DOCTORAL DISSERTATION

# PET neuroimaging in pigs

with focus on anaesthesia, monitoring, radioligand injection and animal welfare


D.V.M., Ph.D.

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Department of Nuclear Medicine & PET

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January 2020


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Department of Nuclear Medicine & PET-Centre

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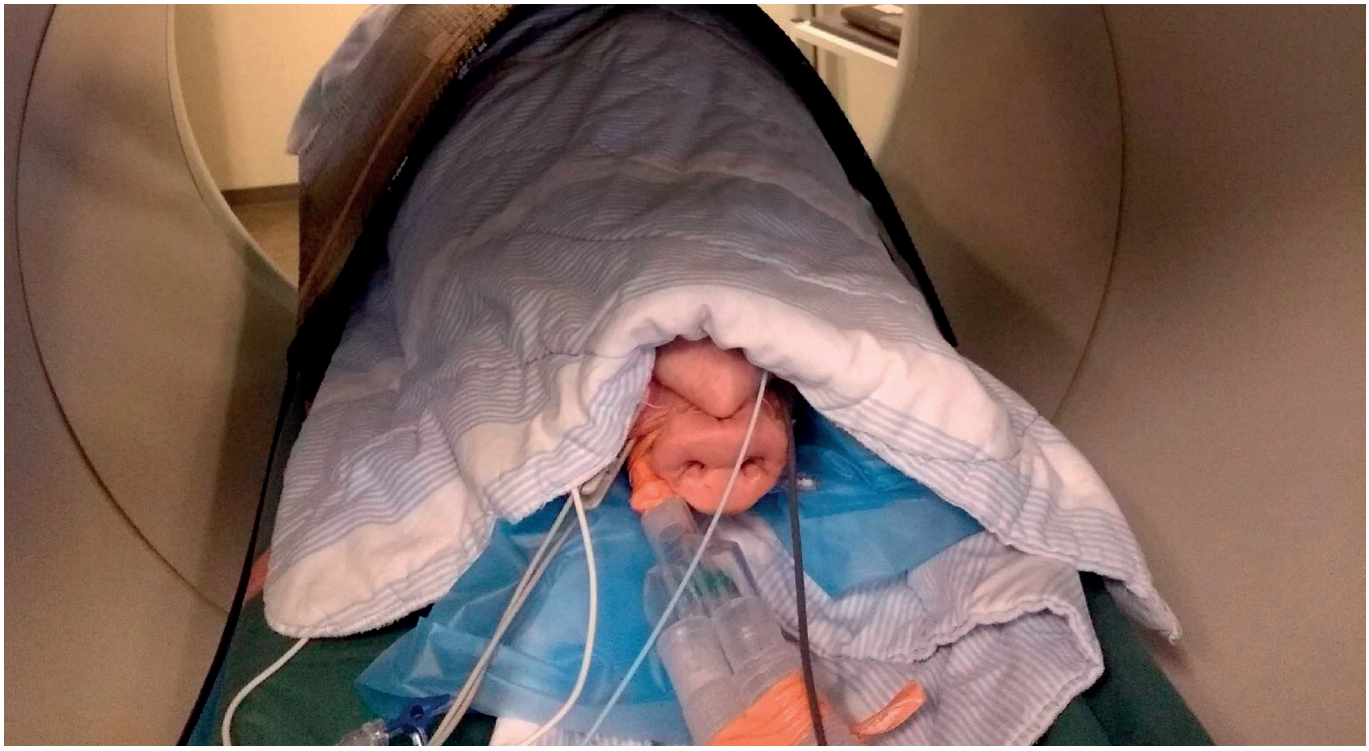
**Cover**: The collage "*From ancient vivisection to modern pig brain scanning*" is made by my friend Andreas Nørgaard Glud, who is known for his humorous and ingenious drawings and he had free hands for this work.

"*Jeg troede du studerende Mineralogie, siden jeg saa den store Mængede Steen her paa dit Gulv. – De findes her alene for Værkets Skyld sagde han. Jeg har bemærket at Tankerne glide bedst fra Pennen, naar man besidder aldeles hensigtsmæssige Skriveredskaber. For at faae gode Penne, maa man have gode Knive, og for at skiærpe dem, maa man besidde ret udsøgte Slibestene. Derfor har jeg, inden jeg gik videre, forskrevet adskillige Steenarter, og rigtigt nok, som du seer, maattet trænge temmelig dybt ind i Mineralogien som et forberedende Studium.*"

The Danish quotation above is taken from the posthumous published novel by Poul Martin Møller (1794-1838) entitled "*En dansk students eventyr*" (*Møller, 1843*). The quotation notes that, despite ten years of work, the licentiate hadn't yet completed his dissertation on the physical and intellectual nature of man, since new aspects needed study. I, too, have previously faced such a dilemma while writing my Ph.D. dissertation in 2001 (*Olsen, 2002*). Then, however, I had a deadline when my grant expired. Now, for the present dissertation, the quotation again bears relevance, in that I have not had a deadline. As a result, after more than eighteen years of experience with animal scanning, I have now finished writing a summary of my studies.

This dissertation is based on my daily work as a laboratory animal veterinarian conducting research at the Department of Nuclear Medicine and PET of Aarhus University Hospital. We carry out positron emission tomography (PET) scanning of brains, livers, kidneys, hearts, lungs and bones in pigs, mice, rats and other species. However, since my appointment in 2002, I have been particularly interested in how best to perform scans by PET of the living pig brain. It soon became clear to me that pigs have a lot to offer in PET brain research, although the literature on how it is best done was very limited. Therefore, I decided to acquire the knowledge that could be useful both for my own work and for that of others who perform PET scanning of laboratory animals. I wanted to gain insight by reusing data from ongoing studies so that I did not sacrifice additional animals for this purpose. The aim was to publish this knowledge, as it could also be relevant for other animal species, scanner types and organ systems, and at conferences I have met researchers with the exact same unsolved problems. These studies have all been based on issues that have arisen during many years of our efforts to scan animals. And even now, there may be many unresolved issues for further work. It is my sincere hope that readers of this dissertation will find inspiration for conducting their own judicious experiments on laboratory animals.

*Aage Kristian Olsen Alstrup, Aarhus, 31st of January 2020.*



# Contents

# 1.0 Papers included in this dissertation

This doctoral dissertation in veterinary science is based on eleven peer-reviewed papers. The papers are listed in chronological order and will be referenced by their numbers in the text (**I-XI**):

**P-I** **Olsen AK**, EM Bladbjerg, AL Jensen & AK Hansen: Effect of pre-analytical handling on haematological variables in minipigs. *Laboratory Animals* 2001, 35, 147-152.

**P-II** **Olsen AK***:* Short-term effects of storage time and temperature on pH, $pCO_2$, and $pO_2$ in porcine arterial blood. *Scandinavian Journal of Laboratory Animal Science* 2003, 4, 30, 197-201.

**P-III** **Olsen AK**, S Keiding & OL Munk: Effect of hypercapnia on cerebral blood flow and blood volume in pigs studied by positron emission tomography. *Comparative Medicine 2006, 56, 5, 416-420.*

**P-IV** **Alstrup AKO**, M Simonsen & AM Landau: Type of anesthesia influences positron emission tomography measurements of dopamine D2/3 receptor binding in the rat brain. *Scandinavian Journal of Laboratory Animal Science* 2011, 38, 3, 195-200.

**P-V** **Alstrup AKO***, AM Landau*, JE Holden, S Jakobsen, AC Schacht, H Audrain, G Wegener, AK Hansen, A Gjedde & DJ Doudet: Effects of anesthesia and species on the uptake or binding of radioligands *in vivo* in the Göttingen minipig. *BioMed Research International* 2013, 808713, 1-9. *: Shared first-author.

**P-VI** **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.

**P-VII** **Alstrup AKO**: End-tidal carbon dioxide ($ETCO_2$) can replace methods for measuring partial pressure of carbon dioxide ($PCO_2$) in pigs. *Laboratory Animal Science Professional 2017, 12, 33-34*.

**P-VIII** **Alstrup AKO**, OL Munk, AM Landau & TP Lillethorup: PET radioligand injection for pig neuroimaging. *Scandinavian Journal of Laboratory Animal Science 2018, 44, 2, 1-5.*

**P-IX** **Alstrup AKO**, NE Zois, M Simonsen & OL Munk: Monitoring variables affecting positron emission tomography measurements of cerebral blood flow in anaesthetized pigs. *Acta Veterinaria Scandinavica 2018, 60, 17, 1-7.*

**P-X** **Alstrup AKO**, P Afzelius, SB Jensen, PS Leifsson, KM Wegener & OL Nielsen: Effects of long-term anaesthesia, blood sampling, transportation, and infection status on hearts and brains in pigs inoculated with *Staphylococcus aureus* and used for imaging studies. *Journal of the American Association for Laboratory Animal Science 2020, 59, 1-11.*

**P-XI** Landau AM, O Noer, **AKO Alstrup**, H Audrain, G Wegener, A Gjedde, DJ Doudet & M Winterdahl: Type of anaesthesia influences [$^{11}$C]MDL100,907 binding to $5HT_{2A}$ receptors in porcine brain. *Molecular Imaging and Biology 2020, 10.1007/ s11307-020-01476-x, 1-8*.

Note: In 2008, my name was changed from **Aage Kristian Olsen** to **Aage Kristian Olsen Alstrup**.

# 2.0 Introduction

## 2.1 Principles of pig neuroimaging

Positron emission tomography (PET) scanning is bridging the gap between preclinical and clinical research, as new tracers and research protocols developed in animal studies can often be translated directly into human trials. In addition to describing the principles behind PET, this section will briefly deal with two other main imaging methods used for animals and humans, namely computerized tomography (CT) and magnetic resonance imaging (MRI), as these scanner modalities are often used consecutively or in one imaging session using combined scanners such as PET/CT or PET/MRI. They can then yield information about anatomy/structure together with physiological processes such as blood flow, blood volume, glucose metabolism, oxygen utilization or receptor binding (*See relevant testbooks: Haacke et al., 1999; Prokop et al., 2003; Bailey, 2005; Matwichuk-Bassett and Berry, 2006, as well as* **R-1** and **R-3**).

PET is a molecular imaging technique that records biological processes after administration of positron-labelled tracers to living individuals (*Bailey, 2005; Matwichuk-Bassett and Berry, 2006*). The course of isotope and tracer generation, PET scanning and data analysis are illustrated in Figure **1.** The positron-emitting isotopes are typically produced on site in a cyclotron, as their radioactive half-lives are short (see Table **1**). Then, the radiochemists produce tracers, where the isotopes are incorporated into organic (e.g. the amino acid [$^{11}$C]methionine) or inorganic molecules (e.g. [$^{15}$O]water). PET radiotracers are synthesized as naturally occurring substances (e.g. [$^{11}$C]glucose) or analogues thereof (e.g. 2-[$^{18}$F]fluoro-2-deoxy-*D*-glucose, [$^{18}$F]FDG), pharmaceuticals (e.g. [$^{11}$C]yohimbine) or other molecules (*see* ***R-3***). The tracers are then administered into the subject, typically as intravenous injections (intraperitoneal in some studies performed on small species) or as lung inhalation of gases (e.g. [$^{15}$O]oxygen and [$^{15}$O]carbon monoxide). The doses are

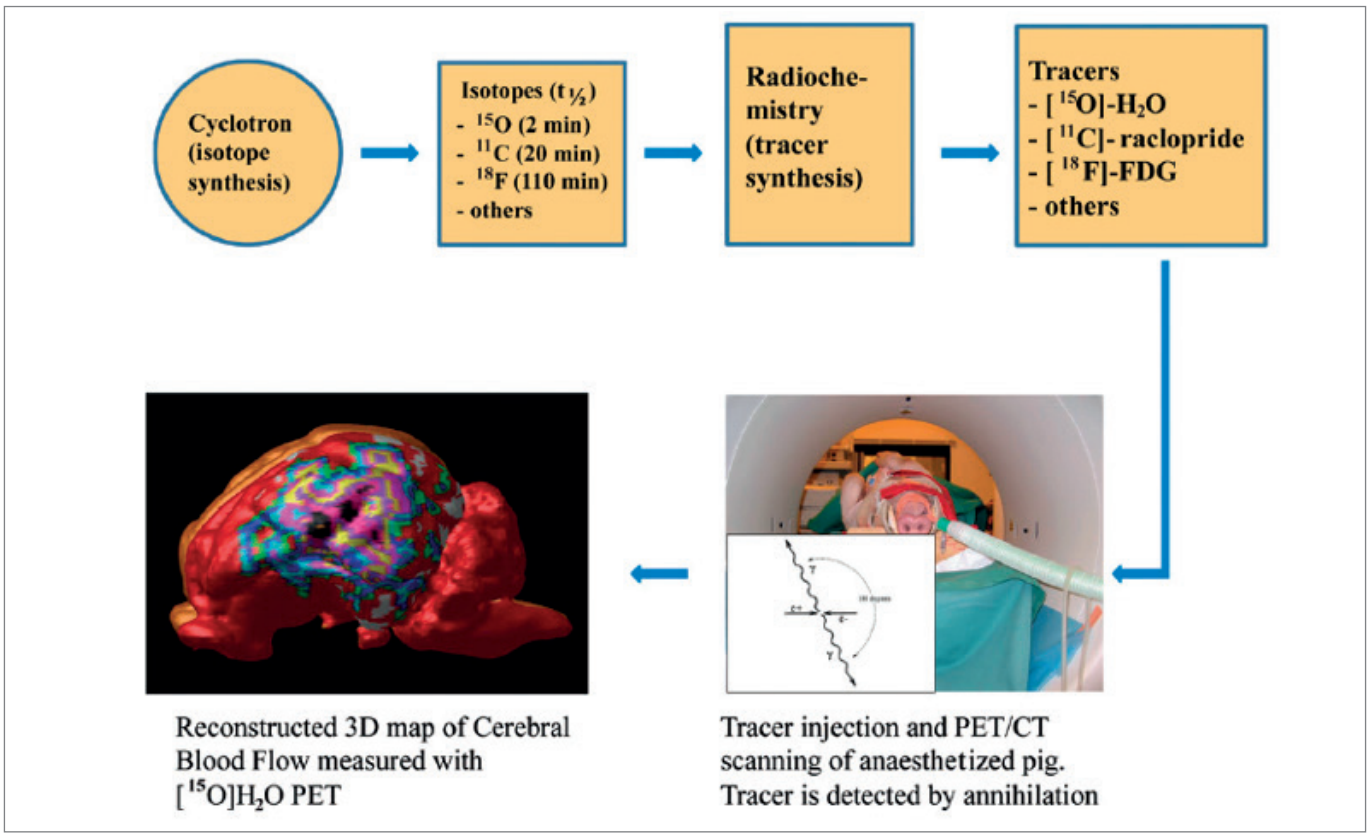



**Figure 1:** Positron emission tomography (PET) scanning of a domestic pig with induced leftsided stroke. Positron-emitting isotopes are produced on site by the cyclotron, and the isotopes are chemically incorporated in tracers, which are intravenously injected into the anaesthetised pig. The scanner detects the two anti-parallel photons (energy at 511 keV) produced by annihilation and reconstructs 3D images of the tracer distribution (Figure **5** in **R-5**).

**Table 1:** Radioactive half-lives of the used positron-emitting isotopes* in this dissertation.

| | | |
|---|---|---|
| [$^{15}$O] | 2.04 minutes (~2 minutes) | used in this dissertation in **P-III** and **P-IX** |
| [$^{11}$C] | 20.3 minutes (~20 minutes) | used in this dissertation in **P-IV, P-V, P-VIII** and **P-XI** |
| [$^{18}$F] | 109.8 minutes (~2 hours) | used in this dissertation in **P-VIII** and **P-X** |

*New tracers can be injected after 5 half-lives of the isotope for the previous tracer. Data for half-lives are from Cherry (2001).

so low that the molecules normally have no pharmacological effect (*Funk et al., 2004; Kung and Kung, 2005*); hence the name *tracers*. However, sometimes the mass effect – also known as the chemical quantity – of the injected dose may affect the imaging outcome. For imaging of receptors, a maximum of one percent of the available receptors should be occupied by the tracer. These restrictions are not as critical in pigs as they are in mice and rats (*Kung and Kung, 2005*), as lower tracer doses are used in pigs (typically 2-25 MBq/kg) compared to small animals (≥15-80 MBq/kg).

The PET scanner detects the two anti-parallel photons that are emitted when a positron annihilates upon contact with an electron in the surrounding tissue. This allows reconstruction of three-dimensional images of the tracer distribution (intensity and anatomical location) within the body. PET scanning can be performed as static scans, where a single recording is performed after the radiotracer has been distributed in the body. Alternatively, PET scanning can be performed as dynamic scans, where PET recording starts at the time of tracer injection. Here, the time course of the concentration of radioactivity in the tissue is recorded by the PET scanner and the time serie of the concentration of radioactivity in blood can be measured in successive arterial blood samples or derived from PET images *(Bailey, 2005; Guo et al., 2007; Jødahl et al., 2019)*. Such measurements can then be used for construction of time-activity-curves and analysed using kinetic models (*Bentourkia and Zaidi, 2007*). PET enables quantitative metabolic measurements (*Logan, 2000; Matwichuk-Bassett and Berry, 2006*).

Compared to CT and MRI, PET has a lower spatial resolution (1½-7 mm, see Table **2**), which affects the ability to differentiate between two objects located next to each other. Here, the relatively large brain and brain regions in pigs is an advantage (*Lind et al., 2007; Ettrup et al., 2010; Bjarkam et al., 2017*). Since PET scanning requires the use of radioactive tracers, the scanned pig is exposed to radioactive radiation limiting how often such scans can be repeated in survival studies – otherwise it risks affecting the pig's physiology (e.g. the immune system).

CT scanning can be performed both in living and dead individuals. CT is performed by a machine that can generate and detect X-rays (*Prokop et al., 2003*). The X-ray tube and detectors are placed on opposite sides of a rotating ring, which surrounds the animal in the scanner. The X-rays may therefore pass through the body and will attenuate at different rates depending on the tissues (e.g. mineralised tissue has a higher attenuation than soft tissue). Based on these differences in attenuation, computer processing can generate a three-dimensional body image based on a series of two-dimensional X-ray images (*Prokop et al., 2003*). Dynamic contrast-enhanced CT has also been used in blood perfusion measurements in pigs (*Winterdahl et al., 2012*). The spatial resolution can be less than ½ millimeter. As CT uses ionizing radiation, the number of times a subject can be scanned is

**Table 2:** Overview of the PET scanners used for pigs and rats in this dissertation.

| Modality | Scanner | Company | Typical Spatial resolution | Papers |
|---|---|---|---|---|
| PET | ECAT EXACT HR-47 | CTI/Siemens Medical Systems, Knoxville, TN | 6.7 mm FWHM (4-7 mm) | **P-III, P-IX** |
| HRRT PET | High-resolution research tomograph | CTI/Siemens Medical Systems, Knoxville, TN | 2.5 mm (1.5 – 3.1 mm) | **P-V, P-XI** |
| PET/CT | Siemens Biograph True point 64 PET/CT | Siemens Medical Systems, Erlangen, Germany | 4.2 mm FWHM (3-5 mm) | **P-VIII, P-X** |
| MicroPET | MicroPET R4 | CTI, Concorde | 2 mm FWHM | **P-IV** |

FWHM: full width at half maximum. HRRT: high-resolution research tomograph. Data based on the data sheets of the scanners and our own experiences.

limited. CT scans are also used for attenuation correction of PET images and to assure proper placement of catheters, electrodes and other implants in the brain prior to PET scanning *(Glud et al., 2019).* CT is used for health monitoring in paper **P-X**. The first imaging of fresh tissue was performed on pig and bullock brains by Godfrey Hounsfield (1919-2004), who later shared the Nobel Prize for such studies (www.nobelprize.org).

MRI scanning can be performed both in living and dead individuals. MRI uses a powerful magnetic field to align a fraction of the nuclear magnetization of the body's hydrogen atoms (primarily water molecules) (*Haacke et al., 1999*) or certain other atoms (e.g. [$^{13}$C]). MRI scanning is based on radiofrequency fields that systematically alter the alignment of the magnetization, so that the hydrogen nuclei will produce a rotation magnetic field that can be detected (*Haacke et al., 1999*). As no X-ray or radiation is involved, pigs can be scanned without health limitations. MRI provides detailed images with less than ½ millimetre spatial resolution. As MRI has a better soft tissue contrast than CT, it is widely used in neurological imaging (*Mier and Mier, 2015*) and prior to stereotatic neurosurgery in pigs (*Sørensen et al., 2011; Ettrup et al., 2012; Glud et al., 2017; Bjarkam, 2020*). Dose optimization of MRI contrast to brain studies has been performed in pigs (*Poulsen et al., 2012*). Functional MRI is based on the blood oxygen level dependent effect of paramagnetic properties of deoxygenated haemoglobin (*Mier and Mier, 2015*), but the air in the pig's extensive sinuses may weaken the quality of functional MRI brain scans (*Alstrup et al., 2013*). Furthermore, MRI-safe equipment is needed, due to the powerful magnetic field. An MRI-based atlas has been used in this dissertation (*Watanabe et al., 2001*). Pigs and other laboratory animals were involved in the early development of MRI scanners – an invention for which the Nobel Prize was later awarded to Paul Lauterbur (1929-2007) and Peter Mansfield (1933-2017) (www.nobelprize.org).

Occasionally, pigs must be moved between scanners, placed close to each other in the same laboratory (*Glud et al., 2019)* or transported between laboratories while the pigs are anaesthetised (*Nielsen et al., 2015; Afzelius et al. 2016A; Afzelius et al. 2016B*). PET/CT scanners are standard for medical PET-imaging today, but also PET/MRI scanners have recently found their way to the clinic (*Shao et al., 1997; Vandenberghe and Marsden, 2015*). Data from PET and CT/MRI scanning can be combined in a time-consuming process called co-registration. With the advent of combined scanners, generating combined structural and functional images has become possible. Since MRI has a number of advantages for soft tissue imaging, these scanners in combination with PET are preferred in brain research (*Vandenberghe and Marsden, 2015*). An overview of the PET scanners used for the studies carried out by the author is given in Table **2**.

## 2.2 Reasons for PET scanning of pig brains

It is often claimed that man (*Homo sapiens*) and pig (*Sus scrofa domesticus*) are phylogenetically closely related, but compared with most other placental mammals, including rodents (e.g. the laboratory rat, *Rattus norvegicus domesticus*), that is not the case (*Kumar and Hedges, 1998; Meredith et al., 2011*). Despite this, the pig and man have great genetic similarity, as shown by Schook and co-workers (2015), and is therefore attractive as a transgenic animal model (see also **R-5**). Pigs were already used in ancient biomedical research, for example in the 2$^{nd}$ century of the Roman Empire, through Galen of Pergamon's (129 AD – c. 216 AD) vivisection of the nervous system in awake animals (*Gross, 1998*), and rediscovered as experimental animals in the early Renaissance (*Vesalius, 1543*). Four Nobel Prizes are based on pig experiments (brain peptide hormones 1977, CT scanner 1979, MRI scanner 2003, and peptic ulcer disease 2005; see www.nobelprize.org). Pigs are increasingly used in biomedical research (*Alstrup and Hansen, 2009*) and are the third most frequently used laboratory animal species (3 % of all laboratory animals) after the mouse (57 %) and the rat (17 %) in Denmark, though the zebrafish in some years surpasses the pig. In 2017, 71 pigs were used for neuroscience, out of a total of 7,758 pigs (*Dyreforsøgstilsynet, 2018),* and pigs are PET-scanned at laboratories in Copenhagen, Odense, Aalborg and Aarhus. The Danish company Ellegaard Göttingen Minipigs A/S (www.minipigs.dk) produces genetically and microbiologically well-characterized Göttingen minipigs for research (*Bollen et al., 2010*), and this has also contributed to the use of pigs in Danish research, as up to 25 % are Göttingen minipigs, while the majority of the rest, mainly used for non-recovery studies, are domestic Danish Landrace-Yorkshire breeds (*Betina Scheef, Dyreforsøgstilsynet, 2018, pers. comm.*). Few inbred pig strains exist, like the Banna minipig (*Cheng et al., 2001*), but they are not available in Denmark – instead outbred pigs are often used as their own control (left-right or pre-post comparisons) for reducing the variation and number of pigs needed in PET studies (e.g. *Lind et al., 2005B; Nielsen et al., 2015; Winterdahl et al., 2019*). See also the left-sided stroke in Figure **1**.

**Table 3:** Basic body and brain biology for rats, pigs and humans used in brain PET scanning studies.

| Parameters<br>Paper number | Rat<br>IV | Domestic pig<br>II, III, VI, VII, VIII, IX, X | Göttingen minipig<br>I, V, VI, VIII, XI | Humans |
|---|---|---|---|---|
| **Body** | | | | |
| Weight [kg] | 0.200-0.500 | 20-300 kg | 15-55 kg | 70-100 |
| Blood [ml/kg] | 65 | 70-80 | 70-80 | 62-67 |
| Lifespan [years] | 2-3 | 10-15 | 10-15 | 70-90 |
| **Brain** | | | | |
| Weight [gram] | 1.5 | 130 | 80 | 1300 |
| Size [mm] | 20 | 60-80 | 50-60 | 150 |
| Type | lissencephalic | gyrencephalic | gyrencephalic | gyrencephalic |

Based on Johnston and Willis (1946), Sharp and Villano (2012), Swindle and Smith (2015) and supplemented with own measurements.

We use pigs in our PET research mainly for practical reasons (see also **R-3**). First, pig brains are of suitable size (6 cm x 5 cm x 4 cm) and volume (75-100 ml) to perform PET studies on the brain, keeping in mind the relatively poor spatial resolution of PET scanners (*Lind et al., 2007*). Brain size of pigs also makes them useful animals for experimental brain surgery (*Sørensen et al., 2011; Ettrup et at., 2012; Glud et al., 2017*). Even though brain sizes are approximately the same at birth, the adult brain is larger in domestic pigs than in Göttingen minipigs, and interestingly, we have shown that the human neurological postnatal development is best reflected in domestic pigs (*Jelsing et al., 2005; Jelsing et al., 2006; Guidi et al., 2011*). Mice and rat brains are often too small to obtain precise anatomical insight into the tracer's binding. Second, several textbooks describe similarities between physiological and pathological processes in pigs and humans (*Tumbleson, 1986; Tumbleson and Schook, 1996*). Although this does not necessarily qualify the pig as an animal model for a specific project (*Wessler, 1976, Hau et al., 1989*), it still indicates that it will often be useful. Third, several pig brain anatomy atlases have been published (*Yoshikawa, 1968; Félix et al., 1999; Watanabe et al., 2001; Saikali et al., 2010; Orlowski et al., 2019*). Recently, a PET template for automatic spatial normalization has been developed for pig brain studies (*Villadsen et al., 2018*). Fourth, many pig brain regions are well-described, such as the brainstem, hippocampus, subcortical and diencephalic nuclei (*Holm and Geneser, 1989; Holm et al., 1992; Østergaard et al., 1992*), and this work continues with several recent publications on the subthalamic nucleus (*Larsen et al., 2004*), substantia nigra (*Nielsen et al., 2009*), hypothalamus (*Ettrup et al., 2010*), telencephalon (*Bjarkam et al., 2017*) etc. as well as electrophysiological studies (*Meier et al., 2018*). In summary, these regional brain studies have shown that the pig brain is largely comparable to human brain. Fifth, pigs are intelligent animals, and it is possible to perform studies to explore relationships between behaviour and neurotransmission (*Lind et al., 2005A; Lind et al., 2005B*), which is essential in long-term studies of neurodegenerative or mood disorders. However, it is a disadvantage that fewer behavioral tests have been developed for pigs than for mice and rats (see also **R-5**). Sixth, repeated blood samples can be drawn to carry out metabolite analyses in porcine studies of new PET radioligands. Up to 10-15 % of total blood volume can be drawn from pigs in PET protocols that require their survival (*Diehl et al., 2001*), and this corresponds to 8-12 ml/kg or approximately 300-450 ml of blood withdrawal from a 40-kg pig. For comparison, few blood samples can be taken from mice and rats with total sampling volumes of up to 0.3 ml and 5 ml blood, respectively. Seventh, pigs can be maintained under anaesthesia for long-term PET studies involving several successive radiotracers administrations (*Rosa-Neto et al., 2004A; Rosa-Neto et al., 2004B; Jensen et al., 2006; Cumming et al., 2007; Nielsen et al., 2015)*. Although long-term anaesthesia of rodents is possible (*Szczensny et al., 2014*), it is our experience that they can only be kept physiologically stable for a few hours, especially if blood samples are taken. Table **3** compares basic biology of rats, pigs and humans.

**R-3** reviews thirty-nine radiotracers that were used until 2010 for PET studies of the living pig brain. Since then, we and other research groups have published results from several other tracers in pig brain studies (e.g. a norepinephine transporter tracer; *Vase et al., 2014*), and have also found new methods for their delivery to the brain by crossing blood brain barrier (*Alstrup et al., 2018)* or intrathecal injection *(Glud et al., 2019*). Such new tracers are often tested in non-recovery pig studies involving blood sampling and long-term anaesthesia. In general, the tested radiotracers are distributed similarly in pig brain as in human brain, which may serve as a proving ground for using pigs in neuroscience studies. A single, but significant, exception exists, namely our failure to identify dopamine

re-uptake sites in pig brains (*Minuzzi et al., 2006*). Table **4** shows the tracers used in this dissertation.

Furthermore, **R-5** reviewed genetically modified pig models for neurodegenerative disorders, i.e. Alzheimer's disease, Huntington's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Spinal Muscular Atrophy and Ataxia-Telangiectasia, and here PET scanning of the pigs can be applied for non-invasive and longitudinal monitoring of disease progression and potential treatment effects. This presupposes, however, that the scans can be performed with minimal impact on the pigs' physiology and welfare. PET scanning has also proven to be particularly suitable for monitoring of non-genetic Parkinsonian pig models (*Dall et al., 2002, Lillethorup et al., 2018A; Lillethorup et al., 2018B; Lillethorup et al., 2018C; Landau et al., 2020*), as well as brain surgery (*Ettrup et al., 2012*), and with the increased potential for somatic cell nuclear transfer cloning and clustered regularity interspaced short palindromic repeat (CRISPR) techniques in pigs, porcine neuroimaging could be of even greater importance in the future (se **R-5** for details).

## 2.3 Practical techniques for PET scanning pigs

The same PET scanners and radioligands are typically used for human patients and volunteers, as well as for pig studies. University hospital scanning centres have experts in most aspects of imaging techniques, and they often have state-of-the-art equipment for imaging human patients. It is of importance to note, however, that handling and imaging of humans and pigs differ in some aspects. In **R-1,** we therefore reviewed scanning protocols used for pigs and other large animals, including anaesthesia and monitoring (these procedures are used in the published papers), see Table **5**. Inhaled anaesthesia (e.g. isoflurane) and infusion anaesthesia (e.g. the ultrashort-acting propofol) are commonly used and suitable drugs for prolonged anaesthesia of pigs (*Becker et al., 1984: Kaiser et al., 2003*). The review also presents techniques for inserting catheters for tracer injection, placing pigs in scanners etc.

**R-2** shows basic techniques for preparation of Göttingen minipigs for experimental procedures (e.g. intubation, transurethral bladder catheterization and vessel catheterization) used in PET scanning. This step-by-step instruction focused on a number of critical procedures that

**Table 4:** PET tracers included in this dissertation.

| Target | Tracer | Administration and comments | Papers |
|---|---|---|---|
| Cerebral blood flow | [$^{15}$O]water | 2 second bolus – similar flow in pigs (50 ml/100 ml/min) as in humans | **P-III** **P-IX** |
| Cerebral blood volume | [$^{15}$O]carbon monoxide | Single inhalation followed by 30 seconds breath-holding | **P-III** |
| Cerebral metabolism | [$^{18}$F]FDG | 30 second infusion – similar glucose metabolism (25 µmol/100 ml/min) as in humans | **P-X** **P-VIII** |
| Dopamine synthesis<br>Dopamine $D_1$ receptor<br>Dopamine $D_{2/3}$ receptor | [$^{18}$F]FDOPA<br>[$^{11}$C]SCH23390<br>[$^{11}$C]raclopride | 30 second infusion ½ hour after capidopa injection<br>30 second infusion<br>30 second infusion – acute changes reflect competition with endogenous dopamine | **P-VIII**<br>**P-V**<br>**P-IV** |
| Noradrenergic alpha-2 receptor | [$^{11}$C]yohimbine | 30 second infusion – low or no peripheral metabolism in pigs | **P-V** |
| Serotonin 2A receptor | [$^{11}$C]MDL100,907 | 30 second infusion | **P-XI** |
| Beta-amyloid plaques | [$^{11}$C]PiB | 30 second infusion – adheres to catheters and only non-specific binding in pig brain | **P-VIII** |
| Peripheral benzodiazepine translocator protein | [$^{11}$C]PK11195 | 30 second infusion – similar brain distribution in domestic as in minipig (neuro-inflammation) | **P-VIII** |

Data mainly based on **R-3** (see this for references) and the published papers.

**Table 5:** Commonly used procedures for PET scanning of our pigs with indication of major challenges.

| **Procedures** | **Blood sampling** | | | | **Hazards and major challenge (Papers)** |
|---|---|---|---|---|---|
| | - | | + | | |
| Premedication, IM | Ketamine + midazolam | | Ketamine + midazolam | | Effects on brain scans? |
| IV access | Ear vein catheter | | Ear vein catheter | | Strict IV access? |
| Induction, IV | Ketamine midazo-lam | Propofol | Ketamine midazo-lam | Propofol | Effects on brain scans? |
| Intubation*+ventilation | Yes | | Yes | | **Ventilation effects on cerebral blood flow? (P-III)** |
| Maintain anaesthesia | Isoflurane | Propofol | Isoflurane | Propofol | **Effect on brain scans? (P-IV, P-V, P-XI)** |
| Monitoring | Yes | | Yes | | **Relevant parameters for neuroimaging? (P-III, P-IX)** |
| Capnography | Yes | | Yes/no | | **Can it replace blood gas measurements? (P-VII)** |
| Blood storage and analysis | No | | Yes | | **Pre-analytical variation? (P-I, P-II)** Useful methods |
| Bladder catheters* | Mostly | | Mostly | | Post-scanning infection and bleeding? |
| Surgery, femoral A+V* | No | | Yes | | Post-scanning pain, infection and bleeding? |
| Wrapping in plastic | Yes | | Yes | | Skin problems? |
| Placing in scanner | Yes (sternal recumbence) | | Yes (dorsal recumbence) | | **Positioning injuries? (P-X)** |
| Tracer injection | Ear vein | | Femoral vein | | **Remnants in ear vein? (P-VIII)** |
| Blood sampling | No | | Yes, femoral artery | | **Effects on brain and welfare? (P-X)** |
| Time of anaesthesia | 1-6 hours (or more) | | 2-8 hours (or more) | | **Effects on brain and welfare? (P-X)** |
| Transport between scanners | Yes | No | Yes | No | **Effects on brain and welfare? (P-VI, P-X)** |
| End of experiment | Mostly survival | | Mostly euthanasia | | Post-scanning welfare? |

A: artery. V: vene. IM: intramuscular. IV: intravenous. Procedures described in **R-1**. *: procedures demonstrated in **R-2**. Major challenges written in **bold** are the topic for this dissertation.

we thought best suited to demonstrate in a video report. Anaesthetic drugs cause respiration depression, and intubation is recommended to avoid hypoxia and hypercapnia, but requires some practice, as the pig has narrow airways and a long snout (*Chum & Pacharinsak, 2012; Swindle and Smith, 2015*), and therefore not always practiced at all laboratories. We have observed that blood lactate is elevated in the pigs when untrained staff have performed the intubation, and in addition, intubation allows assisted respiration, through which stabilising blood gases probably ensure uniform blood flow in the brain. Bladder catheters have several benefits, including prevention of urinary contamination, fluid delivery monitoring and prevention of stress (resulting in tachycardia) due to a full bladder. Some tracers are excreted in urine (e.g. [$^{18}$F]FDG), and could therefore be removed from the scanner field-of-view. Transurethral bladder catheterization can only be performed in female pigs, but in males, diapers can be used to prevent contamination. The superficial veins that are best suited for tracer injection are central and ventrolateral ear veins, and they can easily be cannulated with an over-the-needle plastic catheter (e.g. Venflon) (*Alstrup, 2010; Pairis-Garcia et al., 2014*). This procedure

can be repeated at least 5-6 times, and as it is only slightly invasive, it does not require treatment with antibiotics and analgesia after PET scanning. It is therefore suitable for repeated scans of pig models for neurodegenerative disorders (**R-5**). Alternatively, femoral blood vessels are suitable for tracer injection and blood sampling (when arterial blood sampling is needed). Both the femoral artery and vein can be catheterized after a single skin incision, but the technique requires practice, as it is our experience that the pigs' blood vessels collapse easily. The catheters can also be placed under guidance with ultrasound scanning (*Izer et al., 2017*). In **R-2**, we demonstrate that the open-surgery procedure can be performed without dissecting the two vessels. Instead they are punctured with a Venflon and a guide wire is inserted. This approach may prevent destruction of tissue and enable the procedure to be carried out more quickly, in about 5-10 minutes rather than 10-15 minutes. The same technique can be used in both domestic pigs and minipigs.

## 2.4 Major challenges in PET scanning of pig brains

During functional imaging of the brain, such as PET scanning, it is crucial to keep the subject in good physiological condition (*Rowland and Cherry, 2008*). This is more difficult in pigs than in humans as it is necessary to anaesthetize the pigs, whereas humans can be PET scanned under awake conditions. PET scanning of pigs often takes several hours, including the time of preparatory surgical procedures. In practice, this means that the pigs are often anaesthetised for 2-8 hours, and sometimes significantly longer. At first thought, it would be best to avoid anaesthesia as it is hardly possible to anaesthetize a pig without affecting its brain. However, no reports of PET studies in awake pigs are available. Some other species such as primates, cats and rats have been PET scanned while awake, which avoids side effects of anaesthesia, that might influence the results (*Hassoun et al., 2003; Momosaki et al., 2004*). Awake and freely moving rats are PET scanned in the RatCAP mechanical system (*Schulz et al., 2011*) or by use of a motion tracking system (*Miranda, 2018*), while other laboratory animals (rodents, cats and primates) have been trained for PET scanning without anaesthesia in head-restraining devices (*Momosaki et al., 2004; Perlmutter et al., 1991; Hassoun et al., 2003*). Typically, the authors claim that their animals are not stressed during head restraining. However, that assumption is rarely documented, and it is known from other areas of research that both acute and chronic physical restraint are stressful for animals (*Lasbennes et al., 1986*). It is obvious that stress has negative effects on animal welfare and can alter the outcome of PET brain studies (*Patel et al., 2008*). In addition, the stress level of fixed experimental animals will vary, whereby more animals per study will be required to obtain statistically significant results (*Patel et al., 2008*). It is possible that pigs can be trained for a shorter period of time to lie stress-free in a PET scanner so that they can be scanned in the awake and unrestricted state. However, there are a number of challenges associated with training pigs for such procedures; it is very time-consuming to train animals, and it is hardly possible to keep pigs immobile for several hours. In addition, scans often occur in clinical scanners used for human patients where neither the room nor the scanner may be contaminated by the pigs (e.g. by the much debated methicillin resistant *Staphylococcus aureus*, MRSA bacteria (*Ploug et al., 2015*)), and it will hardly be practical to wrap awake pigs in plastic. Anaesthesia, therefore, seems to be a necessary evil in pig PET studies. **R-4** reviewed published animal studies on effects of anaesthesia in PET neuroimaging. We have reported on the only study showing the impact of anaesthetics in PET neuroimaging of the pig (our published conference abstract for a single tracer prior to **P-V**), whereas several such animal studies have been carried out on mice, rats, cats, dogs and primates. In addition, a minipig study of anaesthesia effects on [$^{18}$F]FDG was published simultaneously with our review (*Lee et al., 2012*). As expected, many of the anaesthetics evaluated in **R-4** affect certain molecular mechanisms in the animal brain. In general, anaesthesia lowers the metabolism of the brain, as shown by 22 % – 66 % reduced uptake of [$^{18}$F]FDG in several animal species (mice, rats and primates), except for medetomindin-tiletamine-zolazepam anaesthesia of dogs (*Lee et al., 2010*). The lowered uptake may not just be due to direct anaesthesia effects on the brain, but anaesthetics may also influence blood glucose and blood lipids, that indirectly can affect tracer uptake and brain metabolism. In the minipig, standard uptake values of [$^{18}$F]FDG are higher for propofol compared to ketamine, tiletamine-zolazepam or propofol/isoflurane anaesthesia (*Lee et al., 2012*).

Furthermore, anaesthesia may have major effects on cerebral blood flow, as it can be twice as high under isoflurane as under propofol anaesthesia (*Todd and Weeks, 1996*). This is particularly problematic when scanning with irreversibly binding tracers, which can be tested with Logan or similar plots (*Logan, 2000*), as their kinetics depend on cerebral

**Table 6:** Anaesthesia effects on PET tracers used for brain metabolism, dopamine, noradrenaline and serotonin neuroimaging in laboratory animals.

| Target and tracers | Species | Anaesthesia | Major effects |
|---|---|---|---|
| **Brain metabolism** | | | |
| [$^{18}$F]FDG | primates | isoflurane | Lower uptake than awake |
| | mice | isoflurane, ketamine-xylazine | Lower uptake than awake |
| | rats | ketamine, ketamine-xylazine, propofol, choral hydrate isoflurane | Lower uptake than awake |
| | dogs | medetomidin/tiletamine/zolazepam | Higher uptake than awake |
| | | propofol/isoflurane | Lower uptake than awake |
| | minipigs | propofol, ketamine etc | Higher uptake during propofol |
| **Dopamine transports** | | | |
| [$^{11}$C]cocaine analogues | primates | ketamine | Higher uptake than awake |
| [$^{18}$F]FECNT | primates | isoflurane dose | Dose-dependent reduction in tracer binding |
| [$^{11}$C]cocaine | rats | isoflurane/alpha-choralose | Faster clearance during isoflurane |
| **Dopamine $D_1$** | | | |
| [$^{11}$C]SCH23390 | rats | chloralhydrate | Increased binding than awake |
| [$^{11}$C]SCH23390 | rats | ketamine | Increased binding than awake |
| [$^{11}$C]SCH23390 | rats | pentobarbital | Decreased binding than awake |
| [$^{11}$C]SCH23390* | minipigs | isoflurane/propofol | Higher binding for isoflurane |
| **Dopamine $D_{2/3}$** | | | |
| [$^{18}$F]fluoroclebopride | primates | isoflurane/ketamine | No differences in uptake and washout |
| [$^{11}$C]MNPA | primates | xylazine-ketamine | Altered binding |
| [$^{11}$C]raclopride | rats | xylazine-ketamine | No differences in binding |
| [$^{11}$C]raclopride* | rats | isoflurane/ Hypnorm-Dormicum | Double binding for isoflurane |
| [$^{11}$C]raclopride | cats | halothane-ketamine | No differences in binding |
| [$^{11}$C](+)PHNO | rats | isoflurane | Enhanced tracer binding |
| [$^{11}$C]PHNO and [$^{11}$C]NPA | rats | isoflurane | Increased receptor binding |
| **Noradrenergic transporters** | | | |
| [$^{11}$C]- and [$^{18}$F]reboxetine analogues | primates | isoflurane/awake | No marked differences |
| **Noradrenergic alpha-2** | | | |
| [$^{11}$C]yohimbine* | minipigs | isoflurane/propofol | No differences |
| **Serotonin transporters** | primates | isoflurane/awake | No marked differences |
| [$^{18}$F]nortropane | | | |
| **Serotonin 1A receptors** | rats | isoflurane/awake | Higher distribution rate in awake |
| [$^{18}$F]FPWay | | | |
| **Serotonin 2A receptors** | minipigs | isoflurane/propofol | Higher strial binding during isoflurane |
| [$^{11}$C]MDL100,907* | | | |

Data from Table **1** in **R-4** (see this review for references) and Lee et al. (2012). *: data from papers **P-IV, P-V** and **P-XI**.

blood flow. Compared to isoflurane and other inhalation anaesthetics, propofol is better in permitting cerebral autoregulation (*Cosmo et al., 2005; Dagal and Lam, 2009*). However, propofol causes a 35 % reduction in brain oxygen metabolism and 39 % reduction in cerebral blood flow in pigs (*Lagerkranser et al., 1997*). In the existing literature (see also Table **6**), there is no consensus about the choice of anaesthetic protocols, but despite this the choice is rarely discussed in papers and very often only briefly described (e.g. *Plisson et al., 2014; Estrada et al., 2016; Hansen et al., 2019*).

Therefore, there is a need to investigate the effects of the anaesthesia protocols on the results of the PET brain scans, and whether knowledge obtained in one animal species can be transferred to others.

Results of PET scanning depend on the physiological condition of the animals, and therefore this must be controlled and documented during neuroimaging. Pigs cannot completely auto-regulate homeostasis during anaesthesia, especially not during long-term procedures, so monitoring of physiological processes is required. Furthermore, basic parameters must be monitored in pigs to assure optimal animal welfare (*Søfteland et al., 1995; Tremoleda et al., 2012*). Only a single study has been performed on monitoring parameters that are relevant to PET scanning of pig brain (*Poulsen et al., 1997*), and there it was shown that partial pressure carbon dioxide ($PaCO_2$) is important for cerebral blood flow measured with [$^{15}$O]water which indirectly also affects the kinetics of other tracers in the brain. However, it was only investigated for a narrow range (4 kPa to 6 kPa), corresponding to normocapnia, and no other monitoring parameters have been investigated. Older review papers suggest monitoring basic parameters (e.g. heart rate, respiration rate and body temperature) during animal imaging (*Balaban and Hampshire, 2001; Colby and Morenko, 2004; Klaunberg and Davis, 2008; Tremoleda et al., 2012*), but these recommendations do not focus on neuroimaging and they are not scientifically substantiated. Actually, this topic is amazingly poorly studied in the veterinary literature, although an increasing number of scientific articles based on imaging techniques in laboratory animals and pet animals are being published. In the existing literature, different monitoring parameters are reported, and even within the same group of researchers there is no consensus. Examples from the Aarhus PET Center include "*physiological functions were monitored continuously*" (*Smith et al., 1999*), "*blood pressure, heart rate, expired air carbon dioxide, and body temperature*" (*Danielsen et al., 1999*) and "*blood pressure, heart rate, $PaCO_2$, body temperature, plasma glucose etc*." (*Poulsen et al., 1997*). Several papers do not mention whether the pigs were monitored during scans. There is a need for further knowledge on which parameters are of importance, so that they can be controlled and deviations can be noted and reported in publications.

In addition to the importance of monitoring parameters on PET scanning results, monitoring is also important for safeguarding animal welfare in recovery studies. Thus, the pigs may have to be scanned for several hours and blood samples repeatedly collected (*Poulsen et al., 1997; Afzelius et al., 2016A; Afzelius et al., 2016B*), and sometimes the pigs are also transported between scanners (*Nielsen et al., 2015*), but the effects of these three stressors in combination have not been studied. Therefore, it is relevant to investigate how scanning procedures affect the pigs and whether it is possible to detect pathological organ changes, both in the cardiovascular system and in the brain, with the usual monitoring parameters. Here, blood lactate, which is a marker of anaerobic metabolism and therefore an indicator of hypoxia, could be a supplementary monitoring parameter that may be helpful during prolonged anaesthesia and transport. In addition to inducing anaemia, intensive blood sampling could also affect the concentration of blood cells. These aspects have never been investigated for pig PET studies. Major breed differences in blood lactate are known from other pig breeds, such as Erhualian pigs versus Pietrain pigs and Rheinhybrid-Pietran versus Landrace-Pietrain crossbreds (*Yang et al., 2012; Hofmaier et al., 2013*), but have never been investigated for Göttingen minipigs and the domestic pigs that are used for PET neuroimaging in Denmark. Furthermore, it is relevant to investigate whether blood lactate is dependent on the weight/age of the pig, the type of anaesthesia and the surgical procedure.

Some of the monitoring parameters are continuously measured (e.g. pulse and body temperature), while others require blood sample analysis (e.g. blood gases and blood cell count). This potentially involves a delayed analysis. Pre-analytical variation is known from human-based data from clinics, where evidence-based recommendations for the storage of blood samples exist (e.g. *Alström et al., 1993; Dukic et al., 2016*). Such recommendations usually also exist for pets (e.g. dogs and cats) as well as the most commonly used laboratory rodents (e.g. mice and rats), while they are inadequately investigated for pigs. Some old pre-analytical blood gas studies have been performed on porcine blood, but they are all based on venous blood samples taken from awake animals with body weights of 80-180 kg and stored for several hours (*Assal et al., 1980; van der Wal et al., 1981; Szenci et al., 1993*), which may differ from the typical pigs used for PET studies. Furthermore, pre-analytical variation in haematology has not been investigated in Göttingen minipigs, but is known in domestic pigs (see *Jain, 1986*). This is particularly relevant for studies of brain diseases with inflammatory components, e.g. white blood count in encephalitis/meningitis/abscesses (*Astrup et al., 2013*), platelet count in stroke (*Amantea et al., 2014*), or for monitoring of intensive blood sampling effects (blood haemoglobin, red blood cell count, white blood cell count, haematocrit and platelet count) during PET neuroimaging of minipigs. Therefore, there is a need for studies specifically aimed at determining the best preclinical handling of pig blood in PET studies.

PET scanning is considered to be a non-invasive method, and intravenous injection of tracers should also be conducted as non-invasively as possible, both to minimize

the effects on the pig's physiology and to ensure optimal animal welfare. In recovery studies, it is an advantage that catheters can be repeatedly placed in the ear veins (*Alstrup, 2010; Landau et al., 2018; Landau et al., 2019; Winterdahl et al., 2019*). Therefore, the ear vein is often used both for anaesthetic infusion and to inject tracers, but the latter can be problematic as the ear is located just outside the brain, and any residues of the tracers in the scanner's field-of-view can affect the scan results. Therefore, there is a need to investigate how residues of tracers in the ears can be minimized. Table **5** summarizes the major challenges for each procedure, and those in bold will be the focus in what follows.

## 2.5 Aims and hypotheses

The studies included in this dissertation are all based on the following principle: The daily work with PET scanning of pig brains has yielded problems which were initially solved through a literature search, and sometimes this work resulted in the publication of reviews (e.g. **R-3** and **R-4**). When it has not been possible to identify a useful solution, the problem has been solved by reusing data. The solution is then used in the subsequent experiments and, when possible, its usefulness was evaluated. To enable this work, the pig studies have, as often as possible, been carried out in a uniform manner throughout the years, and a nearly unchanged anaesthesia journal has been used for several years in which all relevant information has been recorded.

This dissertation is based on the following Need Statement, which should contribute to solve: "*Obtaining relevant knowledge that enables researchers to better perform useful PET scans of pig brains without harming the animals*". Furthermore, it should contribute to greater knowledge of factors of importance for preclinical imaging of experimental animals in general – an issue for many laboratory animal veterinarians, technicians and researchers in their daily work in preclinical research.

More specifically, the aim of this dissertation is to provide research findings in order to optimize procedures for PET scanning of pigs, both to minimize disruptive effects on PET results and to ensure good animal welfare. One important issue is to investigate how anaesthesia affects PET scanning of pig brains as a tool for selecting appropriate anaesthesia protocols. Another issue relates to how the pigs should be monitored during anaesthesia so that critical parameters are controlled and any adverse deviations are noted for later interpretation of results. Capnography is tested as an alternative to measuring carbon dioxide in blood gases. Blood lactate will be further investigated as a possible hypoxia marker during prolonged scanning of pigs. The monitoring parameters should include relevant parameters for the welfare of pigs during prolonged anaesthesia, during intensive blood sampling, and during transport between scanners. We need to know whether certain procedures lead to pathological effects on the brain of pigs, to examine how blood samples used for monitoring should be stored prior to analysis, and to investigate how tracers can best be injected in the ear veins of pigs while minimising residues that may influence PET brain imaging. Finally, the aim is also to show that existing data can be used to optimize future animal studies, the resuse-old-data-principle. This saves animal lives as well as time and money.

**Based on this, the following eight hypotheses are tested in this dissertation:**

**(1)** The choice of anaesthesia may influence the binding of several tracers in the brain (**P-IV, P-V, P-XI**).

**(2)** A number of monitoring parameters correlate with, or even causatively affect cerebral blood flow in pigs measured with [$^{15}$O]water PET (**P-III, P-IX**).

**(3)** Capnography can replace $PaCO_2$-measurements for anaesthesia monitoring in pigs (**P-VII**).

**(4)** Blood lactate concentration differs between pig breed, weight/age, and in response to type of anaesthesia, and surgical procedures (**P-VI**).

**(5)** Long-term anaesthesia, intensive blood sampling and road transport may affect the brain of pigs (**P-X**).

**(6)** It is possible to predict the pathological changes during prolonged anaesthesia with classical monitoring parameters and blood samples (e.g. blood lactate) (**P-X**).

**(7)** Blood samples for measurement of blood gases and blood cell count in pigs should be stored as recommended for human blood (**P-I, P-II**).

**(8)** It is possible to avoid residues of tracers in the ear vein catheter by dissolving the tracer in a large volume of saline and then flushing it with an equivalent volume of saline (**P-VIII**).

These eight hypotheses will be investigated in the eleven published papers.

# 3.0 The choice of anaesthesia in PET studies

Today, there is no gold standard for how to evaluate anaesthesia effects, but the review (**R-4**) shows that three approaches have been used in the literature. One approach involves simply to compare the effects of anaesthetics on PET brain findings. In this setup, groups of animals are anaesthetised with different drugs, but scanned with the same tracer, so the anaesthesia effects can be evaluated (*Kobayashi et al., 1995*). However, it is unknown whether any of the anaesthetics represent the awake state. The advantage of this approach is, nonetheless, that the studies are relatively easy to perform. We have used this approach both in papers **P-IV, P-V** and **P-XI**. A second approach requires PET imaging performed both in anaesthetised and awake laboratory animals (*Noda et al., 2003*). This approach allows an evaluation of the actual effects of anaesthesia, as it can be compared with unanaesthetised animals. The problem is, however, that if the awake animals are fixed during PET scanning, the condition is more likely to reflect animals in a stressed state. Thus, Patel and co-workers showed that physical restraint of awake rats had a marked effect on the binding potential of the dopamine $D_{2/3}$ receptor antagonist [$^{11}$C]raclopride, and that this effect is at least as strong as the effect of anaesthesia (*Patel et al., 2008*). The problems with the two first approaches are solved in the third approach, as it includes tracer injection in freely-moving and unstressed animals that are killed or anaesthetised just prior to PET scanning (*Elfving et al., 2003*). Such animals can be compared with anaesthetised animals, and therefore, this approach provides information on awake brain function. However, it typically requires many animals, scanned at various time points after tracer administration, and it is not possible to produce dynamic PET images for each animal.

## 3.1 Isoflurane versus propofol (P-V, P-XI)

As we – together with many other research groups – use either isoflurane or propofol for anaesthesia in almost all of our pig studies, we decided to compare isoflurane with propofol in two studies involving three commonly used monoaminergic PET tracers in our pig research, namely the dopamine $D_1$-receptor antagonist [$^{11}$C]SCH23390 (*DeJesus et al., 1989*), the alpha-2-adrenergic receptor antagonist [$^{11}$C]yohimbine (*Jakobsen et al., 2006*) and the 5-$HT_{2A}$ serotonin receptor

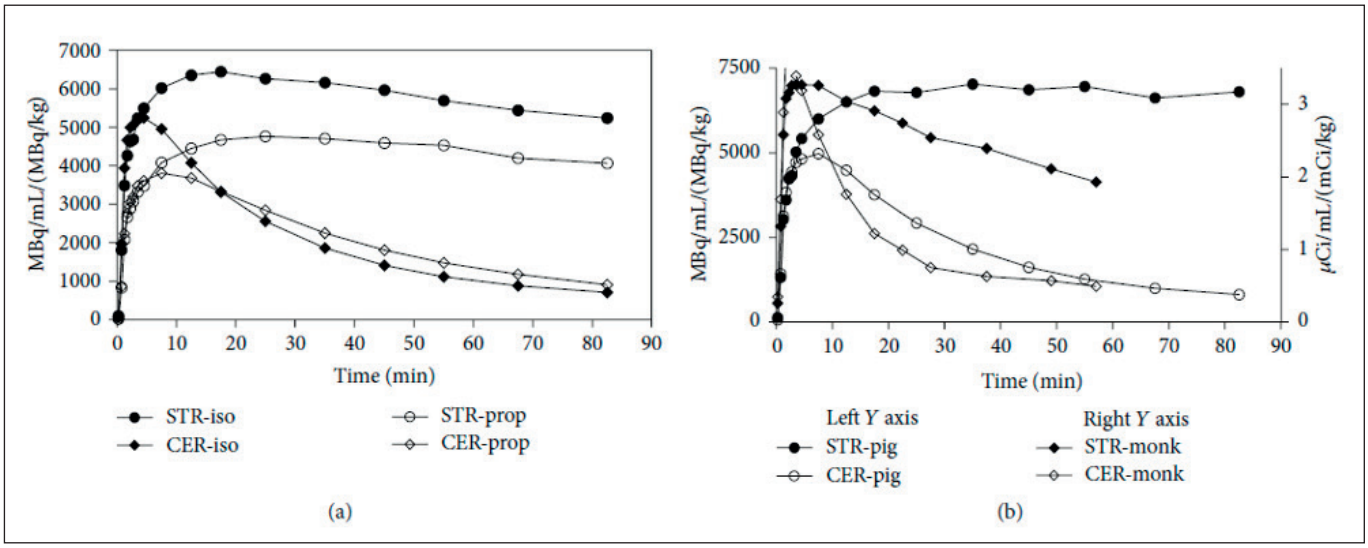



**Figure 2:** Time-activity-curves for [$^{11}$C]SCH23390 in striatum (STR) and cerebellum (CER) during isoflurane (iso) and propofol (prop) anaesthesia in 12 minipigs (**A**) and for comparison a representative minipig (pig) and a rhesus monkey (monk) under isoflurane anaesthesia (*Doudet et al., 2002*) (**B**). Data corrected for injected activity/kg body weight (Figure **2** in paper **P-V**).

**Table 7:** Animals, anaesthesia and monitoring parameters in paper **P-V** and **P-XI.**

| Variable | Isoflurane group | Propofol group | P value |
|---|---|---|---|
| *Göttingen minipigs* | | | |
| Number of pigs: | 6 (8 in **P-XI**) | 6 | - |
| Sex: | female | female | - |
| Weight* [kg]: | 27 ± 2 | 29 ± 4 | NS |
| *Anaesthesia* | | | |
| Premedication (IM): | midazolam+ketamine | midazolam+ketamine | - |
| Induction (IV): | midazolam+ketamine | midazolam+ketamine | - |
| Maintaining: | isoflurane | propofol | - |
| *Monitoring (mean and range)*:* | | | |
| Pulse [beat/min]: | 113 (86-143) | 76 (49-99) | <0.01 |
| $SatO_2$ [%]: | 98 (97-99) | 98 (96-99) | NS |
| Temperature [°C]: | 36.7 (35.5-38.0) | 36.3 (35.9-36.7) | NS |
| *Tracer injections in MBq (mean ± standard deviation):* | | | |
| [$^{11}$C]SCH23390 (**P-V**) | 244 ± 47 | 229 ± 88 | NS |
| [$^{11}$C]yohimbine (**P-V**) | 302 ± 18 | 333 ± 49 | NS |
| [$^{11}$C]MDL100,907 (**P-XI**) | 268 ± 59 | 294 ± 61 | NS |

IM: intramuscular. IV: intravenous. NS: non-significant. $SatO_2$: oxygen saturation. P values are based on ANOVA with Bonferroni correction. *: From **P-V.**

antagonist [$^{11}$C]MDL100,907 (*Landau et al., 2019*). The results of the first two tracers are published in **P-V** and the last tracer in **P-XI** as a brief article. While the exact mechanism of action for isoflurane and propofol is still unknown, both are able to potentiate the gamma-aminobutyric acid receptor and isoflurane also antagonizes the *N*-methyl *D*-aspartate receptor (*Karmarkar et al., 2010*). Furthermore, propofol is known to have a neuroprotective effect (*Fan et al., 2015*). Göttingen minipigs were randomly divided into two groups that were used in different experiments (*Landau et al., 2015; Landau et al., 2018; Landau et al., 2019*). Minipigs, anaesthesia protocols and monitoring parameters for the two papers (**P-V** and **P-XI**) are shown in Table **7**.

The uptake of [$^{11}$C]SCH23390 differed significantly

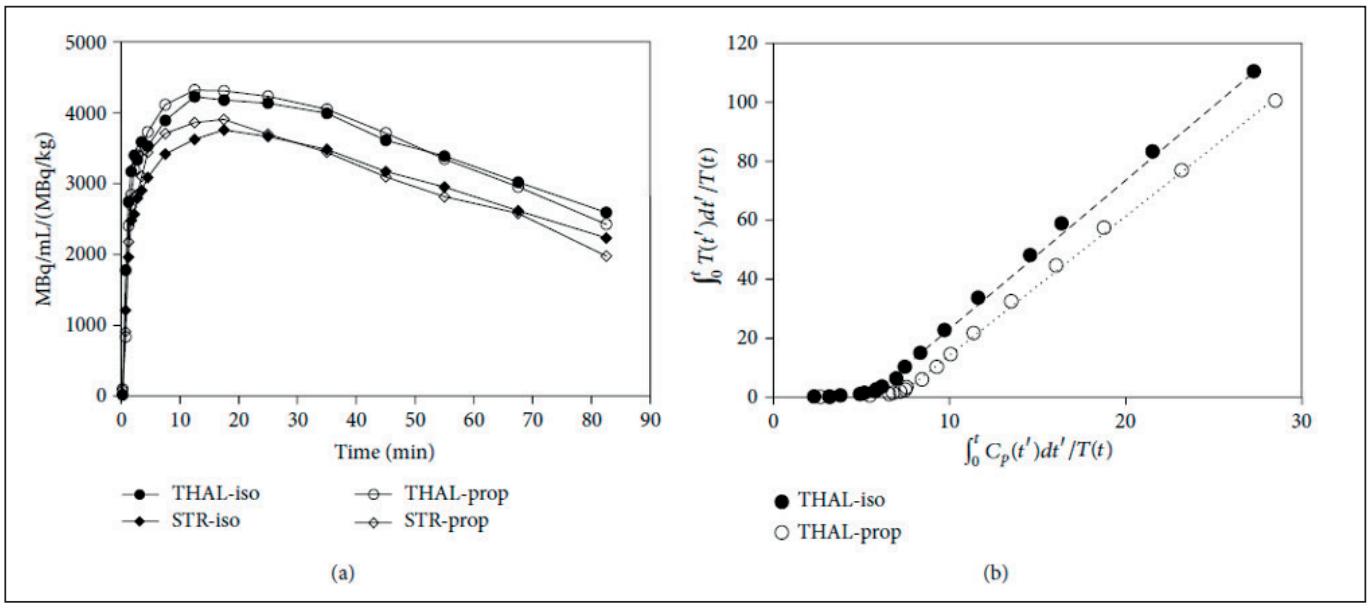



**Figure 3:** Time-activity-curves for twelve pigs (**A**) and Logan plots for two representative minipigs (please, note that the fitted lines are straight) (**B**) for [$^{11}$C]yohimbine binding in striatum (STR), and thalamus (THAL) during isoflurane (iso) and propofol (prop) anaesthesia. Data corrected for injected activity/kg body weight (Figure **1** in paper **P-V**).

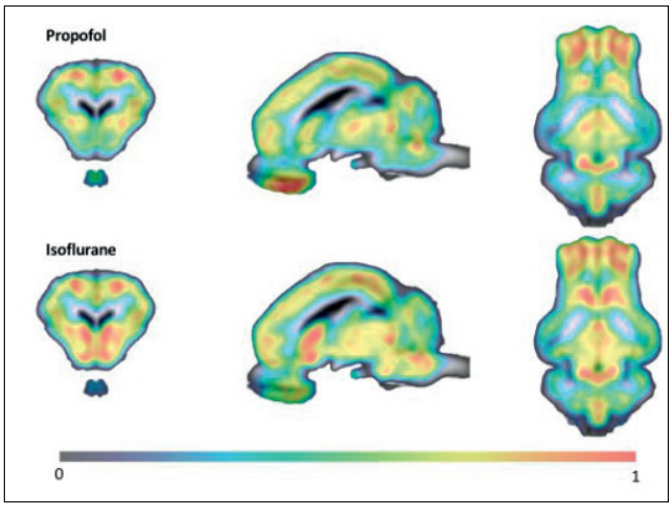



**Figure 4:** Binding of [$^{11}$C]MDL100,907 in Göttingen minipig brains during propofol (N=6) and isoflurane (N=8) anaesthesia. Images are shown as averaged voxel-vise non-displaceable binding potentials in coronal (left), sagittal and axial (right) frames (Figure **1** in paper **P-XI**).

between the two types of anaesthesia during the 90 minutes of scanning (see Figure **2**). The uptake was analysed with two different graphical methods, and they both showed that initial clearance values were higher in the minipigs anaesthetised with isoflurane than in the propofol group, which indicates differences in cerebral blood flow. The uptake was nearly irreversible in striatum based on Logan and Universal Graphical Method plots. This finding differs from earlier published studies in rhesus monkey (*Macaca mulatta*), where a reversible binding was found (*Doudet et al., 2002*), and that was surprising, as we would expect the same kinetics in brain from different species. These results indicate that the kinetics of [$^{11}$C]SCH23390 in pig brain differs from human and non-human primates and is strongly influenced by changes in cerebral blood flow in pigs.

The volumes of distribution of [$^{11}$C]yohimbine were similar in the two anaesthetised groups of Göttingen minipigs, which indicates that the kinetics are insensitive to changes in cerebral blood flow (Figure **3**). With the rapidly reversible binding, the kinetics of [$^{11}$C]yohimbine are independent of the fact that cerebral blood flow is high during isoflurane anaesthesia and low during propofol anaesthesia (*Todd and Weeks, 1996; Lagerkranser et al., 1997*). Instead, the uptake is primarily dependent on receptor density and affinity. Furthermore, the study showed that [$^{11}$C]yohimbine is a suitable neurotracer in Göttingen minipigs, as no metabolism has been detected, while yohimbine is variably metabolized in primates (*Corre et al., 2004*).

Also the binding of [$^{11}$C]MDL100,907 differed between the two types of anaesthesia, especially in the striatum. Under isoflurane anaesthesia, the binding potential was higher than obtained under propofol anaesthesia (Figure **4**). We attribute this difference, at least in part, to the higher blood flow in brain induced by isoflurane, which was substantiated by comparing relative delivery, R1 values, a surrogate marker for cerebral blood flow, under the two types of anaesthesia (Figure **5**): There was significantly higher R1 in isoflurane (1.97 ± 0.17) compared to propofol (1.62 ± 0.07). However, for either anaesthetic no correlations between R1 and binding potential were found in the striatum.

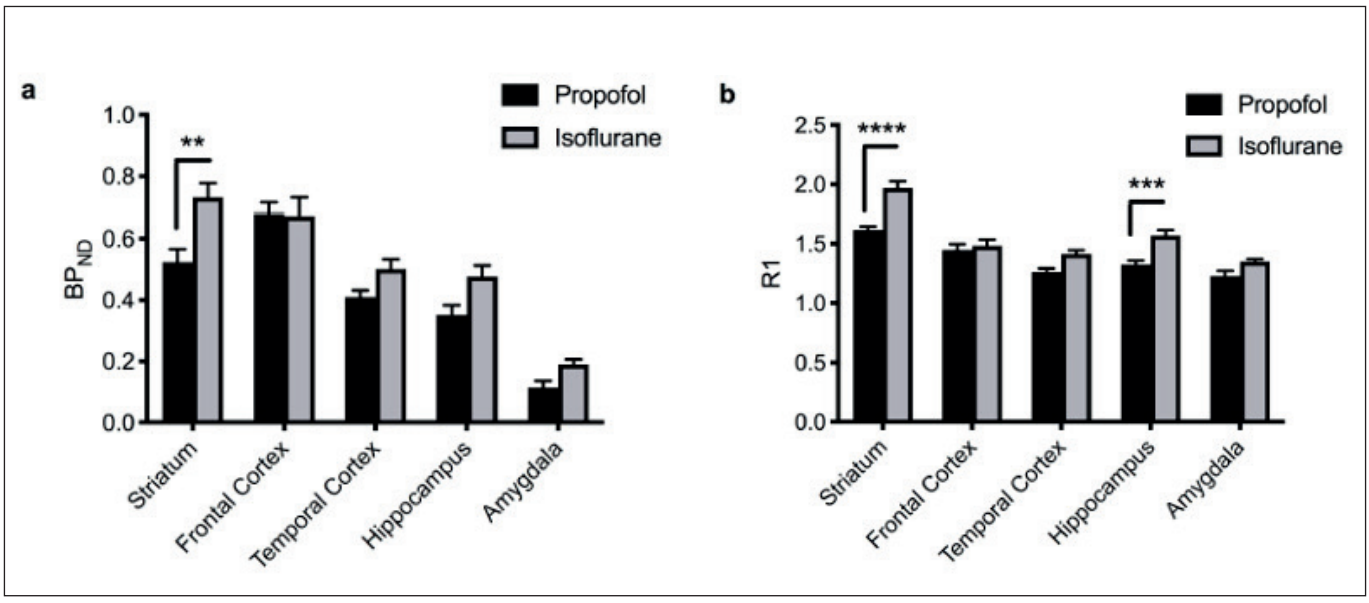



**Figure 5:** Binding potential (BP$_{ND}$) of [$^{11}$C]MDL100,907 (**A**) and R1 blood flow surrogate marker (**B**) in five brain regions during propofol and isoflurane anaesthesia in Göttingen minipigs Two-way ANOVA shows effects of both anaesthesia and region for (**A**) and (**B**). Bonferroni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

## 3.2 Isoflurane versus Hypnorm-Dormicum (P-IV)

Paper **P-IV** is the only study of the eleven included studies that has been performed in a species other than pig. This rat study is included as it demonstrates the marked effects of anaesthesia on tracer binding in the brain and as such, it supplements papers **P-V** and **P-XI**. Furthermore, we use the binding results to compare with typical intervention studies that we have performed in rats and pigs (*Lind et al., 2005B; Pedersen et al., 2007*). Nine inbred male Lewis-rats were randomly anaesthetised with either inhalation or injection anaesthesia before 90 minutes of PET brain scanning with [$^{11}$C]raclopride (Table **8**).

The results (data recorded during 30-90 minutes of steady state) of the microPET scans are shown in Table **8** and Figure **6**. Partial pressure oxygen ($PaO_2$), but not $PaCO_2$, differed between the groups. We found that rats anaesthetised with isoflurane had more than double the binding potential (1.95 ± 0.27) of [$^{11}$C]raclopride compared with Hypnorm-Dormicum (fentanyl-fluanisone-midazolam) anaesthetised rats (0.74 ± 0.24). This difference is higher than the typical effects found in intervention studies. For example, we previously found a 19 % reduction in [$^{11}$C]raclopride striatal binding after treating isoflurane anaesthetised Lewis-rats with 1 mg/kg *d*-amphetamine that increases synaptic dopamine (*Pedersen et al., 2007)*. In Göttingen minipigs, we found a 29 ± 9 % reduction in [$^{11}$C]raclopride striatal binding after treatment with 1 mg/kg *d*-amphetamine (*Lind et al., 2005B*).

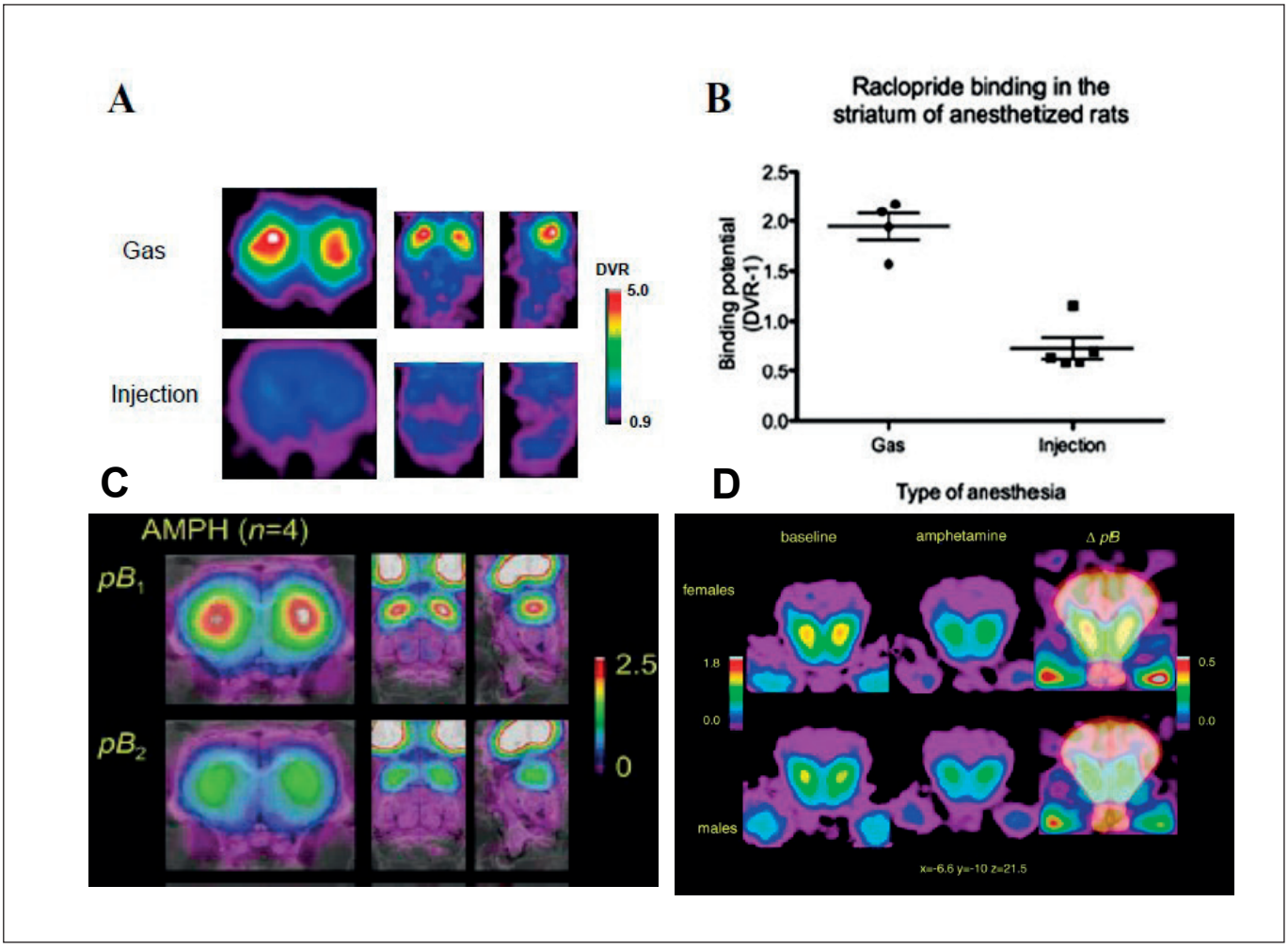



**Figure 6:** Interventional and anaesthetic effects on [$^{11}$C]raclopride in rats and minipigs: Two representative Lewis rats anaesthetised with isoflurane (gas) and fentanyl-fluanisone-midazolam (injection) (**A**). Binding potentials for each of the nine rats (circles represent the four isoflurane anaesthetised rats and squares represent the five fentanyl-fluanisone-midazolam anaesthetised rats) $P < 0.001$ with Student's t-test. Mean ± standard deviation (**B**). Four male Lewis-rats PET scanned under isoflurane anaesthesia with [$^{11}$C]raclopride before ($pB_1$) and after ($pB_2$) treatment with *d*-amphetamine (AMPH) (**C**). Six female and six male Göttingen minipigs PET scanned under isoflurane anaesthesia with [$^{11}$C]raclopride before and after treatment with *d*-amphetamine (**D**). DVR: Distribution volume ratio. pB: binding potential (delta means difference) (Figure **1** in paper **P-IV** supplemented with data from *Pedersen et al., 2007* and *Lind et al., 2005B*).

**Table 8:** Animals, anaesthesia, monitoring and tracer injections in paper **P-IV.**

| Variable | Isoflurane group | Hypnorm-Dormicum group | P value |
|---|---|---|---|
| *Lewis-rats (LEW/Han) (336 ± 37 gram)*<br>Number and sex: | 4 males | 5 males | - |
| *Anaesthesia*<br>Induction<br>Maintenance | box with isoflurane<br>isoflurane on mask | injection subcutaneously<br>1/3 dose every 30-60 minutes | -<br>- |
| *Monitoring*<br>$PaCO_2$ (before)<br>$PaCO_2$ (after)<br>$PaO_2$ (before)<br>$PaO_2$ (after) | <br>5.9 ± 0.5 kPa<br>5.2 ± 1.4 kPa<br>20.1 ± 2.5 kPa<br>18.8 ± 6.4 kPa | <br>7.6 ± 0.6 kPa<br>5.0 ± 0.9 kPa*<br>9.2 ± 1.2 kPa<br>12.9 ± 3.5 kPa | <br>0.05<br>NS<br><0.001<br><0.001 |
| *Tracer injection of [$^{11}$C]raclopride (17 ± 13 MBq)*<br>Scanning | <br>90 minutes | <br>90 minutes | <br>- |

NS: not significant. $PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen. P values are based on Student's t-tests. *: significant (P<0.05, paired Student's t-test) lower than before scanning. Data from paper **P-IV.**

Our results from papers **P-IV, P-V** and **P-XI** strongly indicate that anaesthesia may have a major influence on binding potentials of PET neurotracers of the dopamine and serotonin systems in laboratory animals. A tracer that is unaffected by cerebral blood flow in one animal species can turn out to be strongly dependent on a stable flow in another. Based on papers **R-4**, **P-IV**, **P-V** and **P-XI,** it is not possible to recommend a single useful anaesthetic for PET scanning of pig brains. The studies emphasize, however, that it is crucial that all published studies contain adequate information on anaesthesia protocol, and that knowledge about anaesthesia effects in one animal species cannot uncritically be transferred to other animal species.

# 4.0 Monitoring of pig physiology during PET scanning

Parameters that can be monitored readily during anaesthesia of pigs include the cardiovascular system comprising electrocardiogram, pulse, blood pressure, the pulmonary system including respiration rate, end-tidal carbon dioxide ($ETCO_2$), oxygen saturation ($SatO_2$), body temperature and reflexes, e.g. corneal, palpebral and interdigital (*Swindley and Smith, 2015*). Monitoring may also include arterial blood gases (pH, $PaCO_2$ and $PaO_2$), blood glucose and haematocrit measured in arterial blood samples. Invasive blood pressure (systolic and diastolic blood pressure) is a far more precise method than measuring non-invasive blood pressure in pigs, but requires insertion of a catheter into an artery (*Tuohy et al., 2017*). Pulse oximeters provide both insight into the pulse and $SatO_2$, and they can easily be placed on an ear, tongue or tail (*Alstrup, 2010*). To prevent hypothermia and hyperthermia, body temperature can continuously be measured. This section will investigate the importance of monitoring physiological parameters in pigs during PET scanning of the brain.

## 4.1 Correlations between monitoring and CBF (P-IX)

As already speculated, cerebral blood flow may affect the kinetics of [$^{11}$C]SCH23390, [$^{11}$C]MDL100,907, [$^{11}$C] raclopride and other brain radiotracers. Therefore, in paper **P-IX,** we studied the relationship between monitoring parameters and cerebral blood flow measured with dynamic [$^{15}$O]water PET, together with arterial blood sampling. As [$^{15}$O]water is a diffusion-limited tracer, the cerebral blood flow may be slightly underestimated, but the freely-diffusible [$^{11}$C]butanol is rarely used due to the long radioactive half-life of the isotope (*Herscovitch et al., 1987*). Historical data from PET studies of 37 pigs were collected. All these procedures started with a dynamic PET scan of the brain with [$^{15}$O]water,

**Table 9:** Animals and procedures in paper **P-IX, P-III** and **P-VII.**

| | |
|---|---|
| **Pigs** | Domestic pigs, 3 months, females. Necropsy: free of systemic diseases<br>**P-IX:** 38.1 ± 2.2 kg (mean ± S.D.). N=37<br>**P-III:** 40 kg (39-42 kg). N=4<br>**P-VII:** 40 kg (38-42 kg). N=9 |
| **Anaesthesia** | Premedication (IM) and induction (IV): ketamine and midazolam<br>Maintaining: isoflurane and $N_2O$ |
| **Surgery** | Femoral artery and vein catheters |
| **Monitoring parameters used in the papers** | **P-IX:** pH, $PaCO_2$, $PaO_2$, haematocrit, pulse, systolic blood pressure, diastolic blood pressure, blood glucose, body temperature, anaesthesia time versus cerebral blood flow<br>**P-III:** $PaCO_2$ versus cerebral blood flow and cerebral blood volume<br>**P-VII:** $ETCO_2$ (sidestream) versus $PaCO_2$ |
| **PET scanning** | **P-IX:** [$^{15}$O]water (500 MBq) – PET scanned for 5 minutes with arterial blood sampling from femoral artery after 79-314 minutes of anaesthesia<br>**P-III:** [$^{15}$O]water (800 MBq) and [$^{15}$O]carbon monoxide (800-2,000 MBq) – PET scanned for 5 minutes with arterial blood sampling from femoral artery |

$ETCO_2$: End-tidal carbon dioxide. IM: intramuscular. IV: intravenous. $N_2O$: nitrous oxide. $PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen.

**Table 10:** Mean, standard deviation (STD), Pearson correlation coefficient (r), P-value, step-wise regression (SWR) and number (N) of data for correlations between monitored parameters and cerebral blood flow in domestic pigs.

| **Unit** | **pH** | **$PaCO_2$** [kPa] | **$PaO_2$** [kPa] | **HCT** [%] | **Pulse** [/min] | **SBP** mmHg | **DBP** mmHg | **GLC** mmol/l | **TEMP** [°C] | **TIME** [min] |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mean* | 7.44 | 6.3 | 18 | 30 | 115 | 114 | 76 | 4.9 | 37.7 | 115 |
| *STD* | 0.04 | 0.7 | 5 | 3 | 25 | 14 | 17 | 1.4 | 1.3 | 68 |
| *r* | -0.35 | 0.45 | -0.22 | 0.22 | 0.49 | 0.06 | -0.07 | 0.13 | 0.41 | 0.26 |
| *P value* | **0.064** | **0.016** | 0.28 | 0.29 | **0.024** | 0.80 | 0.76 | 0.53 | **0.05** | 0.11 |
| *SWR* | - | + | - | - | - | - | - | - | + | - |
| *N* | 28 | 28 | 27 | 26 | 21 | 23 | 23 | 26 | 23 | 37 |

Positive correlation coefficients tend to increase together, whereas inverse relationships are observed with negative correlation. P-values prior to correction for multiple comparisons. **Bold** font indicates significant (or borderline significant) correlations before Benjamini Hochberg step-up procedure. $PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen. HCT: haematocrit. SBP: systolic blood pressure. DBP: diastolic blood pressure. GLC: glucose concentration. TEMP: temperature. TIME: anaesthesia length. Data (including legends) from Table **1** and **2** in paper **P-IX**.

before any interventions were performed. As some [$^{15}$O]water PET scans were delayed for reasons not related to the pigs, the effects of anaesthesia time were also included. The basic inclusion criteria for the animals, anaesthesia, monitoring and PET scanning are shown in Table **9**. The following parameters were excluded from the study: electrocardiograms (as we had already measured pulse), respiration rate (as the pigs were mechanically ventilated), $ETCO_2$ (as we used $PaCO_2$ – see section **4.3**), reflexes (as all of them were absent) and blood lactate (as our equipment could not measure that in 2006 and 2007).

As shown in Table **10**, cerebral blood flow (mean 0.54 ± 0.16 ml/ml/min) showed positive correlations with several variables, namely $PaCO_2$, pulse and body temperature, and negative correlation with pH. But after correcting for multiple comparisons (Benjamini-Hochberg step-up procedure), no significant statistical correlations were found between cerebral blood flow and any of the variables. However, a step-wise regression with forward selection showed that $PaCO_2$ (P=0.025) and body temperature (P=0.029) were important predictors of cerebral blood flow. No other parameters add significantly to cerebral blood flow prediction. This indicates that $PaCO_2$ and body temperature must be controlled for maintaining stable cerebral blood flow during PET scanning, so they should be carefully controlled and documented in published papers.

Cerebral circulation is auto-regulated so that cerebral blood flow is maintained between certain levels during varying physiological conditions (*Yoon et al., 2012*). Carbon dioxide is a potent cerebral vasodilator, as it releases local hydrogen ions by buffering with bicarbonate. The highest cerebral blood flow levels were found in pigs with highest $PaCO_2$ concentrations, in agreement with earlier porcine studies (*Poulsen et al., 1997; see also **P-III***). Cerebral blood flow was positively correlated with body temperature in the slightly hypothermic to normothermic pigs, in agreement with other porcine studies showing low cerebral blood flow during severe hypothermia (*Ehrlich et al., 2002; Sakoh and Gjedde, 2003*). This emphasizes the importance of monitoring and controlling body temperature during PET neuroimaging. Pigs become easily hypothermic during anaesthesia if they are not wrapped in blankets and, when necessary, heated with thermostatically-controlled blankets.

We also found that long-term isoflurane anaesthesia was correlated with low $PaO_2$, high pulse and body temperature (from slight hypothermia to normothermia). However, the duration of isoflurane anaesthesia did not show decisive influence on cerebral blood flow, even though isoflurane is known to increase cerebral blood flow. The weakness of this study is that only correlations, and no causal effects, can be determined. In the next section, the effect of one parameter, $PaCO_2$, was investigated experimentally.

## 4.2 Effects of hyper- and normocapnia on CBF (P-III)

The correlation between $PaCO_2$ and cerebral blood flow was shown in paper **P-IX**, but the causal connection was not investigated. The aim of paper **P-III** was to study the effects of $PaCO_2$ on cerebral blood flow measured with [$^{15}$O] water PET scans and cerebral blood volume measured with the true vascular tracer [$^{15}$O]carbon monoxide (*Martin et al., 1987*) in a wider range than the 4 kPa-6 kPa used by Poulsen and co-workers (1997). In paper **P-III,** four anaesthetised pigs (Table **9**) were PET scanned during a broad range of $PaCO_2$ levels (from 5.7 to 24.8 kPa). These levels represent both normocapnia and hypercapnia, which are expected values for properly ventilated pigs and for pigs anaesthetised without the use of respirator during prolonged anaesthesia. Changes in $PaCO_2$ were obtained by down-regulating air delivery from the respirator at least 20 minutes before PET scanning. The results are shown in Tables **11** (normocapnia) and **12** (hypercapnia), and in Figure **7**. Cerebral blood flow increased on average 54 % from mean of 0.48 ml blood/min/liter brain tissue during normocapnia (mean $PaCO_2$ = 6.5 kPa) to a mean of 0.79 ml blood/min/liter brain tissue during hypercapnia (mean $PaCO_2$ = 18.5 kPa). Similarly, cerebral blood volume increased 41 % from 0.061 ml blood/ml brain

**Table 11:** Cerebral blood flow and blood volume estimated by [$^{15}$O]water and [$^{15}$O]carbon monoxide positron emission tomography in pigs during normocapnia (5-8 kPa).

| | $PaCO_2$ | CBF | $V_0$ | CBV |
|---|---|---|---|---|
| Pig 1 | 7.26 | 0.70 | 0.10 | 0.058 |
| Pig 2 | 6.89<br>6.93<br>6.44 | 0.50<br>0.51<br>0.56 | 0.12<br>0.12<br>0.11 | 0.046<br>0.049<br>0.047 |
| Pig 3 | 5.69<br>6.36<br>6.11 | 0.44<br>0.40<br>0.42 | 0.11<br>0.16<br>0.19 | 0.088<br>0.076<br>ND |
| Pig 4 | 6.53<br>6.48 | 0.38<br>0.39 | 0.10<br>0.09 | ND<br>ND |

CBF: cerebral blood flow (ml blood/minutes/ml brain tissue). CBV: cerebral blood volume (ml blood/ml brain tissue). ND: not determined. $PaCO_2$: partial pressure of carbon dioxide (kPa). $V_0$: vascular volume (ml blood / ml brain tissue). Data from Table **1** in paper **P-III**.

**Table 12:** Cerebral blood flow and blood volume estimated by [$^{15}$O]water and [$^{15}$O]carbon monoxide positron emission tomography in pigs during hypercapnia (11-25 kPa).

| | $PaCO_2$ | CBF | $V_0$ | CBV |
|---|---|---|---|---|
| Pig 1 | 18.10 | 0.97 | 0.25 | 0.083 |
| Pig 2 | 15.84<br>17.00<br>17.21 | 0.71<br>0.78<br>0.73 | 0.19<br>0.16<br>0.20 | 0.063<br>0.061<br>0.060 |
| Pig 3 | 20.33<br>20.34 | 0.52<br>0.51 | 0.32<br>0.34 | 0.122<br>0.124 |
| Pig 4 | 11.14<br>22.06<br>24.77 | 0.77<br>0.84<br>0.82 | 0.22<br>0.19<br>0.18 | ND<br>ND<br>ND |

CBF: cerebral blood flow (ml blood/minutes/ml brain tissue). CBV: cerebral blood volume (ml blood/ml brain tissue). ND: not determined. $PaCO_2$: partial pressure of carbon dioxide (kPa). $V_0$: vascular volume (ml blood / ml brain tissue). Student's t-tests show that both CBF ($P<0.05$) and CBV ($P<0.01$) differs from the normocapnia state. Data from Table **1** in paper **P-III**.

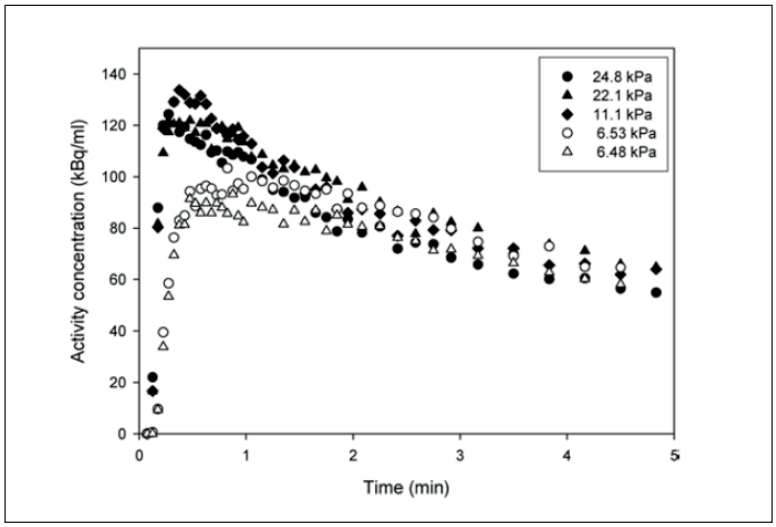



**Figure 7:** Time-activity-curves for five [$^{15}$O]water brain scans in pig number 4 during normocapnia (white symbols) and hypercapnia (black symbols). The curves show increased initial slope during hypercapnia compared to normocapnia (Figure **1** in paper **P-III**).

to 0.086 ml blood/ml brain upon changing from normocapnia to hypercapnia. The increases in blood flow and blood volume were widespread in the brains. The estimated vascular volumes in Tables **11** and **12** differ from cerebral blood volumes estimated with [$^{15}$O]carbon monoxide. Due to the low blood volume in the brain, it was not possible to estimate cerebral blood volume from [$^{15}$O]water.

This study underpins the causal link between increased $PaCO_2$ and increased cerebral blood flow, as suggested in the correlation study (paper **P-IX**). Furthermore, it demonstrates that the $PaCO_2$ concentration has a similar effect on cerebral blood volume (see Figure **8**). Thus accurate control of $PaCO_2$ concentration is crucial in PET brain studies.

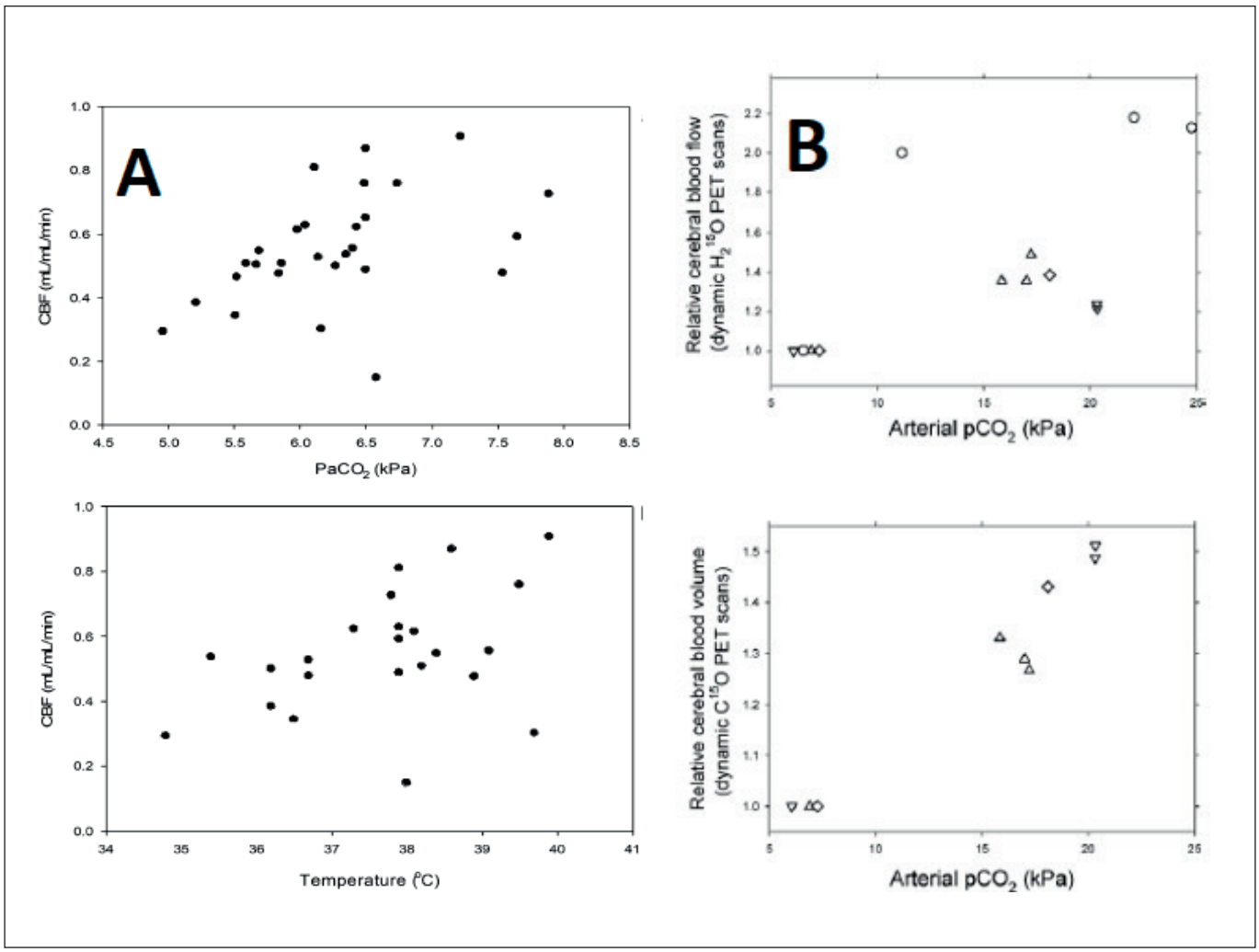



**Figure 8:** Comparison of results from the correlation and the intervention studies. Scatter plots of partial pressure carbon dioxide ($PaCO_2$) and body temperature on cerebral blood flow (CBF) (**A**), and effects of $PaCO_2$ on relative cerebral blood flow and relative cerebral blood volume (**B**) (Figure **1** in **P-IX** and Figure **2** in **P-III**).

## 4.3 Capnography can replace $PaCO_2$ in pigs (P-VII)

As an alternative to $PaCO_2$ measurements, exhaled carbon dioxide can be measured non-invasively by capnography ($ETCO_2$) monitoring in humans and animal species. End-tidal carbon dioxide is a time-saving method that continually provides measurements that can be used for documentation and correction of ventilator settings. Furthermore, capnography can reduce the amount of blood removed from the pig for blood gas analyses during the PET study. This correlation between $ETCO_2$ and $PaCO_2$ has been investigated in 9 domestic pigs (Table **9**) and published as a technical note (paper **P-VII**). Figure **9** shows a plot of $PaCO_2$ as a function of $ETCO_2$. The Bland-Altman plot shows good correlation between the two methods during both hypo-, normo- and hypercapnia. Therefore, $ETCO_2$ can replace measurements of $PaCO_2$ in pigs. The correlation can be described as:

$$\mathbf{PaCO_2\ [kPa] = ETCO_2\ [kPa] + 0.13\ kPa\ (\pm 0.63\ kPa)} \qquad \mathbf{r^2=0.9}$$

These results, first published as an abstract and poster (*Olsen et al. 2007*), are in agreement with the baseline measurements ($PaCO_2$ [kPa] = 0.96 * $ETCO_2$ [kPa] – 0.98 kPa; $r^2$=0.97) on 30-45 kg female Yorkshire pigs used to study severe chest injury during isoflurane anaesthesia (*Isbell et al., 2012* and a similar study by *Belenkiy et al., 2013*).

Taken together, these three studies (papers **P-III**, **P-VII** and **P-IX**) show that measurements of $ETCO_2$ (or $PaCO_2$) and body temperature are relevant, non-invasive methods for obtaining a stable cerebral blood flow during PET scanning. Of course, it is possible that there are other factors that should be monitored to ensure constant blood flow in the brain, and a constant cerebral blood flow does not necessarily give consistent kinetics of all brain tracers.

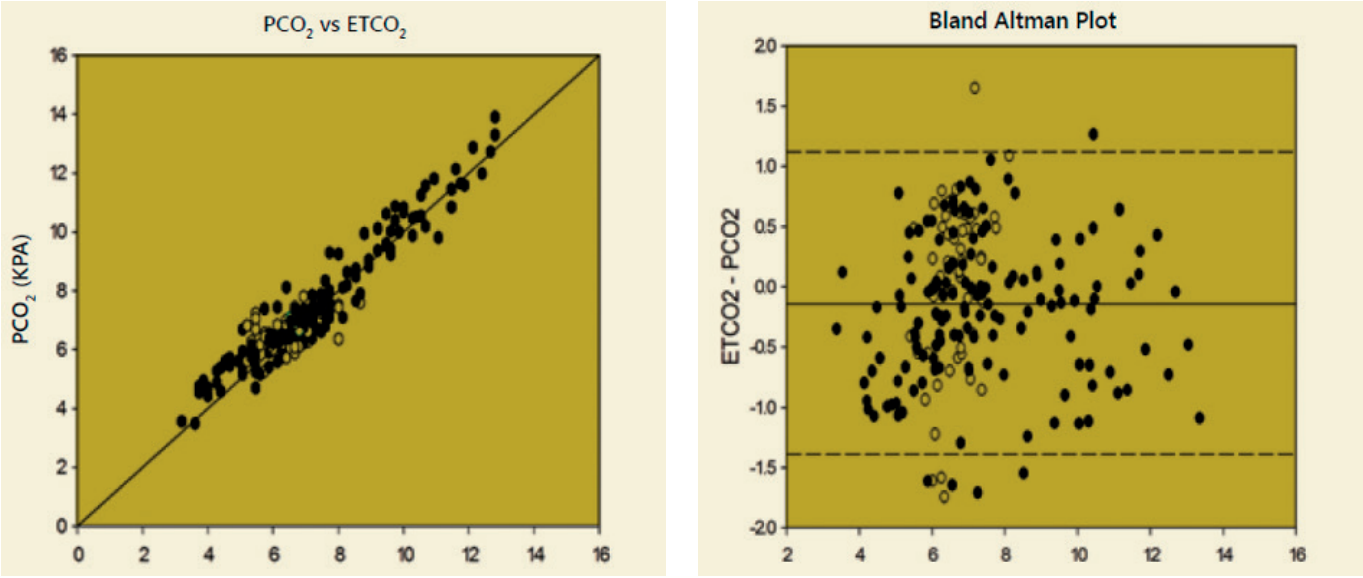



**Figure 9:** The relationship between end-tidal carbon dioxide ($ETCO_2$) and partial pressure carbon dioxide ($PCO_2$) in nine female domestic pigs during anaesthesia. Data were recorded both during normocapnia (o) and hypo- and hypercapnia (•). The left figure shows a plot of $PaCO_2$ measurements as a function of $ETCO_2$ measurements. The right figure is a Bland-Altman plot of the same data (Figure **1** and **2** in paper **P-VII**).

## 4.4 Use of blood lactate for monitoring pigs (P-VI)

Often, blood lactate is a `free' parameter when blood gases are analysed. In human clinical research elevated blood lactate is associated with poor outcome after heart surgery (*O'Connor and Fraser, 2012*). The question is, therefore, whether blood lactate concentration can be meaningfully used for monitoring pigs that are PET scanned, for example, during long-term scans, where the pigs are anaesthetised and placed in the same position for many hours. Initially, there is a need for basic knowledge of blood lactate concentration in pig breeds used in Denmark.

In paper **P-VI,** the effects of breed, weight/age, type of anaesthesia and surgery on blood lactate were evaluated based on former medical records from 81 female domestic and Göttingen minipigs used in a variety of PET studies of different organs supplemented with 11 extra pigs (Table **13** and **14**). Blood was sampled from the femoral artery approximately one hour after anaesthesia induction.

As shown in Table **14,** blood lactate concentrations were significantly higher in Göttingen minipigs than in domestic pigs, whereas anaesthesia type, body weight/age and surgery had no significant effect on blood lactate in domestic pigs. Data did not allow a similar comparison in minipigs.

This study compared, for the first time, blood lactate levels in Danish Landrace-Yorkshire crossbreeds and Göttingen minipigs. The reason why minipigs have higher levels than domestic pigs is still unknown, but it is unlikely due to the age differences between adult minipigs and young domestic pigs included in the study, as no age differences were found in our study (2-5 months) or in the study performed by Hofmaier *et al.* (2013). Blood glucose

**Table 13:** Characteristics of the pigs, anaesthesia and surgery performed in paper **P-VI**

| | |
|---|---|
| **Pig bred** | Domestic pigs (Danish Landrace – Yorkshire crossbreed) N=73 females<br>Göttingen minipigs (from Ellegaard Minipigs ApS) N=19 females |
| **Weight (age)** | 15 kg domestic pigs (2 months)<br>40 kg domestic pigs (3-4 months)<br>70 kg domestic pigs (5 months)<br>35 kg Göttingen minipigs (17 months) |
| **Anaesthesia** | Isoflurane<br>Propofol |
| **Surgery** | Minor: only femoral catheters (N=70) mainly for pig brain studies<br>Major: minor + lapatotomy (N=16), liver surgery (N=4) or craniotomy (N=2) |

Data from paper **P-VI** supplemented with 15 kg domestic pigs (N=11).

**Table 14:** Blood lactate levels (mmol/l; mean ± standard deviation) in pigs.

| Pig breed | N | Anaesthesia | Weight | Surgery | Blood Lactate | P value |
|---|---|---|---|---|---|---|
| Göttingen minipigs | 19 | Isoflurane | 35 kg | Minor | 2.53 ± 1.10 | <0.001 |
| Domestic pigs | 11 | Isoflurane | 15 kg | Minor | 0.75 ± 0.20 | NS |
| 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 |

N: number of pigs. NS: not significant. P values based on Welch-Satterthwaite's t test. Data from Table **1** in paper **P-VI** supplemented with 15 kg domestic pigs.

was somewhat lower in minipigs (3.4 ± 1.2 mmol/l) than in domestic pigs (4.8 ± 1.2 mmol/l), which might indicate increased glycolysis leading to increased blood lactate in Göttingen minipigs. Domestic pigs are here closer to human reference intervals of both blood lactate (0.5-1.6 mmol/l) and blood glucose (3.3-5.7 mmol/l) (*Burtis and Ashwood, 1996*). The observed breed difference may also exist in awake pigs, as we measured 1.8 mmol/l and 2.7 mmol/l in two slightly sedated Göttingen minipigs (27 to 29 kg), while blood lactate was only 0.7 mmol/kg and 0.8 mmol/kg in two slightly sedated domestic pigs (22 to 23 kg). Paper **P-VI** indicate that reference values for blood lactate should be based on pig breed.

Neither type of anaesthesia nor surgery affected blood lactate levels. This may indicate that both isoflurane and propofol are useful drugs for anaesthesia without leading to hypoxia. Note that surgery did not affect blood lactate levels. However, in the two domestic pigs that underwent craniotomy, high blood lactate concentrations were found (1.2 and 1.5 mmol/l), and this perhaps indicates that craniotomy has a greater effect on blood lactate than laparatomy with or without liver surgery. Major brain surgeries (e.g. stereotaxic craniotomy) are always performed during buprenorphine/fentanyl analgesia and local analgesia with lidocain and bupivacain (*Alstrup et al., 2017*).

In the next chapter we will see that blood lactate concentration is fairly stable in anaesthetised pigs, even after many hours of anaesthesia. However, road transportation under suboptimal anaesthesia conditions can significantly increase blood-lactate concentration for many hours.


Monitoring of anaesthetized pigs during PET scanning.

# 5.0 Long-term PET scanning procedures in pigs

## 5.1 Monitoring during long-term experiments (P-X)

How well do pigs used for imaging studies tolerate long-term anaesthesia without it affecting their brains and other organs, and how suitable are classic monitoring parameters to determine this? In paper **P-X**, we had the opportunity to investigate these questions in a series of pig experiments that were performed for the development of new tracers for diagnosing osteomyelitis. Brains were also PET scanned in these pigs. The protocol includes long-term anaesthesia (up to 18 hours), blood sampling (up to 20 ml blood per kg pig), road transportation (up to 1½ hours between two imaging centres) and pigs with or without symptoms of *Staphylococcus aureus* infections (see also *Alstrup et al., 2016*) (Table **15**). This setup, which ended with the euthanasia of all pigs, includes extreme physiological impacts, so potential brain and heart pathology should arise. In addition to the classical monitoring parameters and blood gas measurements, we also measured leukocyte concentrations and performed a series of whole body CT scans as well as static brain [$^{18}$F]FDG PET scans. Necropsy was performed, and brains and hearts were examined histologically. Brain sections were Haematoxylin and Eosin and Fluoro-Jade B-stained (*Schmued and Hopkins, 2000*) to identify micro-pathology and degenerating neurons (hypotic necrosis). As blood lactate was repeatedly measured in the pigs, we also

**Table 15:** Characteristics of the 18 female pigs used in paper **P-X**.

| | **Group I (pig I-IX)** | **Group II (pig X-XVIII)** |
|---|---|---|
| Number | 9 | 9 |
| Pig breed | 20 (and a few 40) kg domestic pigs | 20 kg domestic pigs |
| Pre-treatment seven days before | Inoculation with *Staphylococcus* aureus | Inoculation with *Staphylococcus* aureus |
| Anaesthesia (dorsal recumbence) | 15-18 hours with propofol (one pig died after 6 hours) | 8-14 hours with propofol |
| Blood sampling | 20 ml blood per kg | 14 ml blood per kg |
| Road transportation | 1½ hours from Aarhus to Aalborg | No road transportation |
| Monitoring parameters | Pulse, $SatO_2$, temperature, urine production | Pulse, $SatO_2$, temperature, urine production |
| Arterial blood parameters (every second hour) | $PaCO_2$, $PaO_2$, glucose, lactate, haematocrit | $PaCO_2$, $PaO_2$, glucose, lactate, haematocrit |
| Venous blood parameters (every second hour) | Leukocytes, lymphocytes, neutrophils, eosinophils | - |
| CT- and PET-scannings | 3-5 CT scans + brain 4-5 MBq/kg [$^{18}$F]FDG-PET | 3 CT scans + brain 4-5 MBq/kg [$^{18}$F]FDG-PET |
| Necropsy | Yes, performed by two veterinary pathologists | Yes, performed by two veterinary pathologists |
| Histopathological examination | Brains and hearts | Brains and hearts |

[$^{18}$F]FDG: 2-[$^{18}$F]fluoro-2-deoxy-D-glucose. $PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen. $SatO_2$: oxygen saturation.

**Table 16:** Main results of monitoring, arterial blood analyses and leukocyte counts in paper **P-X**.

| Parameter | Trend |
|---|---|
| **Both groups:** | |
| $SatO_2$ | High (over 97%) throughout the anaesthesia |
| Temperature | Relatively constant (37.4-39.3 °C) throughout the up to 18 hours of anaesthesia |
| Total urine | Continuous production of urine of approximately 115 ml/hour* |
| $PaCO_2$ | Stable (5.9-7.8 kPa) throughout the anaesthesia period |
| Glucose | Limited, non-significant decline from 5.2 mmol/l to 3.5 mmol/l after 18 hours in group I |
| **Group I:** | |
| White blood cells | A non-significant dilution effect during blood sampling (from 15.8 to 12.3 x $10^9$/l)<br>Also, non-significant dilution of lymphocytes and neutrophils, while eosinophia was unchanged |

*: saline was administrated slowly (35-45 drops/minute). $PaCO_2$: partial pressure carbon dioxide. $SatO_2$: oxygen saturation. Data modified from Table **1**, **2** and **3** in paper **P-X**.

saw this as an obvious opportunity to investigate whether high blood lactate is associated with brain hypoxia in pig studies.

Table **16** and Figure **10** show trends in monitoring parameters, arterial blood analyses and leukocyte counts for the included pigs. No systematic differences in the continuous monitoring parameters were found, except a temporary increase in pulse during road transportation. Temperature, urine production and $SatO_2$ were all stable, although the latter was difficult to measure after 16 hours of anaesthesia due to impaired peripheral circulation. While $PaCO_2$ was fairly stable, $PaO_2$ decreased during road transportation and remained low until euthanasia. In contrast, blood lactate increased 5-fold during transport and remained higher than before. Blood glucose decreased slowly, as the pigs were not treated with glucose infusion, but only 1-2 liter saline. Haematocrit only decreased in the pigs with intensive blood collection (and not even significantly), while it was stable in the other pigs. Leukocytes and neutrophils were increased after inoculation. Leukocytes, lymphocytes and neutrophils were non-significantly decreased on the scanning day, probably due to dilution effects caused by the intense blood sampling and saline infusion.

The necropsies showed different degrees of atelectasis in lungs of 17 out of 18 pigs, which was also confirmed by CT scans showing progression during anaesthesia. This may partly be due to the fact that the pigs were placed on their backs during anaesthesia – a position favorable for blood sampling from the femoral artery but not optimal for lung function (*Nyman et al., 1990*). The most common finding in the abdomen was increased fluid found in 13 of the pigs (89 % of the long-term anaesthesia pigs and 44 % in the rest) probably caused by reduced cardiac function. Remarkably, no pathological changes were found in any of the brains, and all Fluoro-Jade-B were negative for neuron hypoxia. Furthermore, the uptake of [$^{18}$F]FDG (Table **17**) was similar in all pig brains and consistent with another published pig study (*Lee et al., 2012*). Even pigs with high blood lactate did not have brain necrosis, perhaps due to the neuroprotective effect of propofol or cerebral autoregulation of blood flow (*Fan et al., 2015; Thomassen et al., 2018*). Of the 18 pigs, two were without symptoms of infections,

**Table 17:** Brain necropsy, histology, Fluoro-Jade-B and median (min-max) [$^{18}$F]FDG uptake.

| Group | Necropsy | Histology | Fluoro-Jade-Color | SUV [$^{18}$F]FDG |
|---|---|---|---|---|
| **I** | No findings (N=9) | No findings (N=9) | All negative (N=9) | 1.8 g/ml (1.5-2.6) (N=9) |
| **II** | No findings (N=9) | No findings (N=9) | All negative (N=8) | 1.9 g/ml (1.4-3.0) (N=9) |

[$^{18}$F] FDG: 2-[$^{18}$F]fluoro-2-deoxy-D-glucose. N: number. SUV: standardized uptake values. No significant differences detected. Data from paper **P-X**.

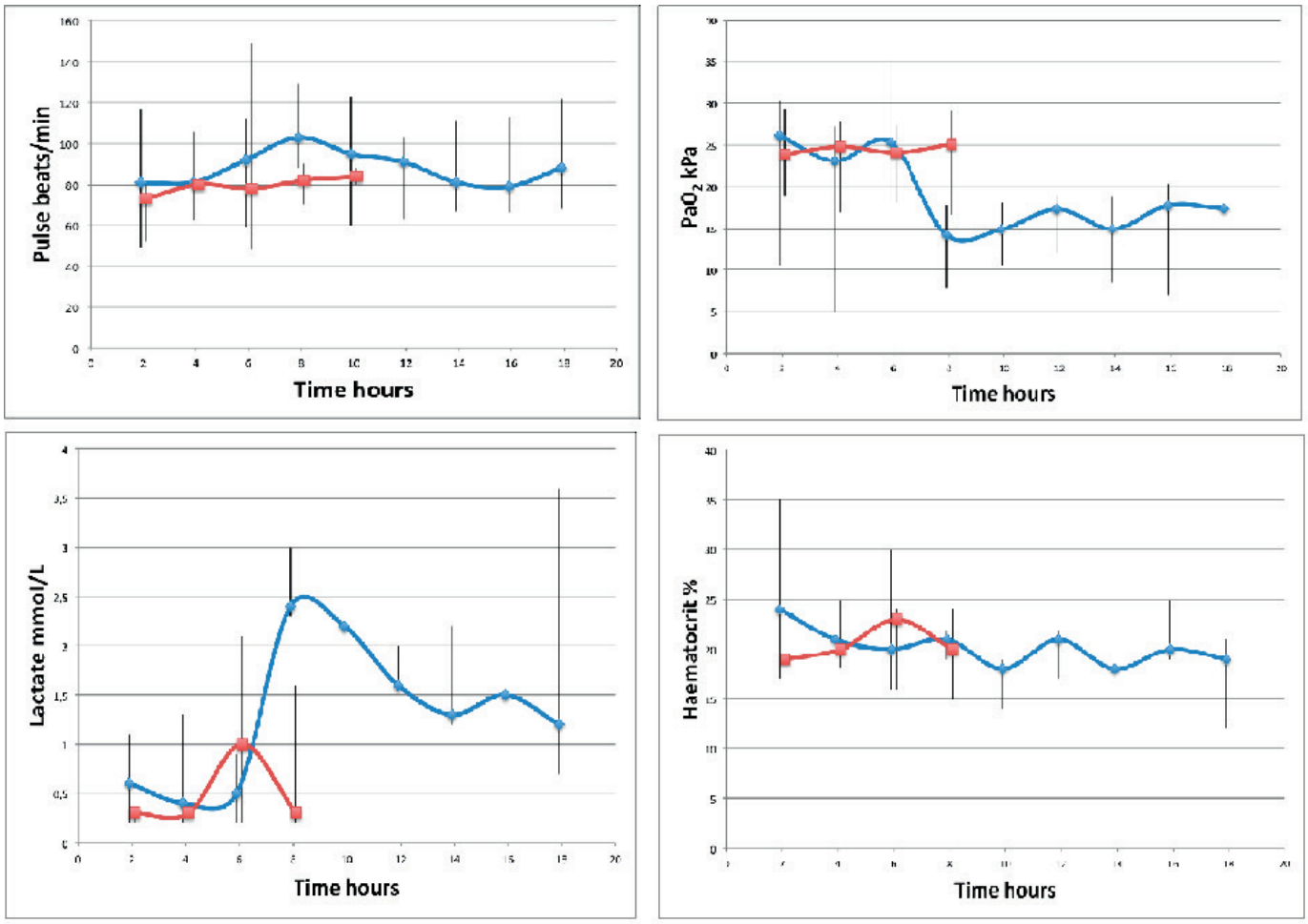



**Figure 10:** Pulse, partial pressure oxygen ($PaO_2$), blood lactate and haematocrit in group I, pigs I-IX (blue curves) and group II, pigs X-XVIII (red curves). Transportation between 6 and 8 hours of anaesthesia in group I, but not in group II. Median and range. Significant differences ($P<0.05$) in pulse, $PaO_2$ and lactate after 8 hours based on Mann-Whitney U-test with Bonferroni correction (Data from Table **1** and **2** in paper **P-X**).

and they did not differ from the other pigs in monitoring and non-infection related pathological findings. The study suggests that brain hypoxia is not a real risk of ordinary PET scanning of pigs which are maintained throughout on artificial ventilation.

This study shows that the cardiovascular system of pigs can be markedly affected by long-term PET scanning procedures, although basic monitoring parameters are unaltered. However, we could not determine whether it is the long-term anaesthesia, blood sampling or road transportation that has burdened the pigs. Probably, a combination of factors is involved. Nevertheless, the findings emphasize that during certain experimental procedures, there is a need for wider monitoring of pigs than just the parameters known to affect PET scans of the brain. Optionally, these studies may be supplemented with CT scanning, which may also detect pathological changes in internal organs. Although the brain was not affected in the pigs, the influence on the heart and other internal organs could still affect the kinetics of brain tracers.

## 5.2 Pre-analytical handling of blood samples (P-I, P-II)

Monitoring of anaesthetised pigs can either take place continuously (e.g. $ETCO_2$) or may be time-shifted (e.g. $PaCO_2$). For the latter, it raises the question of how blood samples should be stored prior to analysis. We have carried out two studies (Table **18**) in pigs in order to assess pre-analytical handling of arterial blood samples used for

**Table 18:** Pigs, techniques and storage variables for papers **P-II** and **P-I**.

| | | Paper | |
|---|---|---|---|
| | | **P-II** | **P-I** |
| **Pigs** | **Breed** | Domestic pig | Göttingen minipig |
| | **Weight/age** | 37-41 kg / 3 months | 18.5-25.0 kg / 9 months |
| | **Sex** | Female | Male (hogs) |
| | **Number** | 6 | 6 |
| **Techniques** | **Anaesthesia** | Isoflurane + nitrous oxide | No anaesthesia. Fixated in backrest |
| | **Sampling** | Femoral artery catheter | Puncture of cranial vena cava |
| | **Anticoagulant** | Heparin | EDTA and CTAD |
| **Storage** | **Time** | 5, 15, 30, 45 & 60 minutes | ½, 1½, 3½, 5½, 7½, 25½ & 27½ hours |
| | **Temperature** | 4 °C & 20 °C | 5 °C & 20 °C |
| | **Variables** | pH, $PaCO_2$ & $PaO_2$ | Blood haemoglobin, red blood cell count, white blood cell count, haematocrit & platelet count |

CTAD: citric acid-theophylline-adenosine-dipyridamole. EDTA: ethylenediamine-tetraacetic-acid. $PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen.

blood gases (paper **P-II**) and venous blood samples used for haematology (paper **P-I**).

Paper **P-II** was performed to investigate the short-term (hour) effects of storage time and temperature on pH, $PaCO_2$ and $PaO_2$ in arterial blood drawn from anaesthetised domestic pigs during PET scanning of the brain. Paper **P-I** was performed to investigate long-term (day) effects of storage time, temperature and anticoagulant on haematological variables in Göttingen minipigs. The difference in storage time between the two studies reflects that most PET research units have access to blood gas analysers, while haematological examinations must often be transported to a central laboratory. Blood gases can be adjusted to compensate ventilator settings quickly, while this is not possible for haematology.

Results for paper **P-II** on the blood gases are shown

**Table 19:** Observed effects of storage time and temperature on pH, $PaCO_2$ and $PaO_2$ in arterial blood samples obtained from domestic pigs.

| **Storage temp.** [°C] | **Time** [min] | **pH** | **$PaCO_2$** [kPa] | **$PaO_2$** [kPa] |
|---|---|---|---|---|
| **4** | 5 | 7.438 (7.413-7.470) | 5.85 (5.39-6.27) | 14.43 (11.50-15.93) |
| | 15 | 7.434 (7.408-7.466)* | 5.96 (5.45-6.34)* | 14.35 (11.91-15.87) |
| | 30 | 7.431 (7.408-7.461)* | 5.97 (5.56-6.26)* | 14.45 (12.17-16.20)* |
| | 45 | 7.435 (7.412-7.466)* | 5.97 (5.51-6.35)* | 14.31 (12.59-15.62) |
| | 60 | 7.438 (7.420-7.475) | 5.87 (5.49-6.16) | 14.90 (12.26-16.01)* |
| Time effects | | P<0.001 | P<0.001 | P=0.002 |
| **20** | 5 | 7.437 (7.424-7.470) | 5.83 (5.41-6.16) | 13.89 (11.62-14.60) |
| | 15 | 7.432 (7.412-7.460)* | 5.87 (5.43-6.20)* | 13.77 (11.79-14.63) |
| | 30 | 7.428 (7.406-7.462)* | 5.90 (5.51-6.31)* | 13.62 (12.68-14.17) |
| | 45 | 7.429 (7.400-7.458)* | 5.89 (5.54-6.33)* | 12.95 (11.14-13.57)* |
| | 60 | 7.429 (7.395-7.461)* | 5.91 (5.60-6.32)* | 11.94 (11.09-13.74)* |
| Time effects | | P<0.001 | P<0.001 | P<0.001 |

$PaCO_2$: partial pressure carbon dioxide. $PaO_2$: partial pressure oxygen. Values shown in table are medians (25-75 percentiles). Variation over time was analysed with Friedman's two-way analysis of variance. *different from baseline values (T=5 minutes) (Wilcoxon's test; P<0.05). Coefficient of variation was 0.3 % (pH), 0.2 % ($PaCO_2$) and 2.2 % ($PaO_2$). Data from Table **1**, **2** and **3** in paper **P-II**.

**Table 20:** Pre-analytical effects of anticoagulants, storage time and temperature on haematological variables in Göttingen minipigs.

| | Anticoagulatia | Temperature [°C] Baseline (median) | Time effects* |
|---|---|---|---|
| **Blood haemoglobin** | EDTA CV = 1.54 % | 5 (8.33 mmol/l) | NS |
| | | 20 (8.39 mmol/l | P=0.05 |
| | CTAD CV = 0.84 % | 5 (7.60 mmol/l) | NS |
| | | 20 (7.45 mmol/l) | NS |
| **Red blood cell count** | EDTA CV = 1.58 % | 5 ($8.29x10^{12}$/l) | NS |
| | | 20 ($8.22x10^{12}$/l) | P<0.05 |
| | CTAD CV = 1.04 % | 5 ($7.52x10^{12}$/l) | NS |
| | | 20 ($7.39x10^{12}$/l) | NS |
| **White blood cell count** | EDTA CV = 2.24 % | 5 ($14.60x10^{9}$/l) | NS |
| | | 20 ($14.45x10^{9}$/l) | P<0.001, higher at 25½ and 27½ hours |
| | CTAD CV = 3.09 % | 5 ($13.30x10^{9}$/l) | NS |
| | | 20 ($13.40x10^{9}$/l) | NS |
| **Haematocrit** | EDTA CV = 2.08 % | 5 (39.5 %) | NS |
| | | 20 (39.4 %) | P<0.001, higher at 25½ and 27½ hours |
| | CTAD CV = 1.24 % | 5 (36.5 %) | NS |
| | | 20 (35.9 %) | P<0.01, higher at 25½ hours |
| **Platelet count** | EDTA CV = 3.10 % | 5 ($597x10^{9}$/l) | P<0.01, lower between 5½ and 27½ hours |
| | | 20 ($640x10^{9}$/l) | NS |
| | CTAD CV = 3.30 % | 5 ($516x10^{9}$/l) | P<0.05, lower at 25½ hours |
| | | 20 ($541x10^{9}$/l | NS |

For all five variables, baseline values were significantly lower in CTAD tubes than EDTA tubes due to the 10 % diluting effects in CTAD. *: Friedmann's analysis of variance and Dunnett's test for comparing with baseline. CV: coefficient of variation. CTAD: citric acid-theophylline-adenosine-dipyridamole. EDTA: ethylenediamine-tetraacetic-acid. NS: non-significant. Data from Tables **1-5** in paper **P-I**.

in Table **19**. There were significant variations in all three parameters for heparinized blood stored at both temperatures. The pH was decreased and $PaCO_2$ was increased at both temperatures. The median $PaO_2$ was variable at 4 °C and decreased at 20 °C. In general, the variations for all three variables were higher at 20 °C than at 4 °C, and these results agree with previous studies performed on pig blood (*Assal et al., 1980; van der Wal et al., 1981; Szenci et al., 1993*). Probably, the decrease in pH is a consequence of hydrogen ion generation from anaerobic glycolysis, and this is also in agreement with the increase in $PaCO_2$. Similarly, the decrease in $PaO_2$ at room temperature is probably due to oxygen

consumption by blood cells. The results of paper **P-II** indicate that pre-analytical time delay can cause increased variation and should therefore be limited as much as possible. If analysis is delayed more than five minutes, the blood sample should be placed on crushed ice or in a refrigerator. However, as already noted, delayed analysis should be avoided, in order to correct ventilation parameters.

Results for paper **P-I** on haematology are summarized in Table **20**. No changes were seen in blood haemoglobin and red blood cell count in citric acid-theophylline-adenosine-dypyridam (CTAD) tubes at both temperatures and in ethylene-diamine-tetraacetic-acid (EDTA) tubes at 5 °C. However, for EDTA tubes at 20 °C, small variations were observed for both variables. White blood cell counts were increased after 25½ and 27½ hours in EDTA tubes at 20 °C, but not at 5 °C and not in CTAD tubes at either temperature. Opposite results were observed for haematocrit and platelet count in EDTA and CTAD tubes; haematocrit was stable at 5 °C, but not at 20 °C, whereas opposite changes took place for platelet count. No clear conclusions can be drawn, but for most variables, the findings indicate that blood should be stored in a refrigerator if the analysis is delayed. Refrigeration is especially important when EDTA tubes are used to determine haematocrit and white blood cell count. CTAD tubes have been developed specifically for platelet studies, as they contain platelet inhibitors, and platelets were also marginally more stable in these tubes.

According to Jain (1986), blood from domestic pigs should be stored in a refrigerator. However, for five of the six minipigs, we found a decrease in platelet count in EDTA tubes stored at 5 °C, which may be due to the non-clinical condition, called pseudothrombocytopenia (*Bizzaro and Bradalise, 1995*). An anecdotal report of platelet agglutination in blood from a minipig has appeared (*Ragan, 1972*). However, as far as we know, we were the first to describe that pseudothrombocytopenia is a common feature of the Göttingen minipig, which has later been confirmed (*Erkens et al., 2017*).

Blood from the femoral artery can be sampled either manually (all tracers, left) or automatically ([$^{15}$O] based tracers, right) in pigs.

# 6.0 PET tracer injection

## 6.1 Tracer injection sites in pigs (P-VIII)

The ear vein is used for intravenous injections of tracers, and it is well-suited for repeated scans of pig models of neurodegenerative brain diseases. However, the short distance between brain and ear vein can be problematic, as both are located in the field-of-view of the PET camera, and radiotracer residues may influence the outcome of the PET scan (local random scatter) (see Figure **11**). This issue, with halo artifacts caused by incorrect scatter correction, is only seen for certain tracers, such as [$^{18}$F]DDOPA, [$^{11}$C]PK11195 (N-butan-2-yl-1-(2-chlorophenyl)-N-methylisoquinoline-3-carboxamide) and [$^{11}$C]PiB (Pittsburgh compound B), that adhere to plastic surfaces (see Table **4**). Particularly problematic are tracers that bind to aggregates, such as [$^{11}$C]PiB used to image beta-amyloid plaques in animal models of Alzheimer's disease (*Snellman et al., 2013*). This might be circumvented partly during image reconstruction by disabling or scaling the correction. Only scatter corrected PET images are quantitative.

In **P-VIII,** we investigated how to avoid highly concentrated tracer residues in the ear catheters. An *in vitro* study was performed with the dopamine synthesis marker [$^{18}$F]FDOPA, which we have previously observed adhere to ear catheters (Venflon) used in our minipigs (*Lillethorup, 2018*). The tracer was diluted with saline to volumes between 5 ml and 50 ml, before doses of approximately 100 MBq were injected into the 22G Venflon. After that, the Venflon was randomly flushed with volumes between 5 and 50 ml of saline. Then, the tracer residue in the Venflon was measured in a gamma counter for one minute, and the results are shown in Figure **12**. We observed a five-fold effect of diluting the tracer prior to injection, from 0.011-0.015 % residual in 5 ml to 0.002-0.003 % residual in 50 ml volume, but no effects of using larger volumes (5-50 ml) for flushing the catheters. The study indicates that tracer volumes of 20 ml or more will reduce contact between tracer and the plastic surface

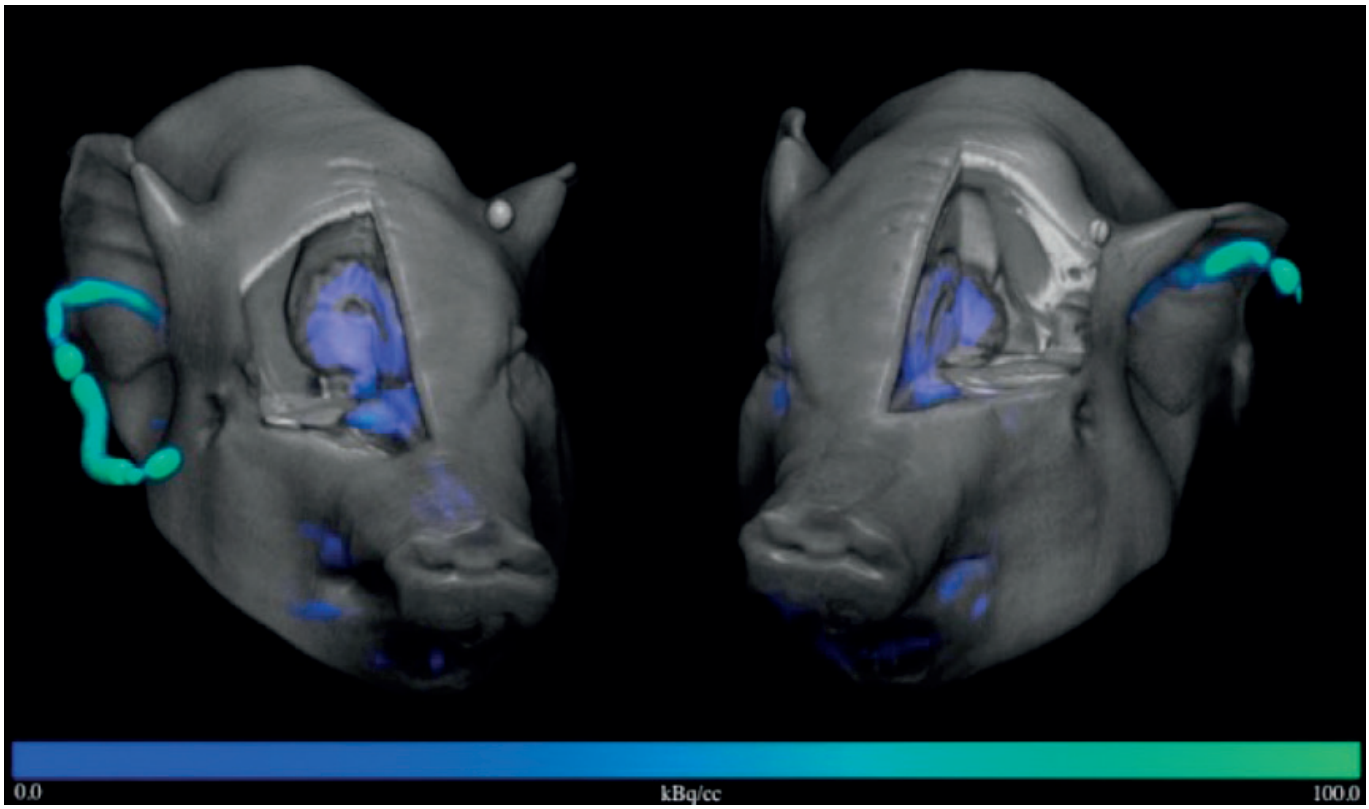



**Figure 11:** PET images showing residues of [$^{11}$C]PK11195, a tracer used for detection of neuroinflammation, in ear catheters in two female Göttingen minipigs (25-28 kg) after injection of 300-400 MBq tracer in a volume of 10 ml and flushed with the same volume saline. Isoflurane was used as anaesthesia (Figure **1** in paper **P-VIII**).

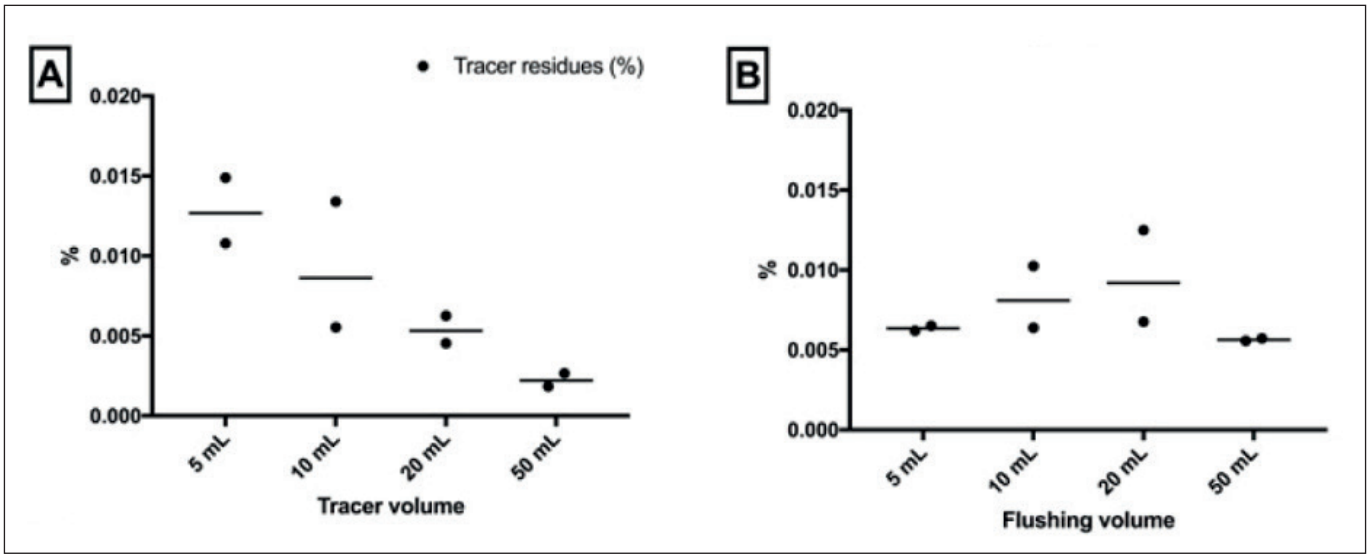



**Figure 12:** The residues of [$^{18}$F]FDOPA shown as % of the injected dose (approximately 100 MBq) in different tracer volumes (5-50 ml) (**A**) and flushing volumes (5-50 ml) (**B**). The *in vitro* study was performed twice (Figure **2** in paper **P-VIII**).

of the catheter, but once the tracer comes into contact with the surface, it cannot be removed by flushing. A few tracers are not chemically stable in saline solution and, therefore, dilution should be done as late as possible prior to injection into the ear vein. Some tracers, such as [$^{18}$F]FDOPA, can be diluted advantageously in phosphate buffer rather than in saline.

These results, obtained *in vitro*, have subsequently been tested in our ongoing Göttingen minipig studies with 20 ml [$^{18}$F]FDOPA, 20 ml [$^{11}$C]SCH23390, 20 ml [$^{11}$C]yohimbine, 20 ml [$^{11}$C]PK11195 and 20/50 ml [$^{18}$F]FDG, and have shown that we can almost avoid the tracer residues in ear catheters. One observed disadvantage of diluting the tracer is, however, that it prolongs the injection time and thereby disrupts the injection of the tracer as a bolus (*Lillethorup, 2018*). For some of the few very `sticky' tracers, such as [$^{11}$C]PiB, we have found that it is better to remove the catheter after injection of the tracer. That can be done quickly, but it is important to ensure a good hemostasis so that blood with tracer does not drip out in the field of view. We measured 0.2%-0.6% of the injected [$^{11}$C]PiB to be left in the Venflon when using a volume of 10 ml and flushing with the same volume of saline. Such PET scans with removal of ear catheters must be performed as the last in a series, unless more than one catheter is placed in ear veins. It is also possible to place catheters in other blood vessels than the ear of pigs. The femoral vein is useful for tracer injections, and this site is an advantageous for minimizing the impact of residue in PET brain studies of pigs.

# 7.0 Discussion

As stated in section 2.5, the following eight hypotheses formed the basis for the research findings presented in this dissertation, and they will together with other aspects be discussed here:

**(1) Confirmed:** The choice of anaesthesia may influence the binding of several tracers in the brain.

**(2) Confirmed:** A number of monitoring parameters correlate with, or even causatively affect cerebral blood flow in pigs measured with [$^{15}$O]water PET.

**(3) Confirmed:** Capnography can replace $PaCO_2$-measurements for anaesthesia monitoring in pigs.

**(4) Partly rejected:** Blood lactate concentration differs between pig breeds, and in response to weight/age, type of anaesthesia, and surgical procedures.

**(5) Rejected:** Long-term anaesthesia, intensive blood sampling and road transport may affect the brain of pigs.

**(6) Rejected:** It is possible to predict the pathological changes during prolonged anaesthesia with classical monitoring parameters and blood samples (e.g. blood lactate).

**(7) Partly confirmed:** Blood samples for measurement of blood gases and blood cell count in pigs should be stored as recommended for human blood.

**(8) Partly confirmed:** It is possible to avoid residues of tracers in the ear vein catheter by dissolving the tracer in a large volume of saline and then flushing it with an equivalent volume of saline.

## 7.1 Anaesthesia – living with the challenges

Anaesthesia may have major effects on the kinetics of several brain PET tracers, but as seen in this dissertation, little is known about these effects in pigs. The first hypothesis can, nevertheless, be confirmed, since the binding of [$^{11}$C] SCH23390 and [$^{11}$C]MDL100,907 were strongly influenced by anaesthesia. The same was observed for [$^{11}$C]raclopride binding in rat brains. No anaesthesia effects on [$^{11}$C] yohimbine were observed in minipigs. Nevertheless, it is important to report anaesthesia protocols in all studies.

The anaesthesia review and our own study with [$^{11}$C] SCH23390 show that it is difficult to predict anaesthetic effects in pig brains, based on knowledge from other species. Furthermore, anaesthesia protocols for rodents cannot directly be applied to pigs: rodents are often anaesthetised with other anaesthetics (e.g. Hypnorm-Dormicum) than pigs, and technically, it is a quite different procedure (*Tremoleda et al., 2012*). While the anaesthesia of pigs is divided up into premedication, induction and maintenance, with different drugs and drug doses, this is often combined to a single drug administration in rodents (*Krinke, 2000*). Further systematic work on testing of various anaesthetic agents on the kinetics of PET tracers in pig brain is needed. Until then, the choice of anaesthesia should provide a good and stable anaesthesia that ensures the welfare of the pig (*Hildebrandt et al., 2008*). The choice of anaesthesia could be based on the following criteria: uniform anaesthetic level for a sufficiently long period of time, safe in use, ensuring good awakening and thus animal welfare and the number of active substances should be minimized so as to minimize the risk of interactions with PET results. Both isoflurane and propofol appear to fulfil these requirements. **R-4** suggests that ketamine anaesthesia of animals can produce neuroimaging results comparable to being awake, but ketamine is not recommended as a mono-anaesthetic in pigs (*Boschert et al., 1996*). In each individual study, consideration should be given to the presence of specific active substances in the anaesthesia, which should be avoided for the sake of the results. In certain cases, specific anaesthetic agents may be omitted when they are specifically known to affect the measured parameters in the brain. Propofol's neuroprotective effect may be both an advantage and a disadvantage, depending on what the study should specifically investigate.

Table **21** shows the use of porcine anaesthesia in the included papers. For all the PET studies, pigs were

**Table 21:** Anaesthesia used in the pig studies of this dissertation.

| Premedication | Anaethesia | Papers |
|---|---|---|
| None | None | **P-I** |
| Ketamine-midazolam | Isoflurane and nitrous oxide | **P-II, P-III, P-VII, P-IX** |
| Ketamine-midazolam | Isoflurane or propofol | **P-V, P-VI, P-XI** |
| Ketamine-midazolam | Isoflurane | **P-VIII** |
| Ketamine-midazolam | Propofol | **P-X** |

premedicated with midazolam and ketamine. This combination has a good and safe sedative effect (we have only very few observed side effects during 1,000-2,000 anaesthesia procedures in pigs), so that it is subsequently possible to place an ear vein catheter for rapid induction of anaesthesia (*Linkenhoker et al., 2010*). We have previously experimented with the tranquilizer azeparone in combination with midazolam, but this combination has proven unsuitable because it prevented the binding of serotonin and dopamine PET tracers in the brain of the pigs (*Donald Smith, pers com., 2002*). The pig-zoletil mixture (see *Alstrup, 2010*) is an obvious alternative, but we have avoided it as it involves several drugs that can potentially affect the results of PET brain scanning. While premedication may affect PET scans made shortly after the onset of anaesthesia, the subsequent effect of anaesthetic drugs administered during brain scanning will be of increasing importance in pig studies. In our published pig papers, PET scans were typically performed 2-3 hours after the induction of anaesthesia.

In our earliest studies, isoflurane was always combined with nitrous oxide, but the hospital chose to stop the delivery of nitrous oxide, and therefore the latest studies were performed without it. A slightly increased dose of isoflurane, due to the absence of nitrous oxide, may have a dose-response effect on cerebral blood flow and hence the kinetics of multiple tracers (*Li et al., 2014*). While nitrous oxide administrated in oxygen may increase cerebral blood flow (*Field et al., 1993*), we have not experienced any major difference after omitting it. Both isoflurane and propofol lead to a gradual increase of the pulse during anaesthesia, but highest during isoflurane. For inhalation anaesthesia, this may be due to reduced cardiac vagal activity, known from a dog study (*Picker et al., 2001*).

## 7.2 Monitoring – management and documentation

Monitoring of anaesthesia is important, as both brain imaging results and animal welfare may otherwise be adversely affected. The monitoring correlation study (paper **P-IX**) showed that $PaCO_2$ and body temperature are both positively associated with cerebral blood flow. This confirmed the second hypothesis, and therefore these two parameters, as a minimum, should be monitored and controlled. Anaesthesia time was significantly correlated with high pulse, low $PaO_2$ and high body temperature, but not with cerebral blood flow. It is interesting that $PaCO_2$ and body temperature were included in some of the earliest papers published more than 20 years ago (*Poulsen et al., 1997; Danielsen et al., 1999*), and should be so in future studies. The importance of controlling $PaCO_2$ was further emphasized in the intervention study (paper **P-III**), where both cerebral blood flow and cerebral blood volume were strongly influenced by variations in $PaCO_2$. It is relatively easy to adjust pH and $PaCO_2$ by changing either tidal volume or respiration rate. Such adjustments will result in changed $PaCO_2$ within seconds to minutes. Body temperature can be controlled through the feedback system, where a temperature sensor in the rectum controls a warming blanket. However, heating and cooling of pigs is a slow process and can take hours. It is therefore crucial to prevent the pig from getting cold or warm already from the beginning. That is best done by covering the pig with blankets in a heated room. The pig's sparse hair cover and poor ability to produce body heat (*Hou et al., 2017*) along with the vasodilatory effects of anaesthetics in the peripheral circulation tend to enhance hypothermia.

One might consider whether a more objective measurement for anaesthetic depth than just reflexes might be useful, but so far, there are no reliable bispectral index monitors on the market that can be used for pigs (*Martin-Cancho et al., 2004; Kurita el al., 2012*).

The capnography study (paper **P-VII**) shows that $ETCO_2$ can replace $PaCO_2$ measurements in pigs, and this confirms the third hypothesis. This is not surprising as the correlation is well-documented in humans (*Satoh et al., 2015*) as well as in other species, such as rats (*Vaghadia et al. 1989*). $ETCO_2$ is an obvious option in studies, such as PET neuroimaging monitoring of disease progression in neurodegenerative models, where arterial blood samples are not available. Furthermore, $ETCO_2$ can be used to identify intubation error, airway obstructions, decreased metabolism and malignant hyperthermia, and monitoring may continue during awakening, where capnography can be used to follow the return of spontaneous breathing (*Ehrenwerth et al., 2013*). One can advantageously supplement $ETCO_2$ and temperature monitoring with measurement of pulsoximetry (pulse and oxygen saturation) that will supplement with information on the cardiovascular system.

As an intermediate metabolite, blood lactate can be used as an indicator of hypoxia and poor-quality anaesthesia (*Bakker and Lima, 2004; O'Connor and Fraser, 2012*). It was explored whether blood lactate also could be a useful parameter for monitoring during anaesthesia of pigs. Not surprisingly, the study showed a very big difference in baseline concentrations in domestic and minipigs. Thus, the fourth hypothesis could only be partially rejected, as there were breed differences, but no differences in response to anaesthesia, weight/age and surgical procedures. In an ongoing study, we show that blood lactate can equally be measured in arterial and venous blood, which may also indicate that ear vein blood is useful (*Hald et al., 2020, in prep.*).

## 7.3 Long-lasting scanning procedures

Paper **P-X** represents an extreme case of a tracer development study, as pigs were long-term anaesthetized with intense blood sampling and road transportation. The idea behind this study was to assess a *worst-case scenario* in order to determine whether extreme experimental conditions would have notable adverse effects on the pig's physiology and body organs, knowing that our usual studies would be less stressful than both of the included groups. All of the pigs were subsequently thoroughly examined by two experienced veterinary pathologists. The findings give rise to some important conclusions relevant for PET scanning of pig brains: First, it was positive that there were no pathological findings and no marked signs of hypoxia in any of the pig brains. Furthermore, the [$^{18}$F]FDG brain uptake was at the same level as observed in other pig studies (*Lee et al., 2012*). Therefore, the fifth hypothesis could be rejected or at least not confirmed, as no brain effects were found. Secondly, however, it was troubling that the classic monitoring parameters (pulse, $SatO_2$, $PaCO_2$, $PaO_2$ etc.) remained unchanged for more than 16 hours, despite the occurrence of pathological changes in the heart and lungs of several of the pigs. This may indicate that classical monitoring parameters are not fully sufficient to assess the condition of the pigs in such extreme PET scans. Blood lactate failed to show changes throughout anaesthesia, except during the road transport, and this may, or may not, relate to the respirator setting. Therefore, the sixth hypotheses may be rejected. Heart and lung pathology was noted, which can indirectly affect PET scan results, for example through affected cerebral hemodynamics. It is also noteworthy that the pathological changes will be expected to affect the welfare of pigs in recovery studies. The study points out that PET brain studies in pigs must not focus solely on effects in the brain, but that we have to look at how the whole animal is affected. Interestingly, it was possible to follow the progression of pathological changes on the series of CT scans, and it is therefore an obvious possibility to supplement classical monitoring with CT scans during such extreme PET scanning protocols. CT scans can also be used to evaluate pig health prior to PET scanning, as described in a case-study on a domestic pig excluded from a PET project on the basis of a CT scan of the thorax that showed undetected pericarditis (*Alstrup, 2015*). The study further shows that necropsy can advantageously be performed after completed animal experiments. Subsequent full-body diagnostic CT scans of our ongoing minipig model of Parkinson's Disease, before and after two hours of PET neuroimaging (sternal recumbence, and without blood sampling), have shown no evidence of any organ pathology.

## 7.4 Pre-analytic handling – importance for documentation

When analyses of blood samples are delayed, the steering value will be correspondingly lower; while a short delay in the analysis will permit adjustment of anaesthesia, a larger delay will be of only documentary importance, since the analysis cannot be used to adjust anaesthesia. This is, for example, a strong argument for measuring $ETCO_2$ rather than $PaCO_2$ in pig studies, as ventilator settings can then be adjusted in real time. It is crucial that the blood samples have been handled correctly so that the results are valid. Paper **P-II** shows that arterial blood samples for blood gas analyses have to be stored in a refrigerator or on crushed ice, if the analyses are postponed. In this way, the samples can be stored at least an hour before analysis, and this is in agreement with recommendations for venous blood samples from other weight groups of pigs (*Haskins, 1977*).

Paper **P-I** shows that prolonged storing of venous blood samples can affect haematology analyses in Göttingen minipigs. Recommendations are, however, limited by the fact that the optimal storage temperature depends on the parameters to be measured. In general, Göttingen minipig blood seems to react similarly to domestic pig blood prior to analysis (*Jain, 1986*), but a significant difference appears for pseudothrombocytopenia observed in minipigs. This phenomenon, where automated measuring equipment fails to provide valid counts of platelets that clot *in vitro,* is known as single cases (1:1,000) for human blood (*Lippi and Plebani, 2012*), but it is even more common (5 out of 6) in Göttingen minipigs. This means that, for example, neuroinflammatory or stroke studies performed in minipigs could lead to erroneous conclusions, if blood samples are collected continuously during the hours of brain PET scans and analyzed only at the end. Recently, and partly based on our study, Erkens and co-workers (2017) further investigated pseudothrombocytopenia in Göttingen minipigs and found that thrombocyte counting should be performed as fast as possible to avoid pseudothrombocytopenia. Interestingly, the effects seemed to be greatest in male pigs and surprisingly also included blood stored by room temperature. Göttingen minipigs may possibly serve as a model for the study of pseudothrombocytopenia in other species, including humans. Based on the two pre-analytical studies, we can only partially confirm the seventh hypothesis that porcine blood should be stored as human blood, and this underlines the importance of performing species-specific studies on the durability of blood samples. Differences exist, even between minipigs and domestic pigs. Not all pigs are created equal.

Blood lactate was not included in the two papers, but based on the same techniques as described for paper **P-II**, we found that arterial blood from 40 kg domestic female pigs can be stored at 5 $^{O}$C for at least one hour, while blood lactate increases at 20 $^{O}$C after just 15 minutes (data to be published). Blood lactate therefore follows the same preanalytical recommendations as in paper **P-II**, which is in agreement with recommendations for human blood samples (*Seymour et al., 2011*). The pre-analytical studies were performed as early as in 2001 and 2003, and since then, we have – as far as possible – followed their recommendations.

## 7.5 Ear vein tracer injection

Injection of the tracers into the ear vein can be problematic, as residues in the catheter can affect the image quality. Dilution of most tracers, such of [$^{18}$F]FDG or [$^{18}$F]FDOPA, in a larger volume of saline (≥ 20 ml) seems to be the simplest solution to limit residues, and we therefore now use this procedure in our own laboratory. The eighth hypothesis could, thus, be partly confirmed for dilution of [$^{18}$F]FDOPA, but there was no effect of increasing the flushing volume. These findings, however, are based only on *in vitro* studies of a single tracer, and other demands may apply to more adhesive traces, such as [$^{11}$C]PiB. In each case with adhesive tracers, therefore, it is necessary to evaluate whether remnants can be detected in the ear catheter when a new tracer is used, and this should preferably be done prior to *in vivo* studies. Alternatively, one of the other procedures may be used, such as removing the ear catheter immediately after injection of the tracer or injecting the tracer into a blood vessel that is as far from the brain as possible, such as the femoral vein. We also plan to test the saphenous veins from lower legs and the abdominal milk vein when we get access to ultrasound equipment for location of these vessels.

## 7.6 The data reuse concept and the 3R's

This dissertation is based on data from our ongoing pig studies that have been carried out in other contexts but, in our opinion, those studies provide independent insight into the suitability of pigs in neuroscience. For example, we used baseline PET scans from different Göttingen minipig studies to compare the effects of anaesthetic agents (papers **P-V** and **P-XI**). The decision to perform this comparison was made so early that a proper randomization of the minipigs could take place, and the two studies were performed during the same period. Therefore, these data did not require use of extra minipigs. In the studies on blood lactate concentration (paper **P-VI**) and cerebral blood flow monitoring (paper **P-IX**), historical data were used, as all medical records from a period were systematically re-investigated. Also, the pre-analytical studies (papers **P-I** and **P-II)** were performed in pigs that we already used for other purposes (*Olsen et al., 2001; Olsen, 2002; Olsen et al., 2002; Bender et al., 2004*). The tracer study, **P-VIII**, was performed *in vitro* and the result tested *in vivo* on ongoing pig studies. Initially, the purpose of **P-III** was to investigate whether [$^{15}$O]carbon monoxide could be used to measure cerebral blood flow, but as this did not prove possible, data could be used in a new context. The rat study, **P-IV**, was performed to test anaesthesia techniques and getting an overview of the image quality of our new microPET scanner. The paired measurements of $PaCO_2$ and $ETCO_2$ (paper **P-VII**) were performed after completion of a simple PET scan of pigs in non-recovery studies, and the study of the consequences of long-term anaesthesia (paper **P-X**) was carried out in an existing osteomyelitis pig study.

This type of research has both advantages and disadvantages. The benefits are straightforward: First, data are almost free, as only some extra work is required to obtain them. Second, from a 3R perspective (*Russell and Burch, 1959*), it is advantageous that no further animal experimentation was performed, as most procedures would have been carried out anyway. In fact, no single pig or rat has been used just to write this dissertation, but still the current data can be used to optimize protocols, so that we can use fewer pigs in the future and use them better.

Conversely, it may also be a disadvantage to conduct research in the reuse-data-way, since most studies were not specifically designed to provide answers to the central questions. For example, it is rarely possible to influence the number of animals, their sex, age and weight. As the design was locked in advance in the pig anaesthetic studies (papers **P-V** and **P-XI**), it was not possible to select the best protocol for comparison of anaesthetic effects. For example, it was not possible to include awake minipigs, and thus, that study cannot answer whether isoflurane or propofol is to be preferred, as awake minipigs were not included. Paper **P-X** was performed on pigs, which were included in a study of *Staphylococcus aureus*, and thus including an element that would only be relevant to few brain scan studies (see, however, *Astrup et al., 2013*). Nor did this study clarify whether the detected organ effects were due to long-term anaesthesia, blood collection or road transport. Clearly, such studies are more descriptive than hypothesis-based.

Despite the limitations, it is my opinion that veterinarians and other researchers can benefit from the knowledge derived from the research that we have carried out, not just to use the obtained results for better neuroimaging in laboratory animals, but also to follow the concept of reusing old data for designing better studies in the future. In this way, we can use old and ongoing studies to explore methodological considerations aimed at optimizing future research procedures. This requires some ingenuity and, of course, also some extra work, but it is my experience that the findings in such studies often turn out to be used extensively. A potential limitation may be that funding is lacking to get the articles published, as many journals switch to open access and will, instead, charge the researchers for the publications (*Sonne et al., 2019*). Method articles, based on our reuse concept, will rarely be included in the research grants as they will be performed and published *ad hoc* and, therefore, not planned in advance of the fund applications. Especially veterinarians will often have access to such data in their daily work and also need the knowledge that they can provide. It is important that the laboratory animal veterinarian has time for such work. Furthermore, the work contributes with first author publications that can be a career advantage for many veterinarians working in human medicine research. I myself have experienced that an independent research profile in laboratory animal science gives respect among other academic peers. Fortunately, many hidden treasure chests of data are waiting to be found!

# 8.0 Conclusions and perspectives

## 8.1 Conclusions

The choice of anaesthesia for pig brain PET protocols is a challenge, as anaesthesia often has a major effect on tracer uptake and binding by reducing brain metabolism, affecting cerebral blood flow and altering receptors and enzymes. The bindings of [$^{11}$C]SCH23390 and [$^{11}$C]MDL100,907, but not of [$^{11}$C]yohimbine, are strongly affected by whether the Göttingen minipigs are anaesthetised with isoflurane or propofol. Similarly, the binding of [$^{11}$C]raclopride in rat brain differs markedly depending on the anaesthetic. The kinetics of [$^{11}$C]SCH23390 is flow-dependent in Göttingen minipigs, whereas this is not the case in primates. Clearly, findings from PET brain studies obtained using anaesthetic on one species, including humans, and non-human primates cannot be assumed to apply directly to another. The choice of anaesthetic for PET brain scanning of pigs may primarily be based on assuring that the depth of anaesthesia is sufficient for good animal welfare, and it should be based on adequate monitoring. Anaesthesia procedures must always be reported in papers.

Monitoring is important during anaesthesia. Both $PaCO_2$ and body temperature are positively associated with cerebral blood flow during isoflurane – nitrous oxide anaesthesia, and therefore these parameters should, as a minimum, be monitored, controlled and reported. The importance of controlling $PaCO_2$ is further emphasized in the intervention study, where both cerebral blood flow and cerebral blood volume are influenced by variations in $PaCO_2$. Capnography can replace the measurement of $PaCO_2$ when it cannot be measured due to lack of an arterial catheter. The hypoxia marker, blood lactate, is highly dependent on the type of pig, but is unaffected by body weight/age, anaesthesia type, or the type of surgery performed. Under the extreme experimental conditions tested in this dissertation regarding prolonged anaesthesia, blood sampling and road transport, no detectable changes were noted thus far in the brain. Furthermore, the circulatory changes could not be detected with traditional monitoring. Anaesthetic length and blood sampling and transportation should be minimized. Arterial blood samples for blood gas measurements and venous blood samples for haematological studies should preferably be analysed rapidly after collection, and, if this is not possible, the storage time and temperature should be carefully considered to avoid errors. If the tracers have to be injected through the ear vein, it is a disadvantage that the injection site is so close to the brain, which can affect the image quality. Diluting the tracer in a large volume (≥ 20 ml) before injection is a simple method to minimize tracer remnants. Alternatively, the ear catheter may be removed after injection of `sticky' tracers or the tracers may be injected into blood vessels further away from the brain. The dissertation shows the value of reusing data for optimising ongoing protocols.

## 8.2 Perspectives

Modern preclinical research is currently in a translational and reproducibility crisis where it is difficult to transfer results from animal studies to human clinic, and where many laboratories cannot reproduce results obtained at other sites (*Cumming et al., 2014; Alstrup and Sonne, 2019*). This dissertation aims at providing small steps towards solving some of these challenges in the neuroimaging of experimental animals. but much more work is certainly required to lessen the crisis.

Systematic work is needed in which the effects of various anaesthetic agents on PET tracers are tested. As a minimum, it could be investigated whether the binding of certain radiotracers for PET neuroimaging is flow-dependent in the pig. As a beginning, a few standard anaesthetics, such as propofol and isoflurane, could be selected together with some of the most commonly used PET brain tracers (e.g. dopamine, serotonin and noradrenaline tracers). Such work is complicated, however, by the fact that results obtained in one species or type of pig are not necessarily found in another. Ideally, well-trained awake pigs should be included, but pigs can hardly be trained to remain motionless for more than a few minutes. Another approach would be to carry out short (e.g. 10-20 minutes) static PET scans after injecting unrestricted pigs with a PET tracer while awake, and then inducing short-time anaesthesia prior to imaging. Such an arrangement with a tracer injection in the awake state may also be useful for repeated PET scans of transgenic and other pigs serving as models for brain

diseases. Studies of the effects of anaesthesia may also provide insight into their mechanisms of action, which are often poorly understood. There is also a need for further studies on the optimal monitoring during anaesthesia. Careful monitoring could remedy some of the problems due to anaesthesia, if these effects can be modelled. The monitoring studies may be followed up with studies investigating causal links between physiological variation, such as body temperature and cerebral blood flow. Furthermore, studies could be performed for tracers other than [$^{15}$O]water, as tracer kinetics can be affected by other factors than just cerebral blood flow. Also, the effects of prolonged anaesthesia and intensive blood sampling should be investigated further, as both can potentially affect the scanning results and animal welfare. Blood sampling can be minimized by using scan images of the carotid artery to establish an input function (*Sari et al., 2017*) or by reinfusion of sampled blood (e.g. SwissTrace System; www.swisstrace.ch). The carotid artery imaging approach may potentially be used for image-based kinetic analyses, but cannot be used to measure metabolites in blood. The dissertation documents that there is a need for the journals to ensure that all papers contain sufficient documentation for anaesthesia and monitoring – a task for both editors and peer reviewers. Here it is an advantage that many journals recognize ARRIVE guidelines (*Kilkenny et al., 2010*).

This work has primarily been based on PET neuroimaging in pigs and supplemented with a single rat study. The principles may also be applied for scanning of other laboratory species. It is probably even more important to control body temperature and $PaCO_2$ in small rodents with spontaneous respiration than pigs, where the anaesthesia and tracer injection issues are also relevant. Anaesthetic effects and monitoring may even be relevant in relation to PET scanning of infants undergoing anaesthesia. The pig has here served as a suitable model, as it has a size that makes it suitable for getting detailed knowledge on how to best scan anaesthetised laboratory animals. Pigs seems to be a good model for this future work.


Towards new horizons: The first pig on its way to our new PET/MR scanner in Aarhus (2019).

# 9.0 Summary of the dissertation

## 9.1 English summary

The aim of this dissertation is to investigate how to best study pig brains by positron emission tomography (PET), and this work was based on eleven original scientific papers (chronologically stated as **I-XI**) supplemented with review articles. PET brain scanning of pigs relies on anaesthesia, which typically can alter brain metabolism, cerebral blood flow and tracer binding. Papers **P-V** and **P-XI** are some of the first published PET study of anaesthesia effects in pig brains, and they show that the kinetics of the dopamine tracers [$^{11}$C] SCH23390 and serotonin tracer [$^{11}$C]MDL100,907, but not of the noradrenaline tracer [$^{11}$C]yohimbine, are different between isoflurane and propofol anaesthesia. The former study furthermore shows that a tracer can be flow-dependent in minipigs, even though that is not the case in primates, and this makes it problematic to transfer data on anaesthesia effects from one species to another. Paper **P-IV**, performed in rats with the dopamine tracer [$^{11}$C]raclopride, demonstrates that anaesthetic effects can be extremely comprehensive, and it is an area that should be further investigated in the future.

Since PET scanning provides functional images, it is crucial that the physiology of the pigs is carefully monitored during anaesthesia. Therefore, we have identified which monitoring parameters that can explain variations in cerebral blood flow measured with [$^{15}$O]water, as this may affect the kinetics of several tracers, such as [$^{11}$C] SCH23390, [$^{11}$C]MDL100,907 and [$^{11}$C]raclopride. Both partial pressure carbon dioxide ($PaCO_2$) and body temperature were positively associated with cerebral blood flow (paper **P-IX**), and the causal relationships for $PaCO_2$ were experimentally investigated (paper **P-III**). Paper **P-VII** shows that capnography can replace $PaCO_2$ in domestic pigs. A potentially useful monitoring parameter is blood lactate, and paper **P-VI** demonstrates that blood lactate concentration differs between domestic pigs and minipigs, but is not affected by age/weight, type of anaesthesia and surgery.

Blood lactate is a marker of hypoxia, and paper **P-X** shows increases during transportation of pigs, whereas this is unaffected by long-term anaesthesia and blood sampling during PET imaging. Furthermore, this study shows that, although there is no hypoxia in the brain, prolonged propofol anaesthesia and blood sampling can affect the cardiovascular system of the pig. In PET brain studies, the analyses of blood samples may often be delayed. Recommendations for handling blood gases and haematological variables are presented in paper **P-I** and **P-II**.

PET tracers are a unique toolbox for the examination of the brain. Paper **P-VIII** discusses how the tracers can be injected in pigs prior to neuroimaging, and an option is to dilute the tracer before it is injected through the ear vein, as this may avoid tracer remnants in the catheter that may influence the outcome of the scan. Overall, this dissertation strives to provide guidance for the planning and performance of PET scans of the pig brain brain, and shows the value of reusing data.

## 9.2 Dansk resume

Formålet med denne afhandling er at undersøge, hvordan man bedst skanner grisehjerner med positron emission tomografi (PET), og dette arbejde er baseret på elleve originale videnskabelige artikler (kronologisk anført som **I-XI**) suppleret med oversigtsartikler samt en dansksproget lægmands-artikel (**D-1**). PET-skanning af grise foregår under brug af anæstesi, som typisk påvirker hjernens metabolisme, cerebrale blodgennemstrømning og sporstof-binding. Artikel **P-V** og **P-XI** er blandt de første publicerede undersøgelse af anæstesieffekter på grisehjernen, og studierne viser, at kinetikken af dopamin-sporstoffet [$^{11}$C] SCH23390 og serotonin-sporstoffet [$^{11}$C]MDL100,907, men ikke noradrenalin-sporstoffet [$^{11}$C]yohimbin er forskellig mellem isofluran- og propofolanæstesi. Førstnævnte studium viser endvidere, at et sporstof kan være flow-afhængig i minigrise, selvom det ikke er det i primater, og dette gør det problematisk at overføre erfaringer med anæstesi-effekter fra en dyreart til en anden. Artikel **P-IV**, der er udført i rotter med dopamin-sporstoffet [$^{11}$C]racloprid, understøtter, at anæstesi effekter kan være ekstremt store, og at det er et område, som bør undersøges yderligere.

Da PET-skanning giver funktionelle billeder, er det afgø-

rende, at grisenes fysiologi overvåges nøje under anæstesien. Vi har derfor undersøgt, hvilke monitoreringsparametre, der kan forklare variationer i den cerebrale blodgennemstrømning målt med [$^{15}$O]vand, da dette vides at påvirke kinetikken af flere sporstoffer, blandt andet andet [$^{11}$C]SCH23390, [$^{11}$C] MDL100,907 og [$^{11}$C]racloprid. Både partiel kuldioxidtryk ($PaCO_2$) og kropstemperaturen er positiv associeret med den cerebrale blodgennemstrømning (artikel **P-IX**), og årsagssammenhængen for $PaCO_2$ er eksperimentelt undersøgt i artikel **P-III**. Artikel **P-VII** viser, at kapnografi kan erstatte måling af $PaCO_2$ hos domesticerede grise. En potentiel vigtig monitoreringsmarkør er blodlaktat, og det bliver i artikel **P-VI** påvist, at blodlaktat-koncentrationen varierer mellem domesticerede grise og minipige, men er uafhængig af alder/vægt, anæstesitype og kirurgi.

Blodlaktat er en markør for hypoxi, og det bliver vist i artikel **P-X**, at den stiger under langvarig transport af grise, men er ellers uændret igennem langvarig anæstesi og blodprøvetagning. Denne undersøgelse viser ydermere, at langvarig propofol-anæstesi og blodprøveudtagning kan påvirke grisenes fysiologi og sundhed, men at hjernen på trods af dette, ikke udsættes for hypoxi. I PET-studier kan analyser af blodprøver blive forsinket. Anbefalinger for håndtering af blodprøver til måling af blodgasser og hæmatologiske variabler bliver præsenteret i artikel **P-I** og **P-II**.

PET-sporstoffer er unikke redskaber til undersøgelse af hjernen. Artikel **P-VIII** diskuterer, hvordan sporstofferne kan injiceres før PET-skanning, og en mulighed er at fortynde sporstoffet inden den injiceres gennem ørevenen, da dette kan minimere rester af sporstoffet i kateteret, som ellers kan påvirke scanningsresultatet. Samlet bidrager afhandlingen til bedre planlægning og udførsel af PET-skanninger af grisehjernen grisehjernen, og viser værdien af at genbruge data. En ikke-videnskabelig, dansksproget oversigtsartikel om emnet er blevet trykt i et veterinært tidsskrift (*Alstrup, 2019*).

# 10.0 Abbreviations

| | |
|---|---|
| 3R | Three R's: replacement, reduction and refinement |
| Bq | Becquerel [decay per second] |
| CBF | Cerebral blood flow |
| CRISPR | Clustered regularity interspaced short palindromic repeats |
| CT | Computerized tomography |
| CTAD | Citric acid-theophylline-adenosine-dypyridam |
| EDTA | Ethylene-diamine-tetraacetic-acid |
| $ETCO_2$ | End-tidal carbon dioxide |
| MBq | Mega becquerel [$10^6$ Bq] |
| MRI | Magnetic resonance imaging |
| MRSA | Methicillin resistant *Staphylococcus aureus* |
| $PaCO_2$ | Partial pressure carbon dioxide |
| $PaO_2$ | Partial pressure oxygen |
| PET | Positron emission tomography |
| R1 | Relative delivery |
| $SatO_2$ | Oxygen saturation |

The chemical names of the tracers appear in the text, but are best known as abbreviations. Abbreviations used in tables and figures are only explained in legends.

Note that throughout the dissertation, numbers are generally stated as mean ± standard deviation.

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Five published review articles, to which I contributed as either first author or co-author, are widely used in this dissertation and are referred to as follows (**1-5**):

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