
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Appl. Sci. 2022, 12(6), 3205
Doi: 10.3390/app12063205
https://www.mdpi.com/2076-3417/12/6/3205

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Vaginal Microbiota Diversity in Response to Lipopolysaccharide in Gilts Housed Under Three Housing Systems
Front. Genet., 08 April 2022
Doi: 10.3389/fgene.2022.836962
https://www.frontiersin.org/articles/10.3389/fgene.2022.836962/full

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Decellularized Organ-Derived Scaffold Is a Promising Carrier for Human Induced Pluripotent Stem Cells-Derived Hepatocytes
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Heart Failure With Preserved Ejection Fraction: Heterogeneous Syndrome, Diverse Preclinical Models
Circulation Research. 2022;130:1906–1925
Doi: 10.1161/CIRCRESAHA.122.320257
https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.122.320257

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No sex-related differences in infarct size, no-reflow and protection by ischaemic preconditioning in Göttingen Minipigs
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Whole blood gene expression within days after total-body irradiation predicts long term survival in Göttingen Minipigs
(2021) 11:15873
Doi: 10.1038/s41598-021-95120-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8342483/pdf/41598_2021_Article_95120.pdf

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Characterization and Pharmacological Validation of a Preclinical Model of NASH in Göttingen Minipigs
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Doi: 10.1016/j.jceh.2021.09.001
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J. Pers. Med. 2022, 12(6), 865
Doi: 10.3390/jpm12060865
https://www.mdpi.com/2075-4426/12/6/865/htm

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BMC Genomics 23, 564 (2022).
Doi: 10.1186/s12864-022-08801-4
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Epigenetic regulation of BAF60A determines efficiency of miniature swine iPSC generation
Sci Rep 12, 9039 (2022)
Doi: 10.1038/s41598-022-12919-6
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Weiterentwicklung der Leberzelltransplantation
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Doi: 10.17169/refubium-33532
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Vagni, P., Airaghi Leccardi, M.J.I., Vila, CH. et al.
POLYRETINA restores light responses in vivo in blind Göttingen minipigs
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Doi: 10.1080/1547691X.2022.2088904
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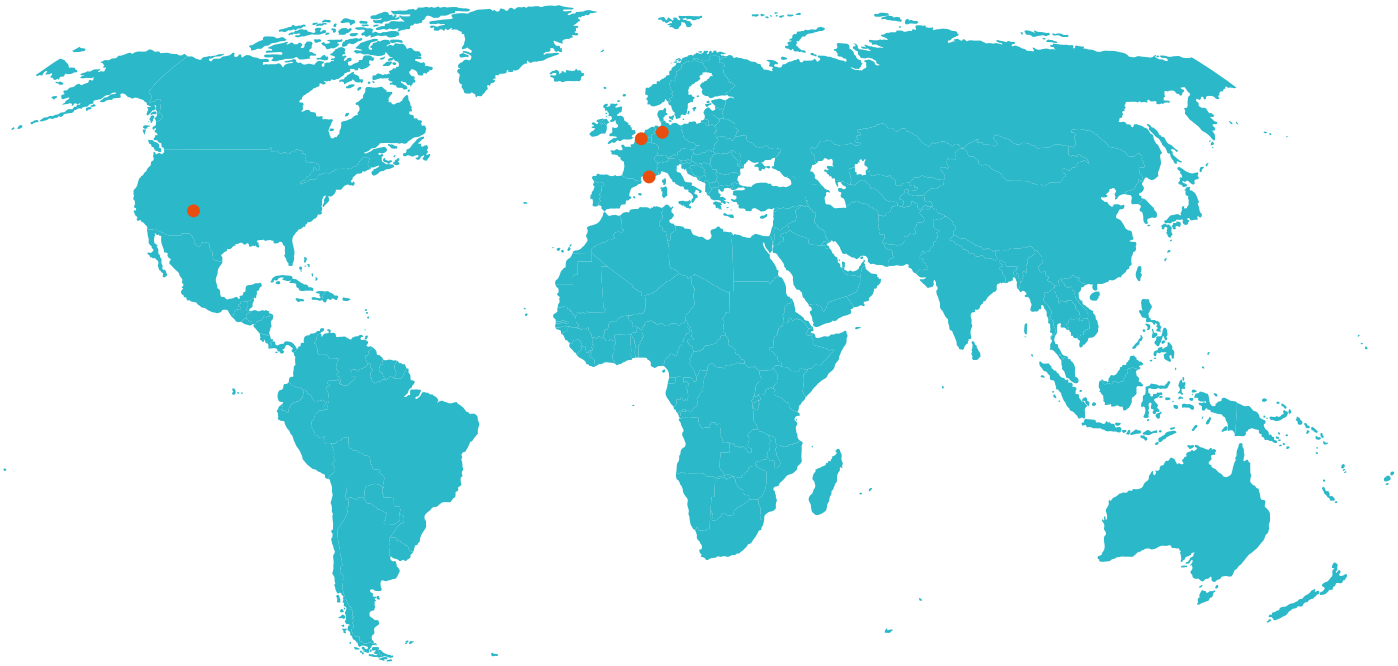
Where to meet us in 2022



CONGRESS / CONFERENCE	DATE	LOCATION
EUROTOX + ICT 2022	18-21 September	Maastricht, the Netherlands
3T Travemünde	20-21 September	Travemünde, Germany
ACT Annual Meeting	13-16 November	Denver, Colorado, United States

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