Ellegaard Göttingen Minipigs A/S 16 March 2021, webinar 10:00-10:40 AM





Imaging techniques for Göttingen Minipigs with PET, MRI and CT

Aage Kristian Olsen Alstrup DVM, PhD, Aarhus University Hospital, Denmark

PET scanning of pigs since 2002

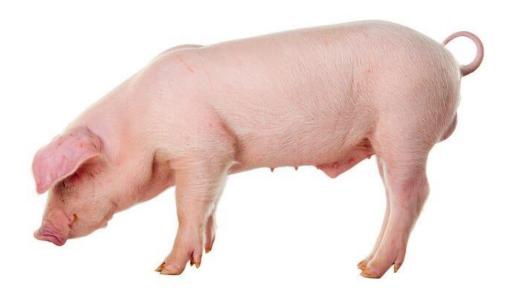
- brain
- liver
- bones
- kidney
- lungs
- heart







We are using both domestic and minipigs





Young domestic pigs Non-recovery studies

Adult Göttingen minipigs Recovery studies





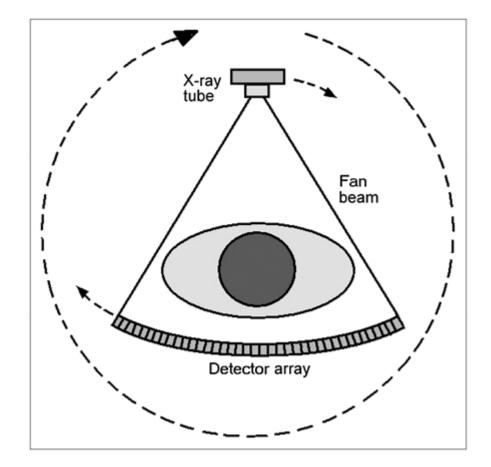
CT scanning

Rotating X-ray tube and detector

Takes few seconds

Dead or alive pigs

Anatomy (bones)







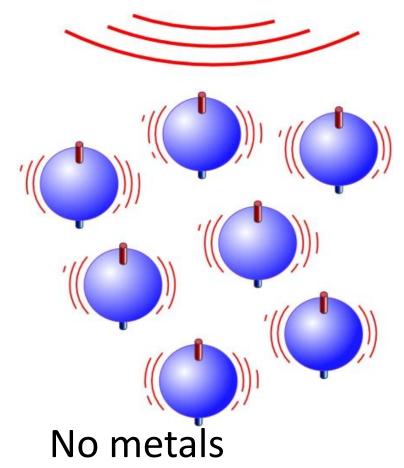
MRI scanning

Radiofrequency field

Takes some minutes

Dead or alive pigs

Anatomy (soft tissue)
Some functional



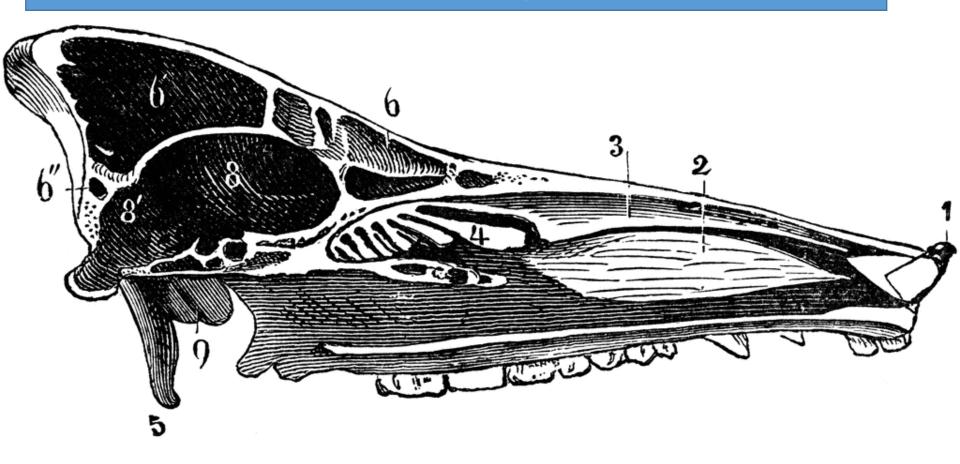








Reduced possibilities for functional MRI scans of the pig's brain







MRI with stereotactic frame used for brain surgery

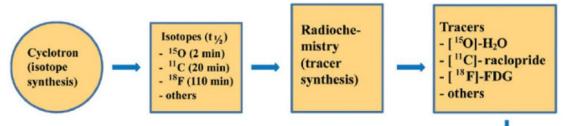






PET scanning

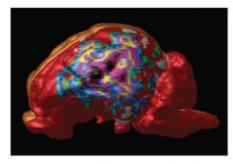
Radiotracers



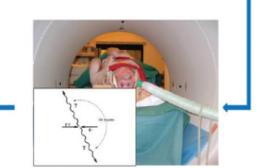
Takes minutes to hours

Only alive pigs

Functional imaging

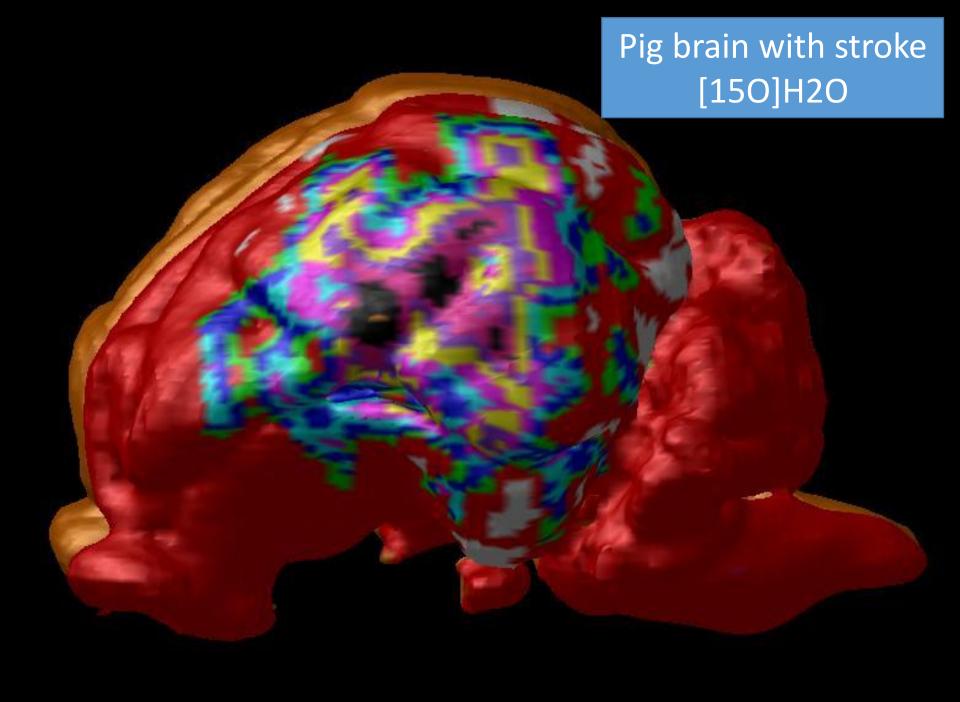


Reconstructed 3D map of Cerebral Blood Flow measured with [15O]H₂O PET



Tracer injection and PET/CT scanning of anaesthetized pig.
Tracer is detected by annihilation





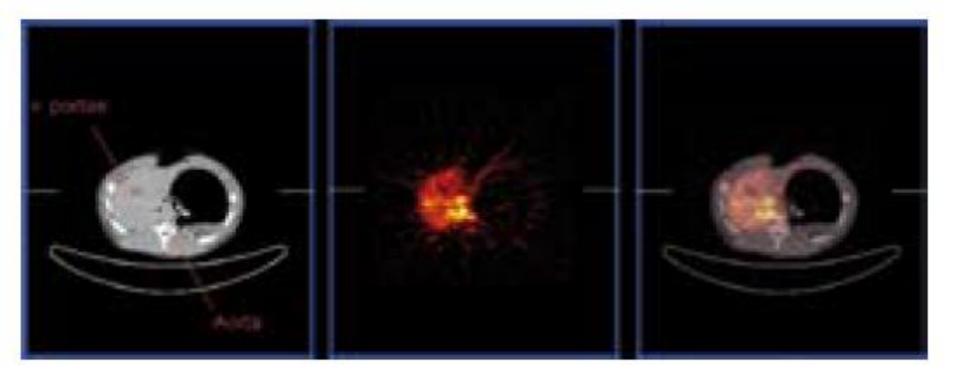


Figure 1. CT (left), C¹⁵O-PET (middle), and fused PET/CT (right) images of the porcine liver region. Without the CT image it is difficult to localize the anatomy of the liver and its supplying vessels.





Procedures for PET/CT scanning



Midazolam + ketamin IM Propofol IV (bolus) Propofol IV (infusion) og isoflurane

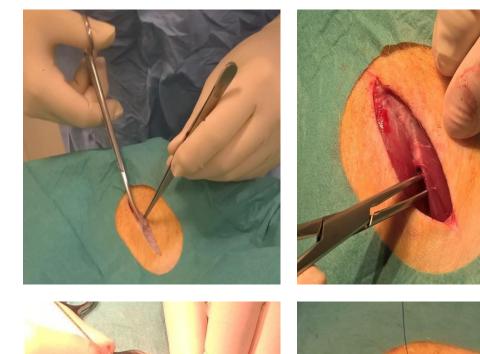
Temperature SatO2+pulse ETCO2

Procedures	Blood sampling						
		-	-	+			
Premedication, IM	Ketamine +	midazolam	Ketamine + midazolam				
IV access	Ear vein cat	heter	Ear vein catheter				
Induction, IV	Ketamine Propofol midazo- lam		Ketamine midazo- lam	Propofol			
Intubation*+ventilation	Yes		Yes				
Maintain anaesthesia	Isoflurane	Propofol	Isoflurane	Propofol			
Monitoring	Yes		Yes				
Capnography	Yes	Yes		Yes/no			
Blood storage and analysis	No		Yes				
Bladder catheters*	Mostly		Mostly				
Surgery, femoral A+V*	No		Yes				
Wrapping in plastic	Yes		Yes				
Placing in scanner	Yes (sternal recu	umbence)	Yes (dorsal recumbence)				
Tracer injection	Ear vein		Femoral vein				
Blood sampling	No		Yes, femoral artery				
Time of anaesthesia	1-6 hours (or more)		2-8 hours (or more)				
Transport between scanners	Yes No		Yes	No			
End of experiment	Mostly survi	ival	Mostly euthanasia				

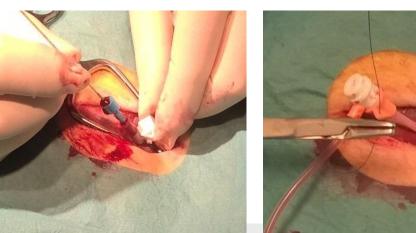


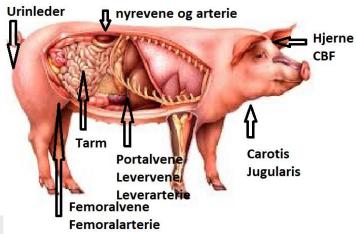


Placing of catheters in arteries and veins





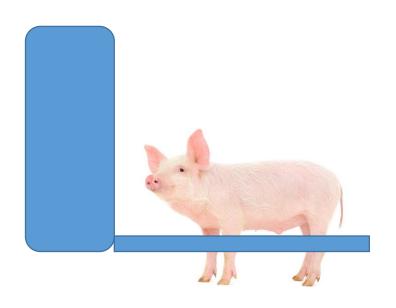


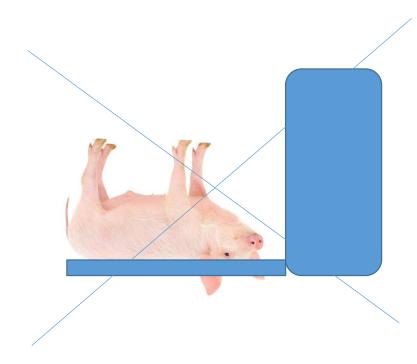








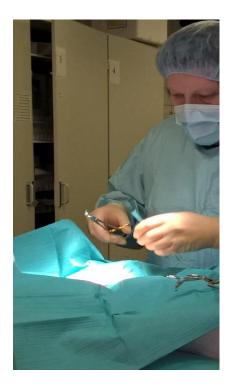








Pig model of osteomyelitis



Inokulation in femoral artery with Stafylococcus aureus



7 days Painkillers



CT, PET and SPECT Monitorering



Necropsy & grafting

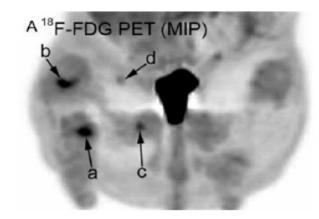


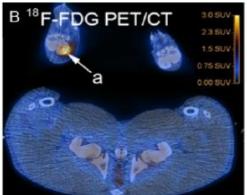


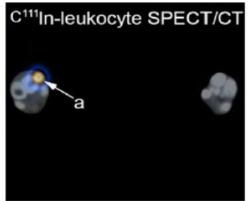
Table 3. Number of gross pathology and/or CT lesions identified by the individual tracers

Lesion	Total	Tracers						
	number	18F-FDG	⁶⁸ Ga-citrate	¹¹ C-methionine	¹¹ C-PK11195	111 In-leukocytes		
Osteomyelitis	5	4	1	2	0	4 ^A		
Soft tissue abscess	5	5	3	4	1	4		
Arthritis	3	0	0	0	0	0		
Enlarged lymph node	5	3	2	2	1	OB		

^AIn one of these four lesions, the patella, the ¹¹¹In-leukocytes had only accumulated in the cortical part of the bone. ⁸Only two lymph nodes were scanned.







Nielsen OL, P Afzelius, D Bender, HC Schønheyder, PS Leifsson, KM Nielsen, JO Larsen, SB Jensen & AK Alstrup: Comparison of autologous (111)In-leukocytes, (18)F-FDG, (11)C-methionine, (11)C-PK11195 and (68)Ga-citrate for diagnostic nuclear imaging in a juvenile porcine haematogenous staphylococcus aureus osteomyelitis model. J. Nucl. Med. Mol. Imaging. 2015, 5, 169-182.





	Tracers lesion	Total number	11C-methionine	11C-donepecil	99mTc-DPD	111 In-leukocytes	18F-FDG
Pig A	Osteomyelitis	3	2	1	0	1	3
	Contiguous periosseous abscess	0	-	640	-	· -	-
	Hematoma/Abscess at inoculation site	0	-	-	-	-	-
	Lymph node enlargement	0	-	-	-	-	-
Pig B	Osteomyelitis	5	5	5	0	5	5
	Contiguous periosseous abscess	0	-	-	-	-	-
	Hematoma/Abscess at inoculation site	1	1	1	0	(1)	(1)
	Lymph node enlargement	1	1	1	0	O	0
Pig C	Osteomyelitis	6	4	3	OA	4 ^A	NTs
	Contiguous periosseous abscess	1	(1)	1	O ^A	(1) ^A	NT®
	Hematoma/Abscess at inoculation site	O	-	-	_A	_A	NTs
	Lymph node enlargement	2	2	1	OA	(1) ^A	NTs
Pig D	Osteomyelitis	4	4	2	0	4	4
	Contiguous periosseous abscess	1	1	(1)	0	1	1
	Hematoma/Abscess at inoculation site	0	2	-	-	-	-
	Lymph node enlargement	3	3	2	0	(1)	1
Pig E	Osteomyelitis	6	4	3	0	5	6
	Contiguous periosseous abscess	2	2	2	0	2	2
	Hematoma/Abscess at inoculation site	1	0	0	0	0	0
	Lymph node enlargement	2	2	2	0	1	2
Total	Osteomyelitis	24	19/24	14/24	0/24	19/24	18/18
	Contiguous periosseous abscess	4	4/4	4/4	0/4	4/4	3/3
	Hematoma/Abscess at inoculation site	2	0	0	0/8	O	0
	Lymph node enlargement	8	8/8	6/8	0/8	3/8	3/6

A: Scans performed on dead pig. B: NT, not tested, as the pig had died.

Afzelius P, AKO Alstrup, HC Schønheyder, P Borghammer, SB Jensen, D Bender & OL Nielsen: Utility of 11C-methionine and 11C-donepezil for imaging of Staphylococcus aureus induced osteomyelitis in a juvenile porcine model: comparison to autologous 111In-labelled leukocytes, 99m Tc-DPD, and 18F-FDG.

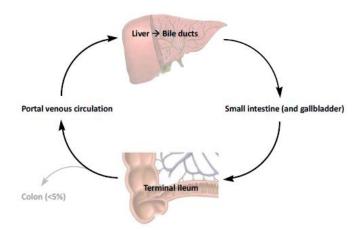
Am. J. Nucl. Med. Mol. Imaging 2016, 30, 6, 286-300.

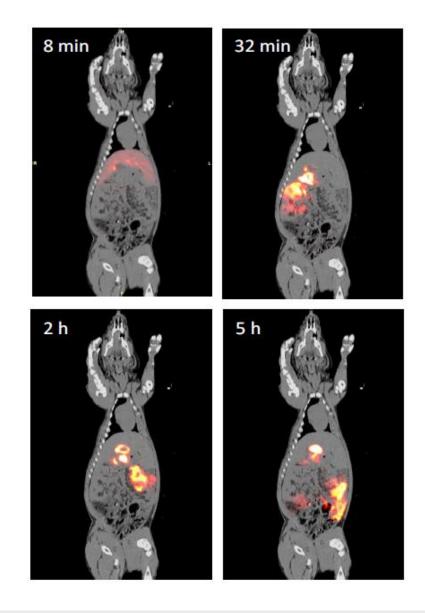




Bile tracers

$N-(4-[^{18}F]fluorobenzyl)-cholylglycine ([^{18}F]FBCGly)$

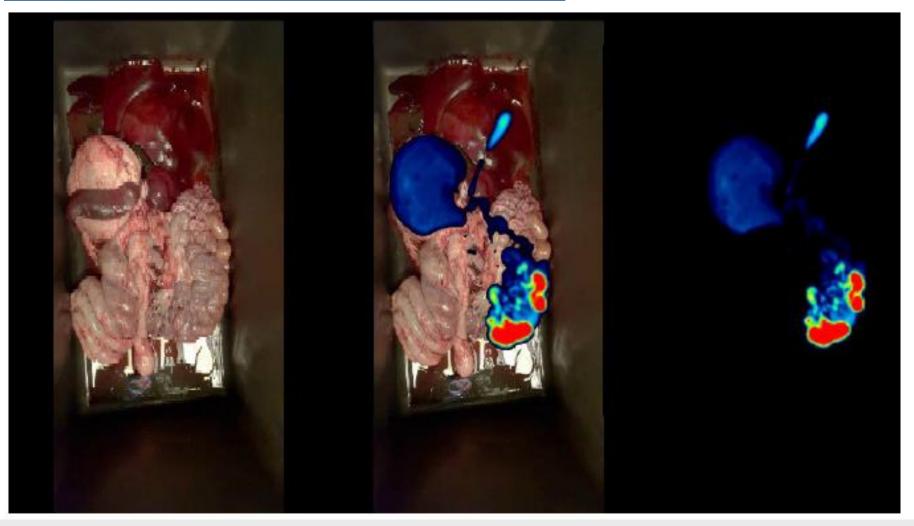








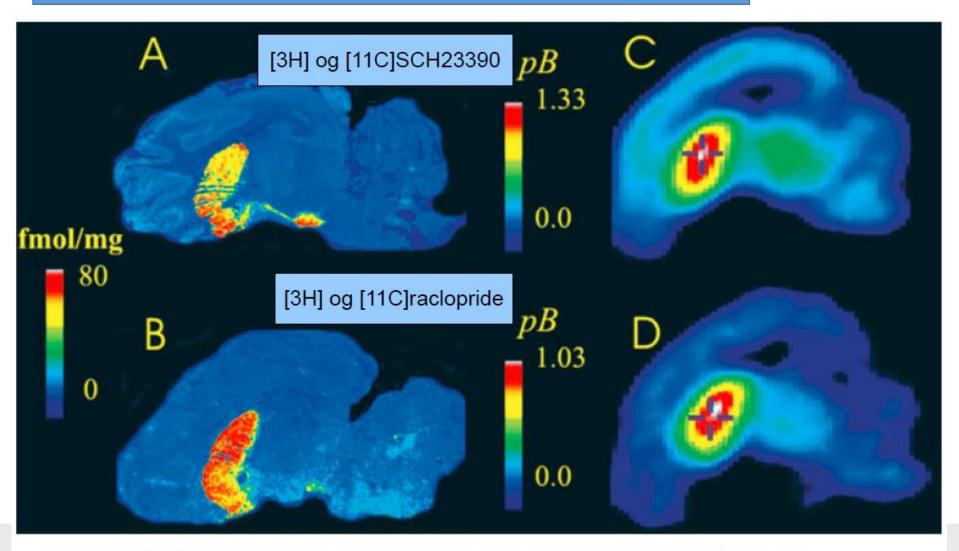
Post mortem: PET scans





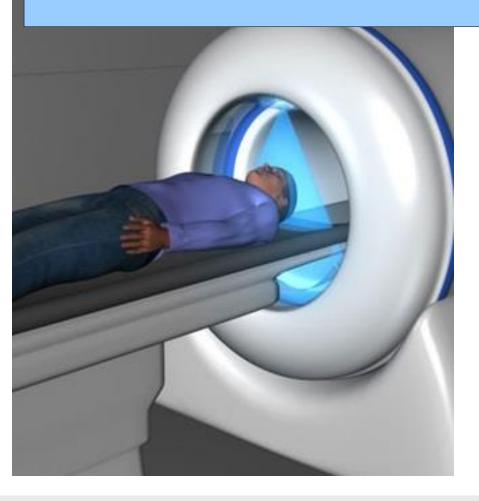


Post mortem: autoradiography



Minuzzi L, AKO Alstrup, D Bender, S Arnfred, R Grant, EH Danielsen & P Cumming: Quantitative autoradiography of ligands for dopamine receptors and transporters in brain of Göttingen minipigs: comparison with results in vivo. Synapse 2006, 59, 211-219.

Anaesthesia makes the difference



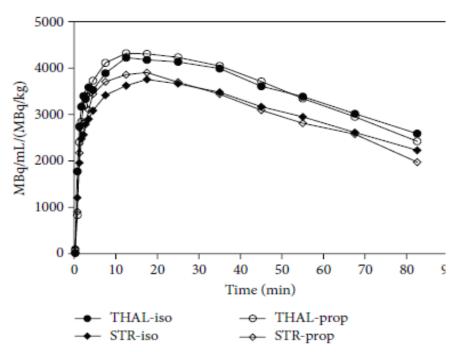




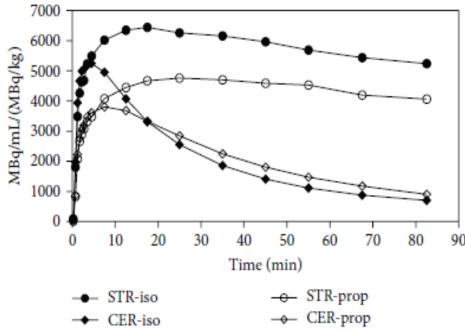


Isoflurane versus propofol i minipigs





[¹¹C]yohimbin (reversibel) Noradrenalin-receptor

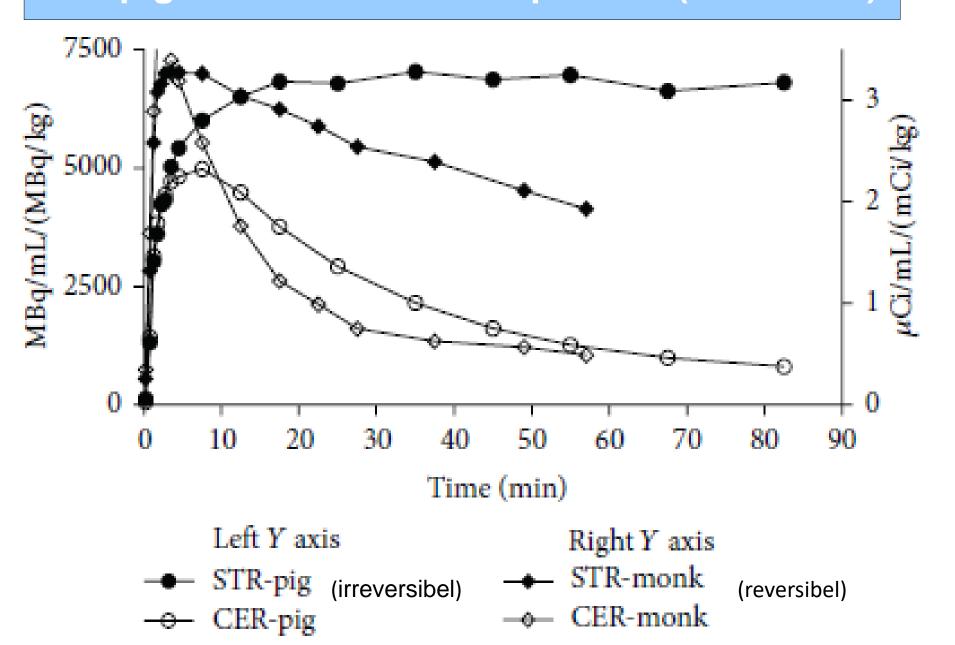


[11C]SCH23390 (irreversibel) Dopamin D₁-receptor



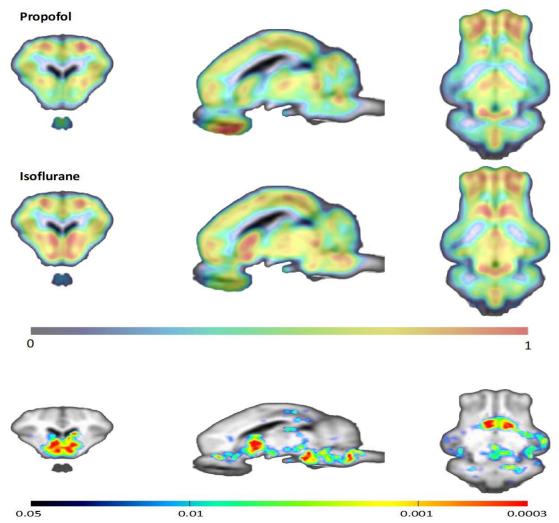


Minipig versus non-human primate (isoflurane)



[¹¹C]MDL100907 (5HT_{2A}-receptors)







[11C]MDL100907 (5HT_{2A}-receptors)

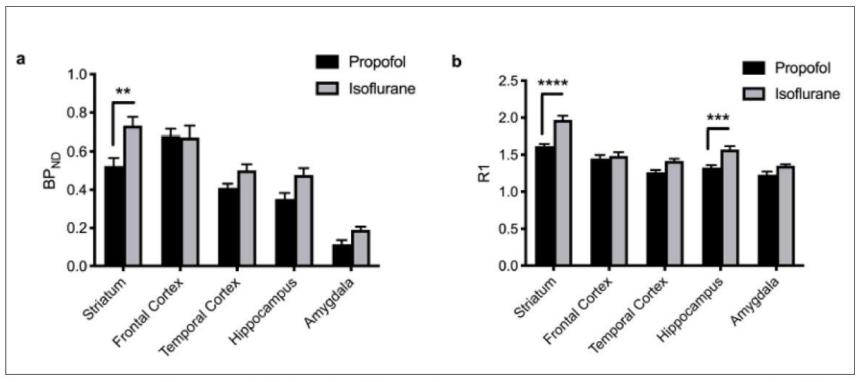


Figure 5: Binding potential (BP_{ND}) of [11 C]MDL100,907 (**A**) and R1 blood flow surrogate marker (**B**) in five brain regions during propofol and isoflurane anaesthesia in Göttingen minipigs Two-way ANOVA shows effects of both anaesthesia and region for (**A**) and (**B**). Bonferroni post-hoc testing of anaesthesia effects: **: P<0.01, ***: P<0.001 and ****: P<0.0001 (Figure **2** in paper **P-XI**).





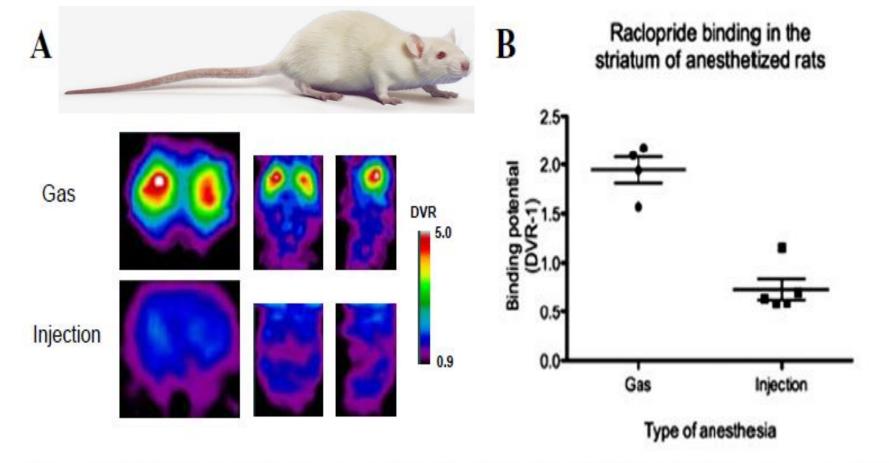


Figure 1. Isoflurane treated rats have significantly higher striatal [\(^{11}\)C]raclopride binding potential than fentanyl-fluanisone-midazolam treated rats. (A) PET images are shown for [\(^{11}\)C]raclopride binding in one representative rat in each group. (B) Binding potential values for [\(^{11}\)C]raclopride are shown for each rat in the experiment, determined by subtracting 1 from the distribution volume ratio (DVR). Circles represent rats treated with isoflurane (N=4) and squares represent rats treated with fentanyl-fluanisone-midazolam (N=5). Binding potential was significantly greater in rats treated with isoflurane (2-tailed student t-test *P<0.001).

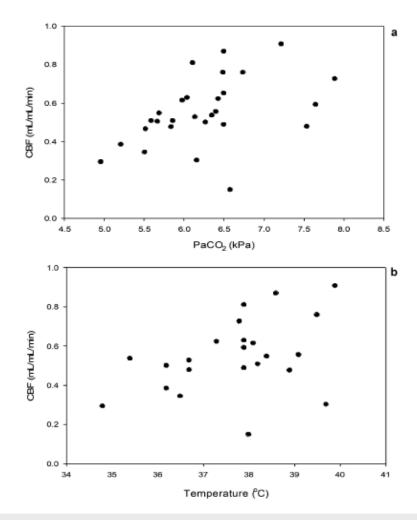


Monitoring and CBF ([15O]H₂O)

Table 1 Estimated cerebral blood flow and measured physiological variables expressed both as mean and median

	Mean	Std dev	Median	Range	N
CBF (mL/mL/min)	0.54	0.16	0.51	0.15-0.91	37
рН	7.44	0.04	7.44	7.35-7.52	28
PaCO ₂ (kPa)	6.3	0.7	6.2	5.0-7.9	28
PaO ₂ (kPa)	18	5	16	10.7-29.0	27
HCT (%)	30	3	30	24.3-35.9	26
$HR (min^{-1})$	115	25	116	53-160	21
SBP (mmHg)	114	14	110	86-142	23
DBP (mmHg)	76	17	70	51-118	23
GLC (mmol/L)	4.9	1.4	4.8	2.3-8.1	26
TEMP (°C)	37.7	1.3	37.9	34.8-39.9	23
TIME (min)	115	68	127	79–314	37

 $\it CBF$ cerebral blood flow, $\it PaCO_2$ arterial carbondioxide tension, $\it PaO_2$ arterial oxygen tension, $\it HCT$ haematocrit, $\it HR$ heart rate, $\it SBP$ systolic blood pressure, $\it DBP$ diastolic blood pressure, $\it GLC$ blood glucose, $\it TEMP$ body temperature, $\it TIME$ duration of anaesthesia, $\it N$ number of observations







	рН	PaCO ₂	PaO ₂	нст	HR	SBP	DBP	ТЕМР	GLC	TIME
CBF	-0.35	0.45	- 0.22	0.22	0.49	0.06	- 0.07	0.41	0.13	0.26
	0.064	0.016	0.28	0.29	0.024	0.80	0.76	0.052	0.53	0.11
	28	28	27	26	21	23	23	23	26	37
pН		-0.82	0.065	-0.014	-0.16	- 0.043	-0.089	- 0.23	0.13	0.01
	-	0.000	0.75	0.95	0.48	0.85	0.69	0.29	0.54	0.96
		28	27	26	21	23	23	23	24	28
Pa CO ₂			- 0.11	0.30	0.24	0.27	0.21	0.34	-0.13	0.10
	-	-	0.59	0.14	0.31	0.21	0.33	0.12	0.56	0.62
			27	26	21	23	23	23	24	28
PaO ₂				- 0.23	-0.60	0.40	0.26	0.035	-0.24	- 0.46
	-	-	-	0.28	0.004	0.059	0.23	0.87	0.27	0.015*
				25	21	23	23	23	23	27
нст					0.45	0.18	0.18	0.22	0.21	0.27
	-	-	-	-	0.046	0.43	0.43	0.33	0.34	0.18
					20	22	22	22	22	26
HR						0.022	0.039	0.33	0.21	0.73
	_	-	-	-	-	0.92	0.87	0.14	0.40	0.000*
						21	21	21	18	21
SBP							0.58	0.27	0.25	0.048
	_	-	-	-	-	-	0.004	0.22	0.28	0.83
							23	23	20	23
DBP								0.019	0.094	- 0.23
	_	-	-	-	-	-	-	0.93	0.69	0.28
								23	20	23
TEMP									0.19	0.53
	_	_	_	_	_	_	_	_	0.42	0.010*
									20	23
GLC										0.44
	_	_	_	-	-	-	_	-	_	0.02
										26





$PaCO_2$ and CBF ([15O]H₂O)

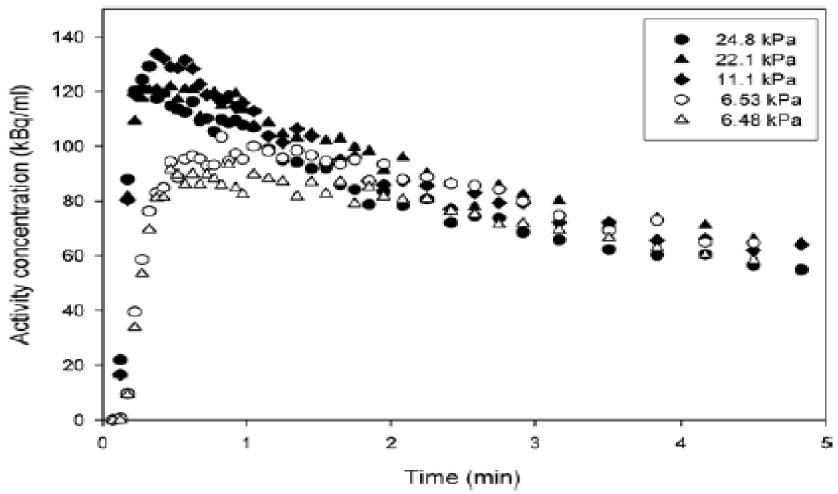


Figure 1. Time–activity curves after five H₂¹⁵O injections in Pig 4. The shape of the activity concentrations as function of time clearly varies depending on whether the pig is normocapnic (white symbols) or hypercapnic (black symbols).

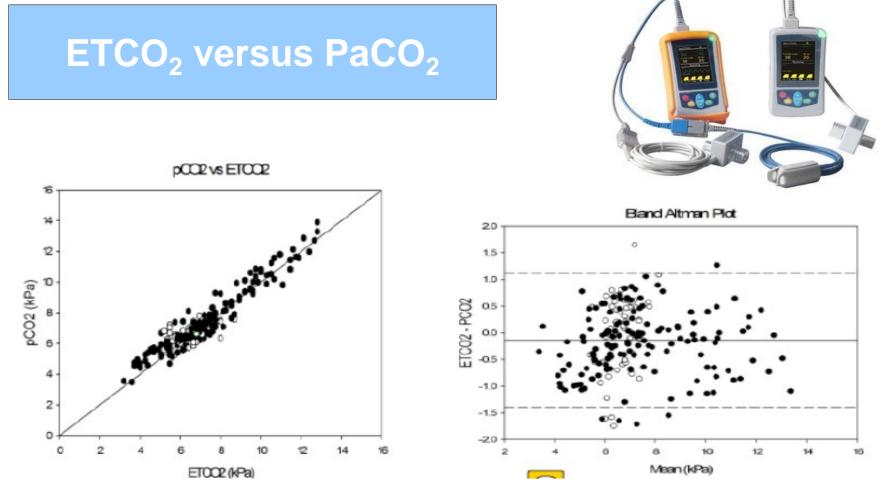


Figure 8: The relationship between end-tidal carbon dioxide (ETCO $_2$) and partial pressure carbon dioxide (PaCO $_2$) in 40 kg female domestic pigs (N=9) during anaesthesia. Data was recorded both during normocapnia (o) and hypo- and hypercapnia (•). The left figure shows a plot of PaCO $_2$ measurements as a function of ETCO $_2$ measurements. The right figure is a Bland-Altman plot of the same data.

Blood lactate in pigs

- Marker for hypoxia

Table 14: Blood lactate levels (mmol/l; mean \pm SD) in pigs.

Pig breed	N	Anaesthesia	Weight	Surgery	Blood Lactate	<u>p</u>
Göttingen minipigs	19	Isoflurane	35 kg	Minor	2.53 ± 1.10	***
Domestic pigs	16	Isoflurane	40 kg	Minor	0.68 ± 0.48	NS
Domestic pigs	16	Propofol	40 kg	Minor	0.77 ± 0.34	NS
Domestic pigs	22	Propofol	40 kg	Major	0.88 ± 0.65	NS
Domestic pigs	08	Propofol	70 kg	Minor	0.71 ± 0.39	NS

***: Significant (P<0.001) differences between Göttingen and domestic pigs. N: number of pigs.

NS: not significant. Data from Table 1 in paper P-VI.





Long-term anaesthesia

Long-term anaesthesia: 15-18 hours

- versus 8-14 hours

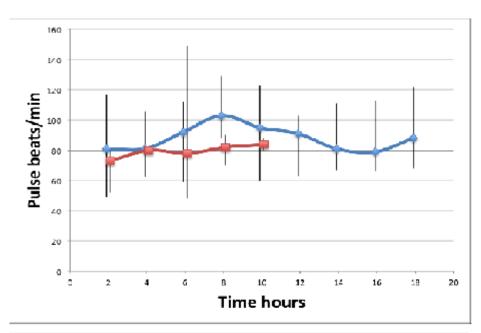
Blood sampling: 20 ml/kg

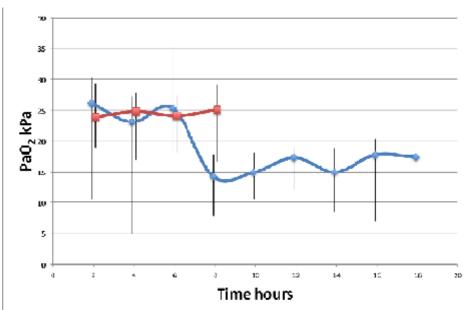
versus 14 ml/kg

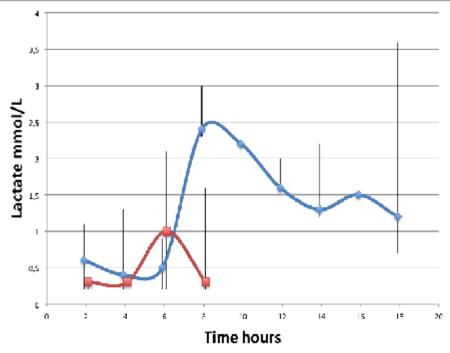
Road transport: 1½ hours

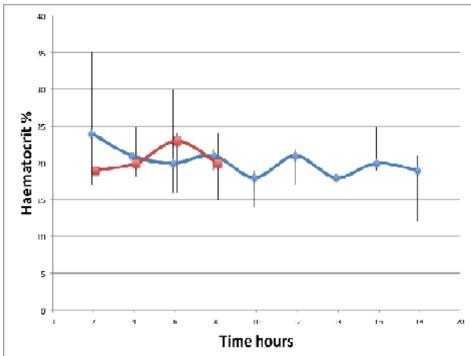
- versus no transport











Atelectasis after long-term anaesthesia

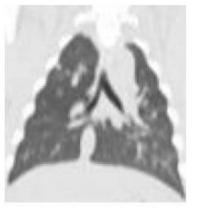
 $2\frac{1}{2}h$

 $4\frac{1}{2}h$

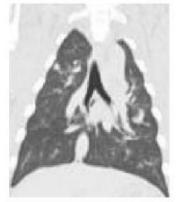
6 h

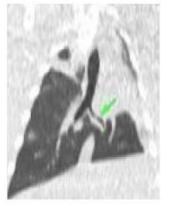
14 h

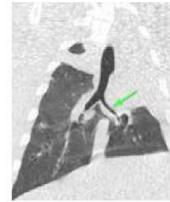
17 h

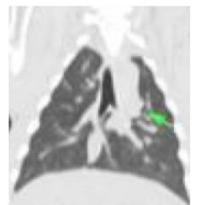


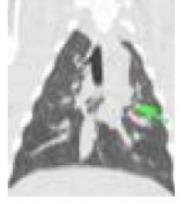


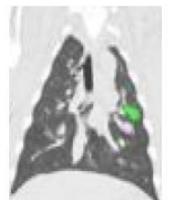


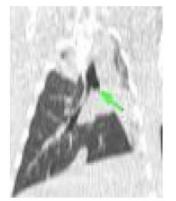


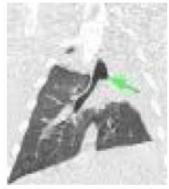
















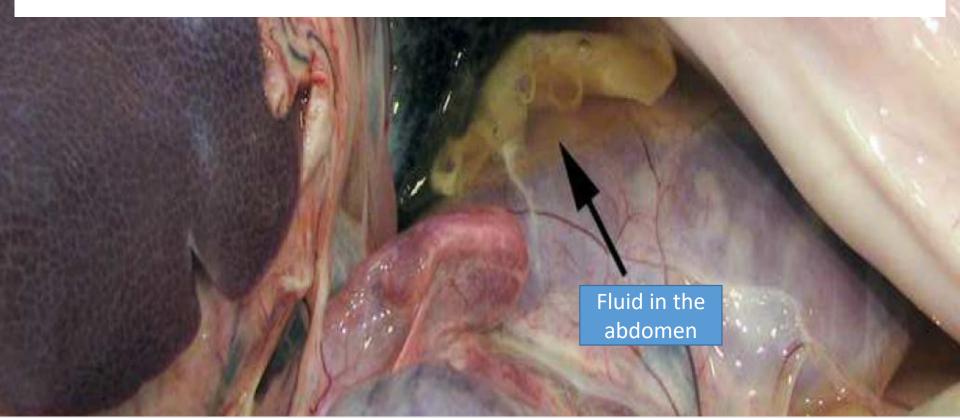


No effects of long-term anaesthesia on brain

Table 17: Brain necropsy, histology, Fluoro-Jade-B and median (min-max) FDG uptake.

Pigs	Necropsy	Histology	Fluoro-Jade-Color	SUV [¹⁸ F]FDG
I-IX	No findings (N=9)	No findings (N=9)	All negative (N=9)	1.8 g/ml (1.5-2.6) (N=9)
X-XVIII	No findings (N=9)	No findings (N=9)	All negative (N=8)	1.9 g/ml (1.4-3.0) (N=9)

SUV: standardized uptake values. FDG: fluorodeoxyglucose. N: number. Data from paper P-X.



Short scans and animal welfare

CT before and after 2 hours of PET scanning

No pathology

14 day periods post-scanning without blood sampling

95 procent without any notes

14 day periods post-scanning with blood sampling

50 procent without any notes (most small bleedings)

14 days periods (controls)

- 98 procent without any notes



Alstrup et al (2021) PET imaging sessions do not cause detectable organ pathology in Göttingen minipigs. In prep.



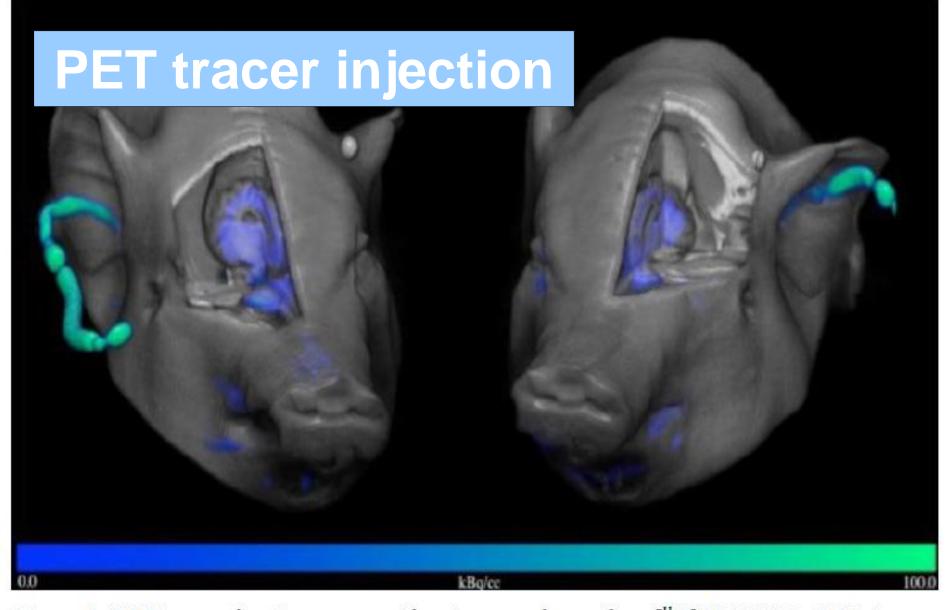


Figure 5: PET images showing tracer residues in ear catheters from [11C]PK11195 in 6 Göttingen minipigs after injection of 300-400 MBq tracer in a volume of 10 ml. The catheters were flushed with 10 ml saline after tracer injection. Figure 1 in Paper X

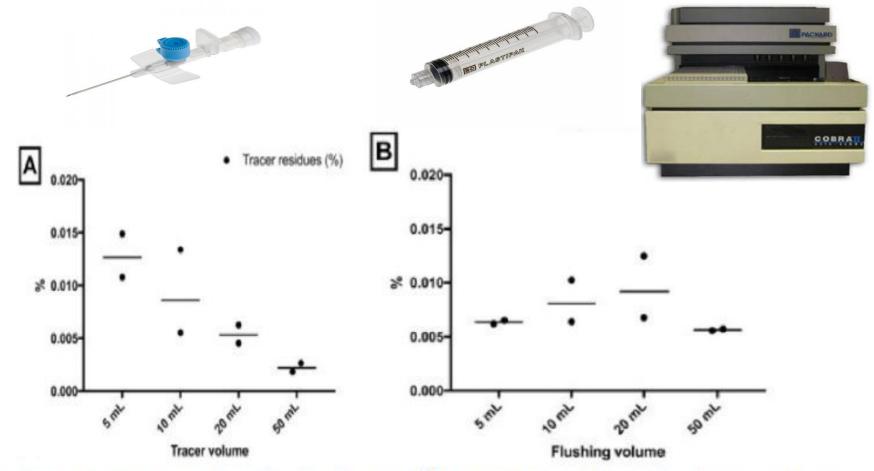


Figure 6: Results of in vitro study with the tracer [18F]FDOPA with different tracer volumes (5-50 ml) (A) and flushing volumes (5-50 ml) (B). The residues of the tracer are shown as % of the injected tracer dose. The study was performed twice.





Thanks for listening





ELLEGAARD

Göttingen Minipigs MR and CT Imaging Atlas

A comprehensive imaging atlas presenting detailed information about the physiology of Cottingen Miniples has been made available for anyone interested in the anatomical evolution of Göttingen Minipigs. This documentation enables a unique opportunity to follow the development of all organs over time and obtain new data.

The images are made from high-resplution CT- and MRI-scans and the atlas covers a wide range of information based on the scans (see page 2). With these, a user-friendly virtual miniplg can be created using freely available software, and organ development analyses can be performed.

Why the imaging atlas is important

The imaging atias fully supports all three parametres of the 3R principles, which contribute with important steps in the development of safer and more effective medicines:

- · Replacement; as the access to and use of the imaging atlas can fully replace the use of new animals in similar studies
- · Reduction; as the use of the maging atias can fully or partly reduce the number of animals used to obtain the same amount of scientific information
- . Refroement: as reducing animal distress is a direct result of a reduced need for operational studies, since knowledge can be obtained through the images, which also supports the definition of age and development-stage



Access to the images

You can gain access to the database with CT- and MRI-scan files free of charge after signing an MTA. The files may be used for non-commercial, internal and/or knowledge building purposes within your organization.

Are you interested?

Contact us on elleggerd@miniples.dk

Background and usability

A total of 12 Göttingen Minipigs were scanned at different ages to follow the anatomical development from the age of 2 to 24

- · Four females were scanned repeatedly, at the ages 2-3 months, 4-5 months, 6-7 months and 12-13 months
- . Two females and two males at the age of 1 year
- . Four females at the age of 2 years



satuo at cr scanner with anaesthetized chringen wininies connected to

Throughout the years, we have received many diverse requests from researchers planning experiments with Göttingen Mihipigs, e.g. the size of the eyeballs at different ages, the development of the teeth, the size and location of certain argans, arteries, and veins.

This atios makes it possible to study the anatomy and the development of growing gragns, the vascular system and bane structures, and makes it easier to choose the right model for each research set-up. Likewise, pre-study considerations, such as age, growth during study period, equipment and probe sizes etc., can he solved beforehand and thereby reduces the use of pilot onimals. in compliance with the 3R principles.

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The value of MRI-scans

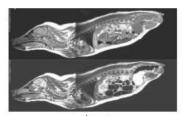
MRI-scans illustrate higher detail in soft tissues and has the ability to change the contrast of the images, which highlight different types of tissue. For example, the signal intensity for fluids is low in T1 weighted images, but high in T2 weighted images. Based on this, most soft tissue structures can be differentiated through either inherent contrast or comparison between the two. The high resolution images depict and enable most organs for segmentation, and sequential scan sets enable a time-evolution curve of all preans.

The value of CF scans

CT-scans are great for imaging the bone structure, good for soft tissue differentiation (particularly with intravenous contrast), and generally provides higher imaging resolution with less motion artifact. The x-ray based CT-scans outline high dense materials very clearly, when segmenting bone structures based on the CT-scans. The cardiovascular system becomes visible through contrast agent administration, and can be segmented with great results.



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MRI-scans snowing sagictal TL weigneed note and sagittal 12 weigneso (portion).



CT scan snowing frontal and distal segmentation of done structure and cardiovascular system.

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Olsen AK et al.: Effect of pre-analytical handling on haematological variables in minipigs. Laboratory Animals 2001, 35, 147-152.

Olsen AK: Short-term effects of storage time and temperature on pH, pCO2, and pO2 in porcine arterial blood. Scandinavia Journal of Laboratory Animal Science 2003, 4, 30, 197-201.

Olsen AK et al.: Effect of hypercapnia on cerebral blood flow and blood volume in pigs studied by positron emission tomography. Comparative Medicine 2006, 56, 5, 416-420.

Alstrup AKO et al.: Type of anesthesia influences positron emission tomography measurements of dopamine D2/3 receptor binding in the rat brain. Scandinavia Journal of Laboratory Animal Science 2011, 38, 3, 195-200.

Alstrup AKO et al.: Effects of anesthesia and species on the uptake or binding of radioligands in vivo in the Göttingen minipig. BioMed Research International 2013, 808713.

Alstrup AKO: Blood lactate concentrations in Göttingen minipigs compared with domestic pigs. Journal of the American Association for Laboratory Animal Science 2016, 55, 1, 18-20.

Alstrup AKO: End-tidal carbon dioxide (ETCO2) can replace methods for measuring partial pressure of carbon dioxide (PCO2) in pigs. Laboratory Animal Science Professional 2017, 12, 33-34.

Alstrup AKO et al: PET radioligand injection for pig neuroimaging. Scandinavia Journal of Laboratory Animal Science 2018, 44, 2, 1-5.

Alstrup AKO et al.: Monitoring variables affecting positron emission tomography measurements of cerebral blood flow in anaesthetized pigs. Acta Veterinaria Scandinavica 2018, 60, 17, 1-7.

Alstrup AKO et al.: Effects of long-term anaesthesia, blood sampling, transportation, and infection status on hearts and brains in Staphylococcus aureus inoculated pigs used for imaging studies. 2019. Submittet til et forsøgsdyrstidsskrift.



