RADIOTELEMETRY MONITORED MEASUREMENTS OF THE EFFECTS OF MEDETOMIDINE-MIDAZOLAM-FENTANYL, ISOFLURANE OR KETAMINE-XYLAZINE ANAESTHESIA ON PHYSIOLOGICAL PARAMETERS IN GUINEA PIGS

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Radiotelemetry monitored measurements of the effects of medetomidine-midazolam-fentanyl, isoflurane or ketamine-xylazine anaesthesia on physiological parameters in guinea pigs

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In tiefer Dankbarkeit und Erinnerung an ”die Chefin”
Julia Henke (1962 – 2016)

Table of Contents

1 Introduction and aims

Guinea pigs (Cavia porcellus, GPs) have been used as an animal model for over 200 years and they continue to be used in the research fields of infectious diseases, allergy, respiration, nervous and immune systems and auditory studies. They are also frequently kept as companion animals and are therefore seen in veterinary practice. In both research and clinical practice, anaesthesia is frequently required. In the clinical routine, GPs are anaesthetized for surgeries and to perform imaging procedures. In the laboratory environment, anaesthesia is required for substance application, device implantation or blood sampling. Anaesthesia research is still needed in GPs, as anaesthesia is known to alter physiological responses. Especially anaesthesia in GPs has been associated with a high mortality risk (1). Knowledge and correct interpretation of the anaesthetic's influences are essential. In practice, this knowledge can save the animal's life, and in laboratory medicine the knowledge about the effects of the anaesthetics is necessary in order to interpret study results correctly. Based on recent literature, 3 anaesthesia options have often been used in the GP: the injectable combination of ketamine and xylazine (KX), the fully antagonizable combination of medetomidine, midazolam and fentanyl (MMF) and the volatile anaesthetic isoflurane (Iso).

There have been reports on the influence of anaesthesia on cardiovascular parameters in GPs (2), but none of these studies has been performed using radiotelemetry technology. This gold standard technique provides many advantages, especially for the critical anaesthesia at the GP. Arterial blood pressure (BP), heart rate (HR) and core body temperature (BT) can be collected continuously without human intervention, such that the critical induction and recovery periods of anaesthesia can be monitored without interruption. The effects of the anaesthetics can be monitored live on an external monitor, whereby one can respond immediately in the case of an emergency. Baseline and long-term recovery values can also be acquired without disturbing the animal and therefore represent more physiological parameters as compared to parameters acquired manually. The radiotelemetry technology is the only method which allows the repeated investigation of BP in the same animal, as the alternative is direct blood pressure cannulation which only provides reliable results for up to one week (3). Therefore, if the BP is of long-term interest, using radiotelemetry reduces the animal number (3R value (3)).

The aim of this research was to investigate to what extent anaesthesia influences hemodynamics, core body temperature and other anaesthesia relevant parameters in the GP. For this purpose,

radio telemetry transmitters were implanted abdominally into GPs. Thereafter, two studies were carried out with the successfully implanted animals. The first experiment tested the effects of one-time anaesthesia with either MMF, KX or Iso and the second investigated the influence of 6 regularly repeated anaesthesias over 3 weeks. The implantation approach of the radiotelemetry device for measuring arterial BP and BT in the GP is theoretically the same as in rats (4), but its performance in GPs is more challenging with high failure rates. As our optimised approach resulted in high long-term survival rates and many of the refinements can also be applied to other surgical procedures, these findings were also published and included in this manuscript.

2 Anaesthesia in the GP

2.1 Anaesthesia - definition and aims

General anaesthesia is defined as an anaesthetic-induced reversible state of unconsciousness (hypnosis), skeletal muscle relaxation (immobilisation) and analgesia (5). So far, no single anaesthetic can provide all the criteria mentioned above, therefore anaesthetics from different substance classes need to be combined. General anaesthesia can be achieved by injection or inhalation anaesthesia and both should meet the following criteria:

- Good adjustability
- Reliable effect
- Large safety margin
- Quick induction and recovery
- Stress-free application for both animal and anaesthetist
- Low impact on physiological parameters (HR, BP, respiratory rate, metabolism)

Good adjustability (a targeted, predictable change of the anaesthetic status and mainly of the narcotic depth) and a quick recovery enable a fast return to self-regulation of physiological functions (BP, HR, BT) and spontaneous behaviours (food and water intake, social contact, movement) (6). The post-anaesthetic energy and water consumption and the ability to counterbalance the temperature losses during anaesthesia are especially important for a full recovery. Applying an anaesthetic, preferably with little stress, should also reliably lead to anaesthesia that is safe for user and animal. The requirements for anaesthesia in the laboratory in experimental procedures differ from those in veterinary practice, as stable hemodynamic parameters are of primary concern in the animal model during an experiment, whereas the animals in veterinary practice are there for therapeutic purposes with a need for a reliable and safe anaesthesia. Examples for the purposes of anaesthetizing GPs are:

- Immobilisation (blood collection from the jugular vein (7), imaging techniques)
- Procedures (castration, radiotelemetry transmitter implantation)
- Therapeutic procedures (fracture treatment (8), dental procedures)

The anaesthesia and analgesia protocol must be tailored to the procedure’s needs and the level of anaesthesia required. However, the choices available for anaesthesia of small mammals are limited, as the combinations used to interfere with the experimental procedures and results or

because of obstacles with drug availability or national drug regulations with specific substances (6).

2.2 GP anaesthesia – a challenge

Small mammal anaesthesia has a higher risk of mortality compared to cats and dogs (9). Both inhalation and injection approaches have been used in GPs, but especially KX led up to 30 % mortality and therefore the GP is considered a high-risk patient (10,11). Even with modern anaesthetics, the anaesthetic/sedative-related death rate in GPs is still at 3.8 % (9).

Challenges with GP anaesthesia begin with determining an accurate body weight which can vary up to 20-40 % depending on the individual's gastrointestinal filling resulting in dosing inaccuracies. Fasting the GPs prior to anaesthesia, on the other hand, leads to hypoglycaemia. Administration routes are limited as GPs have few accessible peripheral vessels, none of which are practicable for conscious intravenous applications, ruling out short-acting hypnotics like propofol. Therefore, for injection anaesthesia, extravasal administration routes (intraperitoneal, intramuscular or subcutaneous) are used which are associated with a slower onset of effect and require small total volumes to avoid tissue irritation. The HR is too fast to be counted with a stethoscope, therefore GP anaesthesia needs special equipment like a high-frequency pulse oximeter for reliable monitoring during the narcosis.

The relatively large body surface to body volume ratio makes GPs prone to anaesthesia related hypothermia which results in prolonged recovery times or even death (11). GPs are very sensitive to all volatile anaesthetics, leading to mucous membrane aggravation, strong salivation and tear production (12), often resulting in impaired breathing. Intubation is also inadvisable, as food particles in the mouth can be inadvertently pushed into the lung with consequent pneumonia (13).

Animal technicians and veterinarians are in many cases not specifically educated and the inconspicuous symptoms of ill GPs are therefore often not detected. As a result, many patients are presented with severe diseases that would have been unproblematic, had they been detected in an earlier stage. A successful anaesthesia in GPs, therefore, requires a detailed knowledge of their species-specific requirements, a careful selection of the best anaesthesia for a given procedure and having the needed equipment to adequately perform the anaesthesia and to monitor physiological status both during anaesthesia and recovery.

2.3 The GP in research

GPs were among the first animals widely used in research laboratories and their first description as an animal model was in 1881, when Robert Koch used 2 GPs to discover Mycobacterium tuberculosis. In the early 1900s, they were used to discover the passive immunisation against tetanus and diphtheria (14). In 1907, the Norwegian professor Holst und the physician Frölich were able to induce scurvy in GPs due to their dietary vitamin C dependency (15). Since then, they have been used in multiple research fields, thereby aiding the discovery of adrenaline, antibiotics, the investigation of blood transfusion and the replacement of heart valves. Their popularity for the use in studies even led to the term „guinea pig“ in the meaning of the German “Versuchskaninchen”. Nowadays, GPs are regularly used in the field of preclinical safety pharmacology, either in an anaesthetised state e.g. for cardiac electrophysiological effect measurements (16) and for evaluation of cardiac safety of drug candidates (17) or in the awake state for the study of electrocardiogram variables (18,19). Further research areas include the investigation of the respiratory, auditory and immune systems and allergy and nutritional research (7). The Bundesministerium für Ernährung und Landwirtschaft (BMEL) publishes annual numbers of animals used in research in Germany (Table 1). Matching the BMELs aim to decrease the animal number used for research purposes, the number of GPs used in animal experiments is steadily declining.

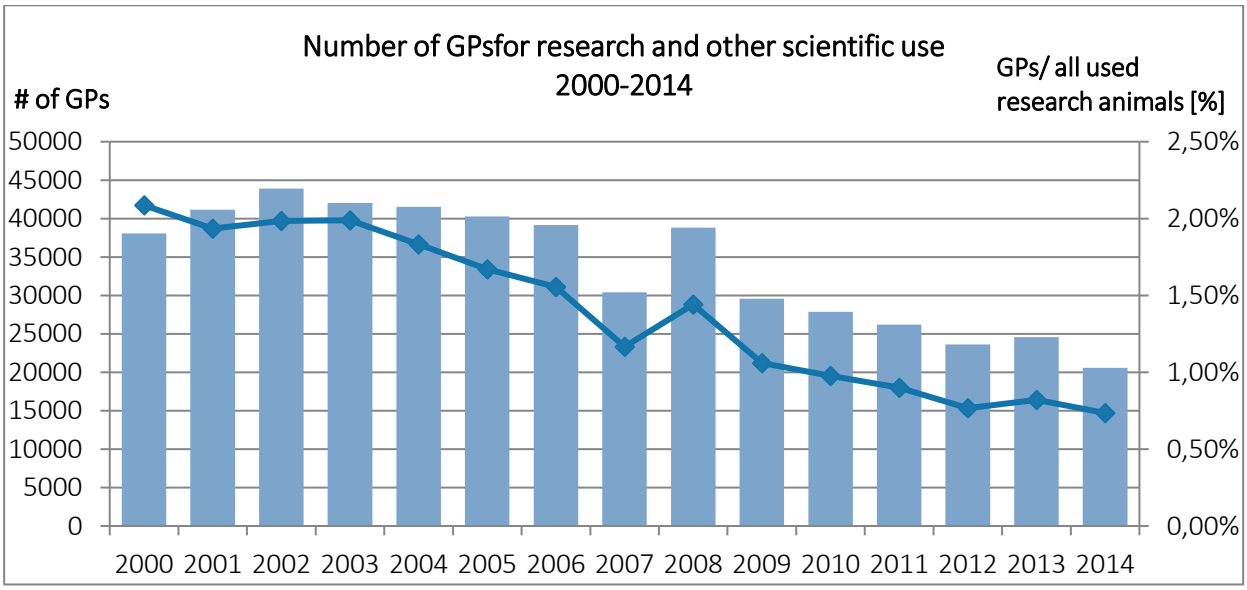

Table 1: (20,21). Bars represent the total numbers of GPs; the trend line represents the course of the percentage share of GPs among all animals used in research.

2.4 Reflexes and evaluation of narcotic depth

The determination of the narcotic depth is the decision criterion on which an operable state is established. It must be checked before every painful procedure which is done by means of the evaluating the reflex responses. Reports and references for the evaluation of reflexes during the anaesthesia in the GP vary widely. Literature reports range from stating only the anaesthetic and dosage, over naming a reflex without defining the testing method or the thereof derived interpretation (8,22), to detailed reports about the performed test and the deduced anaesthesia state (10,11). The correct interpretation of a given reflex response in GPs is admittedly challenging. It requires experience, as the response and the significance vary in the GP (1) depending on such factors as:

- Used narcotic (ketamine induces catalepsy)
- Reflex test performance (intensity, repetition, changing performers)
- Individual condition (BT, illness)

Interpreting multiple test results is, therefore, necessary (23). The first relevant time point during anaesthesia is from when on the animal is immobilised. The second is the onset of surgical tolerance. The hypnosis state (III_1), preceding the surgical tolerance, provides unconsciousness and good muscle relaxation but no analgesia. Pain stimuli in this state can range from heart and respiratory rate increases as far as neurogenic shock (24). Only the surgical tolerance stadium (III_2) provides a strong analgesia. Table 2 shows which tests were described by whom which plane of anaesthesia was tested and in which phase this test was relevant.

Performed tests	Tested plane	Relevant phase	Reference
Righting reflex	Immobilization	II ↔ III_1	Radde, et al. (25) Buchanan, et al. (26) Seidensticker (10) Sloan, et al. (27)
Corneal reflex	Spontaneous reflex arc	III_1 ↔ III_2	Heide (11) Seidensticker (10)
Lid reflex	Spontaneous reflex arc	III_1 ↔ III_2	Radde, et al. (25) Heide (11) Seidensticker (10) Sloan, et al. (27)
Ear reflex (Touching of the outer ear canal with the tip of a clamp)	Spontaneous reflex arc	III_1 ↔ III_2	Radde, et al. (25) Buchanan, et al. (26) Heide (11) Seidensticker (10)
Jaw muscle tone	anaesthesia	III_1 ↔ III_2	Radde, et al. (25)
Hind leg muscle tone	anaesthesia	III_1 ↔ III_2	Radde, et al. (25) Seidensticker (10)
Ear pinch on the **pinna** with a mosquito clamp	analgesia	No reaction ≙ III_2	Buchanan, et al. (26)
Needle/clamp/finger pinching of the interdigital skin/foot pad on the **hind leg**	analgesia	III_2 (28): "too deep" when the response was completely abolished (that was accompanied by severe respiratory depression and cyanosis)	Radde, et al. (25) Jacobson (29) Heide (11) Seidensticker (10) Schwenke, et al. (28)
mosquito clamp pinching of the **interdigital skin** on the **front leg**	analgesia	No reaction ≙ III_2-III_3	Heide (11) Seidensticker (10)
Inguinal reflex (IG) (pinching of the inguinal skin until the 1st ratchet of an artery clamp)	analgesia	No reaction ≙ III_2	Seidensticker (10)
Respiration rate	CNS depression		Schwenke, et al. (28)
mucous membrane colour	perfusion		Schwenke, et al. (28)
Noise sensibility	CNS depression		Seidensticker (10)

Table 2: Performed reflex test, the tested plane, for which phase is it relevant and who described it. Anaesthesia phases (24) II= excitation, III_1= hypnosis, III_2= surgical tolerance, III_3 = depression.

The authors agree that the righting reflex (RR) is the relevant test for the time of immobilization. Thus, a negative RR marks the beginning of induction and the transition from the wake-up to the

recovery period. Before the entrance of surgical tolerance (III_2), analgesia cannot be expected. Therefore, the performed reflex tests on the ear, lid or cornea do not screen for analgesia, but rather provide the anaesthetist with valuable feedback on the narcosis depth on the way to surgical tolerance. Pain-inducing tests only become relevant, when it comes to the differentiation between stage III_1 and III_2. Table 2 shows that the analgesic tests, determining surgical tolerance, are performed on 2 sites, the pinna of the ear and the extremities. There the skin is pinched with the fingers, a clamp or a needle. The desired answer to confirm the surgical tolerance is a very mild or no reaction (24).

2.5 Injectable anaesthetics

A combination of various injectable anaesthetics may be used in GPs depending on

- their utilisation in clinical veterinary practice or the laboratory environment
- the required anaesthesia depth and duration
- the degree of analgesia required
- if the GP is intended to awake from the anaesthesia.

The choice of anaesthesia for use in research protocols is made with a focus on a reliable induction and stable maintenance, a low influence on physiological parameters and a high safety for the user. Ideally, the method should also support a study design in which multiple animals can be kept under anaesthesia simultaneously.

Given these requirements, anaesthetics like urethane (22), pentobarbital (2) or the combination of fentanyl/droperidol/urethane (30) have been used for terminal, long-term studies. These agents were not utilised in veterinary practice due to their disadvantageous side effects like urethane's carcinogenic effect or the very long immobilisation after pentobarbital-induced anaesthesia (294 min, (31)). The following combinations are also described in the literature, but they are not marketed in Germany which limits their use.

- Hypnorm® is a neuroleptic-analgesic combination of fentanyl and fluanisone. If used alone, the analgesia provided is not adequate and, even when used together with a benzodiazepine (midazolam/diazepam), the depth of anaesthesia fluctuates markedly (32).
- Tiletamine/Zolazepam is a combined preparation of a dissociative anaesthetic with longer action than ketamine and a benzodiazepine to counter-balance the muscle tension. In GPs, it was used alone, with an α_2-adrenoceptor agonist or with α_2-adrenoceptor agonist + butorphanol (29).
    - Tiletamine/Zolazepam was tested in dosages from 10-100 mg/kg in GPs, but it did not produce satisfactory analgesia. It may be used for short periods of restraint but it is not suitable for painful procedures (25). Buchanan, et al.(26) tested tiletamine/ zolazepam 40 mg/kg with the addition of 3 α2-adrenoceptor agonists xylazine (5mg/kg), medetomidine (0.5mg/kg) or detomidine (5 mg/kg). They were able to perform ovariohysterectomy in 14 of 30 GPs with the addition of xylazine and in 23 of 29 GPs with medetomidine, whereas no GP lost the ear pinch response when tiletamine/zolazepam was administered

together with detomidine. They concluded that the combination with medetomidine produced a relatively reliable anaesthesia for major surgical procedures which lasted between 168 and 230 min.

- Jacobson (29) studied tiletamine/zolazepam (50/50 mg/mL, 60 mg/kg body weight) with the addition of **xylazine** (5 mg/kg) and **butorphanol** (0.1mg/kg) for deep, long-duration analgesia and rapid recovery with minimal effects on physiological parameters.
- Butorphanol was added to improve the anaesthetic effect because he observed BP effects to the toe pinching test in preliminary anaesthesias with only xylazine. He reports a gentle induction and recovery, long immobilisation (149 min) and surgical tolerance within 10 min in all animals. Deep surgical anaesthesia lasted for 66 min with an additional 43 min on a moderate plane. The cardiovascular system was moderately depressed which was manageable, however, there were substantial effects on respiratory function and blood gas parameters. In summary, the anaesthesia required adjustments to improve the respiratory and blood gas effects and the immobilisation duration.
- **Saffan** is an alfaxalone-alphadolone mixture which was tested intravenously after induction with halothane. Schwenke, et al. (28) report that the transfer to saffan was critical and often accompanied with respiratory arrest and the need for resuscitation. It provided adequate anaesthesia but only when administered close to the lethal dose. Adjusting the correct infusion rate was difficult due to the narrow therapeutic index; therefore, the anaesthesia fluctuated between light and deep.

In contrast to the requirements for experimental procedures, veterinary practitioners focus on different criteria. The adaptability of the narcosis depth is particularly relevant because the animals should only have post-anaesthetic sleep and should return quickly to self-regulation. Furthermore, the safety for animals and users and the long-term effects on the patients are of great relevance. The substances used to fulfil these goals, are described in the following sections.

2.5.1 α_2-adrenoceptor agonists

Alpha$_2$-adrenoceptor agonists are used as analgesic sedatives in veterinary practice and are additionally valued for potentiating the effects of other anaesthetics when used in combinations.
For anaesthesia in the GP, xylazine, medetomidine and recently also dexmedetomidine (the active isomer of medetomidine) are used.

Alpha$_2$-adrenoceptor agonists mimic the effects of the sympathetic nervous system by binding to α_2-adrenoceptors which constitute a family of G-protein–coupled receptors. In the central nervous system (CNS), α_2-adrenoceptors may exist presynaptically, postsynaptically, or both. Blockade of α_2-adrenoceptors, especially presynaptic ones in noradrenergic neurons, promotes the release of norepinephrine. The adrenoceptor subtypes α_{2A} to α_{2D} have been found in the brain (33). They have been isolated centrally (subtype α_{2A}: concentrated in the locus ceruleous, α_{2B}-receptors: only in the thalamus, $\alpha_{2C:}$ mainly in the basal ganglia, hippocampus and cerebral cortex) and in the spinal cord (α_{2A}-receptors: mainly on central terminals of nociceptive primary afferent nerve fibres and in the dorsal horn or lateral spinal nucleus, α_{2C}-receptors: axonal endings on excitatory interneurons) (34).

Alpha$_2$-adrenoceptor agonists induce a dose-dependent sedation. Once the maximal sedative effect is reached, further increases in dose result only in a prolonged, but not deeper sedation (ceiling effect). A deeper level of anaesthesia and analgesia can be achieved by combining α_2-adrenoceptor agonist and opioids. Their receptors are located in the same brain regions and even on the same neurones and can, therefore, activate the same signal transduction systems. Alpha$_2$-adrenoceptor agonists provide a robust muscle relaxation which can counterbalance the catalepsy induced by ketamine. Concerning the cardiovascular system, α_2-adrenoceptor agonists initially lead to hypertension by causing a peripheral vasoconstriction which is then followed by a centrally mediated vasodilation leading to hypotension. With agents like xylazine that have a low α_2-selectivity, the transient hypertension is short and the hypotension long, whereas medetomidine causes a relatively long hypertension (35). With all α_2-adrenoceptor agonists, the HR is distinctly depressed and especially xylazine is known to cause bradyarrhythmias. The central thermoregulation is depressed by α_2-agonists and the lack of muscle activity during anaesthesia rapidly leads to hypothermia. The respiratory rate is lowered centrally but the breathing volume is increased, such that with medetomidine high doses or addition of opioids can result in substantial respiratory depression (36). Side effects of α_2-adrenoceptor agonists are polyuria, reduced pancreatic insulin production with progressive hyperglycaemia and a gastrointestinal motility decrease (37).

The individual reactions to α_2-adrenoceptor agonists differ greatly, depending on the animal's agitation level (36), on the density and location of the α-adrenoceptors and the agent's α_2: α_1 receptor selectivity (38).

Strongly α_2-selective agonists are preferred, because activation of α_1-adrenoceptors is associated with undesirable effects including arousal, restlessness and enhanced movement. Some of the α_2-adrenoceptor agonists activate the imidazoline receptor in the medulla oblongata (Table 3) and that may play an important role for the hypotensive effect (35) observed after α_2-adrenoceptor agonists administration.

	Compound	α_2: α_1 receptor selectivity	I_2 – Imidazoline activity
Agonists	Xylazine	160	No
	Detomidine	260	Yes
	Medetomidine Dexmedetomidine	1620	Yes Yes

Table 3: Alpha$_2$-adrenoceptor agonists used in the GP, their receptor selectivity and their imidazoline activity.

Mechanism of action

Alpha2-adrenoceptor agonists inhibit the presynaptic influx of calcium and neurotransmitters like dopamine and norepinephrine in the CNS. The resultant sedation is mediated in the brainstem and pons by the reduction of norepinephrine release which is needed for arousal. Stress, pain, fear or excitement can interfere with this mechanism, as they increase excitatory endogenous catecholamines (38). The induced analgesia results from interaction with multiple sites in the pain pathway. Alpha$_2$-adrenoceptors in the dorsal horn of the spinal cord are targeted directly and transport the nociceptive signals to the brainstem where they are modulated. Bradycardia develops because the outflow of norepinephrine is reduced and decreased sympathetic tone together with the peripheral vasoconstriction causing an increase in systemic vascular resistance. The inhibition at the interneural level of the spinal cord results in the muscle relaxation. Diuresis is caused by the reduction of antidiuretic hormone and an increased glomerular filtration due to initial hypertension.

Pharmacokinetics

Xylazine and medetomidine are rapidly absorbed after i.m. injection. Peak plasma levels of medetomidine are reached 10 min after s.c. administration in the rat (37). Xylazine and medetomidine are biotransformed in the liver via aliphatic hydroxylation and oxidation and their inactive metabolites are excreted mainly in the urine and, to a small extent, with the faeces (species specific) (5,39).

Field of application

Alpha$_2$-adrenoceptor agonists are applied in both experimental and clinical settings and are used for sedation (0.15mg/kg i.m., Erhardt, et al. (13) and more commonly for general anaesthesia in the GP as they can be combined easily with opioids or with ketamine. In Germany, xylazine (20 mg/mL) and medetomidine (1.0mg/mL) are only approved for use in dogs and cats and therefore need to be used "off-label" for GPs. Owing to its side effects, α_2-adrenoceptor agonists should not be used in patients with cardiovascular, kidney or liver disease (5).

2.5.2 Atipamezole

Atipamezole is an α_2-adrenoceptor antagonist and rapidly and effectively reverses all effects of the α_2-adrenoceptor agonists. It is highly selective for all α_2 subtypes (α_2/α_1, 8526:1 receptor selectivity, (33)) and is therefore preferred over yohimbine. In Germany, it is only sold for the intramuscular use in dogs and cats in a 5mg/mL concentration.

Mechanism of action

Atipamezole blocks the central and peripheral α_2-adrenoceptors, thereby preventing their excitation.

Pharmacological effect

Atipamezole increases the central noradrenaline and serotonin turnover, clinically visible in excitement and agitation, a transient hypotension and tachycardia (34).

Pharmacokinetics

Atipamezole may be applied para- or intravenously, but apart from emergencies, s.c. or i.m. administration should be used to achieve a slower onset of effect. After s.c. administration, it is rapidly absorbed and distributed. Medetomidine can be reversed within 3-7 min when using a 4-6 fold dose of atipamezole. Its half-life is twice as long as that for medetomidine, usually keeping the patient from becoming re-sedated (38). The LD50 is > 30mg/kg after i.v., s.c. or i.p. injection in mice and rats. The elimination half-life is 1.3 h after a single s.c. dose in the rat and it undergoes an extensive first-pass effect in the liver where it is also metabolised (34).

Field of application

In GP anaesthesia, atipamezole is the antagonist of choice for the reversal of α_2-adrenoceptor agonists. Therefore, it is used for partial antagonisation of KX and it is an indispensable part of the MMF antagonisation.

2.5.3 Midazolam

Midazolam is a benzodiazepine with anticonvulsant, anxiolytic and mildly sedative properties and may be competitively antagonised with flumazenil or sarmazenil (distributed in Switzerland). Benzodiazepines act on the $GABA_A$-receptors which are found mainly in the cerebral cortex, hypothalamus, thalamus, limbic system and to a lesser extent in the periphery. This primarily CNS receptor distribution also explains their lack of effect on the circulation and respiration in therapeutic doses (40), however, benzodiazepines may potentiate the respiratory depressive effect of other agents. Unlike diazepam, midazolam is water soluble and can, therefore, be administered in combination with other drugs in a single syringe, as used with MMF (41).

Mechanism of action

Benzodiazepines bind to the $GABA_A$-receptor, a large macromolecule with multiple binding sites which enable synergistic effects with e.g. opioids (40). This binding opens the GABA-activated chloride channels and enables the inflow of chloride ions, thus negatively charging the neurone and making it resistant to excitation. This results in the muscle relaxing, sedative and anxiolytic effect. Different subunits on the $GABA_A$ receptor are likely responsible for these different effects, with the α_2 subunit probably causing the anxiolytic effect. Activation of GABA receptors in the motor neurones and dorsal horn of the spinal cord probably mediate the muscle relaxing effects.

Pharmacokinetics

Due to its good aqueous solubility and therefore the lack of irritating solvents, intra and extra venous (s.c., i.m., i.p.) injection is possible. There is a high bioavailability of around 90 % after i.m. administration. Midazolam is metabolised through α-hydroxylation by cytochrome P450 in the liver into an inactive metabolite which is excreted mainly in the urine (42).

Field of application

Midazolam has been used for sedation (2.5 – 5.0 mg/kg i.m. (13)) but the degree of sedation varies individually and the duration of sedation often does not suffice for an adequate treatment. Therefore, in GPs, it is mostly used in combination with fentanyl and α_2-adrenoceptor agonists within the fully antagonisable anaesthesia (VAA/MMF).

2.5.4 Flumazenil

Flumazenil is a selective benzodiazepine antagonist which can reverse their effects within 1-2 min after i.v. injection by competitively blocking the benzodiazepine binding site on the $GABA_A$-receptor. Depending on the action duration of the used benzodiazepine, additional flumazenil dosing might be required due to its short plasma half-life time.

As the intrinsic activity on the benzodiazepine site is very low, the administration of flumazenil does not induce clinical effects by itself (43). In Germany, it is not marketed for veterinary use and therefore requires an “off-label” use for GPs.

Field of application

Flumazenil is mainly used in GPs for the antagonisation of midazolam in the MMF anaesthesia and, in rarer cases, after benzodiazepine overdosing or to end the sedation. However, the antagonisation of the benzodiazepine in MMF may be omitted (10) which is commonly done because of its high price (255€/ 100 mL, August 2016).

2.5.5 Fentanyl

Fentanyl is a synthetic and selective μ-opioid-receptor analgesic which is approximately 100 times more potent and 1000 times more lipophilic than morphine (35,44). Since 2012 it is distributed for animal use in Germany as fentanyl citrate 50μg for dogs and it underlies the strict regulations for narcotic drugs. Fentanyl may be antagonised with naloxone.

Mechanism of action

Fentanyl selectively acts on μ-opioid receptors in the CNS and in the periphery. Depending on the receptor subtype, different agonistic actions are mediated (Table 4).

Opioid receptor	Agonistic action	Drug
μ_1	analgesia (mainly supraspinal), respiratory depression, low addiction risk, bradycardia, hypothermia, peripheral vasodilation, miosis	morphine, fentanyl, levomethadone, buprenorphine, butorphanol, naloxone
μ_2	Analgesia (spinal), respiratory depression, high addiction risk	

Table 4: (35). Opioid receptors, their agonistic action and the drugs that act on the μ-opioid receptors.

Pharmacological effect

Fentanyl has a short duration of action (20-30 min) (35). This comes with the benefit of quick adjustability, but it is simultaneously unsuitable for long-term anaesthesia. Extravenous and venous administration routes can be chosen. The most relevant side effect in the GP is respiratory depression. Research shows that low opioid doses influence the production of the breathing rhythm and pattern and that higher doses decrease the tidal volume (45).

Pharmacokinetics

Fentanyl is quickly metabolised in the liver and excreted in the urine and bile.

Field of application

Fentanyl is usually not utilised in GPs for analgesia, however, it may be administered during very painful surgeries as an intraoperative bolus injection (0.05-0.1 mg/kg). Its bradycardic side effect may be used to evaluate the degree of analgesia. The most common application is as an analgesic component in the MMF anaesthesia.

2.5.6 Naloxone

Naloxone is a pure opiate antagonist without agonistic properties (35) and a high safety margin. It reverses all pharmacological effects from exogenous and endogenous opioids. Attention must be paid to the post-anaesthetic pain management, as it antagonises not only the respiratory and bradycardic side effects of opioids, but also the analgesia. Therefore, an adequate analgesic coverage with a non-opiate needs to be ensured ahead of time.

Mechanism of action

Naloxone competitively binds to the opioid receptors with the highest affinity to μ-opioid receptors. It can be displaced reversibly from the binding site by addition of an opioid agonist. Substantially higher doses of naloxone are needed for the reversal of the partial opioid agonist buprenorphine (43).

Pharmacokinetics

Reports on the half-life of naloxone vary greatly (43) and depend on the administration route. Rebound-effects after opioid use have been described which can be reversed with additional doses of naloxone (0.003-0.03 mg/kg i.m. or s.c. (46)). Naloxone is mainly excreted via glucuronidation in the liver and excreted in the urine.

Field of application

Naloxone is presently only marketed for human use in Germany. In GP anaesthesia, it is used for the antagonisation of the MMF anaesthesia. It may also be used in emergencies to reverse respiratory depression after opioid administration with a dose of 0.01-0.1 mg/kg (47); it should be administered using the quickest route.

2.5.7 Fully antagonisable anaesthesia (VAA or MMF)

The anaesthesia consisting of **opioid, benzodiazepine and α_2-adrenoceptor agonist** is a relatively new combination anaesthesia which holds the unique benefit of antagonisability of all components. Thus, the MMF anaesthesia can now be terminated similarly fast as it is possible with Iso anaesthesia. The MMF anaesthesia has also been applied in many other small mammals in addition to the GP (rat, mouse, gerbil, chinchilla, hamster (5)). The 3 components are easily combined and they potentiate their equal effects, like the analgesia in α_2-adrenoceptors activation and opioids. That provides the benefit of no or reduced side effects due to the smaller doses required.

Roberts (48) described a fully antagonisable anaesthesia with the i.m. administered agents **fentanyl** (0.05mg/kg), **climazolam** (2.0 mg/kg) and **xylazine** (2.0 mg/kg). She stated a good surgical tolerance for approximately 45 min in GPs. The negative effects on respiration, HR and circulation were completely reversed within 2 min after i.v. antagonisation using **naloxone** (0.03 mg/kg), **sarmazenil** (0.3 mg/kg) and **yohimbine** (2.0 mg/kg).

Henke (1) refined the combination by using **midazolam** (1.0mg/kg) instead of climazolam, as this benzodiazepine is commercially available in Germany and soluble in water in contrast to diazepam. Replacing xylazine with **medetomidine** (0.2 mg/kg) and its substantially higher α_2-adrenoceptor selectivity reduced the α_1-adrenoceptor mediated effects of peripheral vasoconstriction and led to a predictable and effective sedation and analgesia. The antagonist combination was also improved with the benzodiazepine antagonist **flumazenil** (0.1 mg/kg) and the α_2-adrenoceptor antagonist **atipamezole** (1 mg/kg). The following Table 5 lists the currently recommended doses for the agonist and antagonist mixtures in GPs.

Purpose	Product name
agonists	Intramuscular injection (i.m.) in mixed syringe MMF = 1. Medetomidine 0,2 mg/kg + 2. Midazolam 1,0 mg/kg + 3. Fentanyl 0,025 mg/kg
antagonists	Subcutaneous injection (s.c.) in mixed syringe AFN = 4. Atipamezole 1,0 mg/kg + 5. Flumazenil 0,1 mg/kg + 6. Naloxone 0,03 mg/kg

Table 5: Medetomidine-midazolam-fentanyl anaesthesia agonist and antagonist dosages for the use in GPs (13).

Seidensticker (10) studied this combination, also known as “triple anaesthesia“ in 51 GPs in clinical practice and antagonised the animals either fully or partially, namely with or without the benzodiazepine antagonist, here sarmazenil. The fully antagonised GPs regained their RR approximately 5 min earlier and displayed atipamezole related temporary restlessness, whereas some animals in the partially antagonised group displayed a lingering midazolam sedation.

Pros and Cons

The benefits of the MMF anaesthesia include a low-stress induction, as the triple combination does not require a premedication. The GP needs to be injected once or twice, depending if the total injection volume exceeds the amount suitable for a single injection site. The loss of righting is achieved within 3.5 min and they are surgically tolerant after 10 min after induction. During the anaesthesia, the fentanyl provides good analgesia coverage. The surgical tolerance can be maintained for at least 40 min and the duration can be easily extended if needed by injecting one-third of the initial dose. For reversal, the AFN mixture is injected s.c. and the GP will regain a reliable RR within 3-7 min. Injection anaesthesia facilitates transport or repositioning of the animal if needed, compared to inhalation anaesthesia, nevertheless, supplemental oxygen and warmth must be available. A disadvantage of the MMF is the time required until both mixtures are prepared and the necessity to store 6 substances simultaneously. The relatively large dose volume needs to be divided into two injections if the volume exceeds 0.5 mL with the drawback of having 2 injections (1). Additionally, fentanyl is regulated by narcotic drug guidelines and flumazenil is expensive. The latter may be omitted without great disadvantages, depending on the patient (10). The disadvantage of the analgesic antagonisation can be balanced easily by applying metamizole or NSAIDS at the beginning of a painful procedure. For procedures inducing longer or substantial pain, naloxone antagonisation may also be omitted (6).

Field of application

This anaesthesia can be used by both clinicians and researchers, but it has not yet been described in laboratory GP studies, maybe because MMF literature published in English is limited. With the exception of cases needing only very short narcosis or multiple anaesthesias per day, MMF has become the gold standard in Germany for a variety of procedures requiring anaesthesia in GPs, like castration (49).

2.5.8 Ketamine

Ketamine induces a state of “dissociative anaesthesia”, meaning a functional splitting between thalamo-neocortical and the limbic systems. Ketamine dampens the thalamo-neocortical system causing analgesia or better anti-nociception (50). It shows these effects with significantly lower blood concentrations compared to those required for unconsciousness. In addition, there is a marked sedation or even hypnosis occurs. The depression of the thermoregulatory centre makes the animal more susceptible to temperature loss. The pulse rate and BP are markedly increased whereas the respiratory rate is decreased and altered to an apnoeic pattern (51). By stimulation of the limbic system, the skeletal tone is increased (catalepsy) with maintained or even enhanced laryngeal, pharyngeal, lid and corneal reflexes. Ketamine further stimulates the cerebral blood flow, metabolism and intracranial pressure as well as stimulating the superior circulatory and respiratory centres (52).

Mechanism of action

In the CNS ketamine acts as a non-competitive antagonist on the NMDA-receptor, a subtype of the glutamate receptor. Binding to the receptor leads to the inhibition of sodium, potassium and calcium ion fluxes. It also reduces the presynaptic release of the excitatory neurotransmitter glutamate. Ketamine further agonistically interacts with the mu and kappa opioid receptors, but with 10 times lower affinity compared to the NMDA-channel.

Pharmacokinetics

Ketamine has a high bioavailability of 93 % after i.m. injection and, due to its high lipophilicity, passes through the blood-brain barrier easily (52). The pharmacokinetic half-life and duration of effect varies depending on the speed of metabolisation, age and general condition of the patient but are generally short (clinical duration of 30 min for rodents (43)). Ketamine is metabolised in the liver by cytochrome P450 via demethylation and hydroxylation and the metabolites are conjugated and excreted in the urine (51).

Field of application

Currently, ketamine is marketed in Germany as Ursotamin® 100mg/mL for s.c. and i.m. injection for GPs and consists, like the other veterinary ketamine preparations, of both the R and S isomers. Meanwhile, the S-isomer, with a 3-4 fold higher NMDA-receptor affinity, was introduced to the human drug market. Experiences with its use in the GP have not yet been published.

Intramuscular injection of ketamine has been described as painful and cause of muscle necrosis (53) due to the low pH of 3.5-5.5 in all marketed ketamine preparations in Germany. In the GP, ketamine when used alone does not induce a satisfactory anaesthesia and is, because of its other effects, also not used for analgesia. In the veterinary practice and in experimental procedures, it is often combined with α_2-adrenoceptor agonists or benzodiazepines, however, even these combinations are associated with a prolonged recovery and catalepsy (26).

2.5.9 Ketamine and α_2-adrenoceptor agonist combination

Ketamine combined with xylazine (KX) is the most commonly used injection anaesthesia in the GP and has been performed in veterinary practice and in the experimental laboratory with varying dosages. The difficulties with this combination are reflected in the divergent literature reports concerning anaesthesia depth and duration, number of injections required and difficulties with post-anaesthetic recovery. Although the partial antagonisation of xylazine is possible with either yohimbine or atipamezole, according to the literature it is not often practiced, possibly because of the reported ketamine hangover after xylazine removal (1). The following references summarize the anaesthesia experiences with KX use in non-terminal studies (Table 6).

Ketamine [mg/kg]	Xylazine [mg/kg]	Purpose/ experience	Reference
35 60 87	5 8 13	i.p., 87/13 combination can be used for restraint and mildly painful procedures but not for major operative procedures. Dose dependent reduction of body temperature and respiratory rate	Radde, et al. (25), 1996
40 60	5 5	i.m., both dosages failed to provide adequate anaesthesia to perform ovariohysterectomy	Buchanan, et al. (26), 1998
80-100	2-3	i.m., "risky" anaesthesia extension, interdigital reflex response often persisting	Henke (1), 1998
20	5	i.v. infusion, surgical anaesthesia was attained at low doses. During the experiment, the animals didn't experience adverse side effects	Schwenke et al. (28), 2004
30	5	i.m. provided 30-50 min of anaesthesia for procedure requiring restraint (echocardiography)	Çetin, et al. (54), 2005
30	2.5	s.c./i.m./i.p., suitable for routine procedures up to 45 min (bleeding from the vena cava)	Dang, et al. (31), 2008
15 20	5 8	Ketamine i.p., xylazine i.m., abdominal ECG radiotelemetry implantation	Stemkens-Sevens, et al. (55), 2009

Table 6: A recent literature overview on KX use with recovery in GPs.

Ketamine (40 mg/kg) paired with **medetomidine** (0.5 mg/kg) or **detomidine** (5 mg/kg) was tested by Buchanan, et al. (26) which caused a quick onset of anaesthesia that lasted for 118 –196 / 110 – 167 min but did not produce adequate analgesia for ovariohysterectomy. Combining ketamine with **dexmedetomidine** (active isomer of medetomidine) is also an interesting option. The dosage recommendation of ketamine (40 mg/kg) and dexmedetomidine (0.25 mg/kg) i.p. was issued by Flecknell (56), however, so far literature lacks experience statements for the use in GPs.

Pros and Cons

KX offers the advantages of using 2 inexpensive active ingredients which are easily combined and provide the user with a patient that can be easily moved or repositioned. Also, the application of KX is, apart from the risk of self-injection, safe for the operator. Disadvantages include the unreliable induction phase of anaesthesia without obvious reasons for the different individual responses and it can cause muscle necrosis after i.m. injection (53). Catalepsy may occur during recovery and there is a long post-anaesthetic recovery with the need for close physiological monitoring. There are also reports of ambiguous reflex responses during anaesthesia (1).

Field of application

KX may be used for many procedures requiring anaesthesia in the GP (see Table 6). Within veterinary medicine, KX is used with great success in many animal species (cat, dog, cattle), it is readily available and inexpensive. Therefore KX may comprehensively also be applied to GPs by a user with little GP experience, someone who is unaware of the new options in the anaesthesia field or for whom the MMF anaesthesia is not profitable. This occurs in common practices with a low small mammal share or in the research field with long-term established study designs which asks for a special design or reproducibility.

2.6 Inhalation anaesthesia

Another method of inducing anaesthesia is the exposure to the active ingredient with the breathing air. Inhalation anaesthesia provides very quick anaesthesia level adjustability that is not possible with injectable anaesthetics. The inhalation anaesthetics methoxyflurane (57), halothane (58), enflurane (59), isoflurane (Iso) and sevoflurane (11) have been tested in the GP (see following paragraph). As explained below, Iso remains as the only realistic option from this selection and is therefore dealt with in more detail in the following subchapter.

The induction of volatile anaesthetics in GPs is performed by exposing the whole animal to the narcotic gas (Fig 1). After the loss of the RR the anaesthesia can be maintained by further administration using a nose cone (Fig 2). Macedo, et al. (8) combined ketamine (15 mg/kg) and butorphanol (1mg/kg) for the induction of anaesthesia and maintained a surgical level of anaesthesia for fixation of a fracture with Iso (no stated vol. %) thereafter. They describe the premedication and maintenance as uneventful.

Fig 1: Whole body chamber used for the induction of Iso in GPs.

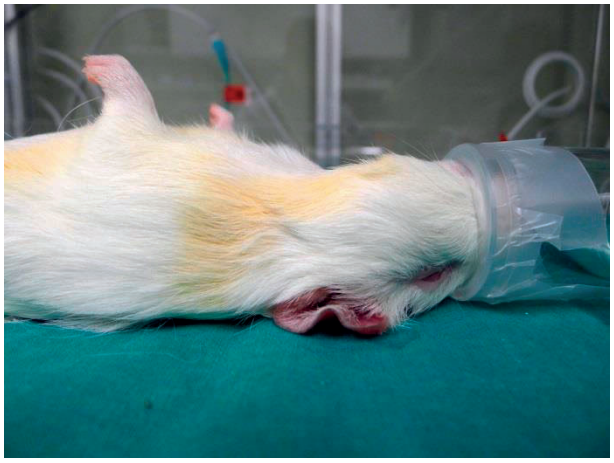

Fig 2: Nose cone used for the maintenance of Iso anaesthesia in GPs.

Cooke (58) experimented with halothane in the GP (2.25 vol. % maintenance) and reports a “return to consciousness” after 10 min. Seifen, et al. (59) determined the MAC (minimal alveolar concentration, 50 % of patients do no longer show a reaction to a pain stimulus) of halothane with 1.01 vol. %. It leads to extreme dose dependent cardiovascular (52 % decrease in mean arterial blood pressure; 30 % decrease in HR, (60)) and respiratory depression and increases bronchosecretion. The 20 % degradation in the liver produces highly toxic metabolites which can cause liver cell necrosis (60). Further disadvantages are its lack of analgesic effect, mild hypnosis and

unsatisfactory relaxation (61). In Germany it is no longer used in the GP, but it was recently chosen for anaesthesia in a Japanese study (62).

The newest inhalation anaesthetic in veterinary medicine is sevoflurane which provides the quickest on- and offset of anaesthesia and has been investigated by Heide (11) in the GP. It required 7.8 vol. % for a loss of the RR which was accomplished after approximately 4.5 min and 7 vol. % to maintain the anaesthesia. Premedication with atropine prevented a saliva production in the induction phase. Even with premedication, 16 of 22 GPs developed gasping breathing up to 25 min after induction and had to be resuscitated with oxygen and tilting. The respiratory rate decreased to 21 breaths per minute. Based on this outcome, Heide (11) advised against the use of sevoflurane in the GP.

2.6.1 Isoflurane

Iso is a halogenated methyl ether with 5 fluorine ions and was synthesized for the first time by Ross Terell in 1965. It is a clear, volatile liquid and is neither explosive nor inflammable. The rather pungent ether-like smell is reported to cause aversive reactions in animals.

Pharmacological effect

Iso is strongly hypnotic and muscle relaxing, but not considerably analgesic in clinical doses (61). Seifen, et al. (59) determined the MAC of Iso with 1.15 vol. %. In the cardiovascular system, there is a decreased peripheral resistance with a resultant fall in systemic BP, reflex tachycardia (43) and a reduction of renal blood flow with lower glomerular filtration rate. The respiration is dose dependently depressed and the gas irritates the airways causing salivation and bronchosecretion (11,61).

Mechanism of action

The detailed mechanism of action of volatile anaesthetics is not yet fully understood. It is, however, known that the immobilisation is primarily mediated through the depression of spinal neuron networks. Grasshoff, et al. (63) found that glycine (39 %) and $GABA_A$ (36 %) receptors were mainly responsible, but that glutamate receptors and potassium channels also caused some of the relaxation. The hypnosis is produced in the CNS by modulation of nicotinic acetylcholine, the potentiation of inhibitory $GABA_A$-receptors und the depression of excitatory glutamate transmission (Campagna et al., 2003). The changes in blood flow result from multiple factors: the relaxing action of

anaesthetics on the vascular smooth muscle, the possible mediatory role of nitric oxide (64), MAP (mean arterial blood pressure) decrease through vasodilation, influence of indirect metabolic mechanisms following the reduction of cardiac work, pressure-flow self-regulation, and time-dependent vascular adaptation (65). The respiratory function is impaired by the inhibition of the respiratory control systems like the feedback of central respiratory centres, chemoreceptors, pulmonary reflexes and neuronal input thereby altering the oxygen supply and the CO_2 elimination (66).

Pharmacokinetics

Iso is administered with the breathing air by means of a special vaporizer. Its high lipid solubility allows a quick entrance into the blood stream and further on into the brain. The low blood solubility ensures the quick on- and offset of effect, clinically observed by a fast adjustability. In the GP the induction concentration is 4.5 vol. % which is then lowered depending on the individual ReR and reflex responses. Iso is almost fully exhaled and only 0.17 % is degraded over atoxic metabolites.

Field of application

Iso is used in common practice and in the laboratory field and is licensed for GP use in Germany. In research it is frequently used to anaesthetise GPs for short periods of time or repeatedly on the same day. Toxicology studies benefit from the low metabolisation it offers. As small amounts of Iso evaporate into the surrounding air during every anaesthesia (e.g. machine or patient piece leakage) which affects the anaesthesia operator, special working safety measures need to be met such as suctions or the exclusion of especially endangered individuals (expecting mothers). Also, the transportability of animals under Iso is more challenging as the gas needs to be constantly applied.

Specialities in the GP use

Literature reports on Iso anaesthesia in the GP are very limited. The most detailed research has been done by Heide (11). She confirmed older reports on the necessity of premedicating the GPs which she performed with 0.04 mg/kg. This decreased the fluid secretion to the mucous but did not prevent thick mucous formation after 30 min which became clinically noticeable in breathing sounds. Atropine also averagely increased the pulse rate by 42 bpm.

Multiple researchers (67–69) subjected GPs to Iso to perform abdominal radiotelemetry transmitter implantation. Apart from performing the implantation, the reference does not state if premedication was used, how long the anaesthesia was maintained or if there were any problems.

2.7 Radiotelemetry

Telemetry originates from the Greek words for “far” and “measure” and means acquiring information from a distance. The parameters are collected by sensors on the measuring point and are reported to another spatially separated location. With radiotelemetry the acquired information is transferred via radio waves. Figure 3 shows the fully implantable radiotelemetry device (PhysioTel ™ HD-S11, DSI, St. Paul, USA) for small mammals that was used in all GPs in this study.

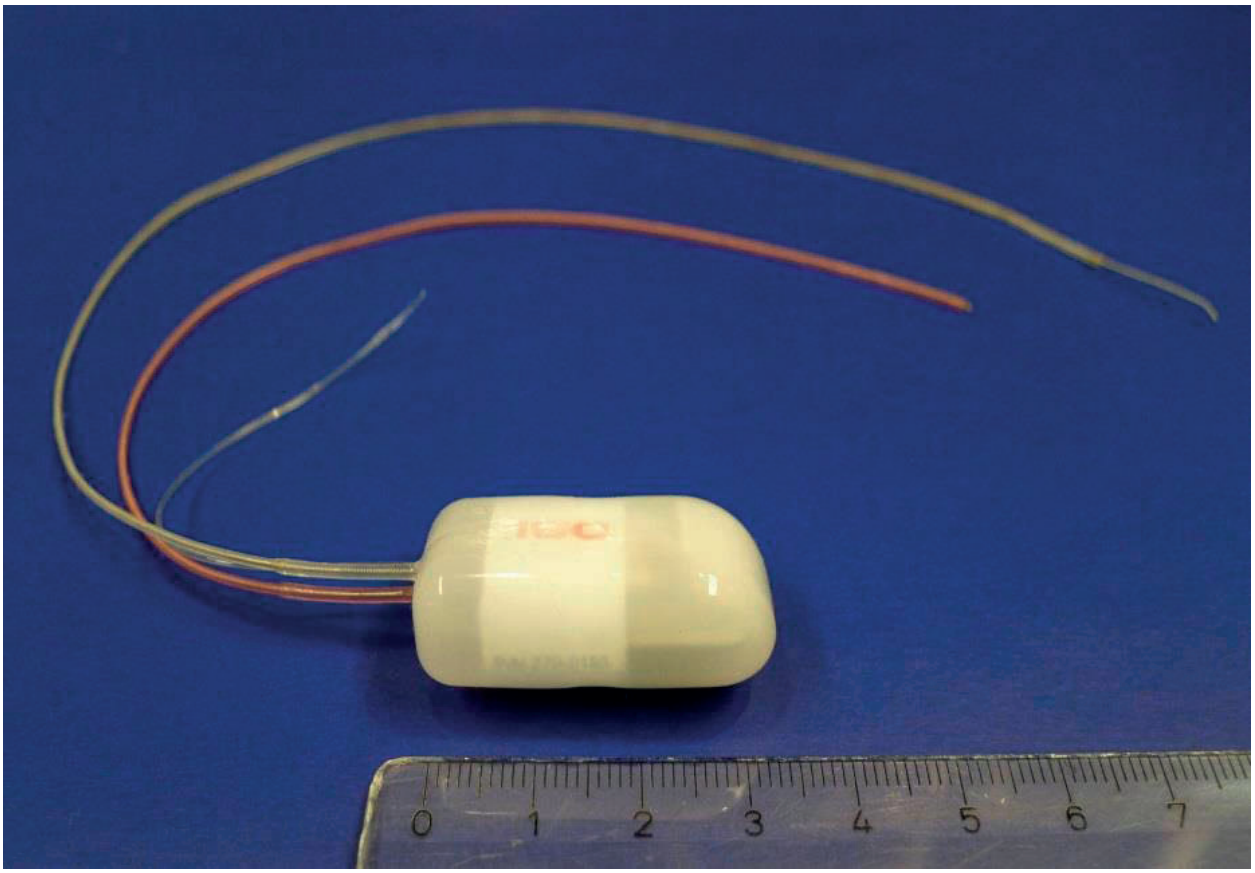

Fig 3: Fully implantable radiotelemetry device (PhysioTel ™HD-S11, DSI, St. Paul, USA) for small animals, 8 g, 5.9 cc volume, minimum animal weight 175 g, 2 months warranted battery life. Transmitter body with 2 ECG leads (long white and red) and one blood pressure catheter (transparent led).

The red and the white leads are ECG cables with an inboard wire and outside insulation. The BP catheter is made out of plastic, it is fluid filled and the last centimetre is gel filled. The body is covered with a silicone that provides 4 eyelets on the side facing down with which it can be sutured to the inner abdominal wall. Figure 4 shows the location of the implant in the GP. Due to the material properties the BP catheter is not visible. The cable loops in the inguinal and the tracheal

area are installed during surgery to allow some leeway during movement or in the process of animal growth. The small loops at the end of the wire enable a better ECG signal compared to straight wire ends.

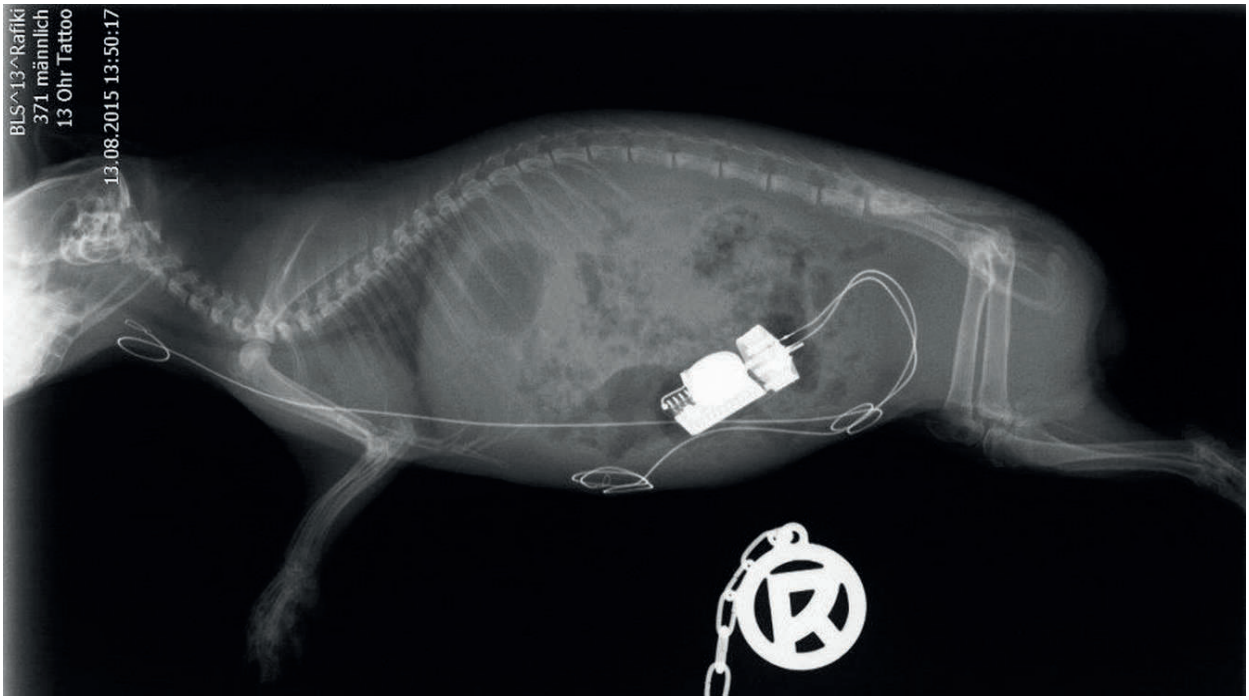

Fig 4: Radiotelemetry implant in a GP. The device body is implanted in the abdomen; both ECG leads are fixed subcutaneously, one over the trachea and the other over the pectoral muscle. The blood pressure catheter is fluid filled, made out of plastic and is therefore not visible.

The implantation approach is basically the same as described in rats (4) but species specific differences in anatomy (e.g. thinner and more embedded abdominal aorta) and physiology (need for absolutely sterile environment) complicate the procedure in the GP.

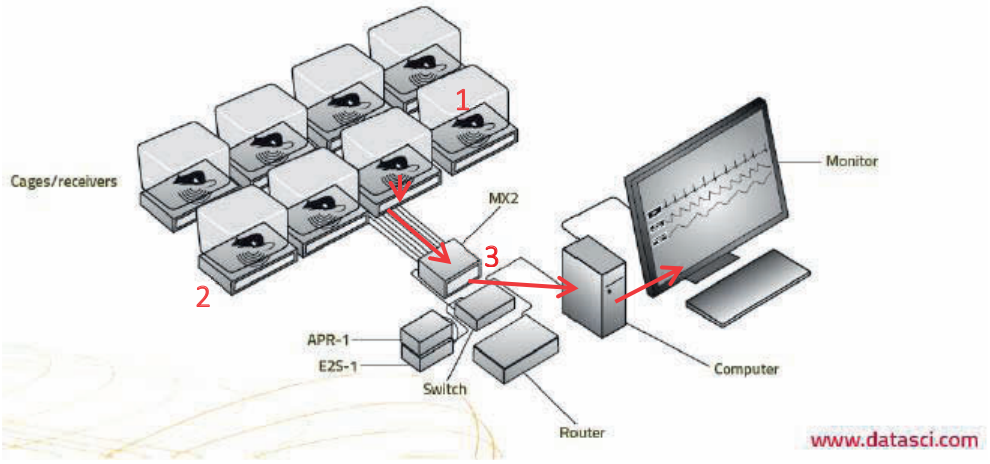

Fig 5: Radiotelemetry set up for 8 animals by DSI. 1= device inside the animal, 2= receiver plate, 3= matrix.

Therefore we published our detailed approach in the GP in a separate paper (publication 1) instead of adding it to the single anaesthesia description (publication 2). Explanation of the approach is therefore skipped at this point. After successful implantation, the GP can be used in the setup (Fig 5) with places for 8 animals as marketed by Data Sciences International (DSI, St. Paul, USA).In the beginning, the individual GPs' device is powered on with a magnet and from then on, the animals', BP passed along the gel filled tip and fluid filled cable, is recognized by a sensor inside the device body (Fig 5.1). For this type of device, the BP signal is audible with a radio. A transmitter modulates the BP signal into a frequency modulation (FM) wave which now carries the BP signal via radio waves without the need for a direct connection. The radio waves are registered with an antenna in the receiver plate which is located below the animals' cage (2). The receiver plate sends the signal to a matrix (3) which demodulates the FM back into a voltage that can then be observed on a monitor display. The ECG and activity values are acquired alike. With this device type, the values can be observed in real time, providing an excellent tool for monitoring anaesthesia.

The telemetry technology has existed for more than 50 years, but only the development of affordable, smaller and more reliable devices in the last 15 years has allowed it to enter the laboratory field (3,4). It has proven itself as a very versatile tool with many benefits. Above all, it eliminates artefacts from restraining the animal (4) and allows the investigation of a wide range of areas such as physiological, behavioural, welfare and pharmacological research (55). It further reduces the animal numbers used in research as one animal can be used repeatedly. Measurements can be performed continuously, even outside of usual working hours (70) and over a long period of time(71). It is considered the gold standard for acquiring reliable measurements of cardiovascular parameters (70) and BT (53).The technique has been validated versus the BP measurement with an exteriorized catheter in the GP (72). This is especially relevant for BP measurements as the alternative is having to euthanize the animal after direct carotid artery cannulation. Also, the implanted animals can still be group housed. These benefits and the large number of acquired measuring points per animal reduce the animal number by 60-70 % in single studies and up to 90% in multiple studies (4) thereby adding greatly to the 3R idea.

A great disadvantage of BP radiotelemetry is the related cost. The installation of a comparable setup requires 16 transmitters for small animals, 8 receiver plates, a modulation matrix and an analysis software worth a total of approx. 175.000 € on top of unspecific requirements such as a measuring room, a small animal surgery room and a computer for monitoring. The implantation

of a radiotelemetry device with a BP catheter in GPs requires advanced surgical skill and is accompanied with a lower long-term survival rate compared to the rat. Also, the GP BP model requires surgery and a long lead time, in our setting at least 5 weeks, before it can be used in a study for the first time. The GPs need to be ordered and delivered, they need to acclimatize to the new setting, the surgical implantation has to be performed and needs to be successful in a sufficient number of animals. Thereafter, a recovery period of at least 1 week is required. A faster entry in a research study is not reasonable as the implantation influences contradict the use of the animal as a reliable model organism (71). Late onset infections, unrealistic measuring results or fighting among the GPs can further reduce the number of usable animals after the critical early stage. Furthermore, in the field of safety pharmacology the predictability of results acquired in the GP for humans is questioned as the GP physiologically exhibits only about half of the BP seen in humans. Out of the sum of these reasons, the BP implanted GP is rarely used in the real research environment.

2.7.1 Data analysis

To be able to show the received anaesthesia signals on a computer display, we used one of several data analysis programs, Notocord-hem™ (NOTOCORD Systems, Croissy-sur-Seine, France). In figure 6, the parameter courses of HR, BT and BP are shown during Iso anaesthesia in a male GP.

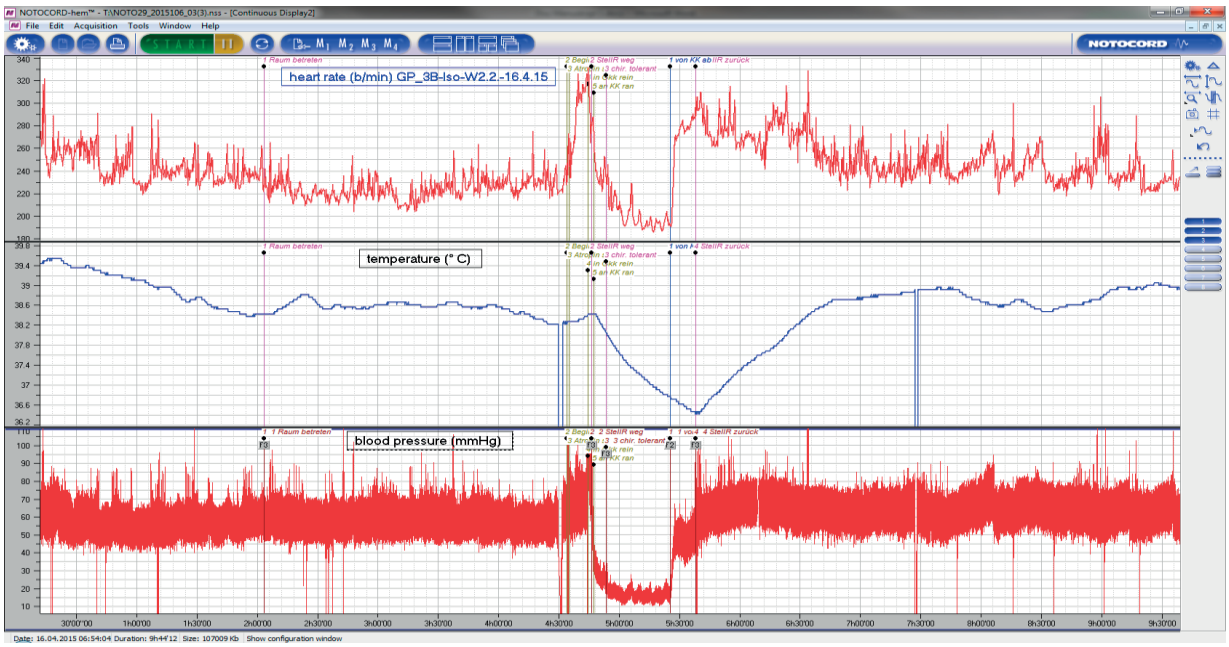

Fig 6: Screenshot of an Iso anaesthesia signal read-out with Notocord-hem™ in a male GP, monitored by an implanted BP radiotelemetry device.

The label, colours, axis scaling and the shown interval can be adjusted individually among many other options. With the keys F1-F3 relevant time points can be set. F3 (pink) was used for notes like “entering the room”, F1 (yellow) for application of atropine or exposure to Iso and F2 (blue) arked the end of Iso exposure. Figure 7 shows the continuous display in which the ECG and the BP can be monitored in real time. The data points were then transferred with the add-in “Analysis ToolPak” to Microsoft Excel. Each anaesthesia was edited in a separate file and all of them were given to the internal statistic department for mathematically correct table and graph design.

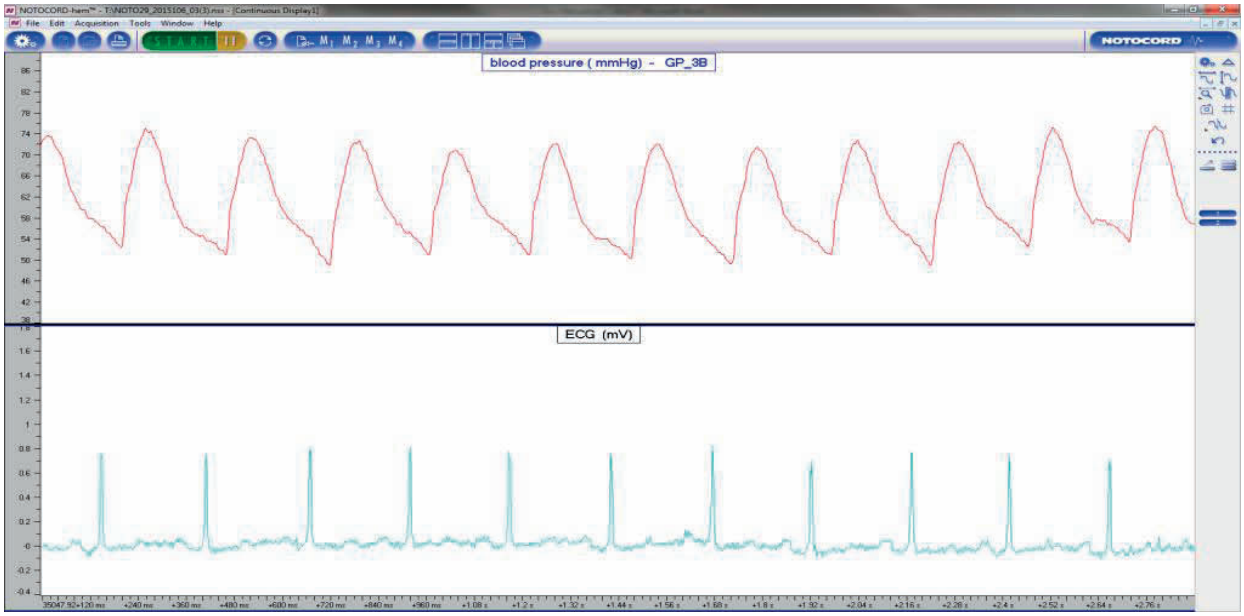

Fig 7: Continuous display in the Notocord-hem™ software during the signal read-out of a radio-telemetry signal implanted into a GP.

2.7.2 Setup

The anaesthesias were performed in a closed room, furnished with the radiotelemetry receiving equipment. The required anaesthesia devices were integrated into the existing setup, such that the whole process of the narcosis (baseline, induction, maintenance, recovery) was performed in the same room without re-moving the animals. The single cages were placed directly over 2 com-bined receiver plates as one device provided a dome-shaped signal range of approx. 30 cm. The device signals interfered with each other if they came to close because they all transmit their sig-nals on the same frequency. Moreover, no metallic materials could be placed between the animal and the receiver. For that reason neither the water heating tub used during our implantation nor an electrical heating mat could be used during the anaesthesia. The receiver plates were covered

with plastic water heating mats (Fig 8 & 9) attached to smoothly adjustable water baths which were protected by surgical drapes. An Iso extraction hood was positioned over one working place (Fig 8).

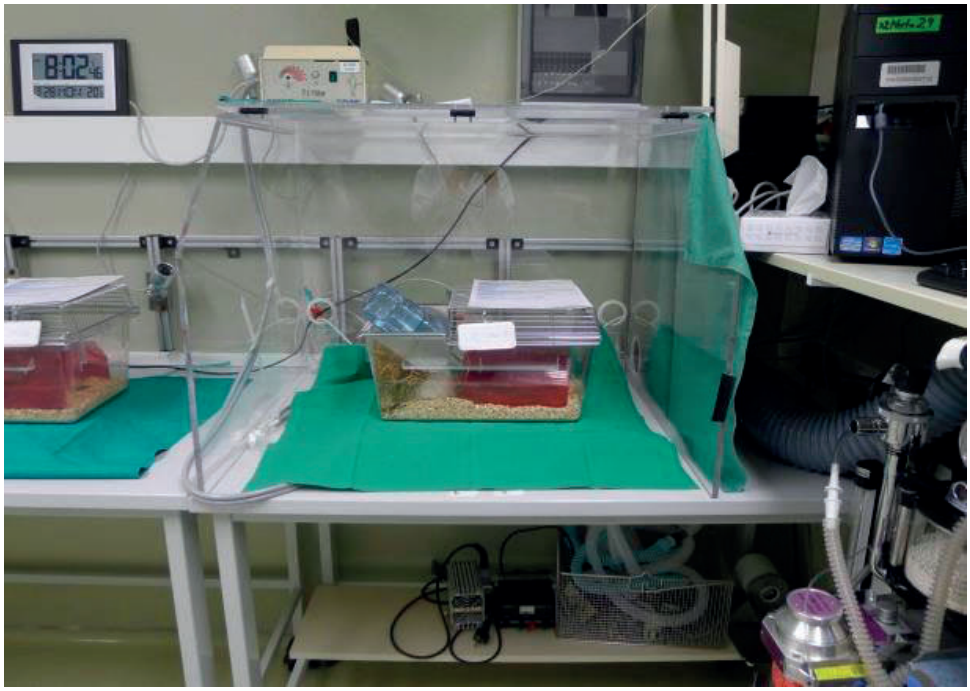

Fig 8: Suction hood for the performance of Iso anaesthesia in GPs.

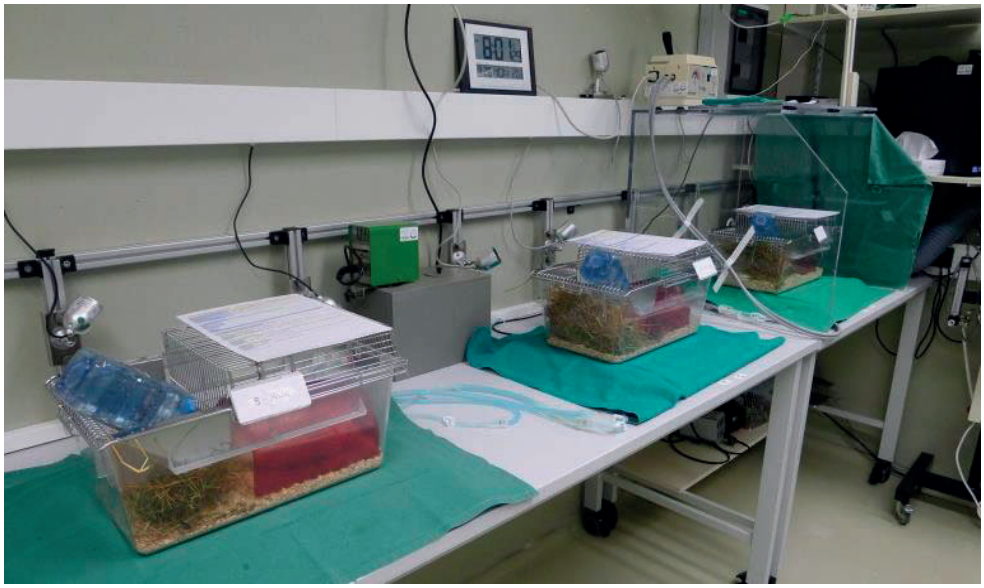

Fig 9: Set up for a radiotelemetry monitored anaesthesia study in male GPs.

Each single cage was labelled and equipped with bedding, hay, food pellets, a water bottle and the home cage shelter. We observed a faster calm down with transferring the scent of the home cage to the new one. The GPs were sitting calmly in their cages except for short initial movements. Since the exact anaesthesia procedure is described in publication 1, no further description is given here.

2.7.3 Blood pressure

The term refers to the arterial BP. It is the product of the heart minute volume (which is HR x beating volume) and the total peripheral resistance. Maintaining it is one of the most important physiological functions. It ensures the perfusion of all living organs and tissues, the exchange of metabolic products and the oxygen supply. The main organs that suffer from BP deviation are the CNS, heart and kidneys (73). There is the invasive and the non-invasive method to measure the BP. The non-invasive oscillometry is inexpensive and can be performed quickly on awake animals. But this system is very susceptible to movement faults, detects incorrect values at shallow pulse waves and is in general less accurate and reliable compared to an invasive approach (4). Since 2008 the newest generation of non-invasive BP measurement, the high definition oscillometry (HDO) (74) has been used in research with good results in many species (75). It enables the performance of a direct high frequency analysis of the incoming pulse signals with a 32 bit processor. Also an electronically controlled valve ensures the linear and pulse adaptive air deflation. So far this technique has not been tested in GPs. Invasive BP measurements, as performed in this study, are achieved by inserting a catheter into a primary artery and thereby directly measuring the pulse waves. Our used catheter type is fluid filled with the sensor positioned in the device body. The intra-arterially placed tip is sealed with silicone gel to prevent blood from entering and it is coated with an antithrombotic layer to prevent a thrombus formation. With the direct measurement, the sensor unit can be fully implanted as in our study resulting in living freely moving GPs that can be used in studies several times.

In the GP, the BP is an especially interesting parameter as they exhibit a comparatively low arterial BP of around 64 mmHg (MAP (68,76)) which is then further depressed during anaesthesia. For the parameter acquisition the pulse wave is measured continuously. The highest and the lowest graph point of every pulse wave depict the systolic ($P_{systolic}$) and diastolic ($P_{diastolic}$) value (Fig 10), while the mean BP is calculated with the use of equation 1.

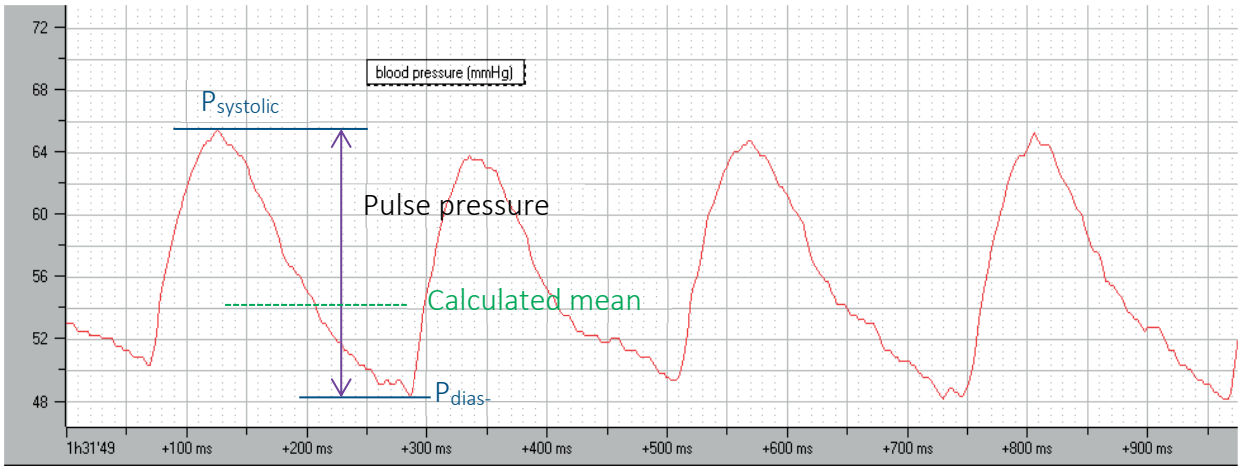

Fig 10: MAP signal in a male GP, measured by an intra-abdominally implanted radiotelemetry transmitter.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Equation 1: Arithmetic mean equation for the calculation of mean blood ar-

2.7.4 Heart rate

The HR is defined as the number of ventricle contractions per time, usually per minute (beats per minute = bpm). It is a relevant monitoring parameter during anaesthesia and many anaesthetics act depressively on the cardiovascular system. Therefore the HR provides a differentiation criterion between stage III_1 and the surgical tolerance stadium III_2 also enabling the evaluation of the analgesic coverage. It can be measured by auscultation with a stethoscope, but this approach does not yield scientifically representative results of a GPs fast HR. The pulse oximetry is another non-invasive method which acquires the pulse frequency via the HR. Devices specially designed for the high HRs of small laboratory animals have been introduced to the market for some time. The electrical heart activity is measured in an electrocardiogram (ECG) with subcutaneously implanted electrodes. With radio-telemetry there are 2 options of acquiring the HR, either by counting each BP wave, or each spike of the QRS complex in the ECG. Because we knew of the low BP and the further depression especially with volatile anaesthetics, we questioned the reliability of counting every pulse wave. The experts in the telemetry group recommended drawing the HR from the parameter which is less susceptible to interferences and therefore the ECG was chosen as the basis for HR count.

2.7.5 Core body temperature

Core body temperature refers to the temperature in the highly metabolic and visceral parts of head and torso of a homoeothermic organism. The BT is regulated by the CNS in the region of the anterior hypothalamus and is therefore also affected by anaesthetics mostly in a depressive manor. Additionally, the disadvantageous body-volume to surface-ratio and the fast metabolism of all small mammals requires a tight temperature monitoring. The measured values for BT are dependent on the location where the temperature is measured and the technique used for the measurement. The common measuring point for temperature measurement in the GP is in the rectum which can be performed with either a conventional fever thermometer or a room thermometer with a flexible sensor tip. This method is quick, inexpensive and applicable in awake GPs. If performed correctly the rectal temperature values are comparable to the ones acquired using an implanted radio-telemetry device (4), however rectal temperature measuring mistakes can easily occur. The thermometer tip can be placed into the perianal sac or into faeces instead of the rectum lumen, or the measurement time may be too short which leads to incorrectly low temperatures. Also the stress of restraint is known to increase the body temperature. In radio-telemetry, the temperature sensor is embedded inside of the abdominally implanted device body and collects measurements multiple times per second. The above mentioned sources of error in the rectal measuring approach are thereby prevented.

3 Publication 1

"Successful implantation of an abdominal aortic blood pressure transducer and radio-telemetry transmitter in guinea pigs - Anaesthesia, analgesic management and surgical methods, and their influence on hemodynamic parameters and body temperature"

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Successful implantation of an abdominal aortic blood pressure transducer and radio-telemetry transmitter in guinea pigs — Anaesthesia, analgesic management and surgical methods, and their influence on hemodynamic parameters and body temperature

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ABSTRACT

Introduction: Guinea pigs (GPs) are a valuable cardiovascular pharmacology model. Implantation of a radio-telemetry system into GPs is, however, challenging and has been associated with a high failure rate in the past. We provide information on a novel procedure for implanting telemetry devices into GPs and we have measured the hemodynamics (arterial blood pressure, BP and heart rate, HR) and core body temperature (BT) in the 24 h after surgery.
Methods: Male Hartley GPs (Crl:HA, 350-400 g, 6.5 weeks, n = 16) were implanted with a radio transmitter abdominally and were then monitored continuously (HR, BP and BT) for 24 h after surgery.
Results: 13 of 16 GPs (81%) survived the surgery. Surgery duration was 94 min (min) (range: 76–112 min) and anaesthesia duration was 131 min (range: 107–158 min). GPs lost body weight until 2 days after surgery and then regained weight. Mean arterial BP increased from 33.7 mm Hg directly after surgery to 59.1 mm Hg after 24 h. HR increased from 206 bpm directly after surgery to 286 bpm at 8 h and fell to 251 bpm at 24 h after implantation. BT was 36 °C directly after surgery, fell to 35.4 °C until regaining of the righting reflex and then stabilized at 38.5 °C after 24 h.
Discussion: A high survival rate in telemetered GPs is possible. We achieved this through a procedure with minimal stress through habituation and planning, continuous warming during anaesthesia, an optimal anaesthetic and analgesic management, efficient surgical techniques and vitamin C supplementation.

1. Introduction

The guinea pig (GP) is a valuable small animal model for studying cardiovascular pharmacology, particularly when evaluating electrocardiogram-derived (ECG) parameters. GPs express the myocardial human-Ether-a-go-go Related Gene channel which rats do not. Its dysfunction or pharmacological blockade can cause the potentially fatal "long QT syndrome". Therefore, drug candidates can be tested for their effect on QT prolongation in the GP (Katagi et al., 2015). Also, compared to larger animal models, GP have a low body weight (BW) and hence need less test substance and have lower costs for their purchase and housing (Hess, Rey, Wanner, Steiner, & Clozel, 2007).

The direct, intravascular measurement of aortic blood pressure and the ECG using radio-telemetry has established itself as the model-of-choice for testing drug candidates for possible cardiovascular effects. It allows the acquisition of data from conscious, unrestrained animals, with little human influence and over long periods of time (Kurtz, Griffin, Bidani, Davisson, & Hall, 2005). Core body temperature (BT), locomotor activity and biopotentials (EEG, electromyogram; (Leon, Walker, DuBose, & Stephenson, 2004) can likewise be measured. However, abdominal radio transmitter implantation into GPs has proved to be very challenging and has been associated with high failure rates.

Guinea pigs are one of the most difficult rodents to anaesthetize safely (Schwenke & Cragg, 2004), with a limited choice of acceptable anaesthetics and the need for a well prepared surgery. The high surface to body volume ratio of the GP, combined with the inadequate thermoregulatory control mechanisms and the thermoregulatory depression of anaesthetics, causes a rapid BT loss during anaesthesia (Buchanan, Burge, & Ruble, 1998). Open abdominal surgery leads to a particularly fast temperature loss, making external body warming

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essential (Kiyatkin & Brown, 2005). The body weight (BW) of GPs can vary widely as the intestinal filling accounts for up to 20–40% of the total BW, resulting in dosing inaccuracies of any injectable anaesthetic agent (Henke, 1998). The GP low resting mean arterial BP of 63 mm Hg (DePasquale, Ringer, Winslow, Buchholz, & Fossa, 1994)(DePasquale et al., 1994), the anatomically deeply embedded and fragile abdominal aorta (Provan, Stanton, Sutton, Rankin-Burkart, & Laycock, 2005) and the large caecum (Popesko, Rajtová, & Horák, 1992) additionally impede BP catheter placement into the abdominal aorta. Thus, multiple factors have contributed to the low success rate of this method in the GP.

During the implantation, the mentioned complications (multifactorial temperature loss, relatively risky anaesthesia, low arterial BP and fragile vascular structure) likely lead to changes in HR, BT and BP. So far, there is no data on the surgery's impact on the hemodynamic values in the first 24 h after radio transmitter implantation in GPs.

We provide a detailed description of a successful implantation approach into GPs, including anaesthesia, analgesia and surgical technique. We further report for the first time, data for BP, HR and BT during the critical first 24 h after abdominal surgery.

The following information can be utilized in any other abdominal surgery in the GP, both in experimental models and in curative approaches.

2. Methods

All experiments and procedures were performed in accordance with the German Animal Welfare Act (Art. 3 G v. 28.07.2014 I 1308) and the regional council for animal welfare.

2.1. Housing and acclimatization

Sixteen male Hartley GPs (Charles River Laboratories, Sulzfeld, Germany) (Crl:HA, delivery weight 350–400 g, average age of 6.5 weeks) were housed for 19 days prior to the radio-telemetry device implantation in groups of 2–3 in cages (EHRET TERULAN THF 1776) containing wooden bedding material (Lignocel FS14, Rettenmaier & Söhne, Rosenberg, Germany) and 2 red transparent plastic shelters.

Cage bedding changes were performed twice weekly. The GPs received 20 g/animal of pelleted, commercially available diet (3410 complete feed, KLIBA NAFAG, Provimi Kliba Sa., Kaiseraugst, Switzerland) and a large amount of autoclaved hay daily. Tap water was available *ad libitum*. The animal room was maintained at 20 ± 2 °C and 55 ± 10% relative humidity with an air change of at least 15 cycles/h. The light–dark cycle was 12:12, starting (5:30) and ending (17:30) with a dimmer phase of 30 min. For background acoustic habituation, radio music was simultaneously switched on and off with the lights. BW and the general condition of each animal were monitored daily. Beginning two weeks before the implantation, the animals were handled and acclimatized daily to being held, single housed for two hours in Makrolon® Type III cages with identical enrichment to their home cages and to being placed into the radio-telemetry data acquisition room. The GPs received 20 mg of Vitamin C in an aqueous solution orally for 7 days prior to and for 14 days after the implantation.

All habituation handling, medication, surgery and post-operative care was done by the same veterinarian.

2.2. Implantation of the radio transmitter system

Before the surgery, all electrical devices were checked for faultless function. On the morning of the surgery, the room and materials were arranged before the arrival of the animal.

The GP was removed from its home cage, weighed, examined clinically (checked for aberrant posture, behaviour, eyes, nose, fur), given the first oral dose of antibiotic dissolved in drinking water (enrofloxacin 10 mg/kg; for detailed medication and supplement list see Table 1) and singly placed into a Makrolone® III cage, containing cellulose bedding, 1 red shelter, pelleted food, autoclaved hay and a water bottle. The animal was then transported to the surgical preparation area.

After an average of 45 min after enrofloxacin medication, the GP was injected intramuscularly (i.m.; *m. semimembranosus/m. semitendinosus/m. biceps femoris*) with MMF (Henke, 2010; medetomidine 0.2 mg/kg, midazolam 1.0 mg/kg, fentanyl 0.025 mg/kg, see Table 1) and was returned to the cage until the righting reflex (RR, the animal rights itself when placed on its back) had disappeared. An additional one third of the initial MMF dose was given during the surgery to maintain surgical tolerance, which was assessed by evaluation of a mildly positive reaction to foot withdrawal (withdrawal of hind leg to a fingernail pinch on 1 toe with extended hind leg) and inguinal reflex (the hind leg is kicked as a response to a pinch in the inguinal region with a curved clamp up to the first catch). For surgical preparation the GP was then placed in dorsal recumbency on a heating mat, the abdominal and neck area was shaved

Table 1
Medication and vitamin supplement used for abdominal radio transmitter implantation and for pre- and post-surgical treatment in guinea pigs.

Purpose	Product name	Brand name/manufacturer
Anaesthesia-agonists	MMF = Medetomidine[1] 0.2 mg/kg + Midazolam[2] 1.0 mg/kg + Fentanyl[3] 0.025 mg/kg intramuscularly (i.m.) in mixed syringe	[1]DOMITOR®, 1 mg/mL, Orion Corporation, Espoo, Finland [2]Dormicum® 5 mg/mL, Roche Pharma AG, Grenzach-Wyhlen, Germany [3]Fentanyl®-Janssen 0.1 mg/2 mL, JANSSEN-CILAG, Neuss, Germany
Anaesthesia-antagonists	AFN = Atipamezole[4] 1.0 mg/kg + Flumazenil[5] 0.1 mg/kg + Naloxone[6] 0.03 mg/kg i.m. in mixed syringe	[4]ANTISEDAN® 5 mg/mL, Orion Corporation, Espoo, Finland [5]Flumazenil HEXAL® 0.1 mg/mL, HEXAL AG, Holzkirchen, Germany [6]Naloxon Inresa 0.4 mg/mL, Inresa Arzneimittel, Freiburg, Germany
Local anaesthetic	Lidocaine hydrochloride 0.9 mL/animal, subcutaneous (s.c.)	Xylocain® 1%, AstraZeneca, Wedel, Germany
Antibiotic	Enrofloxacin 10 mg/kg, oral (p.o.)	Enrotron® 100 mg/mL oral solution for drinking water dilution, aniMedica, Senden-Bösensell, Germany
Analgesia/anti-inflammation	Meloxicam 0.4 mg/kg, s.c./p.o.	Metacam® 2 mg/mL, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany Metacam® oral suspension 1.5 mg/mL, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany
	Metamizole 80 mg/kg i.m. p.o.	Novalgin® 1 g/2 mL, Sanofi-Aventis Deutschland, Frankfurt am Main, Germany Metamizol HEXAL® oral drops, 500 mg/mL, HEXAL AG, Holzkirchen, Germany
Supplement	Pure L-ascorbic acid 20 mg/day solved in sodium chloride solution, p.o.	Vitamin C Pulver, dm-Drogerie Markt, Karlsruhe, Germany

Picture 2. Aseptically prepared male guinea pig in the self-designed, adjustable water heating tub (Pic. 3) at the beginning of radio transmitter implantation.

and the cropped hair removed with a vacuum cleaner. Next it was injected with meloxicam 0.4 mg/kg subcutaneously (s.c.) and with metamizole 80 mg/kg i.m. and eye lubricant was applied. Lidocaine hydrochloride (0.9 mL s.c.) was infiltrated for local anaesthesia along the *linea alba* and the neck incision site.

The incision sites were disinfected with Propanol (Kodan®-spray) and iodine solution (Betaisodona®). The animal was moved to the surgical area and placed onto a surgical drape over a specially designed heating tub, warmed with adjustable water temperature circulation (Pictures 2 and 3). The animal's nose was inserted into a nose cone with 0.7 L/min oxygen flow.

After the surgeon's hand disinfection and gowning, the animal's head and feet were covered with surgical drapes and the incision sites with an adhesive surgical incision drape. All sterile supplies (see "other sterile equipment" Table 2) and instruments (Picture 1.) were

Picture 3. Self-designed, adjustable water heating tub for heat supply in guinea pigs during surgery. Aluminium surface and a notch for the nose cone.

prearranged on the table and a 23G needle was bent to an 80° angle as an *aorta abdominalis* insertion tool.

The foot withdrawal and inguinal reflex were re-evaluated and the mid-line was then incised in the *linea alba* from below the sternum to the umbilical region with the scalpel. The muscle layer was lifted and cut with the scissors. Two Backhaus towel forceps were inserted to spread the muscle and skin layer.

The intestine was carefully moved cranially and held in position using an unfolded, warm, moistened (sodium chloride solution) gauze and a spring-loaded retractor. The *aorta abdominalis* was exposed (from distal to the renal arteries to the aortic bifurcation) and dissected free of surrounding tissue with gentle strokes of dry cotton tips and the dissecting forceps. The two micro clamps, clean swabs applicators, dissecting forceps and vessel cannulation forceps were prearranged on the table to save preparation time while the aorta was clamped. One micro clamp was positioned distal to the *Aa. renales* and one cranial to the *arteria mesenterica caudalis* (Popesko et al., 1992) to stop blood flow. When the surrounding tissue was dried with cotton tips, the aorta was punctured between the micro clamps with the 80° angled needle. The angled needle was slightly lifted and the catheter tip was advanced underneath the needle and into the vessel cranially for approximately 1 cm and secured in place with a drop of uncoloured tissue glue. A square 0.25 cm^2 cellulose patch with a slit to the mid-point was placed roof-like over the catheter insertion site for additional support and protection.

The micro clamps were carefully removed but immediately repositioned in case of bleeding. Otherwise, the correct catheter placement was confirmed by an acoustic pulsatile signal from the radio receiver. Next, the gauze was removed and the intestine repositioned, before the telemetry device body was sutured to the caudal inner right abdominal cavity wall with a non-absorbable needle-thread-combination using single knots.

Both ECG cables were passed through the caudoventral abdominal muscle wall (red to the left, white to the right) with the 14 G needle and the abdominal cavity was closed using the non-coloured absorbable needle-thread-combination Vicryl™ 3–0 and a *Reverdin* suture on the muscle layer. The muscle layer was dabbed with diluted iodine solution.

Three subcutaneous pouches were made lateral to the skin — caudal left and right and cranial left. The bent tunnel tube was advanced s.c. parallel to the skin incision on the left up to the sternum. The red cable was inserted into the bent tunnel tube and the tube was pulled in the cranial direction. The tunnelled cable was adjusted to the body length with additional movement range. The wire end was looped and tied with the MERSILENE™ thread. The 14 G needle was lead through the *m. pectoralis*, the tied wire loop was inserted into the needle lumen. The needle was removed positioning the wire loop i.m. and it was tied to the muscle with a single knot. Exposed cable parts were rolled up and tucked in the left subcutaneous skin pouches.

The neck skin was incised with one scissor cut and the tissue was dissected until the trachea was visible under the *m. trachealis*. The white cable was tunnelled s.c. using the same method with the bent tunnel tube to the neck incision site where it was shortened, similarly looped and sutured to the *m. trachalis*.

The neck skin was closed with 3 single knots of the non-coloured Vicryl ™ 3–0 and the abdominal incision with the same thread but using an intracutaneous suture technique.

At the end of the surgery up to 20 mL/kg warmed sodium chloride solution were distributed s.c. in multiple injection sites and the drapes and nose cone were removed. The wounds were cleaned and sprayed with wound regeneration aiding product (Wund-Pflege-Spray, Dr. Schaette, Bad Waldsee, Germany) and the GP was individually numbered using an ear tattoo.

The implanted GP was then placed into a Makrolone® type III single cage, equipped identically to the home cage, which was positioned onto a water warming mat over 2 combined radio-telemetry receiver plates in the nearby telemetry measuring room.

Table 2
Surgical, sterile and non-sterile equipment list for radio transmitter implantation in guinea pigs.

Surgical preparation equipment:
1× hand-held vacuum cleaner
1× Electrical hair clipper, Isis, Aesculap®,Suhl, Germany
1× Eye Lubricant, VitA-Pos®, URSAPHARM, Saarbrücken, Germany
15+ sterile gauze swabs, 7.5 × 7.5 cm, type 17, 12-ply
1× Alcoholic disinfection spray, (2-Propanol, 1-Propanol, Biphenyl-2-ol) Kodan® Tinktur forte farblos antiseptic, Schülke & Mayr, Norderstedt, Germany
Iodine Solution, Betaisodona®-solution, Mundipharma GmbH, Limburg (Lahn), Germany

1 surgical instrument sterilization container with:
1× needle holder
1× dissecting forceps medium point, curved
1× Mayo scissors
1× vessel cannulation forceps for 0.5-1 mm catheter diameter
1× gillies tissue forceps, 1 × 2 teeth
1× curved mosquito clamp
2× Backhaus towel forceps
1× autoclavable magnet
3× micro clamp HD-S, S&T HD-S, Fine Science Tools GmbH, Heidelberg, Germany
1× tunnel tube, self-built, 20 cm length, 3 mm diameter, 10° angle
1× spring-loaded retractor, maximal spread 4 cm, Fine Science Tools GmbH, Heidelberg, Germany

1 Swab sterilization container with:
5+ gauze swabs, 7.5 × 7.5 cm, type 17, 12-ply
30+ small one-sided cotton tips 15 cm
1 × 0.25cm^2 large cellulose patch, Small Animal, DSI, St. Paul, MN, USA
10+ twisted gauze sponges, plum size

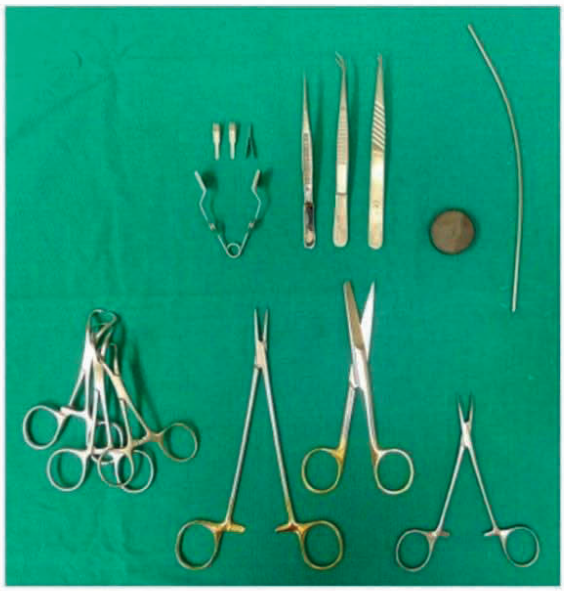
Other sterile equipment:
1× radio-telemetry transmitter, PhysioTel® HD-S11, DSI, St. Paul, MN, USA, weight 8 g, 2 biopotential cables (red and white) 30 cm length, 1 BP fluid-filled tip catheter 8 cm length.
1× non-coloured absorbable needle-thread-combination (skin), Vicryl ™, 3–0, FS-2, Ethicon®, Johnson-Johnson AG, Spreitenbach, Switzerland
1× non-coloured absorbable (muscle), Vicryl ™, 3–0, SH-1 plus, Ethicon®, Johnson-Johnson AG, Spreitenbach, Switzerland
1× non-absorbable needle-thread-combination, MERSILENE *, 3–0, SH Plus, Ethicon®, Johnson-Johnson AG, Spreitenbach, Switzerland
1× surgical incision drape 30 × 20 cm, Raucodrape®, Lohmann & Rauscher International, Rengsdorf, Germany
1 × 100 mL 0.9% NaCl (sodium chloride) bottle, AlleMan Pharma, Rimbach, Germany
1× Histoacryl ® uncoloured tissue glue, B.Braun Surgical, Rubi, Spain
1 × 20 mL syringe
1 × 5 mL syringe
1× surgical disposable scalpel 23 blade
3 × 23 G needle
1 × 14 G needle
4× surgical drapes
1× glass bowl (with 5 mL iodine solution + 20mLNaCl)

Non-sterile equipment:
1× radio receiver
1× heating mat
1× water bath warmed heating tub, self-designed with guinea pig shaped depression (Picture 2)
1 × 55 °C warm water bath for NaCl solution bottle
iodine solution, Betaisodona®-solution, Mundipharma GmbH, Limburg (Lahn), Germany

After 5 min of measuring surgical tolerance anaesthesia values, the animals' anaesthesia was antagonized with AFN (atipamezole 1.0 mg/kg, flumazenil 0.1 mg/kg, naloxone 0.03 mg/kg in a mixed syringe) s.c. in the axillary region. The warming mat was kept on until the animals' BT was at least 38 °C. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial blood pressure (MAP), HR and BT were continuously monitored for 24 h after antagonization.

The anaesthesia duration (from the first injection of MMF until AFN antagonization) and surgery duration (from the first incision until the abdominal skin wound was closed) were noted.

HR values were derived from the ECG.



Picture 1. sterile surgical tools for abdominal radio transmitter implantation in guinea pigs. For detailed description see Table 2.

2.3. Postoperative monitoring

Twenty-four hours of analgesia were covered with the pre-surgical meloxicam injection and with oral applications of metamizole 80 mg/kg (Metamizol HEXAL® oral drops) given every 4–6 h. Meloxicam (0.4 mg/kg) and enrofloxacin (10 mg/kg) were continued orally for 2 more days at 24 h intervals. All oral medication was dissolved in drinking water and applied using sterile 1 mL syringes.

The animal's condition was monitored at least twice daily for at least 7 consecutive days post-surgery with special focus on BW, temperature (firstly BT then rectal in the home cage), posture and fur appearance, food intake, defecation, wound healing and hind leg movement. The GPs were weighed daily from their arrival to 14 days after transmitter implantation. The wound healing progress was assessed and it was noted when the abdominal wound was free of scabs.

3. Results

Sixteen GPs underwent surgery and 15 successfully survived the implantation. One GP had to be euthanized intra-operatively due to excessive bleeding when the *aorta abdominalis* was punctured.

Two of the surviving 15 GPs had to be euthanized post-operatively, one at 2 days and one at 6 days after surgery. Thus, overall, 13 of 16 animals (81% survival rate) survived the surgery. Twelve of the 13 GPs

Table 3
Division of the 24 h after radio-telemetry transmitter implantation in guinea pigs. AFN = anaesthesia antagonist, atipamezole, flumazenil, naloxone. Righting reflex = the animal can right itself when placed on its back.

Phase	Definition	Time frame (0–24 h)
I	Begin of data acquisition – AFN s.c.	0 min–5 min
II	AFN s.c. – regained righting reflex	5 min–mean 10 min (Min 7–Max 14)
III	Regained righting reflex – 480 min	Individual (mean 10 min)–8 h
IV	481 min–960 min	8:01 h–16 h
V	961 min–1440 min	16:01 h–24 h

showed stable health and physiological parameters for at least 10 weeks after implantation (see Table 4). The one GP was excluded due to stagnation of weight gain and overall clinical condition.

In 4 of 13 animals the technique for data acquisition directly after implantation malfunctioned and resulted in an n = 9 in phase I and II (see Table 4).

In the 15 completed surgeries, the anaesthesia lasted an average of 131 min, minimally 107 and maximally 158 min. The surgery duration was on average 94 min with a minimum of 76 and a maximum of 112 min. The total data acquisition time of 1440 min was divided into phase intervals I to V (see Table 3).

The values for SAP, MAP and DAP rise in phases I and II and stay comparatively stable from phase III until the end of the measurement after 24 h (see Table 4 and Fig. 1). The blood pressure values at 10 weeks are equivalent to those at 24 h (Table 4).

Values are averaged over the defined phase interval (Table 3). AFN = atipamezole, flumazenil, naloxone; SAP = systolic arterial pressure; MAP = mean arterial pressure; DAP = diastolic arterial pressure.

Table 4
Blood pressure values (SAP, MAP, DAP), heart rate (HR) and core body temperature (BT) in male Hartley guinea pigs during recovery phases I–V and at 10 weeks after implantation. Data was acquired continuously over 24 h using the radio telemetric measurements after the abdominal transmitter implantation had ended. Values are averaged over the defined phase intervals (see Table 3). Data at 10 weeks are averaged over 15 min. SD = standard deviation.

Parameter	Phase	n	Mean	SD
Systolic arterial pressure (SAP)	I	9	39	7
[mm Hg]	II	9	45	8
	III	13	69	5
	IV	13	66	3
	V	13	70	5
	at 10 weeks	12	70	5
Diastolic arterial pressure (DAP)	I	9	29	5
[mm Hg]	II	9	32	6
	III	13	46	4
	IV	13	45	2
	V	13	48	4
	at 10 weeks	12	51	4
Mean arterial pressure (MAP)	I	9	34	6
[mm Hg]	II	9	38	7
	III	13	58	4
	IV	13	56	3
	V	13	59	4
	at 10 weeks	12	60	4
Heart rate (HR)	I	9	206	16
[bpm]	II	9	247	31
	III	13	269	25
	IV	13	287	24
	V	13	252	18
	at 10 weeks	12	218	17
Core body temperature (BT)	I	9	36	1.2
[°C]	II	9	35.4	1.2
	III	13	37.4	1
	IV	13	38.7	0.4
	V	13	38.5	0.4
	at 10 weeks	12	38.8	0.2

HR rose from a mean of 206 bpm in phase I to 246 bpm in phase II and further to 269 bpm in phase III. It reached its maximum during phase IV (hours 8 to 16) with 286 bpm and then decreased to 251 bpm in phase V (see Fig. 2). The HR at 10 weeks is lower and the BT is slightly higher than in phase V (Table 4).

The GPs gained on average 32.6 g BW from their arrival day up to day −15 (+9.4%), 24.2 g from day −14 to −8 (+6.1%) and 19.5 g from day −7 to the day before the surgery (+4.7%). From the day 0 until 2 they lost on average 53.6 g (−11.9%). They started regaining weight from day 3 until 7 with 33.8 g (+8.3%) and 41.1 g (+9.3%) from day 8 to 14. BW continued to increase steadily after 14 days post-surgery (Fig. 3).

The GP abdominal wounds were free of scabs by 8 days after surgery (min.7, max. 10, n = 14).

No incision wounds had to be re-sutured. Visible remains of suturing material were removed without complication at 14 days after implantation. No signs of infection or automutilation of the wound were noted.

4. Discussion

With our optimized approach we reached the highest long term survival rate (81%) published so far for abdominal radio-telemetry implantation in GPs (other survival rates: Hess et al., 2007: 68%; Provan et al., 2005: 0%). Provan et al. described a 50% animal loss intraoperatively due to a ruptured aorta during cannulation. We did not encounter this, but we also noticed the higher vascular fragility of the aorta in comparison to the rat. Both of the GPs that needed to be euthanized showed a severe lameness in both hind legs. A perforated abdominal aorta with a worsening general condition plus seroma and a thrombus in the distal abdominal aorta (similar to the report by (Hess et al., 2007) were found to be the cause of the lameness.

We cannot determine one most important factor for the higher success rate we report here, but multiple refinements likely contributed to the higher survival rate. Since the surgical approach in the GP is essentially the same as in rats, the crucial differences are likely the need for special handling, care and medication.

GP are extremely sensitive to stress caused by their environment, which will also affect the measured hemodynamic parameters. We allowed the GPs an increased acclimatization time of 19 days. At arrival, we saw clinical signs of distress such as "freezing" (Lee, Pellom, Oliver, & Chirwa, 2014), running hectically for cover or fighting vigorously for release. With daily training and quiet handling, those behaviours decreased significantly.

We additionally worked quietly and with minimal personnel, and prepared rooms and materials before the animals' arrival. Agitated animals require additional doses of MMF for surgical tolerance anaesthesia which results in a high BP with a greater risk of bleeding. The one GP euthanized intraoperatively was excited before the surgery and had to be dosed repeatedly to reach a surgical tolerance level, ending in a total MMF dose of 1.7 times the usual. This likely led to the severe bleeding which consequently led to the animal's euthanasia.

We administered medications orally where possible to avoid the pain and stress associated with skin injections. We noticed severe defensive reactions to s.c. injections, such as high pitched squealing and shrugging to escape. The GPs anticipated being injected when held and stayed alarmed until returned to the cage, making a stress-free injection almost impossible.

GPs lack an enzyme that catalyses the conversion of glucose to vitamin C (Hess et al., 2007) and therefore benefit from vitamin C supplementation. Oral administration of vitamin C (20 mg/day (Hamel, 2002)) was more reliable than supplementation in drinking water, as freshly operated animals had a lower food (see weight decrease in Fig. 3) and water intake. With vitamin C, the GPs appeared to recover faster, however, further studies are needed to support that conclusion.

A GP's general condition can decline rapidly within one day such that examinations should be performed at least once daily for at least

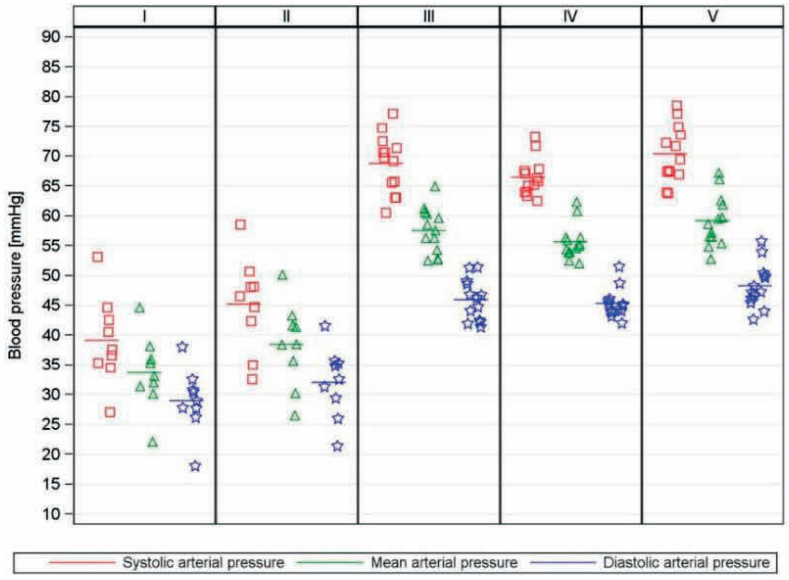


Fig. 1. Blood pressure values (SAP, MAP, DAP) in male Hartley guinea pigs over 24 h after radio transmitter implantation divided in phases I–V; Phase I–II n = 9, Phase III–V n = 13, each mark represents one guinea pig, lines show the group mean.

7 days after surgery. The BW is a helpful parameter to assess the post-surgical recovery, as a reduced food and water intake can result in a decline of up to 10% BW in 24 h. Pre-surgical fasting is likewise hazardous due to the GP's high metabolic rate (Sawyer, 2008). Hess et al. reported a 3–13% BW loss in the first week after surgery and a return to "normal" after two weeks. Our method resulted in an average loss of 53.6 g (−11.9%) BW until the second day after surgery (Fig. 3) and an increase thereafter. Our choice of metamizole regimen instead of tramadol (1×/day over 2 days after surgery, Hess et al.) is a likely explanation for the faster gain. A single tramadol dose has a half-life of 2.9 h in rats, 1.1 h in mice and 1.45 h in dogs (Matthiesen, Wöhrmann, Coogan, & Uragg, 1998). It is therefore unlikely that post-surgical pain can be treated adequately for 24 h in GPs with the tramadol dose used.

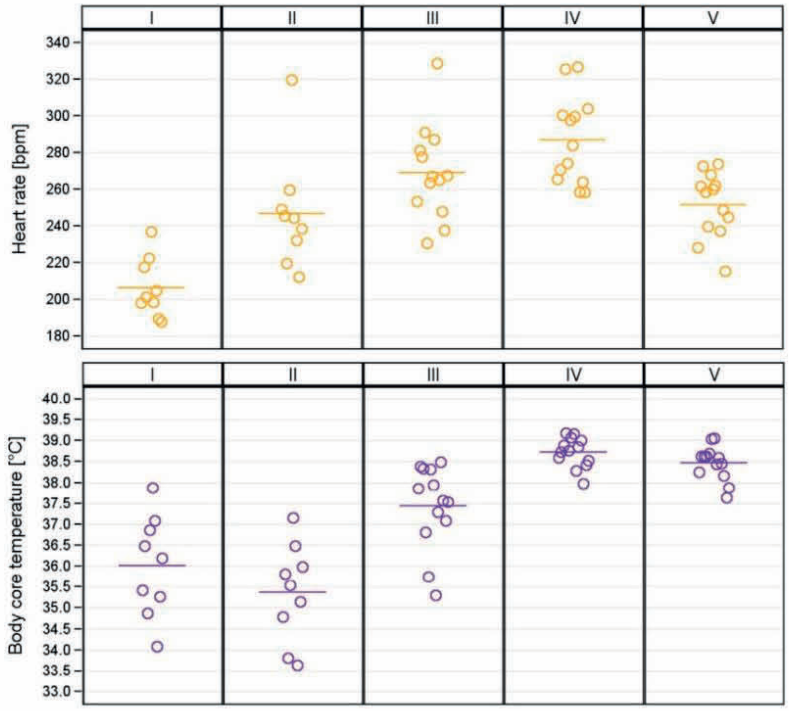


Fig. 2. Heart rate (HR, orange) and core body temperature (BT, violet) in male Hartley guinea pigs over 24 h after radio transmitter implantation, phase I–II n = 9, phase III–V n = 13, each circle represents one guinea pig, lines show the group mean. Values are averaged over the defined phase intervals (Table 3).

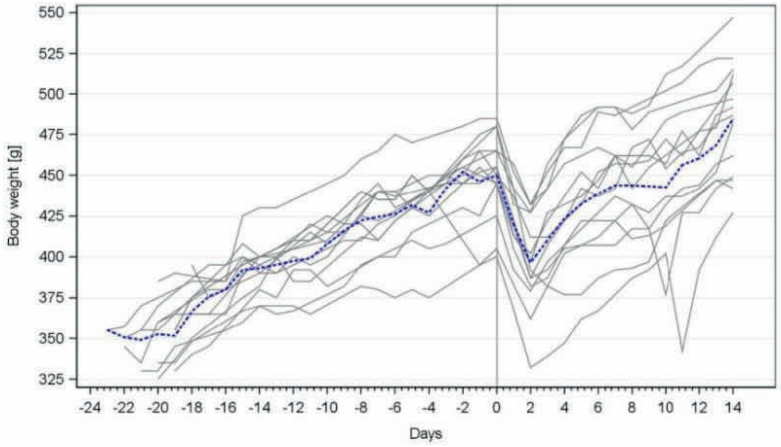


Fig. 3. Individual (n = 13, grey) and mean (blue) body weight (BW, [g]) of male Hartley guinea pigs in relation to the day of radio-telemetry implantation (day 0) from the animal delivery day to the facility until 14 days after surgery. After day 0 the telemetry implant weight (8 g) was subtracted from BW.

Metamizole acts as an analgesic, spasmolytic and antipyretic, has very little gastrointestinal or kidney side effects and a fast onset after oral application (Tacke, Henke, & Erhardt, 2008). We additionally observed clinical improvements in agility and food intake in the first 15 min after metamizole administration.

In contrast to Hess et al. (2007), we chose MMF instead of isoflurane anaesthesia. Isoflurane use in GPs causes severe bronchosecretion and salivation leading to breathing difficulties. Atropine premedication 10 min before isoflurane anaesthesia decreases these symptoms transiently (Heide, 2003) but the effect only lasts for approximately 20 min after which thickened mucous inhibits the breathing (Henke, 2010). Heide (2003) and Henke (2010) advise isoflurane only for short term anaesthesia and for less painful procedures, since isoflurane has only a weak analgesic effect (Erhardt, Henke, Haberstroh, & Kroker, 2004).

MMF doesn't cause airway constrictions, the anaesthesia onset is fast (animals are operable after 15 min after induction, (Henke, 2010), extending the anaesthesia through additional dosing is unproblematic (Henke, 1998) and transport in an anaesthetized state is easy. Analgesic coverage is superior to isoflurane, with the strong analgesic fentanyl which we find well suited for this procedure. AFN also antagonizes the fentanyl, therefore early non opioid analgesia (here metamizole) is crucial for an effective postoperative analgesia. Taking all factors into consideration, we consider MMF the anaesthesia of choice for radio transmitter implantation in GPs.

A major factor for a quick recovery is adequate warming. Anaesthesia significantly decreases the thermoregulatory ability with consequent need for external warming (Albrecht, Henke, Tacke, Markert, & Guth, 2014; Buchanan et al., 1998; Kagstrom, Laumola, Poijes, Johansson, & Ericson, 2012). Starting from the loss of the righting reflex, GPs stop being able to regulate or generate body heat, so minimizing anaesthesia duration is important for maintaining BT. Retaining the existing warmth is essential, especially as the enlarged surface during open abdominal surgery increases BT loss. Moving the large intestine outside the abdomen during catheter implantation should be avoided to prevent even more BT loss and to prevent the intestine from drying out. Continuous warming of as much body surface area as possible is the most beneficial approach. Cold GPs will consume neither water nor food and will start thermoregulatory shivering only after the RR has returned. Unnoticed BT decreases will lead to a sedation like state which finally results in death. We achieved a greater body surface warming than with a standardly used heating mat (Heide, 2003) by placing the GP in a self-designed, adjustable water heating tub with an aluminium surface (Picture 2, Picture 3, 39 °C on the animal fur) during surgery.

Despite our efforts to retain as much BT as possible (continuous warming, warming tub, warmed sodium chloride solution s.c., short anaesthesia times), the postoperative BT was 36 °C (Fig. 2; physiological values 38.7–39.1 °C; (Akita, Ishii, Kuwahara, & Tsubone, 2001). The BT decreased further to 35.4 °C in phase II (time from AFN s.c. to regaining

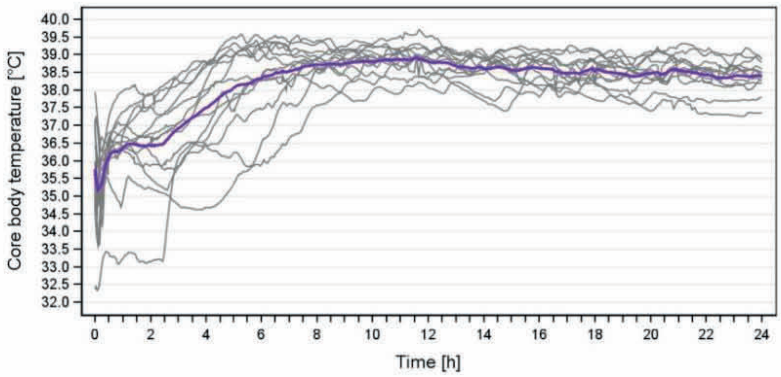


Fig. 4. Mean (violet) and individual (grey) values of core body temperature (BT) in n = 13 male Hartley guinea pigs over the course of 24 h following the implantation. Values were acquired telemetrically and measurements started directly after radio-telemetry transmitter implantation had ended.

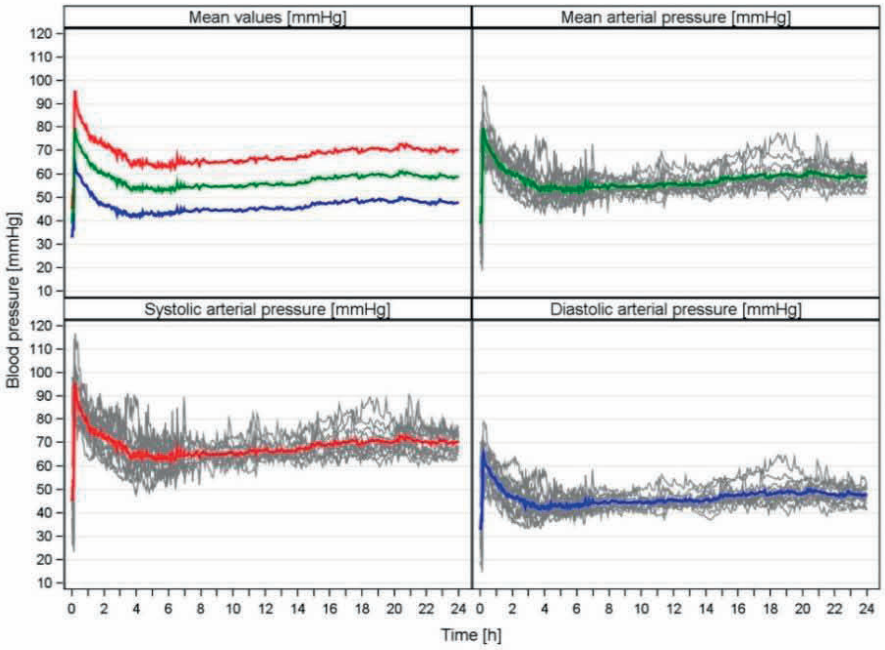


Fig. 5. Telemetrically measured abdominal arterial blood pressure in 13 male Hartley guinea pigs for 24 h after abdominal radio transmitter implantation. Systolic arterial pressure (red), mean arterial pressure (green) and mean diastolic arterial pressure (blue) are presented as individual courses (grey) and the mean course (highlighted). Upper left: comparison of highlighted blood pressure means. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the RR; Table 3) although the cages were placed on warming mats. Thus, even with measures to maintain BT, it can be only partly prevented. In our study the BT stabilized by 8 h after surgery (equals phase IV-V; Figs. 2 & 4). BT values below 37.5 °C at 8 h or after should be considered too low. A renewed examination and external warming should be performed until the animal has reached a BT of 38.5 °C.

The effect of the catheter and transmitter implantation on hemodynamic values is unknown. Mean BP rose sharply after anaesthesia antagonization, assumably due to the administration of AFN to antagonize the anaesthesia. BP then decreased to 57 mm Hg (MAP, phase III) at 8 h and remained stable from then on, ending with a MAP of 59 mm Hg (Fig. 5).

HR also increased as a result of AFN administration (Fig. 6) and reached its highest level after 8 h but then declined until the end of the 24 h measurement. A possible explanation for the longer lasting increase in HR is the almost linear correlation between BT and HR (Fig. 6, (Wilber & Zeman, 1968). HR increased simultaneously with the increase of BT through shivering (see brown section in HR curve in Fig. 6). At 38.5 °C (Akita et al., 2001 reported physiological BT of 38.7–39.1 °C) the shivering stopped, the HR stabilized and then decreased to 251 bpm (phase V, Table 4). With normothermic conditions restored, the HR is regulated by an increasing parasympathic tone, which is the main influence on HR in the temperature range from 29 to 39 °C (Walsh, 1969). A HR of 251 bpm at 24 h post-surgery is within the physiological range of the telemetrically measured HR in awake GP as reported by Akita et al. (2001, 239 to 257 bpm).

Evaluating the course of HR after implantation is a valuable screening tool, as it gives insight in both the BT and the successful recovery process (see Fig. 6).

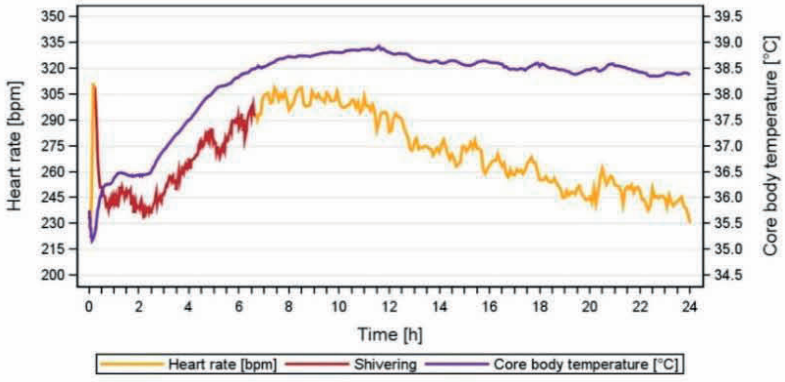


Fig. 6. Means of heart rate (HR) and core body temperature (BT) in 13 male Hartley guinea pigs for 24 h after radio-telemetry surgery. The regained righting reflex allows the BT to increase through shivering (see red section in HR curve) with parallel increase of HR. After normothermic values were restored (from 8 h onward) the HR is down-regulated to physiological values by the parasympathic nervous system.

Table 5
Recommendations for the handling of guinea pigs (GP) during acclimatization, before, during and after the radio transmitter surgery. MMF = midazolam, medetomidine, fentanyl; BT = core body temperature.

Do's (and their rationale)	Don'ts (and their consequences)
Acclimatization period	
• Catch the GP on the first attempt; hold it securely with the feet in contact with a surface (reduces stress to a minimum)	• Chase the GPs around their cage, letting them escape several times
• 10 min/day human contact is enough to acclimatize the GP to being handled	• Overexert the GP with long or repeated handling (they will lose body weight)
Pre-operative period	
• Minimize the time from MMF injection to the first incision (the shorter the total anaesthesia time, the more BT can be maintained)	• Work too slowly (requires more anaesthetic)
• Inject the incision site with a local anaesthetic (it will stop the stimulation of peripheral nerves during s.c. tunnelling)	• Omit the local anaesthesia (GP will twitch repeatedly during s.c. manipulation)
Surgery/radio-telemetry transmitter implantation	
• Choose the suitable anaesthesia for the intended procedure (reliably applicable with good intraoperative analgesia and fast postoperative recovery)	• Use an unfamiliar anaesthesia (uncontrollable side effects), inadequate analgesia (decreased postsurgical food and water intake, pain!) long postoperative recovery (requires intensive monitoring and additional support)
• Be careful with the tissue surrounding the *a. abdominalis*, the *m. psoas major* and the *n. genitofemoralis* during the dissection of the aorta	• Dab and dry the *m.psoas major* and embedded nerves repeatedly (can cause muscle and nerve irritation and hind leg paralysis)
• Thoroughly dissect the aorta from the connective tissue (clamps will thereby hold tighter, and it is easier to see if the catheter tip is really placed correctly in the aorta)	• Dissect quickly or omit this step (high risk of rupturing either the thin walled *v. cava* or the aorta, tissue glue might not be leak-proof)
• Clamp only the *a. abdominalis* and do so as briefly as possible (minimize distal tissue oxygen shortage)	• Clamp the *v. cava caudalis* unnecessarily, or leave the clamps for longer than 10 min (increases the chance of thrombosis)
• Use tissue glue only on dry surfaces and apply only sparingly ➔ read product instructions!	• Use tissue glue on moist surfaces or apply multiple drops "to be safe" (tissue glue doesn't stick satisfactorily to wet surfaces, too much glue can cause disturbed wound healing)
• Hold the telemetry transmitter securely in your hand when approaching with the magnet for signal testing	• Leave the transmitter lying loosely (the transmitter will be pulled towards the magnet and could rip the catheter tip out)
• Suture the abdominal skin wound completely intracutaneously and hide both knots (undisturbed wound healing)	• Suture with thread showing, (GPs start nibbling on the external thread parts ➔ infection, restitching, prolonged wound healing)
• Clean the wounds well (aids post-operative wound evaluation)	• Leave the wounds bloody or iodine coloured
Post-operative period	
• Stay with the GP until it has regained its righting reflex (enables quick intervention in case of emergency)	• Focus on other work or cleaning (no possibility of noticing or aiding the animal if necessary)
• Evaluate the following: body weight, feeding, pain symptoms, general posture, use of all feet, defecation and urine passing	• Only look fleetingly inside the cage (unnoticed pain, lameness, missing food or water uptake, intestinal obstruction)
• Provide continuous pain medication (many GPs will start feeding again directly after metamizole dosing)	• Leave large gaps in the pain medication or stop it to soon (adverse animal welfare, prolonged regeneration time, less food and water intake)

Value comparisons in Table 4 show similar BP values. Therefore the BP in GPs can be considered stable from 24 h until at least 10 weeks after implantation. The BT at 10 weeks (38.8 °C) is slightly higher than 38.5 °C at 24 h but ranges within normothermic values. The difference is more likely due to the still minimally decreased BT at 24 h (ongoing recovery after surgery), than to deviating values at 10 weeks.

The HR is significantly lower than reported by Akita et al., probably because the GPs were measured repeatedly in the single cages for multiple hours during the 10 weeks. This long-term habituation likely leads to a lower baseline HR at 10 weeks (Masini, Day, & Campeau, 2008).

We applied refinements for improved wound healing, including the use of non-coloured suturing material, 3 days of oral enrofloxacin and using no wound gel (ProntoVet®, B.Braun Vet Care, Tuttlingen, Germany). In test animals we used violet-coloured suturing material, only 1 dose of enrofloxacin on the day of surgery and we applied wound gel after surgery. Many of those animals had to be re-sutured and some wounds showed redness or swelling and a prolonged healing time. The GPs licked their wounds when wound gel was applied.

Like (Cunliffe-Beamer, 1993) we confirm the absolute need for aseptic surgical technique. Although this should be practised during any surgical procedure, GP compared to rats have an even lesser tolerance for unsterile technique, likely resulting in suture infections or late developing abscesses.

Handling the GP intestine as little as possible and very gently, is rewarded with a faster post-surgical food intake and an overall faster clinical recovery. More recommendations are summarized in Table 5.

In summary, we demonstrate the possibility of successful abdominal radio-transmitter implantation into GPs for long term data acquisition in the conscious and unrestrained state. Implanted GPs should be back within their hemodynamic physiological range at 8 h for BP and BT and at 24 h for HR.

The combination of multiple refinement factors likely resulted in the high survival rate. Those factors include a minimal stress exposure through habituation and planning, continuous warming during anaesthesia, choice of an optimal anaesthetic and analgesic management, optimized surgical techniques and vitamin C supplementation.

The described refinements constitute a valuable contribution to the three Rs' principals of humane experimental technique (replace, reduce, refine) by (Russell & Burch, 1959).

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4 Publication 2

"Comparison of Physiological Parameters and Anaesthesia Specific Observations during Isoflurane, Ketamine-Xylazine or Medetomidine-Midazolam-Fentanyl Anaesthesia in Male Guinea Pigs"

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PLOS ONE

RESEARCH ARTICLE

Comparison of Physiological Parameters and Anaesthesia Specific Observations during Isoflurane, Ketamine-Xylazine or Medetomidine-Midazolam-Fentanyl Anaesthesia in Male Guinea Pigs

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Abstract

Guinea pigs (GPs) are difficult to anaesthetize successfully, the choices for anaesthesia are limited and physiological parameters are likely to be influenced substantially under anaesthesia. We implanted blood pressure radio-telemetry devices into 16 male GPs and subjected them to anaesthesia with ketamine-xylazine (KX), medetomidine-midazolam-fentanyl (MMF) or isoflurane (Iso, plus atropine premedication) in a randomized order with a 7 day interval between anaesthesias. Each anaesthesia lasted 40min, after which Iso was discontinued, MMF was fully antagonized with atipamezole-flumazenil-naloxone and KX was partially antagonized with atipamezole. Hemodynamics were recorded continuously for at least 240min after induction and the GPs were monitored for respiratory rate, reflex responses and specific observations until regaining of their righting reflex (RR). Blood for glucose testing was taken from the ear at 7.5, 20 and 40min during anaesthesia. Recovery time was short with MMF and Iso but long for KX. MMF induced only a transient blood pressure drop after antagonization, whereas Iso caused a marked hypotension during maintenance and KX led to moderate hypotension after antagonization. MMF and Iso produced tolerable heart rate changes, but KX led to long term post-anaesthetic bradycardia. Hypothermia occurred with all anaesthesias, but the GPs returned to normothermia the fastest under MMF, followed shortly by Iso. KX, however, caused a profound and prolonged hypothermia. The respiration was depressed with all anaesthesias, substantially with MMF (-41%) and KX (-52%) and severe during Iso maintenance (-71%). Blood glucose with MMF and KX increased throughout the anaesthesia, but the values remained within reference values with all anaesthetics. The reflex responses character and strength varied between the anaesthetics. In conclusion, MMF is the anaesthetic of choice and Iso may be used for short, non-painful procedures. We advise against the use of KX in GPs.

policies on sharing data and materials. None of the authors currently serve as editorial board members to PLOS ONE, have acted as an expert witness in relevant legal proceedings or currently sit on a committee for an organization that might benefit from the publication of this data.

Introduction

Anaesthesia is regularly required in guinea pigs (*Cavia porcellus*, GPs). They are considered to be one of the most difficult rodents to anaesthetize safely [1], and only few anaesthetics show satisfactory effect [2]. It is known that anaesthesia significantly alters physiological parameters [3], but so far in GPs its' influence has not been investigated thoroughly. We implanted radio-telemetry devices having a blood pressure transducer into male GPs and continuously measured the effect of 3 commonly used anaesthetics during anaesthesia induction, maintenance and recovery. We investigated the influence on hemodynamic parameters, core body temperature, respiratory rate (ReR), blood glucose, reflex responses and anaesthesia-related observations.

Materials and Methods

Housing and acclimatization

This research was approved by the Regierungspräsidium Tübingen, Germany under the approval number 12–038. For anaesthesia isoflurane, the combination of medetomidine-midazolam-fentanyl and ketamine-xylazine were used. Euthanasia was performed with pentobarbital. All experiments and procedures were performed in accordance with the German Animal Welfare Act (Art. 3 G v. 28.7.2014 I 1308) and the regional council for animal welfare.

Sixteen male albino Hartley guinea pigs from Charles River Laboratories (Sulzfeld, Germany, delivery body weight (BW) 350-400g/ age of 6.5 weeks) were housed for 19 days prior to the radio-telemetry implantation in groups of 2–3 (EHRET TERULAN THF 1776) with wooden bedding material (Lignocel FS14, Rettenmaier & Söhne GmbH + Co.KG, Rosenberg, Germany, change 2x/week). Two shelters per cage, 20g/GP of pelleted diet (3410 complete feed, KLIBA NAFAG, Provimi Kliba Sa., Kaiseraugst, Switzerland) and a large amount of autoclaved hay were supplied daily. Tap water was available *ad libitum*. The animal room was kept at 20±2°C, 55±10% and had an air change of 15 cycles/h. Light-dark cycle was 12:12 ± dimmer phases of 30min. A radio played music for acoustic habituation when lights were on. BW and general condition of each animal were monitored daily. The animals were acclimatized daily to being handled and to being single housed.

Radio-telemetry transmitter implantation

The perioperative antibiotic and analgesic medication with enrofloxacin (10mg/kg, Enrotron 100mg/mL, aniMedica GmbH, Senden-Bösensell), meloxicam and (0,4mg/kg, Metacam, Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany) and metamizole (80mg/kg, Novalgin, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany), as well as the implantation of the radio-telemetry transmitter (DSI, PhysioTel HD, HD-S11, DSI, St.Paul, MN, USA) was performed as described previously [4]. The surgery was carried out under MMF anaesthesia [5] (medetomidine 0.2mg/kg, midazolam 1.0mg/kg, fentanyl 0.025mg/kg, see Table 1) and the same dosage was also used for the following MMF anaesthesias.

Study design

Thirteen GPs were each subjected to anaesthesia with Iso (Forene100%, AbbVie Deutschland, Ludwigshafen, Germany), KX (Ketasol -100 ad us.vet., Dr. E. Graeub AG, Bern, Switzerland; Rompun 2% Bayer Vital GmbH, Leverkusen, Germany) or MMF using a randomized cross-over design with a 7 day interim between the anaesthesias (day 0, 7, 14). BP, HR and BT were continuously measured via radio-telemetry. A maximum of 2 GPs per day were anaesthetized sequentially. Baseline values for both GPs were acquired before the first anaesthesia started and the GPs were premedicated at 10min before induction. After 40min, MMF was antagonized with AFN,

Table 1. Medication used during radio-telemetry transmitter implantation and for MMF anaesthesia in male guinea pigs.

Purpose	Product name	Brand name / Manufacturer
anaesthesia-agonists	Intramuscular (i.m.) in mixed syringe MMF = 1. Medetomidine[1] 0,2mg/kg +2. Midazolam[2] 1,0mg/kg + 3. Fentanyl[3] 0,025mg/kg	1. DOMITOR, 1mg/mL, Orion Corporation, Espoo, Finland. 2. Dormicum 5mg/mL, Roche Pharma AG, Grenzach-Wyhlen, Germany. 3. Fentanyl-Janssen 0.1mg/2mL, JANSSEN-CILAG, Neuss, Germany
anaesthesia-antagonists	Subcutaneous (s.c.) in mixed syringe AFN = 4. Atipamezole[4] 1,0mg/kg +5. Flumazenil[5] 0,1mg/kg + 6. Naloxone[6] 0,03mg/kg	4. ANTISEDAN 5mg/mL, Orion Corporation, Espoo, Finland. 5. Flumazenil HEXAL 0.1mg/mL, HEXAL AG, Holzkirchen, Germany. 6. Naloxon Inresa 0,4mg/mL, Inresa Arzneimittel, Freiburg, Germany

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KX was partially antagonized with atipamezole (0.15mg/kg ANTISEDAN, Orion Corporation, Espoo, Finland) and Iso exposure was stopped. A total measuring cycle consisted of 2h habituation (the last 15min were used as the baseline values), 10min premedication, 40min of anaesthesia and wake-up and recovery phases as long as needed for each individual. Data was acquired for at least 240min after induction. All procedures were done by the same veterinarian.

Experimental Protocol

The GPs were weighed, examined (condition of fur, eyes, nose, posture and behaviour) and each placed into a single cage (Makrolon type III, EHRET Labor- und Pharmatechnik, Emmendingen, Germany) equipped like the home cage. They were transported to the data acquisition room with lighting, climate and radio settings the same as in the home cage room. Telemetry receiver plates were covered with a heating mat, one cage was placed on top and the data acquisition was started. After 2h of alone habituation time, the first GP was lifted out of its cage and premedicated in the dorsal neck (see 2.5–2.7). The heating mat was turned on (39°C) until the GP had recovered to a BT of at least 38°C. Anaesthesia was induced (see detailed description for each treatment below), the GP was placed on its back on the heating mat and eye lubricant (VitA-Pos, URSAPHARM, Saarbrücken, Germany) was administered after loss of the righting reflex (RR). Pure oxygen (0.7mL/min) was supplied through a nose cone from loss of RR to antagonization, after which the GP was replaced to its single cage in dorsal recumbency to assess the time until RR returned. During anaesthesia, reflex responses (see Table 2)

Table 2. Reflex tests, observations and responses during guinea pig (GP) anaesthesia.

Name	Definition for positive response
Righting reflex (RR)	The GP is able to right itself when placed on its back
Lid response (LR)	Blink to touching of the eyelid
Ear Reflex (ER)	Ear twitching to a skin touch of the ear canal entrance
Foot withdrawal reflex (FWR)	The GP withdraws its extended hind foot to a fingernail pinch on one toe
Inguinal reflex (IR)	The GP kicks a hind leg, when pinched up to the first clamp catch in the inguinal region lateral to the nipples
Muscle tone	Evaluation of counter pull to hind leg extension
Chewing	Chewing movements without food or water uptake
Shivering	Rhythmical torso and lower back muscle contractions
Piloerection	Ruffled instead of sleek fur
KX only: sedation	Head drooping, staggering walk, reduced responsiveness, squinted eyes, delayed reflexes
Iso only: Cleaning, eye watering, blinking	Tearing eyes, squinting/blinking, fore feet rubbing over the ears and nose, anogenital region licking
Iso only: respiratory sounds	Breathing related rattle, coughing over the trachea and lung, audible with or without stethoscope
Skin colour	Evaluated on the eye lids, ears, lips, front feet and hind feet

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and ReR (visual assessment of breaths per minute) were evaluated in 2.5min intervals from 0-15min and 40-55min and in 5min intervals from 15-40min (0min = induction) or until the RR was regained (10min interval from 60-. . .min).

Reflex responses were rated from–to +++. No reaction was considered a negative (-) result, a minimal response tested ±, a mild one as +, a delayed/reduced response as ++ and a physiological response as +++. Only–and ± responses to FWR and IG were considered surgically tolerant. Hectic movements, running and restlessness were defined as excitation. Blood glucose (BG) was measured during the anaesthesia (OneTouch Ultra2, LifeScan Europe, Zug, Switzerland) at 7.5, 20 and 40min, with blood taken from an ear prick with a blood lancet (Solofix B. Braun, Melsungen, Germany). In case of apnoea, the GP was tilted back and forth for ventilation and was replaced to the nose cone after regular breathing was restored. GP was warmed via water filled gloves, if BT dropped below 35°C. The depth of anaesthesia was divided into reflex-dependent intervals (Table 3).

NOTOCORD-hem was used for the telemetric data acquisition. Values between premedication (-10min) until 60min after induction were averaged in 20sec intervals and thereafter in 2.5min intervals.

No statistical evaluation was done. Baseline and anaesthesia values are presented descriptively (Tables 4, 5 and 6).

MMF anaesthesia

For premedication 0.4mL/kg of sodium chloride (in this case as a placebo) were injected s.c. into the dorsal neck region (Fig 1).

The GP was immediately returned to the single cage. MMF was mixed in one syringe but the dose was divided, if the total volume exceeded 0.5mL. Injections were given i.m. into the caudal part of the femoral muscle of one/both hind legs and the GP was then returned to its cage. After 40min the antidote AFN was injected s.c.

Iso anaesthesia

Atropine (Atropinum Sulfuricum 1.0mg Eifelfango, Bad Neuenahr-Ahrweiler, Germany) was diluted with sodium chloride to 0.1mg/mL and 0.4mL/kg (0.04mg/kg) of the solution was injected s.c. (as described for MMF). A circular whole body chamber (BC) with an inserted liquid absorbent cloth (Fig 2) was prefilled with 99% O_2 and 4.4% Iso (measured by Criticare Poet II Patient Monitor, Soma Technology Inc., Bloomfield, USA).

The gas flow was interrupted, the box turned vertical and the GP was lowered, hind legs first, into the chamber. The lid was closed, the BC repositioned horizontally and the gas flow

Table 3. Reflex dependent anaesthesia interval definition for anaesthesia with MMF, isoflurane and KX in male guinea pigs.

Anaesthesia interval	Definition
I = induction	From MMF, KX injection or isoflurane exposure to loss of RR
II = non-surgical tolerance	From loss of RR to mildly positive (±) foot withdrawal and inguinal reflex
III = surgical tolerance	From (±) foot withdrawal and inguinal reflex to (partial) antagonization
IV = wake-up	From (partial) antagonization to regaining of RR
V = recovery	From RR until 240min

For reflex definition see Table 2. RR = righting reflex, MMF = medetomidine-midazolam-fentanyl, KX = ketamine-xylazine.

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Table 4. Baseline and anaesthesia values during medetomidine-midazolam-fentanyl (MMF), isoflurane (Iso) and ketamine-xylazine (KX) anaesthesia in male guinea pigs.

Parameter	Treat-ment	N	Baseline	Induction	Non-surgical tolerance	Surgical tolerance	Wake-up	Recovery
SAP	MMF	11	66.9 ± 2.7	79.0 ± 12.4	69.5 ± 15.6	68.5 ± 13.2	39.8 ± 7.7	72.2 ± 3.6
[mmHg]	Iso	13	66.3 ± 5.0	91.9 ± 8.6	43.0 ± 11.2	20.6 ± 7.7	47.3 ± 11.9	70.4 ± 5.7
	KX	7	68.3 ± 3.4	78.1 ± 10.7	61.2 ± 14.6	66.0 ± 8.6	50.6 ± 6.4	61.5 ± 4.1
DAP	MMF	11	48.1 ± 2.7	57.0 ± 7.9	51.2 ± 10.0	50.3 ± 8.6	28.8 ± 5.5	52.3 ± 3.1
[mmHg]	Iso	13	48.4 ± 3.8	67.4 ± 7.1	32.2 ± 8.0	16.4 ± 6.7	35.4 ± 9.2	50.6 ± 3.5
	KX	7	48.5 ± 4.7	55.1 ± 7.5	44.2 ± 10.0	47.7 ± 5.8	37.5 ± 4.5	47.9 ± 4.3
MAP	MMF	11	57.6 ± 3.3	67.4 ± 9.7	59.8 ± 12.5	58.8 ± 10.8	34.3 ± 6.5	62.0 ± 3.2
[mmHg]	Iso	13	56.9 ± 3.7	78.8 ± 7.8	37.6 ± 9.6	18.5 ± 7.2	41.4 ± 10.4	60.2 ± 4.4
	KX	7	58.9 ± 3.2	66.0 ± 9.1	52.4 ± 12.2	56.1 ± 7.2	43.8 ± 5.6	54.1 ± 4.1
HR	MMF	11	245.8 ± 18.2	277.3 ± 25.4	233.5 ± 8.2	214.2 ± 10.0	240.6 ± 20.7	288.4 ± 20.8
[bpm]	Iso	13	247.0 ± 16.0	291.8 ± 29.1	275.2 ± 20.9	244.9 ± 16.0	278.0 ± 15.8	284.0 ± 10.9
	KX	7	237.9 ± 11.1	263.3 ± 20.9	218.4 ± 9.0	197.0 ± 7.8	206.8 ± 28.9	212.1 ± 9.5
BT	MMF	11	38.9 ± 0.2	38.6 ± 0.3	38.3 ± 0.4	37.0 ± 0.5	36.3 ± 0.7	38.6 ± 0.6
[°C]	Iso	13	38.9 ± 0.4	38.9 ± 0.4	38.7 ± 0.4	37.0 ± 0.4	35.8 ± 0.6	38.5 ± 0.3
	KX	7	39.0 ± 0.2	38.6 ± 0.3	38.4 ± 0.4	37.5 ± 0.3	36.6 ± 0.7	37.5 ± 0.7

Mean and SD values, baseline = average of 15min before induction of anaesthesia, SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, BT = core body temperature.

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restarted. The GP remained in the BC until loss of RR and of voluntary movement. During the induction in the BC only visual observations could be obtained. Gas inflow was redirected to the nose cone (inflow of 0.7mL O_2, 3% Iso), the GP was removed from the BC and placed in a dorsal position on the warmed receiver plate with the nose inserted into the nose cone.

Table 5. Respiratory rate during anaesthesia with medetomidine-midazolam-fentanyl, isoflurane and ketamine-xylazine in male guinea pigs.

Induction	2.5min		5min		7.5min		10min		12.5min		15min	
Treatment	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**
MMF	10	83.2 ± 14.5	11	73.5 ± 9.3	11	71.6 ± 9.0	11	67.7 ± 9.9	11	71.3 ± 13.1	11	69.5 ± 12.0
Iso	12	92.0 ± 18.8	13	76.9 ± 13.3	13	65.5 ± 11.1	13	50.5 ± 16.9	13	42.9 ± 11.4	13	37.2 ± 11.0
KX	6	61.3 ± 19.0	7	56.6 ± 6.7	7	57.1 ± 7.2	7	56.6 ± 4.3	7	55.4 ± 3.6	7	57.7 ± 5.1
Maintenance	**20min**		**25min**		**30min**		**35min**		**40min**			
Treatment	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**		
MMF	11	70.2 ± 12.9	11	69.5 ± 14.3	11	67.6 ± 13.0	11	66.5 ± 13.1	11	65.8 ± 12.3		
Iso	13	34.9 ± 13.2	13	31.4 ± 10.8	13	32.6 ± 12.4	13	28.9 ± 12.7	13	37.4 ± 14.5		
KX	7	56.0 ± 4.6	7	55.4 ± 6.3	7	54.9 ± 6.0	7	54.9 ± 6.0	7	56.6 ± 4.9		
Recovery	**42.5min**		**45min**		**47.5min**		**50min**		**52.5min**		**55min**	
Treatment	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**
MMF	11	64.0 ± 17.3	11	63.3 ± 14.6	10	89.2 ± 23.9	11	93.5 ± 18.9	11	90.2 ± 18.0	9	91.6 ± 13.6
Iso	13	53.2 ± 9.9	13	60.6 ± 10.2	13	62.2 ± 11.5	13	71.1 ± 16.8	12	75.3 ± 17.7	12	76.7 ± 13.6
KX	7	49.7 ± 5.6	7	48.6 ± 7.1	7	44.0 ± 8.9	7	41.7 ± 8.6	7	41.7 ± 8.6	7	42.9 ± 5.5
	60min		**65min**		**70min**		**80min**					
Treatment	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**	**n**	**mean ± sd**				
KX	7	53.1 ± 14.4	7	50.3 ± 14.2	7	62.3 ± 20.4	7	65.7 ± 14.4				

Mean and SD of respiratory rate [1/min], MMF = medetomidine-midazolam-fentanyl, Iso = isoflurane, KX = ketamine-xylazine.

doi:10.1371/journal.pone.0161258.t005

Table 6. Blood glucose level during anaesthesia in male guinea pigs.

Unit	Treatment	n	7.5min	20min	Increase [%]	40min	Increase [%]
[mmoL/L]	MMF	11	6.1 ± 1.0	8.7 ± 1.9	+ 41.9	11.1 ± 3.3	+ 27.7
	Iso	13	7.3 ± 1.8	7.7 ± 2.1	+ 5.3	7.9 ± 2.4	+ 2.5
	KX	7	6.2 ± 1.7	8.0 ± 2.2	+ 28.9	10.0 ± 4.1	+ 25.3

Averaged means and SD of blood glucose levels, MMF = medetomidine-midazolam-fentanyl; Iso = isoflurane; KX = ketamine-xylazine.

doi:10.1371/journal.pone.0161258.t006

KX anaesthesia

Premedication was the same (sodium chloride) as used with MMF. Ketamine (75mg/kg) and xylazine (15mg/kg) were mixed in a single syringe. For induction, the GP was positioned upright, hind feet touching the ground and KX was injected intraperitoneally into the dorsal abdomen 1cm lateral to the midline. Intraperitoneal application was selected to avoid muscle tissue necrosis and due to the large injection volume. The GP was returned to the single cage until it had lost its RR. At 35min a maximum of 10mL of 39°C warmed 0.9% sodium chloride solution was distributed s.c. over the abdomen to compensate for dehydration. At 40min xylazine was antagonized with 0.15mg/kg atipamezole s.c. into the axillary region.

Results

Three out of 16 implanted GPs were euthanized before the beginning of the anaesthesia study, 2 because of hind leg lameness at 2 and 6 days after surgery and 1 due to excessive

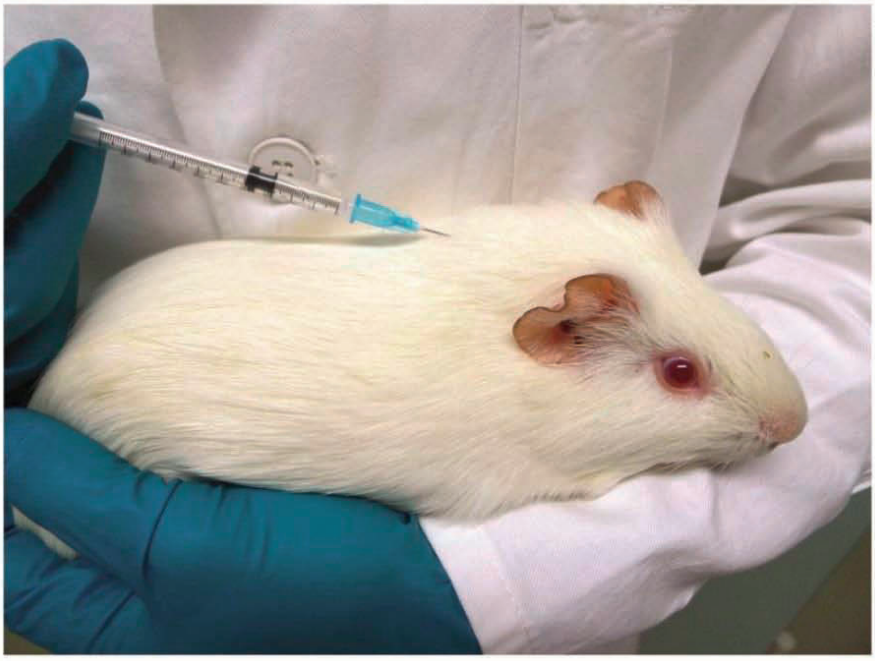

Fig 1. Subcutaneous injection in the neck of a guinea pig.

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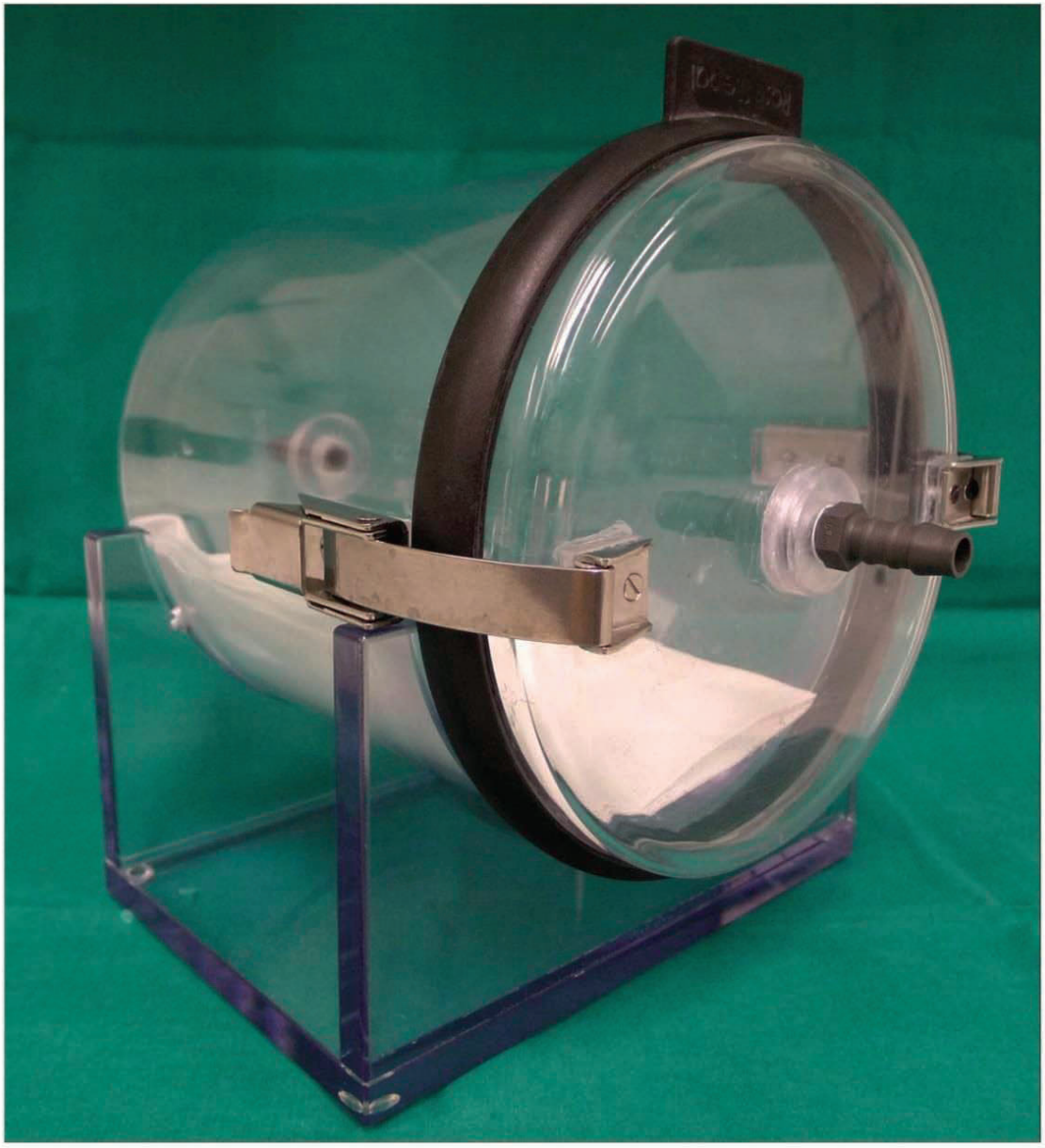

Fig 2. Circular whole body chamber for isoflurane induction in guinea pigs.

doi:10.1371/journal.pone.0161258.g002

intraoperative bleeding. Thirteen guinea pigs entered the study and all animals survived the anaesthesia study.

With MMF anaesthesia, 2 animals didn't reach the defined surgical tolerance state (reflex status > ± for FWR and RR) and they were excluded from the hemodynamic data analysis. With KX anaesthesia, 3 animals never lost RR and 3 more didn't reach surgical tolerance; these 6 animals were also excluded from the hemodynamic evaluation. Consequently, anaesthesia with MMF could be assessed with 11 GPs, 13 for Iso but only 7 GPs for KX. Two GPs showed apnoea under Iso anaesthesia while attached to the nose chamber.

Anaesthesia duration

During anaesthesia induction, non-surgical tolerance and surgical tolerance durations differed only marginally between the 3 anaesthetic regimens (Fig 3).

Induction and non-surgical tolerance were short with a range between 6min for KX and 9.2 min for MMF. Significant differences were seen in the speed until regaining of RR (wake up). MMF had the quickest wake up phase, followed closely by Iso, whereas GPs with KX needed by far the longest time until they regained their RR (59.7min).

Baseline and anaesthesia values

Baseline values before each anaesthesia were similar apart from a slightly lower HR with KX (238bpm, see (Table 4).

MMF

During MMF anaesthesia, after a short induction peak in MAP and HR, MAP ranged within baseline values (Fig 4, MAP 3–4) and HR slightly below (Fig 4, HR B-C) during maintenance.

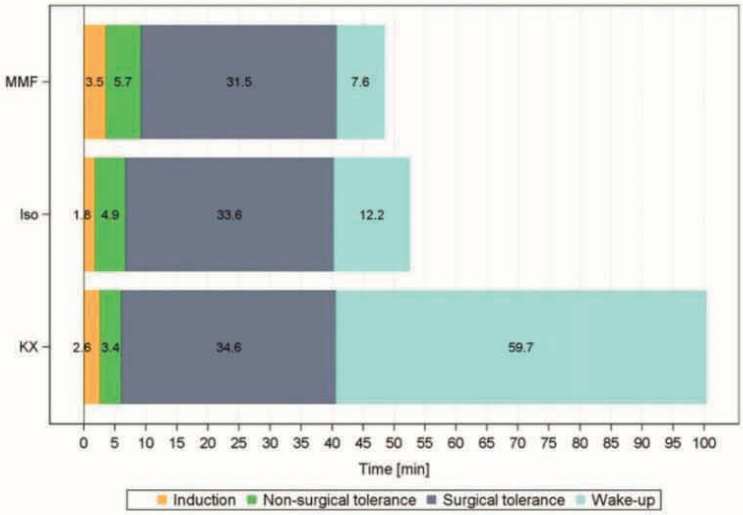

Fig 3. Duration of anaesthesia with MMF, Iso and KX in male guinea pigs. MMF = medetomidine-midazolam-fentanyl, n = 11; Iso = isoflurane, n = 13; KX = ketamine-xylazine, n = 7; interval definition see Table 3.

doi:10.1371/journal.pone.0161258.g003

Three min after antagonization, the HR increased until 51.3min (mean RR regained at 48.3min). After AFN, MAP resumed baseline levels at 147.5min (7) and HR after 182.5min (F). BT decreased continuously until shortly before RR was regained (Fig 4, BT I-II) and rose from there on to return to baseline (>38.7°C) after 102.5min (III).

Iso

MAP decreased abruptly (-43% to baseline) within 3.3min after induction (Fig 5, MAP 2). The MAP declined even further to very low levels (18 mmHg, -69%) until 40min (3) but increased steeply after Iso exposure was stopped, reaching baseline values at 53.7min (4) and remaining stable thereafter. The HR peaked when placing the GPs into the BC (Fig 5, HR A), but it fell again quickly (B). It slightly rose again, with the transfer to the nose cone (C) then decreased continuously during maintenance to slightly below baseline HR (E). The HR immediately rose after stop of Iso exposure until 54min (F) then decreased continuously but baseline HR values were not reached before 240min (G). BT fell progressively (Fig 5, BT II) until after regaining of

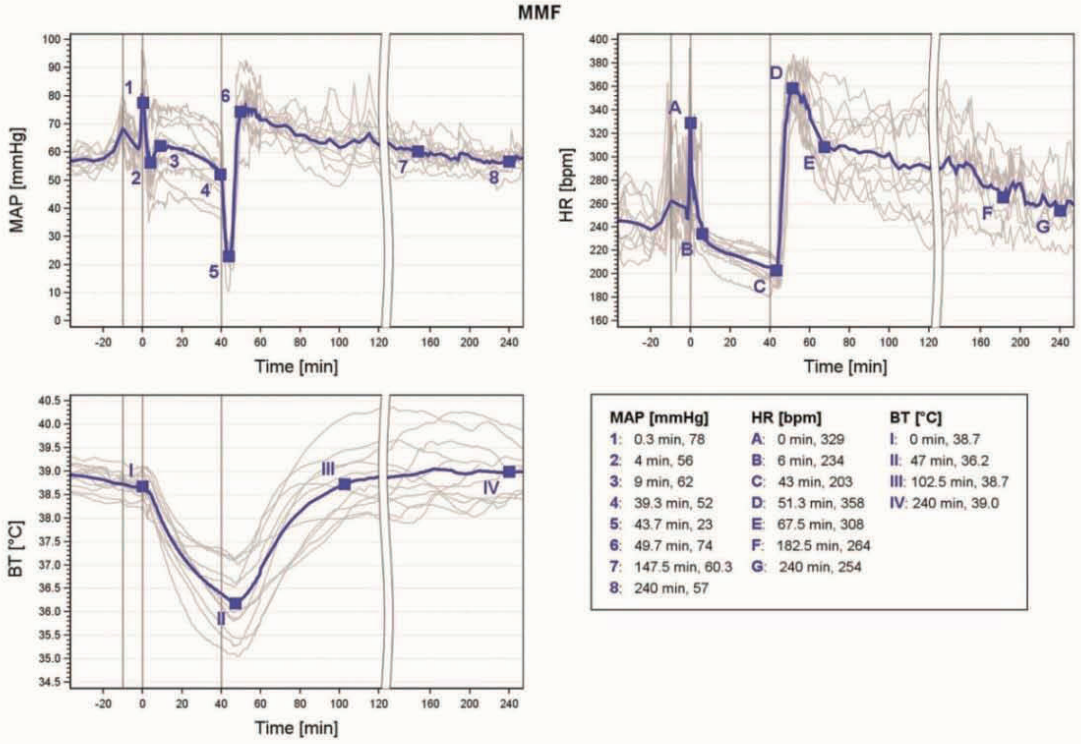

Fig 4. MAP, HR and BT during MMF anaesthesia in male guinea pigs. Mean (blue) and individual arterial pressure (MAP), heart rate (HR) and core body temperature (BT) during medetomidine-midazolam-fentanyl (MMF) anaesthesia in 11 male guinea pigs. 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ antagonization at 40 min.

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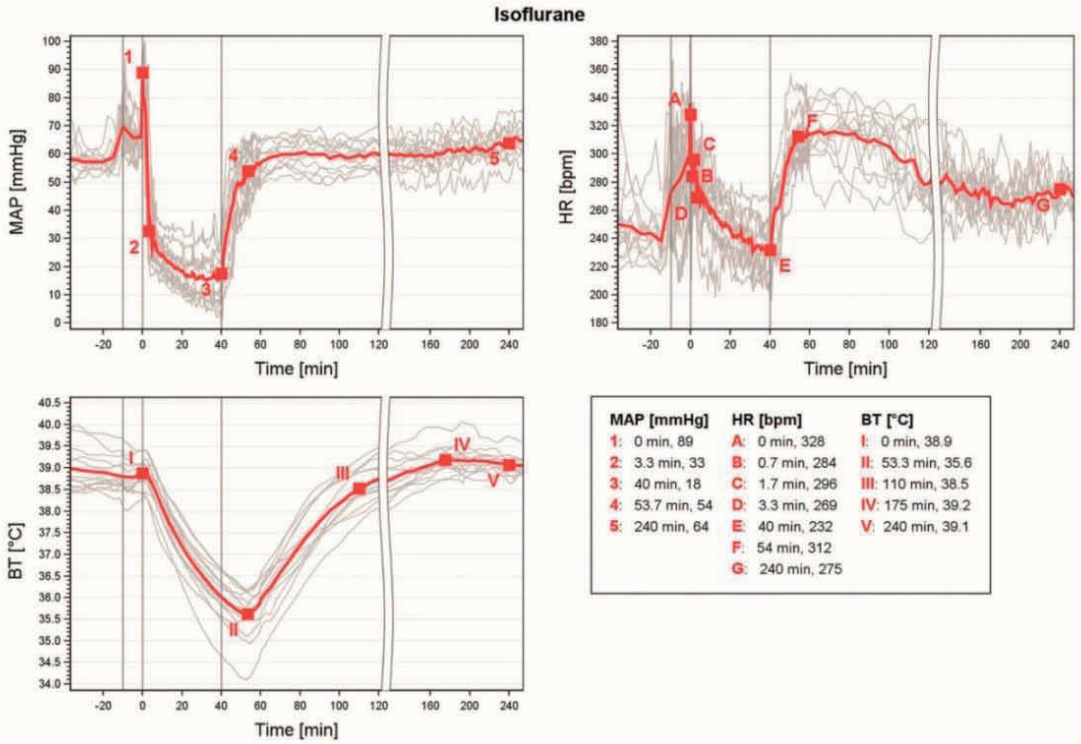

Fig 5. MAP, HR and BT during isoflurane anaesthesia in male guinea pigs. Mean (red) and individual arterial pressure (MAP), heart rate (HR) and core body temperature (BT) during isoflurane anaesthesia in 13 male guinea pigs. 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ end of exposure at 40 min.

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RR at 52.5min. It increased after 53.3min and reached stable baseline BT values after 110min (III).

KX

For KX the MAP values varied substantially (Fig 6). During maintenance the MAP increased steadily from slightly below to near baseline values at 40min (4). Atipamezole induced a fall (-39%, 5) followed by a slow advance with wide individual variance. Baseline values were regained at 102.5min (6). The MAP remained stable but slowly decreased to end slightly below baseline MAP. HR dropped after induction until 12.3min (Fig 6, HR B) and remained stable during maintenance. It decreased slightly following atipamezole (D) and thereafter varied widely during recovery until 147.5min (E). From there on the mean HR increased gradually but baseline HR values were not reached until 5.25h after induction (not included in Fig 6). The BT continuously decreased after KX induction (Fig 6, BT I) and it fell further after atipamezole antagonization with individual variance. In 2 GPs the BT dropped below 35°C during

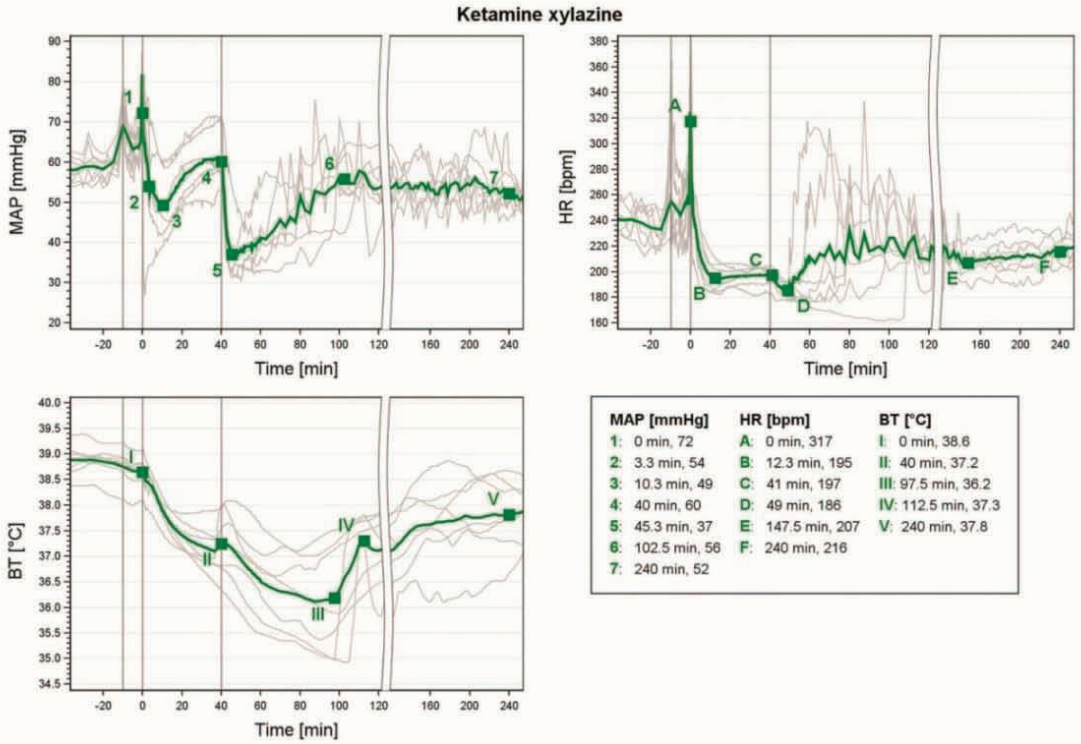

Fig 6. MAP, HR and BT during KX anaesthesia in male guinea pigs. Mean (green) and individual arterial pressure (MAP), heart rate (HR) and core body temperature (BT) during ketamine-xylazine (KX) anaesthesia in 7 male guinea pigs. 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ partial antagonization at 40 min.

doi:10.1371/journal.pone.0161258.g006

recovery and they received external warming. Mean BT gradually increased but all GPs were still hypothermic at 240min after induction (baseline BT 39±0.2°C). Three GPs reached 38.8°C after 5h (307.5, 317.5 and 332.5min), the other 4 GPs had not reached pre-anaesthetic BT levels at 8h.

Respiration

All drug combinations induced a significant decrease of ReR under anaesthesia (Table 5), which was substantial with MMF (-41%, baseline 114brpm,) and drastic in Iso (-71%). GPs in the BC showed irregular breathing and defence movements during Iso (slowly and deeply → held breath → steadily and more shallow), such that an averaged ReR of the first 2.5min was not representative for that period. ReR was also reduced markedly (-52%) but stably under KX maintenance, yet fell further after the administration of atipamezole. With MMF, the ReR increased rapidly after the GPs had regained their RR, whereas with Iso, ReR progressively increased once the Iso was discontinued.

One GP showed apnoea during Iso maintenance and mild respiratory breathing sounds were auscultated in 4 GPs. Reflex testing (FWR, IG) led to a short term ReR increase with all three anaesthesias.

Blood glucose

Mean BG values increased during the use of all three anaesthetic agents. MMF led to the largest BG increase (especially during induction), followed by KX. Iso increased the BG only modestly (Table 6). All acquired BG values remained within physiological ranges (5-16mmol/L, 89-287mg/dL, [6]).

Reflexes and observations

With KX, only 7/13 GPs became surgically tolerant, but those who did fell asleep without displaying excitation. During maintenance they showed an increasing exophthalmos, piloerection and a high muscle tone with sharp reflex responses. After atipamezole the GPs became cataleptic and sedated in dorsal recumbency (Fig 7) and responded only temporarily to reflex testing with twitching and chewing.

It required multiple attempts before RR was achieved and thereafter the GPs showed only a mild thermoregulatory shivering and neck located piloerection. After antagonization the reflex responses varied instead of progressing continuously and the GPs remained sedated long after the return of RR.

Iso exposure led to surgical tolerance in all 13 GPs. During BC induction, the GPs appeared highly stressed (strong defensive reactions for approximately 20sec before slowing and

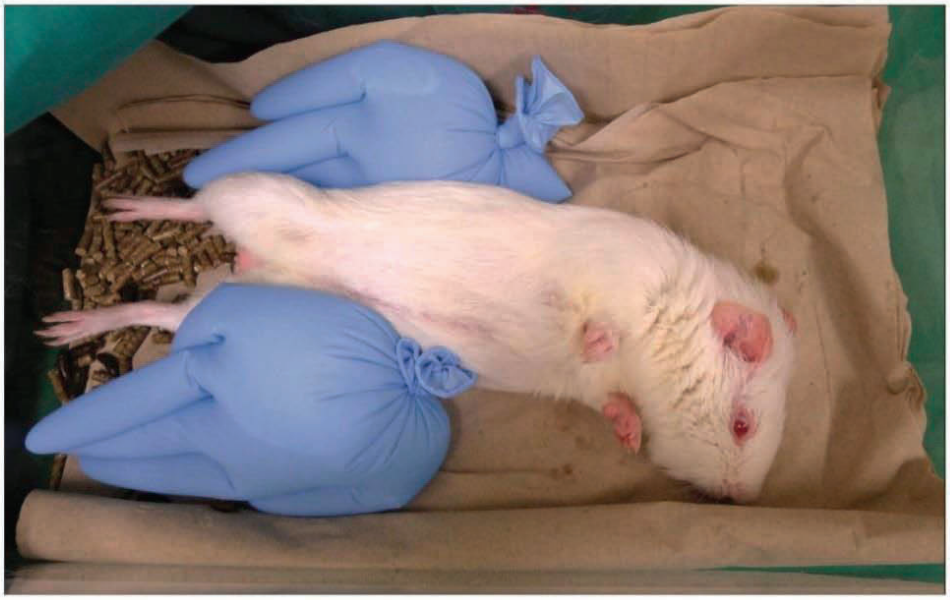

Fig 7. Recovery from ketamine-xylazine anaesthesia in a guinea pig. Cataleptic male guinea pig during recovery after partial ketamine-xylazine anaesthesia antagonization with atipamezole with warming gloves to compensate hypothermia.

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slumping to the bottom of the BC). The skin turned pale and they urinated before losing RR. Throughout the Iso exposure, the skin turned pink, the FWR and IG were weak or delayed with a low muscle tone, but the responses intensified when Iso was stopped and the skin colour normalized. After regaining of RR, multiple GPs showed a hind limbs paresis, although they were walking with their front feet and strong thermoregulatory shivering started, combined with a distinct piloerection.

With MMF 11/13 GPs achieved surgical tolerance. During induction, GPs only displayed excitations when disturbed while falling asleep. Throughout MMF the ear reflex remained ±, the muscle tone was high and FWR and IG were strong. The reflex responses intensified quickly and progressively after antagonization and the GPs abruptly regained their RR, followed by a short hyperactive phase. At induction the skin colour turned pink, then faded to pale during maintenance and turned pink once more at AFN administration before it normalized thereafter. Thermoregulatory shivering and piloerection were pronounced in the first 30min after return of RR.

In all anaesthesias, reflex responses intensified in following order during recovery: FWR and IG, ear reflex, lid reflex, RR. After antagonization or Iso exposure stop, RR and skin colour returned from the head to the back and the GPs displayed chewing from + FWR and IG onwards.

Discussion

In this study we have acquired continuous radio-telemetry data concerning the effect of MMF, Iso and KX on hemodynamics and other physiological parameters during anaesthesia in male GPs and we have also described anaesthesia specific observations. As expected, all anaesthetics had an effect on the parameters and we saw substantial differences between them.

The difficulty in anaesthetizing GPs is due to a combination of factors. The choice of anaesthetics is limited, as a venous induction in the awake state is not viable or the anaesthetic does not provide for a quick recovery to enable fast body temperature and energy regulation. Also the evaluation of anaesthetic depth requires experience, anaesthesia and species specific knowledge, as reflex responses and reactions to anaesthetics vary between anaesthetic agent, individual and intra-individually, especially with KX [2]. Therefore GPs profit from a quickly reversible anaesthetic, close monitoring during and after anaesthesia and a qualified and experienced anaesthetist.

The anaesthetic should be chosen on the considerations of 1) safety, 2) convenience, 3) reliability of the anaesthetic effects, 4) short, low-stress induction and recovery time, 5) the influence of hemodynamic parameters and 6) quickly adjustable anaesthetic depth.

We chose the three paravenous anaesthetics KX, MMF and Iso as they were the most used in recent studies. Until now, KX has been the most commonly used anaesthesia in GPs, which has been administered in various dosages (25-120mg/kg for ketamine and 0.2-13mg/kg for xylazine), but was often reported to lack reliability and frequently needed additional dosages to achieve satisfactory surgical depth [2, 7]. Iso is the most used inhalational anaesthetic in laboratory animals, due to its good hypnotic and muscle relaxing effect, the minimal metabolization and the quick on- and offset of anaesthesia [8]. It does however require atropine premedication in GPs, because they react to Iso with heavy bronchosecretion, salivation and tear production [9] in addition to the dose-dependent respiratory depression.

MMF is an established anaesthesia in Germany which combines midazolam's anticonvulsive and sedative action, fentanyl's strong, short-acting analgesic effect, with medetomidine's sedative, analgesic and muscle relaxing effects [8]. The single effects are potentiated, enabling a

dose reduction of each component and thereby creating fewer side effects. Its benefits are a fast induction, easy extension and a full antagonization with a quick recovery.

Anaesthesia duration

The 40min anaesthesia duration was chosen as this time span allows the performance of many (surgical) procedures and it allows comparability to past studies [9, 10]. Ideally, the time until reaching the desired anaesthetic depth is short, maintenance duration can be varied as needed and time until regained RR is rapid. A short induction is beneficial as the sensitive excitatory anaesthesia phase II [8] is passed quickly and more anaesthesia time is saved for the planned procedure. Rapid post-anaesthetic return to RR speeds the return to auto-regulation of physiological parameters, especially of BT and BG. All 3 anaesthetics had a short duration until surgical tolerance, but with KX only 7/13 GPs reached an operable reflex depth. Wake-up durations for MMF and Iso were relatively short, such that the GPs' were able to return to body temperature auto-regulation and normal behaviour quickly after anaesthesia stop.

By comparison, KX, even with partial antagonization, required almost 1h until return of RR and recovery thereafter was unreliable, resulting in the need for continued monitoring. Omitting atipamezole, however, led to yet longer durations until regaining of RR (240min after KX induction in mice, 300min after KX induction in rats; [11]; [12]). In summary, MMF and Iso allow quick and reliable induction until desired anaesthetic depth, a flexible maintenance time and a fast return to stable physiological conditions. By contrast, even partially antagonized KX leads to an undesirably long post-anaesthetic recovery and is therefore not advisable for procedures requiring a reliable recovery.

Blood pressure

The BP guarantees the adequate perfusion of major organs (brain, heart, kidney and liver) and is therefore tightly regulated. Preferably, anaesthesia should have little effect on BP. Our data shows, however, that all 3 anaesthetics influence the BP markedly (Fig 8).

The transient peak of BP associated with induction was most pronounced with Iso due to the comparatively higher handling stress and the defence reactions to the narcotic gas. Iso's vasodilatory effect led to symptoms of cold feet, pale mucous membranes and weak pulse, which classify as worthy for acute intervention because of risk for vital organ damage [13]. In comparison, rats with Iso only show a mild MAP decrease [10]. Minimal metabolization allows a quick return to a stable MAP after discontinuation in both species.

KX and MMF showed little deviations from baseline MAP, especially MMF had desirable maintenance values. Marked effects are seen in KX and MMF upon administration of atipamezole. In MMF, it reversed the medetomidine hypertonia [14], and we could not observe disadvantageous effects caused by the BP drop. KX produced tolerable BP values until atipamezole induced a moderate hypotension that the GP could only gradually reverse.

Overall, MMF's effect on BP is acceptable, whereas significant hypotension occurs during Iso maintenance and after partial KX reversal.

Heart rate

A regular HR ensures constant oxygen supply to vital organs and can also be used to assess anaesthetic depth and analgesia. The transiently increased HR reflects the handling and injection stress and, shortly thereafter, drug-induced effects influence the HR (Fig 9).

With Iso, despite the severe hypotension, GPs displayed only a mild and declining tachycardia, likely due to Iso's inhibition of the HR baroreceptor reflex [15], which is reversed when Iso is stopped, resulting in a steep increase in HR. The mild bradycardia during MMF maintenance

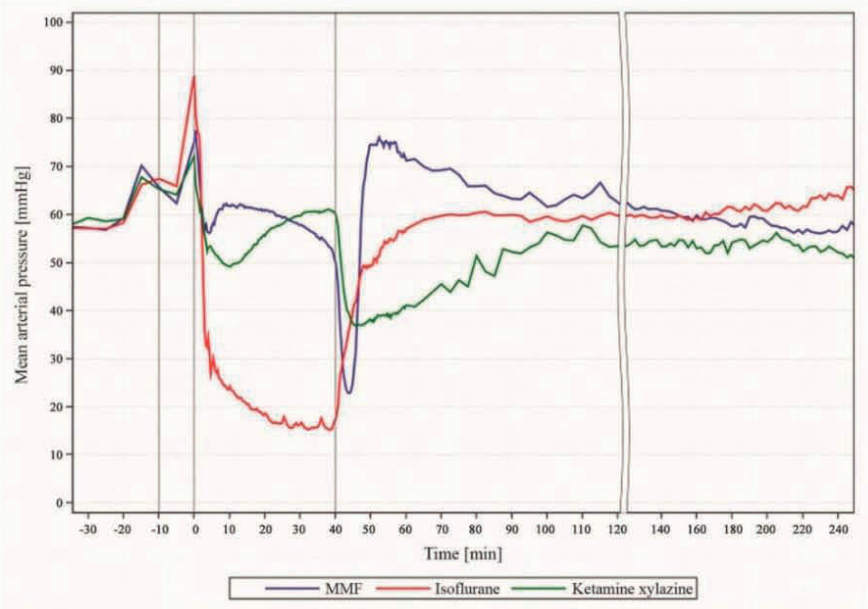

Fig 8. MAP course during MMF, KX and Iso anaesthesia in guinea pigs. Mean arterial blood pressure (MAP) course during anaesthesia in male guinea pigs with medetomidine-midazolam-fentanyl (MMF, n = 11), isoflurane (n = 13) and ketamine-xylazine (n = 7). 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ end of exposure at 40 min.

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is uncritical and may be the result of the potentiated bradycardic medetomidine effect by fentanyl [16]. The steep HR increase upon antagonization results initially from the atipamezole component in AFN and is intensified after 7.6min by the regained RR (Fig 3), resulting in a transient tachycardia (max. HR 358 bpm at 51.3min, Fig 4D). Similar HR values with Iso and MMF from 70min onwards suggest that HR is no longer under direct pharmacological influence, but may be elevated as a result of thermoregulatory shivering [17].

KX induced a very stable HR from 12.3 until 41min in GPs (Fig 6B and 6C) but with medium bradycardic values, whereas rats showed only a minimally decreased HR with KX [10]. After atipamezole antagonization the HR declines further (-11 bpm, Fig 6C and 6D), despite the drop in pressure which would usually cause a reflex tachycardia as seen with MMF antagonization. The remaining ketamine induces a catalepsy, preventing the RR return and with that a movement-induced HR increase.

In conclusion, Iso produced values closest to physiological HR range, closely followed by MMF. Antagonized KX, however, resulted in an anaesthetic and long term post-anaesthetic bradycardia and should therefore only be considered when a long lasting bradycardia is acceptable, but with substantial effects on other physiological parameters.

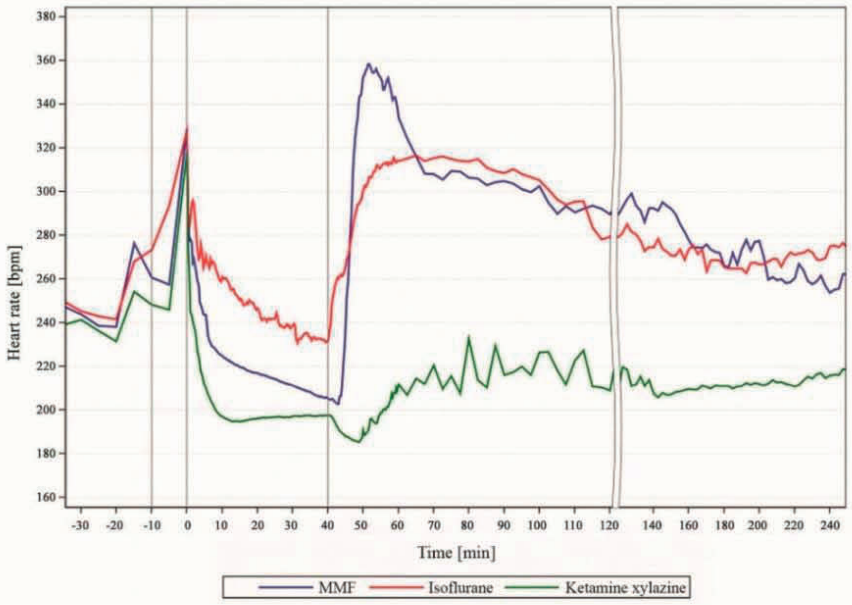

Fig 9. HR course during MMF, KX and Iso anaesthesia in guinea pigs. Heart rate (HR) course during anaesthesia in male guinea pigs with medetomidine-midazolam-fentanyl (MMF, n = 11), isoflurane (n = 13) and ketamine-xylazine (n = 7). 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ end of exposure at 40 min.

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Core body temperature

Anaesthesia often leads to hypothermia, especially in small mammals. Their large body surface-to-volume ratio and absent BT production due to lack of movement during anaesthesia, are amplified by the pharmacological depression of central thermoregulation and by ventilation with cold gases [8].

Hypothermia leads to a slowed metabolism during anaesthesia with a prolonged recovery or even post-anaesthetic death [18].

We also observed a rapid BT loss, despite the use of water heated mats (temperature losses KX: -2.4°C in 97.5min, Iso: -3.3°C in 53.3min, MMF: -2.5°C in 47min). The time course of the BT loss was similar with all 3 anaesthetic agents up to 15min after induction. BT with MMF decreased until AFN was administered and would likely have continued to decrease, had the anaesthesia not been antagonized (xylazine-fentanyl-climazolam, 33.6°C at 45-90min after induction; [19]). During Iso, BT is lost faster (Fig 10) due to redistribution of body warmth from the body core to the periphery caused by peripheral vascular dilation [20].

We can only hypothesize, why the BT remains the highest at 40min with KX. A combination of insulating factors, namely the high muscle tension, peripheral vasoconstriction and a lower blood flow to the periphery by a reduced heart rate, may result in a slowed BT loss. Further studies are needed to explore the precise mechanism of action of KX on the BT in GPs.

After discontinuation of Iso or antagonization of MMF with AFN, the GPs started thermoregulatory shivering and increased their BT steadily, in contrast to KX, where the catalepsy

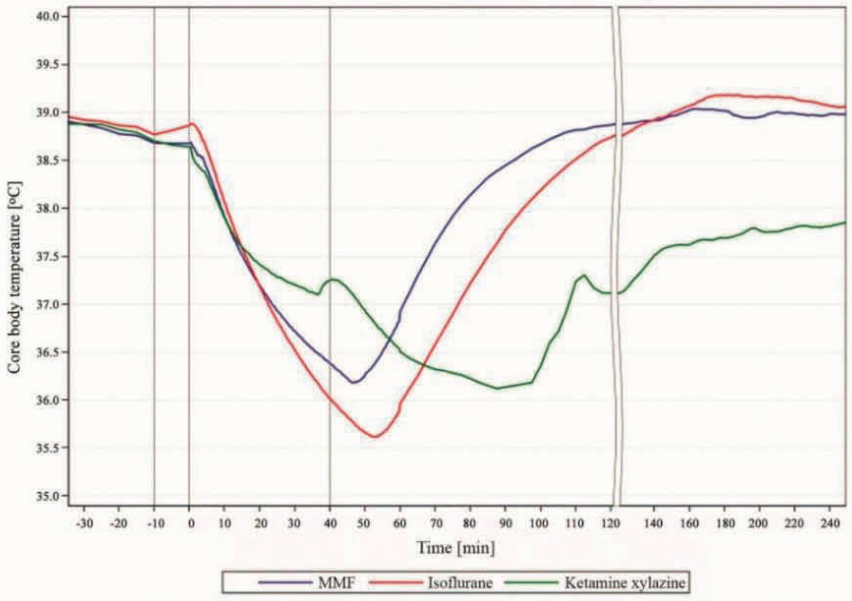

Fig 10. BT course during MMF, KX and Iso anaesthesia in guinea pigs. Core body temperature (BT) course during anaesthesia in male guinea pigs with medetomidine-midazolam-fentanyl (MMF, n = 11), Isoflurane (n = 13) and ketamine-xylazine (n = 7). 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ end of exposure at 40 min.

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impeded both shivering and physiological movement. Only 3/7 KX GPs had returned to baseline BT after over 5h. Considering this, the 2 hypothermic GPs would probably have died eventually without additional warming.

Apparently water heated mats can only slow the BT loss during anaesthesia, such that further BT retaining methods like short anaesthesia times and warming of a larger body surface area are required. A close post anaesthetic BT monitoring and optional external warming are especially essential with KX use. In summary, MMF led to the least BT loss and the fastest return to physiological levels, followed closely by Iso, whereas KX led to a prolonged and only slowly reversible hypothermia.

Respiration

GPs often react with hypoventilation to anaesthesia [9], however mechanical ventilation is not recommended as remnant food particles are easily transferred into the lung during intubation [21]. Therefore, reliable spontaneous breathing is crucial. Iso use is especially critical, as the GPs' long air passages are exposed to Iso's respiratory depressive, bronchodilatory and hypersalivatory properties. The water secretion to the saliva in the lower respiratory tract can be transiently reduced by atropine premedication but thick mucous accumulates nevertheless. Comparatively high Iso concentrations are therefore needed to reach the mucous membrane. The GP's airways and mucous membranes were irritated by Iso, resulting in irregular breathing during induction (Fig 11, [9]).

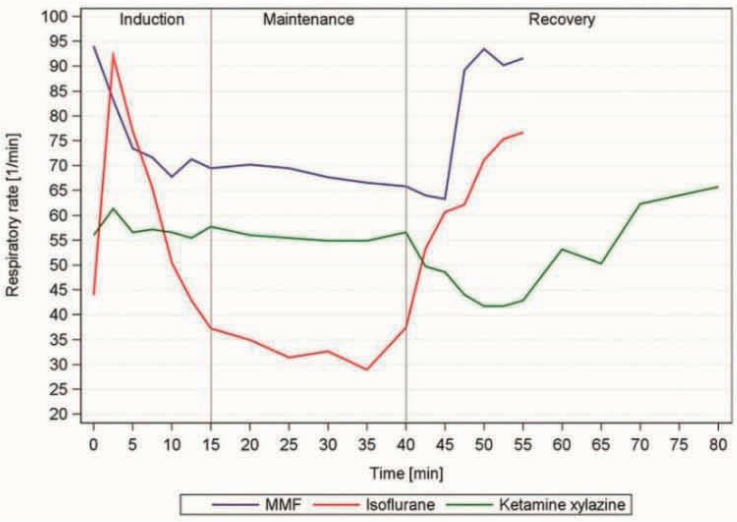

Fig 11. Respiratory rate course during MMF, KX and Iso anaesthesia in male guinea pigs.
MMF = medetomidine-midazolam-fentanyl; n = 11, Iso = isoflurane, n = 13; KX = ketamine-xylazine, n = 7. Anaesthesia was lifted after 40min.

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During maintenance balancing anaesthesia depth and hypoventilation proved challenging and adjustments were often done in 0.1% Iso concentration steps. The decrease in ReR during Iso maintenance of more than 2/3 compared to physiological ReR is partly mediated through enhancement of central $GABA_A$ receptor responses [22]. More than 50% ReR decrease compared to physiological values indicates impending respiratory failure and the need for treatment [23], which we observed during Iso and post-anaesthetic KX. We supplemented O_2 to prevent hypoxia and used the lowest Iso concentrations possible, but lengthy Iso anaesthesia with the observed hypotension and respiratory depression could lead to hypoxic tissue damage.

The hypoventilation during KX is the result of the potentiation of ketamine's respiratory depression by xylazine [24]. In the recovery phase (Fig 11) the respiratory stimulating atipamezole effect is overcome by the high muscle tension and sedation, which impede physiological ReR.

With MMF, the medetomidine induced hypoventilation is potentiated by fentanyl, as action potential transmission from the pre-Bötzinger complex in the ventrolateral medulla is inhibited, leading to skipped inspirations and irregular breathing [25]. Xylazine-fentanyl-climazolam anaesthesia in GPs led to a similar but slightly lower ReR decrease (52 brpm, [19]) compared to MMF, suggesting that xylazine depresses ReR more than medetomidine.

However, the potentiation of the single components with MMF and thereby achieved component dose reduction, avoids severer respiratory side effects, making it the anaesthesia of choice concerning effects on respiration.

Blood glucose

A BG increase can be induced by acute stress or the anaesthetic agent and values need to be interpreted accordingly. In a preliminary study pre-anaesthetic BG measurements were

attempted in awake GPs, but this led to severe defence reactions and resulted in delayed anaesthesia induction. Therefore, BG tests in awake GPs were not performed in this study. Although GPs are easily stressed, catecholamine-induced hepatic glycogenolysis probably did not cause the rise (Fig 12) with MMF and KX, as BG levels were tested for the first time after loss of consciousness and α_2-adrenoceptor agonists reduce catecholamine levels.

However, comparative stress-hormone blood levels and pre-anaesthetic BG are needed to confirm this hypothesis. Looking at pharmacological effects, xylazine inhibits the insulin secretion on the β-cells in the pancreas [26] and ACTH and corticosterone are decreased. Also, plasma glucagon and growth hormone levels increase during KX [27]. Therefore, the rise in BG with KX likely originates from an insulin shortage, reducing the uptake of glucose into the cells and an increased hepatic glucose production and secretion.

Concerning MMF, medetomidine induces hyperglycaemia, which could be lowered by adding midazolam in a cat model [26]. Fentanyl passively increases the BG level by inhibiting glucose-stimulated insulin release, which was seen in rat pancreatic islet cells [28].

The greater BG increase in MMF compared to KX (see Table 6) could be explained by medetomidine's higher α_2-selectivity (1620 medetomidine:160 xylazine (Virtanen, 1988)). Clinically, GPs with MMF are able to compensate for the BG increase with a quick return to food consumption 30min after AFN administration [18]. BG measurements after anaesthesia are needed to assess and interpret the post-anaesthetic development. Concerning our BG data, Iso produced the smallest increase but all values were within the physiological range, so no anaesthetic can be clearly favoured.

Reflexes and observations

The assessment of reflex responses is an essential tool to evaluate anaesthesia depth and phase and should be performed regularly by the same person and in combination with evaluation of

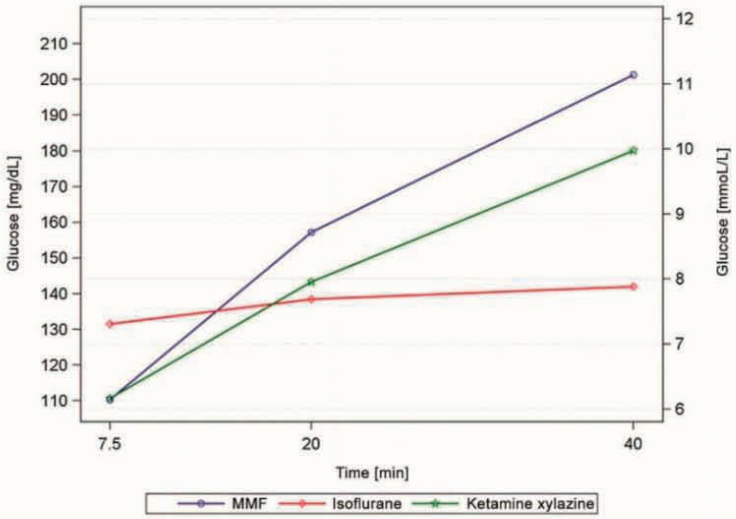

Fig 12. Blood glucose during MMF, KX and Iso anaesthesia in male guinea pigs. MMF = medetomidine-midazolam-fentanyl; n = 11, Iso = isoflurane, n = 13; KX = ketamine-xylazine, n = 7.

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ReR and HR. The RR marks a successful induction and completes the wake-up phase and the FWR and the IG allow a conclusive assessment of surgical tolerance in the GP [19, 29]. Stress- and painful stimuli (repeated reflex testing, loud noises, touching) during induction should be avoided as they prolong the time until loss of RR. Testing for FWR, instead of the interdigital reflex examines a deeper consciousness disconnection via pain character (bone pain vs. skin pain) and it allows simultaneous muscle tone assessment. Species and anaesthetic agent specific knowledge is essential for the adequate interpretation of reflex responses. We, like Seidensticker [18], observed anaesthesia-related differences in reflex response character and speed. Whereas reflex responses were almost exaggerated during MMF anaesthesia, they were distinct but comparatively reduced with KX and generally slow and weak under Iso anaesthesia. We chose an anaesthetic depth with a mildly positive FWR and IG, as a complete response loss leaves ReR as the only indicator, which is especially questionable with Iso. It also removes the assessment of the dose safety margin until death of the animal. Iso's effect of delayed and weak reflex responses, combined with an observed transient hind limb paresis directly after regaining of RR (also reported by Heide [9]), made us question the significance of FWR and IG for anaesthetic depth evaluation during Iso.

Under Iso, motoneuronal excitability in the spinal cord is suppressed [30], which may play an important role for the surgical immobility property. As GPs regain motor function after anaesthesia from head to back, the altered synaptic transmission could explain the temporary muscle paresis. With KX the anaesthesia was unreliable in its induction, achieving and in the duration of a surgical plane (only 7/13 GPs achieved surgical tolerance). The GPs needed intensive monitoring after antagonization, with reduced behaviour and abnormal posture even after return to RR.

MMF administration in GPs was rapid, tolerated well and the reflex responses were predictable and consistent during maintenance. After AFN the GPs returned quickly to pre-anaesthetic behaviour and posture. As AFN also reverses fentanyl, painful procedures need to be treated with NSAIDs or metamizole following the anaesthesia to provide adequate analgesia. For the sake of this study, reflex responses were tested every 2.5 min during induction, but this should be omitted in the standard setting, as it causes an unnecessary disturbance without adding beneficial information. Instead, we suggest monitoring for the loss of RR and assessing the FWR and IG for the first time 2min thereafter. Also premedication for MMF and KX was only necessary for comparison in this study, so this stressor can normally also be eliminated.

MMF was the pre- and post-surgically least stressful choice for the animal and allowed reliable insights concerning anaesthetic depth and stage for the anaesthetist.

Conclusion

Based on our data, MMF is the anaesthesia of choice in GPs for any procedure that exceeds a short, not painful immobilization, for which Iso would be a possible option. HR influence was acceptable and BP, BT, ReR impact, reflex responses and the low anaesthesia related stress were more beneficial for the GP compared to Iso and KX. Iso offered the benefits of quick induction and reliable anaesthesia and could therefore be used for short and not painful procedures (blood withdrawal, examination). However, dramatically low BP and ReR, as well as fast BT loss and uncertain reflex responses make it undesirable for procedures longer than 10min.

KX results were not satisfactory during anaesthesia and disadvantageous in the post-anaesthetic phase, which is why we advise against KX use in GPs.

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Author Contributions

Conceived and designed the experiments: SS JH.

Performed the experiments: SS.

Analyzed the data: SS JH BG.

Wrote the paper: SS BG ST.

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5 Publication 3

"Repeated anaesthesia with isoflurane and medetomidine-midazolam-fentanyl in guinea pigs and its influence on physiological parameters"

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RESEARCH ARTICLE

Repeated anaesthesia with isoflurane and medetomidine-midazolam-fentanyl in guinea pigs and its influence on physiological parameters

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Abstract

Repeated anaesthesia may be required in experimental protocols and in daily veterinary practice, but anaesthesia is known to alter physiological parameters in GPs (*Cavia porcellus*, GPs). This study investigated the effects of repeated anaesthesia with either medetomidine-midazolam-fentanyl (MMF) or isoflurane (Iso) on physiological parameters in the GP. Twelve GPs were repeatedly administered with MMF or Iso in two anaesthesia sets. One set consisted of six 40-min anaesthesias, performed over 3 weeks (2 per week); the anaesthetic used first was randomized. Prior to Iso anaesthesia, atropine was injected. MMF anaesthesia was antagonized with AFN (atipamezole-flumazenil-naloxone). Abdominally implanted radio-telemetry devices recorded the mean arterial blood pressure (MAP), heart rate (HR) and core body temperature continuously. Additionally, respiratory rate, blood glucose and body weight were assessed. An operable state could be achieved and maintained for 40 min in all GPs. During the surgical tolerance with MMF, the GPs showed a large MAP range between the individuals. In the MMF wake- up phase, the time was shortened until the righting reflex (RR) returned and that occurred at lower MAP and HR values. Repeated Iso anaesthesia led to an increasing HR during induction (anaesthesias 2–6), non-surgical tolerance (anaesthesias 3–6) and surgical tolerance (anaesthesias 4, 6). Both anaesthetics may be used repeatedly, as repeating the anaesthesias resulted in only slightly different physiological parameters, compared to those seen with single anaesthesias. The regular atropine premedication induced HR increases and repeated MMF anaesthesia resulted in a metabolism increase which led to the faster return of RR. Nevertheless, Iso's anaesthesia effects of strong respiratory depression and severe hypotension remained. Based on this increased anaesthesia risk with Iso, MMF anaesthesia is preferable for repeated use in GPs.

KG does not alter our adherence to PLOS ONE policies on sharing data and materials. None of the authors currently serve as editorial board member to PLOSONE, have acted as an expert witness in relevant legal proceedings or currently sit on a committee for an organization that might benefit from the publication of this data.

Introduction

Repeated anaesthesia may be required in experimental protocols and in daily veterinary practice. It is known that anaesthetic agents significantly alter the animals' physiological parameters [1, 2]. For guinea pigs (*Cavia porcellus*, GPs), single medetomidine-midazolam-fentanyl (MMF), isoflurane (Iso) or ketamine-xylazine (KX) anaesthesia caused substantial effects on physiological parameters [3]. MMF produced comparably few and acceptable deviations from physiological values and Iso, with its quick induction and reliable onset of effect, was found to be primarily useful for short-term and non-painful procedures. KX however led to a prolonged wake-up phase, associated with catalepsy and hypothermic recovery, such that it is not recommendable for use in GPs.

Based on these results, the present study investigated the effects of recurrent MMF and Iso anaesthesias on physiological parameters in GPs. The duration of defined anaesthesia phases, mean arterial blood pressure (MAP), heart rate (HR), core body temperature (BT), respiratory rate (ReR), blood glucose (BG) and body weight (BW) were investigated. Overall, few deviations from the course of the individual MMF and Iso anaesthesia were expected, based on the outcome of repeated MMF and Iso use in the rat [4]. The repeated i.m. injections could lead to local tissue alteration and possibly to a poorer resorption of the MMF anaesthesia. Furthermore, the GPs high susceptibility to stress [5] may lead to behavioural changes in the course of the experiment. The used radio-telemetry technique was particularly valuable for our study as it offered continuously measured data with low human intervention [6] and the possibility to repeatedly anaesthetize the same individual.

Materials and methods

1.1 Housing, acclimatization and radio-telemetry implantation

All experiments and procedures were performed in accordance with the German Animal Welfare Act (Art. 3 G v. 28.7.2014 I 1308) and the regional council for animal welfare. This research was approved by the Regierungspräsidium Tübingen, Germany under the approval number 12–038. Necessary euthanasia was performed with pentobarbital.

Sixteen male albino Hartley GPs from Charles River Laboratories (Sulzfeld, Germany, delivery BW 350–400g/ age of 6.5 weeks) were housed for 19 days prior to the radio-telemetry implantation in groups of 2–3 (EHRET TERULAN THF 1776). Wooden bedding material (Lignocel FS14, Rettenmaier & Söhne GmbH + Co.KG, Rosenberg, Germany, change 2x/ week) and two shelters were supplied per cage. The GPs received 20g/GP of pelleted diet (3410 complete feed, KLIBA NAFAG, Provimi Kliba Sa., Kaiseraugst, Switzerland), a large amount of autoclaved hay daily and tap water *ad libitum*. The animal room was kept at 20±2˚C, 55 ±10% with an air change of 15 cycles/h. The light-dark cycle was 12:12 ± dimmer phases of 30 min. A radio played music for acoustic habituation when lights were on. BW and general condition of each animal were monitored daily. The radio-telemetry transmitter implantation procedure (DSI, PhysioTel® HD, HD-S11, DSI, St. Paul, MN, USA) in the GP as well as the pre- and postoperative treatment, have already been described in detail in a previous publication [7]. Therefore, only a brief description is given here. Prior to the implantation, the GP was weighed, examined clinically and given the first dose of antibiotic (oral enrofloxacin 10 mg/kg, Enrotron® 100 mg/mL, aniMedica, Senden-Bösensell, Germany). The GPs received 20 mg of Vitamin C (oral aqueous solution, Vitamin C Pulver, dm-Drogerie Markt, Karlsruhe, Germany), 7 days prior to and for 14 days after the implantation. The animal was transferred to the surgical preparation area, where anaesthesia was induced with intramuscular (i.m.; m.

Table 1. Medetomidine-midazolam-fentanyl anaesthesia dosage and antagonization for guinea pig anaesthesia.

Purpose	Product name	Brand name / Manufacturer
anaesthesia- agonists	MMF	[1] DOMITOR®, 1mg/mL, Orion Corporation, Espoo, Finland [2] Dormicum® 5mg/mL, Roche Pharma AG, Grenzach-Wyhlen, Germany [3] Fentanyl®-Janssen 0.1mg/2mL, JANSSEN-CILAG, Neuss, Germany
	Intramuscular (i.m.) in mixed syringe	
	1 Medetomidine[1] 0.2mg/kg + 2 Midazolam[2] 1.0mg/kg + 3 Fentanyl[3] 0.025mg/kg	
anaesthesia- antagonists	AFN	[4] ANTISEDAN® 5mg/mL, Orion Corporation, Espoo, Finland [5] Flumazenil HEXAL® 0.1mg/mL, HEXAL AG, Holzkirchen, Germany [6] Naloxon Inresa 0.4mg/mL, Inresa Arzneimittel, Freiburg, Germany
	Subcutaneous (s.c.) in mixed syringe	
	4 Atipamezole[4] 1.0mg/kg + 5 Flumazenil[5] 0.1mg/kg + 6 Naloxone[6] 0.03mg/kg	

MMF/AFN anaesthesia dosage also used during radio-telemetry transmitter implantation.

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semimembranosus/ m. semitendinosus/ m.biceps femoris) MMF injection ([8]; medetomidine 0.2 mg/kg, midazolam 1.0 mg/kg, fentanyl 0.025 mg/kg, see Table 1).

After the loss of the righting reflex (RR), the abdominal and throat incision sites were prepared and lidocaine hydrochloride (0.9 mL s.c., Xylocain® 1%, AstraZeneca, Wedel, Germany) was injected. The GP received meloxicam (0.4 mg/kg subcutaneous/ s.c., Metacam® 2 mg/mL, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) and metamizole (80 mg/kg i.m., Novalgin® 1 g/2 mL, Sanofi-Aventis Deutschland, Frankfurt am Main, Germany) injections. Thereafter, the GP was transferred to the surgical area and provided with oxygen (0.7mL oxygen inflow) and heat (water bath set to 40˚C). The abdomen was incised in the mid-line, the intestine was carefully moved cranially and the aorta abdominalis was dissected free of surrounding tissue. The radio-telemetry transmitter tip was inserted into the abdominal aorta between the caudal renal artery and the aortic bifurcation [9] and was fixed in place using uncoloured tissue glue (Histoacryl® uncoloured tissue glue, B.Braun Surgical, Rubi, Spain). The telemetry device body was sutured to the abdominal cavity wall. The two ECG cables were passed through the abdominal muscle wall and the abdominal cavity was closed. One ECG cable was led beneath the skin to the chest and tied to the m. pectoralis. The skin over the throat was incised and the m. trachealis was dissected free. The second ECG cable was led subcutaneously to the throat and the abdominal incision was closed. The second ECG cable was attached to the m. trachealis and the throat incision was sutured. Thereafter, the anaesthetized GPs were transferred to the telemetry data acquisition room, antagonized with AFN (atipamezole 1.0 mg/kg, flumazenil 0.1mg/kg, naloxone 0.03mg/kg, Table 1). Additional heat was supplied until the GP's BT was at least 38˚C. The animal was monitored for 24 h with radio-telemetry and personal observation. Thereafter, the GP was returned to its home cage. The initial 24 h of analgesia were ensured by the pre-surgical meloxicam injection and by oral applications of metamizole 80 mg/kg (Metamizol HEXAL® oral drops, 500 mg/mL, HEXAL AG, Holzkirchen, Germany) given every 4–6 h. Meloxicam (0.4 mg/kg) and enrofloxacin (10 mg/kg) were continued orally for 2 more days at 24 h intervals. The GP was examined twice daily for 7 consecutive days after surgery and the BW was checked daily until 14 days after the implantation. There was a 41/42 d rest between the implantation and the first anaesthesia repetition. The dosages from Table 1 were also used for the subsequent MMF anaesthesias.

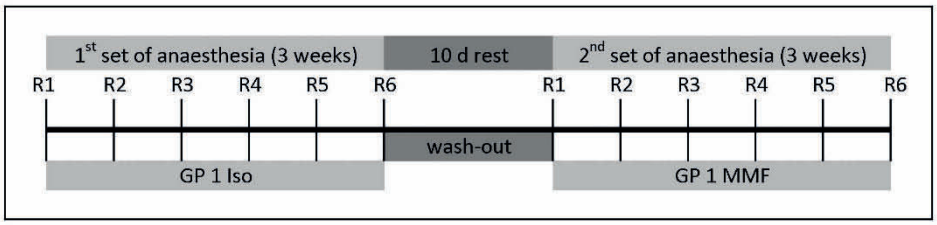

Fig 1. Repeated anaesthesia time schedule. Each GP was anaesthetized 6 times over 3 weeks with either MMF or Iso (in this example, first Iso), followed by a wash-out phase of 10 d. Thereafter the same GP was anaesthetized with the other substance (here MMF) for 6 repetitions. R1 = anaesthesia repetition #1, Iso = Isoflurane, MMF = medetomidine-midazolam-fentanyl.

https://doi.org/10.1371/journal.pone.0174423.g001

1.2 Study design

Thirteen GPs were subjected to anaesthesia twice per week over 3 weeks, either exclusively with Iso or with MMF (Fig 1), resulting in 6 recurring anaesthesias with the same anaesthetic agent, (henceforth each anaesthesia repetition will be described as "round"/R).

The sixth anaesthesia repetition was followed by a wash-out period of 10 d, in which no experiment was conducted. After the wash-out period, each GP was submitted to a second set of 2 anaesthesias per week, performed over 3 weeks, but this time with the other anaesthetic. In total, each GP received 12 anaesthesias. Depending on the day of the week, there were either 2 or 3 days between the anaesthesias (Table 2). All animals in a common home cage were anaesthetized on the same day, but with individually assigned anaesthesias (Table 2, home cage groups: GPs 1–2, 3–4, 5–7).

Which anaesthesia was applied first for each animal, was randomly assigned in a 2x2 cross-over order. The course of the anaesthesia procedure with the weighing and examination of the GPs before anaesthesia, the telemetry room set up, premedication, anaesthesia induction, reflex testing and response evaluation and the anaesthesia phase duration were the same as described in a previous publication [3]. Each GP was placed into a single cage and was transferred into the data acquisition room. The telemetric measurement began with 120 min of habituation time in the measuring room (Fig 2), during which the animals, to be anaesthetized that day, had no human contact.

Table 2. Daily schedule of repeated anaesthesia with MMF or Iso in GPs.

Week 1							Week 2		
Mon	**Tue**	**Wed**	**Thu**	**Fri**	**Sat**	**Sun**	**Mon**	**Tue**	**Wed**
1 Iso	5 Iso		1 Iso	5 Iso			1 Iso	5 Iso	
2 MMF	6 MMF		2 MMF	6 MMF			2 MMF	6 MMF	
3 MMF	7 MMF		3 MMF	7 MMF			3 MMF	7 MMF	
4 Iso			4 Iso				4 Iso		
R 1	R 1		R 2	R 2			R 3	R 3	
	2 d interim			3 d interim				2 d interim	
		2 d interim			3 d interim				

The daily schedule shows the first half of 3 weeks of repeated anaesthesia application, using the first 7 GPs as an example. Iso = Isoflurane, MMF = medetomidine-midazolam-fentanyl, R1 = anaesthesia repetition #1.

https://doi.org/10.1371/journal.pone.0174423.t002

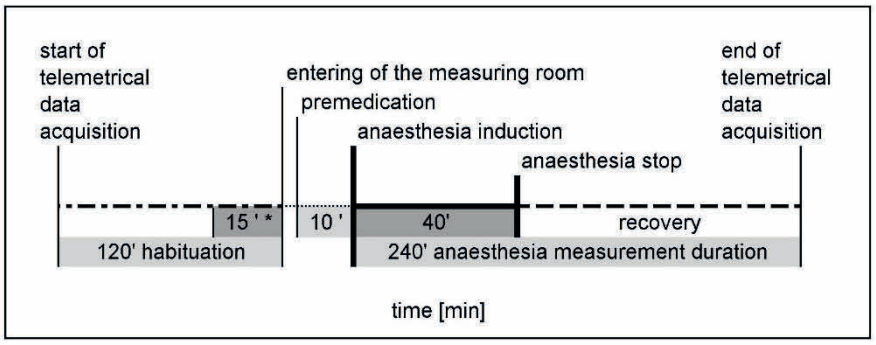

Fig 2. Course of one anaesthesia repetition with either MMF or Iso. * The parameters during the last 15 min of the habituation time were averaged as daily baseline values for that GP.

https://doi.org/10.1371/journal.pone.0174423.g002

After 120 min, the room was entered and the GP was premedicated in the dorsal neck region. Atropine (0.04mg/kg, Atropinum Sulfuricum 1.0mg Eifelfango®, Bad Neuenahr-Ahrweiler, Germany) was injected s.c. prior to Iso anaesthesia and 0.4mL/kg sodium chloride were applied as a placebo before MMF anaesthesia. Ten min after premedication, anaesthesia was induced with either Iso or MMF. For Iso, the GP was placed into a pre-filled whole body induction chamber with 4.4 vol. % Iso (Forene®100%, AbbVie Deutschland, Ludwigshafen, Germany) and 99% O_2 (measured by Criticare Poet II Patient Monitor, Soma Technology Inc., Bloomfield, CT, USA). After the GP had lost its RR, it was placed on its back on a water heated mat (set to 39˚C) and attached to a pre-flowed nose cone. At the beginning, the Iso concentration was 3±0.15 vol. % with 0.7mL O_2 inflow in the nose cone, but it was gradually reduced to 2.3±0.16 vol. % in the course of anaesthesia. MMF anaesthesia (Table 1) was induced with an i.m. injection into the caudal part of the femoral muscle of one hind leg, or split and injected into both hind legs, if the total volume exceeded 0.5mL. The animal was returned to its single cage until it had lost its RR. Thereafter, it was placed on its back on a water heated mat, and was attached to a pre-flowed nose cone with 0.7mL O_2 inflow. With both anaesthetic agents, the anaesthesia was stopped after 40 min. Iso was discontinued and MMF anaesthesia was antagonized with AFN s.c. in the chest region. The GP was disconnected from the nose cone and was placed into its home cage in dorsal recumbency. For each GP, the parameter baselines of the day for were averaged from the last 15 min of the habituation time (Fig 2). The baselines for all animals due that day, were collected before the start of the first anaesthesia. During the entire data acquisition, BP, HR and BT were continuously recorded via radio-telemetry. The respiratory rate (ReR) and the reflex responses were monitored manually at an interval of 2.5 min between 0–15 min (0 = induction) and 40–55 min, and at 5 min between 15 and 40 min. The ReR was counted visually and breathing sounds were auscultated with a stethoscope over the lungs and trachea. The anaesthesia depth was evaluated by assessing the RR, lid reflex, foot withdrawal reflex and inguinal reflex (Table 3).

Blood glucose (BG) values were acquired during the anaesthesia at 7.5, 20 and 40 min (One-Touch Ultra2, LifeScan Europe, Zug, Switzerland) with blood taken from an ear prick with a blood lancet (Solofix® B.Braun, Melsungen, Germany).

Table 3. Anaesthesia phase definition.

Anaesthesia phase	Definition
I = induction	From MMF or isoflurane exposure to loss of RR
II = non-surgical tolerance	From loss of RR to mildly positive (±) foot withdrawal and inguinal reflex response
III = surgical tolerance	From (±) foot withdrawal and inguinal reflex to antagonization/ exposure stop
IV = wake-up	From antagonization/exposure stop to regaining of RR
V = recovery	From RR until 240 min

Reflex response dependent anaesthesia phase definition for anaesthesia with MMF and isoflurane in male guinea pigs. RR = righting reflex, MMF = medetomidine-midazolam-fentanyl.

https://doi.org/10.1371/journal.pone.0174423.t003

1.3 Statistical analysis

NOTOCORD- hem™ was used for the telemetric data acquisition and the raw data was further evaluated with MS Excel. Values between premedication (-10 min) until 55 min after induction were averaged in 20 sec intervals, the values before and thereafter in 2.5 min intervals. The statistical evaluation was performed with SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA). The analysis was done separately for each parameter (anaesthesia duration, MAP, HR, BT, ReR, BG, BW) and for each anaesthetic (Iso and MMF). For MAP, HR and BT, the differences between the 1st anaesthesia and the subsequent ones were analysed by an analysis of covariance (ANCOVA) with factors "round" and "baseline" as covariates. An analysis of variance (ANOVA) was used to analyse anaesthesia duration, BG and ReR. The significance level was set at $\alpha = 5\%$, therefore a p-value ≤ 0.05 was considered to be statistically significant. The adjusted mean mentioned in the following tables is based on ANCOVA with factor round and baseline as covariates. Rounds 2 to 6 were compared to round 1. Positive mean difference values indicate an increase compared to round 1.

Results

Sixteen GPs were implanted, 3 were euthanized before the study began and 1 was excluded from the procedure, due to a lack of BW gain and a poor general condition.

Therefore, 12 GPs entered the study and all animals survived the complete protocol. In round 2 during repeated Iso, one GP had to be excluded from MAP, HR and BT analysis due to a computer program failure. Overall, the repeated application of both MMF and Iso reliably led to an operable state in all animals and with each round of anaesthesia. The MMF dosage and Iso inflow concentration were not increased throughout the repetitions. There was no indication that the first set of anaesthesias had an impact on the second anaesthesia set.

2.1 Anaesthesia duration

The induction and non-surgical tolerance with MMF was short and consistent (Table 4) and accounted for between 24.5–27.7% (10.1–11.2 min) of the total anaesthesia time. With Iso, these phases were even shorter and made up between 15.5–19.3% (6.3–7.8 min) of the 40 min anaesthesia duration. All animals remained surgically tolerant during all anaesthesias until 40 min after induction.

With repeated MMF anaesthesia, the wake-up duration decreased significantly, especially from round 1 to 2, and then again in rounds 5 and 6, whereas the Iso wake-up duration

Table 4. MMF and Iso anaesthesia phase durations during guinea pig anaesthesia.

Narcosis phase	Round	Treatment	Adjusted mean	Mean diff.	P value	Treatment	Adjusted mean	Mean diff.	P value
Induction [min]	1	MMF	3.39			Iso	1.37		
	2		3.95	0.56	0.1305		1.43	0.07	0.6177
	3		3.4	0.01	0.9847		1.38	0.02	0.9014
	4		3.54	0.15	0.7458		1.31	-0.06	0.6648
	5		3.24	-0.15	0.7514		**1.09**	**-0.28**	**0.0447**
	6		2.98	-0.41	0.3942		1.15	-0.22	0.1113
Non-surgical tolerance [min]	1	MMF	6.74			Iso	4.94		
	2		7.23	0.49	0.5922		**6.37**	**1.43**	**0.0259**
	3		7.08	0.33	0.765		4.67	-0.27	0.7305
	4		7.03	0.28	0.8131		4.87	-0.07	0.9288
	5		7.58	0.84	0.4969		*6.53*	*1.59*	*0.0716*
	6		7.18	0.44	0.7248		6.31	1.37	0.1271
Surgical tolerance [min]	1	MMF	31.14			Iso	34.33		
	2		*29.28*	*-1.86*	*0.0898*		**32.70**	**-1.63**	**0.026**
	3		30.05	-1.09	0.4133		34.28	-0.06	0.9435
	4		29.75	-1.39	0.3364		34.43	0.10	0.9068
	5		29.65	-1.49	0.3208		32.98	-1.35	0.1221
	6		30.27	-0.88	0.5661		*32.86*	*-1.48*	*0.093*
Wake-up [min]	1	MMF	9.37			Iso	10.48		
	2		**6.22**	**-3.15**	**0.0025**		10.60	0.13	0.8106
	3		**6.29**	**-3.08**	**0.0052**		11.35	0.88	0.1723
	4		**6.38**	**-2.99**	**0.0069**		10.03	-0.44	0.5194
	5		**4.9**	**-4.47**	**0.0001**		10.69	0.22	0.7593
	6		**5.56**	**-3.81**	**0.0007**		10.40	-0.08	0.9167
Recovery [min]	1	MMF	180.23			Iso	181.43		
	2		183.95	3.73	0.5333		182.68	1.25	0.8009
	3		190.45	10.23	0.139		*170.82*	*-10.60*	*0.0896*
	4		187.03	6.81	0.3391		178.96	-2.47	0.7141
	5		191.92	11.69	0.1071		183.49	2.07	0.7685
	6		182.54	2.32	0.7475		180.95	-0.48	0.9472

Duration of 'anaesthesia phases [min], adjusted mean with mean differences and p values over 6 rounds of repeated anaesthesias with medetomidine-midazolam-fentanyl (MMF) and isoflurane (Iso) in 12 male guinea pigs. **Bold** = p value $\leq$ 0.05, *italic* = 0.05 < p value $\leq$ 0.10.

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remained constant in the course of the rounds. In round 6, the wake-up duration with MMF was approximately half the time compared to that with Iso. A further division of the MMF wake-up phase into "duration from antagonization to the lowest MAP" and "duration from lowest MAP to return of RR", indicates that the time between the antagonization and the lowest MAP remained stable (between 3.33 and 2.64 min), but that the duration between the lowest MAP and the return of RR decreased from 5.72 to 2.08 min (-64%) from round 1 to 6 (Table 5).

2.2 Mean arterial blood pressure, heart rate and core body temperature

MMF. During surgical tolerance, the MAP in round 1 was significantly higher compared to the following rounds. We also observed a large individual variation of MAP during the surgical tolerance phase, which was not seen in the baseline values (Fig 3). The adjusted mean of all animals during surgical tolerance was 60.1–66.5 mmHg, the lowest individual MAP was

Table 5. Faster return to the righting reflex in the course of repeated MMF anaesthesia.

	Duration from antagonization to lowest MAP [min]		Duration from lowest MAP to return of righting reflex [min]	
Round	Mean duration	SD	Mean duration	SD
1	3.33	1,14	5,72	3,03
2	2,89	1,40	3,00	1,53
3	3,06	1,29	2,92	1,84
4	2,89	1,09	3,11	2,01
5	2,64	1,31	1,94	1,01
6	3,17	1,08	2,08	1,20

Duration shortening from the lowest MAP point to the return of the righting reflex over 6 rounds of repeated medetomidine-midazolam-fentanyl anaesthesia in 12 male guinea pigs. SD = standard deviation.

https://doi.org/10.1371/journal.pone.0174423.t005

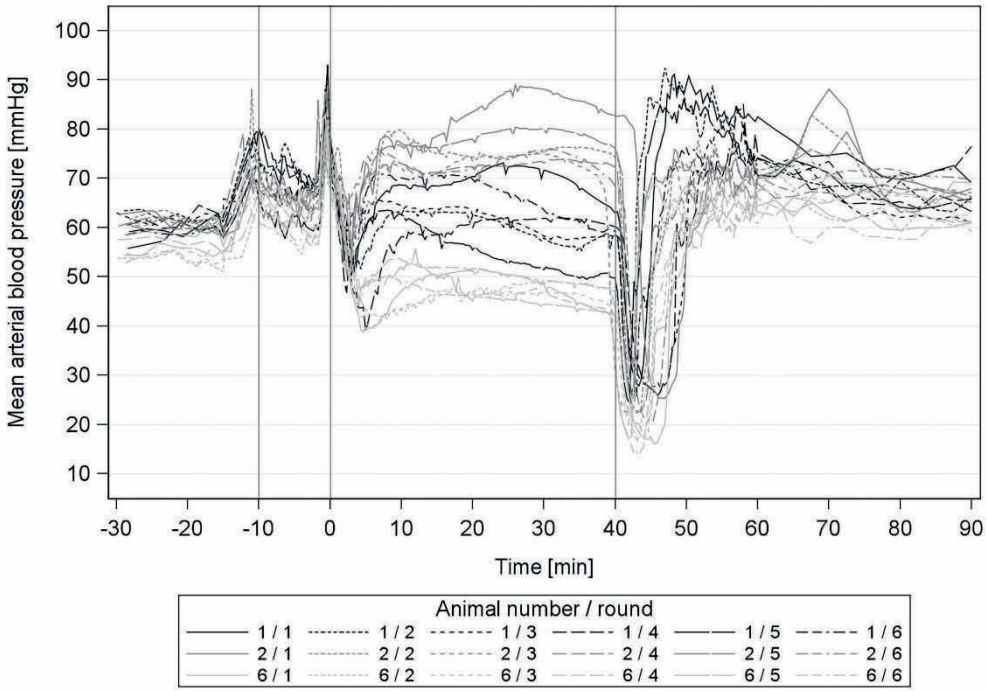

Fig 3. Mean Arterial blood Pressure (MAP) variance during surgical tolerance in male guinea pigs. Mean arterial blood pressure (MAP) variance during surgical tolerance in 3 group representative male guinea pigs during 6 repeated anaesthesias with medetomidine-midazolam-fentanyl. 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ antagonization at 40 min.

https://doi.org/10.1371/journal.pone.0174423.g003

Table 6. Return to the righting reflex at lower MAPs in the course of repeated MMF anaesthesia in guinea pigs.

		MAP at antagonization [mmHg]		MAP low point [mmHg]		MAP at return of righting reflex [mmHg]	
Round	n	Mean	SD	Mean	SD	Mean	SD
1	12	61.91	15.08	24.39	8.66	67.90	14.59
2	12	53.85	14.34	23.17	6.32	59.88	13.51
3	12	55.76	10.66	24.39	6.94	58.13	15.29
4	12	53.43	9.23	23.38	4.09	60.42	11.57
5	12	53.07	13.18	24.64	5.09	57.04	14.20
6	12	53.02	14.21	21.90	7.38	52.64	11.75

Lowering of mean arterial blood pressure (MAP) at antagonization and return to the righting reflex at lower MAP in the course of 6 rounds of repeated medetomidine-midazolam-fentanyl anaesthesia in 12 male guinea pigs. SD = standard deviation.

https://doi.org/10.1371/journal.pone.0174423.t006

45.1–49.4 mmHg and the highest 72.6–86.0 mmHg. However, the GPs stayed within their blood pressure range throughout all rounds (Fig 3, GP 2 repeatedly exhibited a high surgical MAP).

In the wake-up phase the GPs showed decreasing MAP values in rounds 2–6 compared to round 1. With closer inspection of the wake-up phase, the MAP value at antagonization in round 1 is approximately 8 mmHg higher compared to round 2–6 (Table 6). The MAP low point after the antagonization was however consistent (24.39–21.9 mmHg) throughout all rounds. With consecutive rounds, the GPs returned to their RR at a lower MAP value. Noticeable value reductions were seen from round 1 to 2–5 and then again to round 6.

HR during repeated MMF anaesthesia was only significantly altered in the wake-up phase. It decreased from round 1 to 2–5 (-13.9 to -23.1 bpm) and then further to round 6 (-30.2 bpm, Table 7). At the time of antagonization, the HR was the same throughout rounds 1–6, but at return of RR, the HR decreased from rounds 1 to 2–6 (Table 8).

There was no significant difference in BT between the rounds with repeated MMF anaesthesia except for a 0.3˚C rise in the wake-up phase of round 4 (Table 7).

Iso. During the preanaesthetic baseline, the MAP values during round 2–6 of repeated Iso were increased (+2–3.6 mmHg) compared to round 1 (Table 9). The MAP values were also higher in rounds 2, 4 and 6 of the wake-up phase.

The HR was increased during induction (+16.4–23.9 bpm, round 2–6), the non-surgical tolerance (+12.4, +21.3, +16.8 bpm, round 3–6) and surgical tolerance (+13.1 bpm, rounds 4, 6).

The BT was not altered significantly, except from a reduction of 0.2˚C during round 2 of surgical tolerance.

2.3 Respiratory rate

There was no major change of ReR during the repeated anaesthesias. Until min 7.5, the drop in ReR was comparable between MMF and Iso. Thereafter, the ReR remained stable (approx. 67 brpm) with MMF anaesthesia until antagonization. In comparison, the ReR continued to decrease with Iso anaesthesia to, on average, 48.2 brpm at 15 min, ending with approx. 39.2 brpm at 40 min (Fig 4).

With repeated Iso anaesthesia, 6 of 12 GPs developed breathing problems in at least one of the rounds. Two of the 6 had to be removed temporarily from Iso exposure due to apnoea in

Table 7. Parameter values over 6 rounds of repeated medetomidine-midazolam-fentanyl anaesthesia in guinea pigs.

		Preanaesthetic baseline		Induction		Non-surgical tolerance		Surgical tolerance		Wake-up		Recovery	
Parameter	Round	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value
SAP [mmHg]	1	69.0		75.3		71.6		78.0		49.0		72.7	
	2	69.9 (0.8)	0.3565	74.2 (-1.1)	0.6311	69.6 (-2.0)	0.3962	**72.2 (-5.8)**	**0.0290**	**40.9 (-8.1)**	**0.0021**	72.7 (0.1)	0.9399
	3	70.0 (0.9)	0.3633	75.1 (-0.2)	0.9481	69.9 (-1.7)	0.5744	72.3 (-5.7)	0.1003	**41.8 (-7.2)**	**0.0284**	71.0 (-1.7)	0.2920
	4	70.5 (1.4)	0.1792	75.3 (-0.1)	0.9822	69.1 (-2.5)	0.4736	*71.3 (-6.8)*	*0.0901*	**41.0 (-8.0)**	**0.0280**	71.4 (-1.2)	0.4899
	5	70.5 (1.4)	0.1823	77.5 (2.2)	0.5306	72.0 (0.4)	0.9140	73.2 (-4.9)	0.2595	**40.3 (-8.7)**	**0.0234**	72.9 (0.3)	0.8883
	6	**72.0 (3.0)**	**0.0063**	76.1 (0.8)	0.8190	69.2 (-2.4)	0.5486	71.0 (-7.0)	0.1248	**38.0 (-11.0)**	**0.0061**	72.3 (-0.4)	0.8592
DAP [mmHg]	1	50.9		55.2		53.2		56.6		35.0		55.3	
	2	51.0 (0.2)	0.8064	54.5 (-0.7)	0.6537	51.5 (-1.8)	0.2919	**52.4 (-4.3)**	**0.0226**	**29.3 (-5.7)**	**0.0019**	55.3 (0.0)	0.9820
	3	50.8 (-0.1)	0.9070	55.0 (-0.2)	0.9228	51.9 (-1.3)	0.5489	52.7 (-3.9)	0.1117	*30.6 (-4.4)*	*0.0571*	53.7 (-1.6)	0.1336
	4	51.1 (0.3)	0.7416	55.0 (-0.2)	0.9358	51.1 (-2.1)	0.3980	*51.7 (-4.9)*	*0.0826*	**29.8 (-5.2)**	**0.0470**	53.7 (-1.6)	0.1753
	5	50.8 (-0.1)	0.9450	56.1 (0.9)	0.6984	52.7 (-0.5)	0.8650	53.2 (-3.4)	0.2643	*29.7 (-5.3)*	*0.0570*	54.7 (-0.6)	0.6482
	6	*52.6 (1.7)*	*0.0594*	55.2 (0.0)	0.9955	51.0 (-2.3)	0.4375	*51.3 (-5.4)*	*0.0976*	**27.2 (-7.7)**	**0.0085**	54.8 (-0.5)	0.7091
MAP [mmHg]	1	59.1		64.7		61.7		66.5		41.9		63.6	
	2	59.7 (0.6)	0.3966	63.8 (-0.9)	0.6231	59.6 (-2.0)	0.3071	**61.4 (-5.2)**	**0.0218**	**35.0 (-7.0)**	**0.0016**	63.5 (-0.1)	0.9222
	3	59.5 (0.4)	0.6457	64.5 (-0.2)	0.9228	60.0 (-1.7)	0.5197	*61.5 (-5.1)*	*0.0879*	**36.1 (-5.8)**	**0.0345**	61.8 (-1.8)	0.1691
	4	59.9 (0.8)	0.3495	64.6 (-0.1)	0.9549	59.2 (-2.5)	0.4111	*60.4 (-6.2)*	*0.0722*	**35.3 (-6.6)**	**0.0320**	61.9 (-1.7)	0.2486
	5	59.7 (0.6)	0.4932	66.2 (1.5)	0.6002	61.4 (-0.2)	0.9445	62.2 (-4.4)	0.2383	**34.9 (-7.0)**	**0.0329**	63.1 (-0.4)	0.7849
	6	**61.5 (2.4)**	**0.0109**	65.0 (0.3)	0.9274	59.1 (-2.5)	0.4687	60.1 (-6.5)	0.1026	**32.5 (-9.4)**	**0.0064**	62.9 (-0.7)	0.6794
HR [bpm]	1	226.7		261.7		225.7		210.5		254.4		265.8	
	2	228.8 (2.1)	0.5751	265.4 (3.7)	0.4985	222.7 (-3.0)	0.1564	209.7 (-0.9)	0.6769	**240.5 (-13.9)**	**0.0444**	267.6 (1.8)	0.6549
	3	231.7 (5.0)	0.2448	262.9 (1.2)	0.8601	221.7 (-4.0)	0.1498	209.4 (-1.1)	0.6799	**235.6 (-18.7)**	**0.0133**	258.2 (-7.6)	0.1428
	4	230.4 (3.7)	0.4017	265.5 (3.8)	0.6089	223.7 (-2.0)	0.5175	212.0 (1.4)	0.6414	**234.3 (-20.1)**	**0.0091**	262.6 (-3.2)	0.5798
	5	226.1 (-0.6)	0.8987	258.4 (-3.3)	0.6687	222.5 (-3.2)	0.3417	210.3 (-0.3)	0.9386	**231.3 (-23.1)**	**0.0030**	261.6 (-4.2)	0.4916
	6	227.8 (1.1)	0.8078	259.3 (-2.4)	0.7592	220.0 (-5.7)	0.1113	206.4 (-4.2)	0.2203	**224.2 (-30.2)**	**0.0002**	258.6 (-7.2)	0.2624
BT [°C]	1	38.8		38.6		38.4		37.5		37.0		38.9	
	2	38.8 (0.0)	0.7484	38.7 (0.0)	0.5717	38.4 (-0.0)	0.9960	37.5 (0.0)	0.8777	37.1 (0.2)	0.2907	38.9 (-0.1)	0.5378
	3	38.8 (0.0)	0.8988	38.8 (0.1)	0.1577	38.5 (0.1)	0.2996	37.6 (0.1)	0.4581	37.2 (0.2)	0.1443	38.9 (-0.0)	0.8172
	4	38.9 (0.1)	0.5295	38.8 (0.1)	0.1457	38.5 (0.1)	0.1995	37.7 (0.2)	0.1294	**37.3 (0.3)**	**0.0346**	39.1 (0.2)	0.2534
	5	38.8 (0.0)	0.6763	38.7 (0.1)	0.3509	38.5 (0.1)	0.6249	37.6 (0.1)	0.5366	37.2 (0.2)	0.1272	39.0 (0.1)	0.6369
	6	38.8 (-0.0)	0.8131	38.7 (0.1)	0.4260	38.5 (0.1)	0.5380	37.6 (0.1)	0.6797	37.1 (0.2)	0.2727	38.9 (-0.1)	0.7166

Adjusted mean with mean differences and p values over 6 rounds of repeated medetomidine-midazolam-fentanyl anaesthesia in 12 male guinea pigs. **Bold** = p value $\leq$ 0.05, *italic* = 0.05 < p value $\leq$ 0.10. SAP = systolic arterial pressure, DAP = diastolic arterial pressure.

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one round and were tilted back and forth for ventilation and the other 4 displayed varying degrees of aggravated tracheal respiratory sounds towards the end of the anaesthesia in at least one round.

2.4 Blood glucose

The BG levels during anaesthesia with MMF and Iso were within the same range at 7.5 min after the onset of anaesthesia. With repeated MMF anaesthesia the BG values at 20 and 40 min were significantly higher during rounds 2, 4 and 6 compared to round 1 (Table 10). With repeated Iso anaesthesia, the BG values remained stable (40 min: 6.04–7.53 mmol/L).

Table 8. Return to the righting reflex at lower heart rates in the course of repeated anaesthesia in guinea pigs.

		HR at antagonization [bpm]		HR at return of righting reflex [bpm]	
Round	n	Mean	SD	Mean	SD
1	12	204.16	10.96	343.28	22.62
2	12	206.91	9.82	320.69	34.10
3	12	206.69	9.00	320.29	43.21
4	12	207.42	8.15	316.93	36.19
5	12	205.65	9.14	314.95	48.27
6	12	201.53	10.22	312.45	30.11

Return to the righting reflex at lower heart rate (HR) during the wake-up phase in the course of 6 rounds of repeated medetomidine-midazolam-fentanyl anaesthesia in 12 male guinea pigs. SD = standard deviation.

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2.5 Body weight

At the beginning of this study the GPs had a mean BW of 614±36.6g. With repeated MMF anaesthesia, the GPs gained on average 53.7 g from round 1 to 6 with an increase per round of between 7.1 and 13.8 g. The weight increase during repeated Iso anaesthesia from round 1 to 6 was 54.9 g and the GPs gained between 7.2 and 14.7 g per round. Significant BW variations between the rounds were not seen.

2.6 Clinical observations

Observations describe subjective and qualitative descriptions of anaesthesia-related behaviours. Duration and quantitative statements about the observed behaviours must be confirmed in further ethogram studies. During the induction phases with MMF, the GPs did not exhibit excitation behaviours if they could fall asleep without disturbance (noise, touch).

In the course of the repeated anaesthesias, the GPs developed increasingly strong defensive reactions against being caught and the neck injections, which they expressed through pronounced flight behaviour, blunt ruffled fur and loud squealing when caught. During Iso anaesthesia, shortly after the transfer to the nose cone, all GPs displayed reddened sclerae and multiple GPs developed a loud and pounding heart beat for 5–7.5 min. The return of RR was preceded by chewing motions and partially by teeth grinding sounds. Directly after the return of the RR, several GPs dragged their hind feet behind them for about 20 sec, although they were able to walk with their front feet. Within a few minutes thereafter, they also showed cleaning motions on the head, face and the anogenital region. Thereafter, during the recovery phase, they sat still, shivered and squinted their eyes before finally returning to their normal behaviour.

Discussion

In this study, we anaesthetized 12 male GPs with implanted radio-telemetry devices repeatedly with MMF and Iso to assess whether repeated anaesthesia altered the duration of anaesthesia phases, the physiological parameters (MAP, HR, BT, ReR, BG), BW increase and the behaviour during the anaesthesia. In general, in our single and in our repeated anaesthesia study, the times for anaesthesia phase duration and the preanaesthetic parameter values were comparable, therefore those values can be considered as reliable reference values.

Table 9. Parameter values over 6 rounds of repeated isoflurane anaesthesia in guinea pigs.

		Preanaesthetic baseline		Induction		Non-surgical tolerance		Surgical tolerance		Wake-up		Recovery	
Parameter	Round	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value	Adj. mean (mean diff)	p value
SAP [mmHg]	1	67.2		101.0		45.7		17.6		40.2		72.9	
	2	*69.0 (1.8)*	*0.0782*	100.7 (-0.3)	0.8938	*39.9 (-5.7)*	*0.0910*	18.1 (0.5)	0.7114	**45.4 (5.2)**	**0.0113**	73.1 (0.2)	0.8556
	3	**71.2 (4.0)**	**0.0026**	99.7 (-1.3)	0.6672	45.9 (0.2)	0.9650	20.0 (2.3)	0.2111	*44.8 (4.6)*	*0.0978*	72.8 (-0.1)	0.9099
	4	**70.3 (3.1)**	**0.0299**	102.4 (1.4)	0.6713	48.9 (3.2)	0.5017	19.8 (2.2)	0.3310	**47.2 (7.1)**	**0.0357**	74.7 (1.8)	0.1921
	5	*69.8 (2.7)*	*0.0749*	101.7 (0.6)	0.8571	44.3 (-1.4)	0.7790	17.5 (-0.1)	0.9590	43.5 (3.3)	0.3726	73.7 (0.8)	0.5872
	6	**71.7 (4.5)**	**0.0043**	103.9 (2.8)	0.4357	45.1 (-0.6)	0.9144	20.3 (2.7)	0.3278	**49.2 (9.0)**	**0.0290**	75.0 (2.1)	0.2122
DAP [mmHg]	1	49.5		74.0		33.8		13.3		30.1		53.3	
	2	50.5 (1.0)	0.1319	73.7 (-0.3)	0.8491	*29.7 (-4.1)*	*0.0890*	13.9 (0.6)	0.6257	**34.0 (3.9)**	**0.0204**	53.7 (0.4)	0.5472
	3	**51.9 (2.4)**	**0.0037**	73.2 (-0.8)	0.7147	34.1 (0.3)	0.9284	15.2 (1.9)	0.2589	33.4 (3.2)	0.1516	53.5 (0.2)	0.8011
	4	**51.4 (1.9)**	**0.0311**	74.5 (0.6)	0.8060	35.9 (2.1)	0.5274	15.2 (1.9)	0.3402	*34.9 (4.8)*	*0.0743*	*54.9 (1.6)*	*0.0902*
	5	*51.2 (1.7)*	*0.0553*	74.4 (0.5)	0.8523	32.2 (-1.6)	0.6551	12.9 (-0.4)	0.8636	31.8 (1.7)	0.5742	54.3 (1.0)	0.3308
	6	**52.3 (2.8)**	**0.0031**	75.6 (1.7)	0.5102	33.0 (-0.8)	0.8184	15.2 (2.0)	0.4163	*36.0 (5.9)*	*0.0693*	*55.2 (1.9)*	*0.0899*
MAP [mmHg]	1	57.4		86.5		39.8		15.5		35.2		62.5	
	2	**59.4 (2.0)**	**0.0162**	86.3 (-0.2)	0.9212	*34.8 (-4.9)*	*0.0866*	16.0 (0.5)	0.7199	**39.7 (4.5)**	**0.0145**	62.7 (0.2)	0.8081
	3	**60.7 (3.3)**	**0.0019**	85.5 (-1.0)	0.6923	40.0 (0.2)	0.9579	17.6 (2.0)	0.2570	39.0 (3.8)	0.1253	62.4 (-0.0)	0.9674
	4	**59.9 (2.5)**	**0.0243**	87.5 (1.0)	0.7200	42.3 (2.6)	0.5209	17.4 (1.9)	0.3740	*41.0 (5.8)*	*0.0532*	64.0 (1.5)	0.1733
	5	*59.6 (2.2)*	*0.0583*	87.1 (0.6)	0.8398	38.1 (-1.6)	0.7012	15.2 (-0.3)	0.8872	37.6 (2.4)	0.4716	63.2 (0.8)	0.5342
	6	**61.1 (3.6)**	**0.0027**	88.8 (2.3)	0.4468	39.0 (-0.8)	0.8581	17.8 (2.3)	0.3761	**42.5 (7.4)**	**0.0444**	64.2 (1.8)	0.1904
HR [bpm]	1	226.3		280.1		272.6		238.5		274.1		262.4	
	2	224.0 (-2.3)	0.4939	**296.5 (16.4)**	**0.0023**	275.8 (3.2)	0.5447	239.4 (1.0)	0.8030	278.1 (4.0)	0.3884	261.2 (-1.1)	0.7117
	3	227.7 (1.4)	0.7117	**304.0 (23.9)**	**0.0009**	*285.0 (12.4)*	*0.0623*	244.2 (5.7)	0.2568	279.4 (5.3)	0.3466	259.2 (-3.2)	0.4214
	4	223.3 (-3.0)	0.4532	**300.2 (20.1)**	**0.0134**	**293.9 (21.3)**	**0.0043**	**251.6 (13.1)**	**0.0259**	*284.2 (10.1)*	*0.0972*	261.0 (-1.3)	0.7615
	5	219.7 (-6.6)	0.1000	**299.8 (19.7)**	**0.0260**	**288.8 (16.3)**	**0.0345**	247.4 (8.9)	0.1555	277.6 (3.5)	0.5717	*254.4 (-7.9)*	*0.0932*
	6	224.1 (-2.2)	0.5869	*296.5 (16.5)*	*0.0791*	**289.4 (16.8)**	**0.0326**	**251.6 (13.1)**	**0.0486**	*284.8 (10.7)*	*0.0913*	*253.2 (-9.2)*	*0.0609*
BT [°C]	1	38.8		38.8		38.7		37.4		36.6		38.7	
	2	38.8 (0.0)	0.6463	38.8 (-0.1)	0.5015	38.6 (-0.1)	0.1889	**37.2 (-0.2)**	**0.0477**	36.4 (-0.1)	0.2355	38.6 (-0.0)	0.6799
	3	38.9 (0.0)	0.6006	39.0 (0.1)	0.1281	38.8 (0.1)	0.1237	37.5 (0.1)	0.4091	36.6 (-0.0)	0.9503	38.6 (-0.1)	0.2810
	4	38.9 (0.1)	0.4234	38.9 (0.1)	0.5210	38.8 (0.1)	0.4946	37.4 (-0.0)	0.9471	36.6 (-0.0)	0.9875	38.7 (0.0)	0.7558
	5	38.9 (0.1)	0.5154	38.9 (0.1)	0.2968	38.7 (0.0)	0.6866	37.3 (-0.0)	0.6894	36.5 (-0.0)	0.7455	38.7 (0.0)	0.6377
	6	38.8 (0.0)	0.6577	38.9 (0.1)	0.3409	38.7 (0.0)	0.6648	37.4 (0.0)	0.7077	36.7 (0.1)	0.4491	38.7 (0.1)	0.3021

Adjusted mean with mean differences and p values over 6 rounds of repeated isoflurane anaesthesia in 12 male guinea pigs. **Bold** = p value $\leq$ 0.05, *italic* = 0.05 < p value $\leq$ 0.10. SAP = systolic arterial pressure, DAP = diastolic arterial pressure.

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3.1 Effects on anaesthesia duration

Repeating the MMF and Iso anaesthesia caused only mild effects on the anaesthesia duration and the short time differences between the rounds (induction, non-surgical tolerance) can be considered clinically irrelevant (Fig 5).

The observation that MMF anaesthesia required a longer time than Iso until the surgical tolerance was reached (in GPs +3.7 min), was also described in rats [4]. The time differences probably originated from the different routes of application, as an inhaled anaesthetic provides a faster onset of action compared to an i.m. injection [10]. With Iso, the anaesthesia depth also strongly depends on the applied concentration. As this needs to be adjusted individually based on the ReR, the time until surgical tolerance is achieved, can also differ individually.

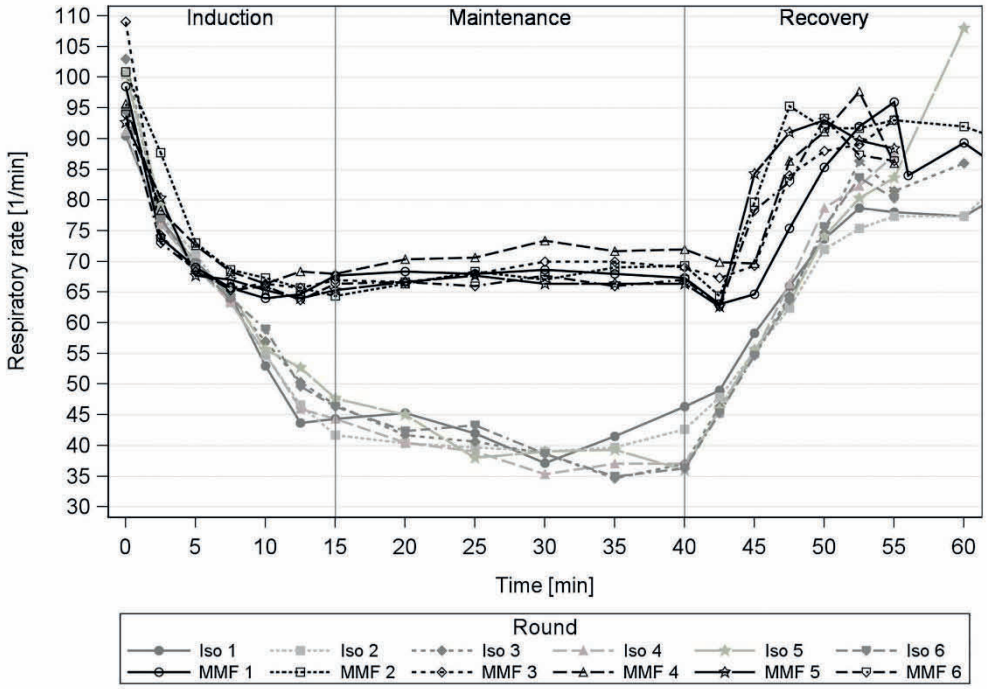

Fig 4. Respiratory rate course during repeated anaesthesia in guinea pigs. Repeated anaesthesias (6 times with an interval of 3 or 4 days) with isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) in 12 male guinea pigs.

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Table 10. Variations of blood glucose values during repeated medetomidine-midazolam-fentanyl anaesthesia in guinea pigs.

Time [min]	Round	Adjusted mean	Mean diff.	p value
MMF 20	1	8.70		
	2	**10.89**	**2.19**	**0.0018**
	3	9.16	0.46	0.5859
	4	**10.96**	**2.26**	**0.0172**
	5	8.75	0.05	0.9588
	6	**10.73**	**2.02**	**0.0451**
MMF 40	1	12.40		
	2	**15.12**	**2.72**	**0.0025**
	3	14.22	1.82	0.1062
	4	**15.93**	**3.53**	**0.0064**
	5	13.21	0.81	0.5501
	6	**16.43**	**4.03**	**0.0051**

Increase of blood glucose values [mmol/L] in round 2, 4 and 6 at 20 and 40 min during 6 repeated medetomidine-midazolam-fentanyl (MMF) anaesthesias in 12 male guinea pigs. Results based on the ANOVA for repeated measurements—comparison versus round 1. **Bold** = p value $\leq$ 0.05, *italic* = 0.05 < p value $\leq$ 0.10.

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Fig 5. Anaesthesia duration in 6 repeated anaesthesias with Iso and MMF male guinea pigs. Duration of anaesthesia intervals [min] in 6 repeated anaesthesias with isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) in 12 male guinea pigs.

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Before the study, we speculated that repeated i.m. MMF injections might result in a tissue change accompanied by a worsening resorption, but, as the non-surgical tolerance with MMF was not prolonged with multiple use, that suspicion was not confirmed.

The shortening of the wake-up phase is the most significant effect that occurred in the course of the MMF repetitions. This can be considered a beneficial development, as it enables a faster return to self-regulation. The time until the MAP low point after the antagonization was not shortened, but thereafter, the GPs returned to their RR at lower MAP and HR values. (Table 5). This effect is presumably due to a faster metabolism, since MMF and AFN are metabolized in the liver [11] and can therefore accumulate and induce a pharmacological tolerance. Such an effect is not expected from Iso, since it is only minimally metabolized (0.17%).

This number and interval of anaesthesia repetition did not lead to a strong deviation from the usual anaesthesia phase durations with MMF or with Iso. Since the anaesthetics themselves

lead to short induction and wake-up phases, both anaesthetics may be used repeatedly concerning the anaesthesia durations.

3.2 Effects on mean arterial blood pressure, heart rate and core body temperature

MMF. Repeating MMF anaesthesia affected the MAP in the surgical tolerance phase and the MAP and the HR in the wake-up phase. The absolute reduction, however, was rather small and of little clinical relevance. Overall, the GPs exhibited only a mild hypotension during MMF anaesthesia, which is desirable, concerning their relatively low physiological MAP (57 mmHg) [3]. The individually different BP values occurred independently of the number of anaesthesia repetitions and an adverse influence of a high or low BP course could not be determined for the individual. A group-consistent BP cannot be expected during the MMF anaesthesia in the GP (Fig 3). The BP effects are likely caused by the component medetomidine, which induces a peripheral vasoconstriction through α_{2B}-adrenoceptors [12] in favour of a high central aortic blood pressure The large inter-individual range of BP during MMF maintenance could originate from differing densities of α_{2B}-adrenoceptors in the peripheral vessels. GPs with a low MAP during maintenance (animal 6 Fig 3) might exhibit a larger number of receptors, such that the dosage of 0.2mg/kg medetomidine was not sufficient to block all receptors. Alternatively, the peripheral vessels in those animals with lower MAP may be less vasoconstrictive than in other individuals, which could be tested by MAP measurements in peripheral vessels during MMF anaesthesia.

The MAP reduction in the surgical tolerance phase (Table 7, Fig 6) and in the wake-up phase after round 1 of repeated MMF anaesthesia is likely caused by a tolerance effect.

For the surgical tolerance phase, this might have been achieved through a down regulation of receptor density or a weakening of signal transduction. Our results showed that the MAP minimal pressure values in the wake-up phase of approximately 23±6 mmHg (averaged over all rounds, Table 6) occur independently of the anaesthesia repetitions. After the low point in arterial pressure, the GPs returned to the RR with increasing anaesthesias at lower MAP and HR values (Tables 6 and 8). Apparently, MMF anaesthesia and AFN antagonization repetition intervals of 3 or 4 days pharmacologically affected the regulation of the RR, possibly through a faster biotransformation. Both medetomidine and atipamezole are metabolized by hydroxylation through the enzyme cytochrome P450 [13] and the repetitive administration may have caused this enzyme complex to be up-regulated. This hypothesis is supported by the comparatively large reduction step from round 1 to 2 and smaller effects with further rounds.

Iso. Overall, the repetitions of Iso anaesthesia caused only minor and clinically negligible changes to the MAP, HR and BT of the GPs. The MAP and HR values after premedication with atropine were slightly higher from the 2nd Iso repetition on, compared to the 1st anaesthesia (Figs 6 and 7). This was also visible during Iso anaesthesia maintenance for HR. Additionally, the HR values significantly increased in the induction ($\geq$ round 2, Fig 7), the non-surgical tolerance ($\geq$ round 3) and in the surgical tolerance phase ($\geq$ round 4) with growing repetitions (Fig 7), which suggests a faster metabolism of atropine with repeated atropine applications.

It is possible that either a small residual atropine volume remained in the local fat tissue, thus increasing the dose threshold for a visible effect. There may also be an increase in the muscarinic receptor density. In comparison, rats do not require atropine premedication prior to Iso anaesthesia and showed a HR decrease in the course of the anaesthesias [4]. On the basis of the results in rats, the effect of the repeated atropine application may mask the influence of repeated Iso anaesthesia in the GP. However, omitting atropine is not recommended in the

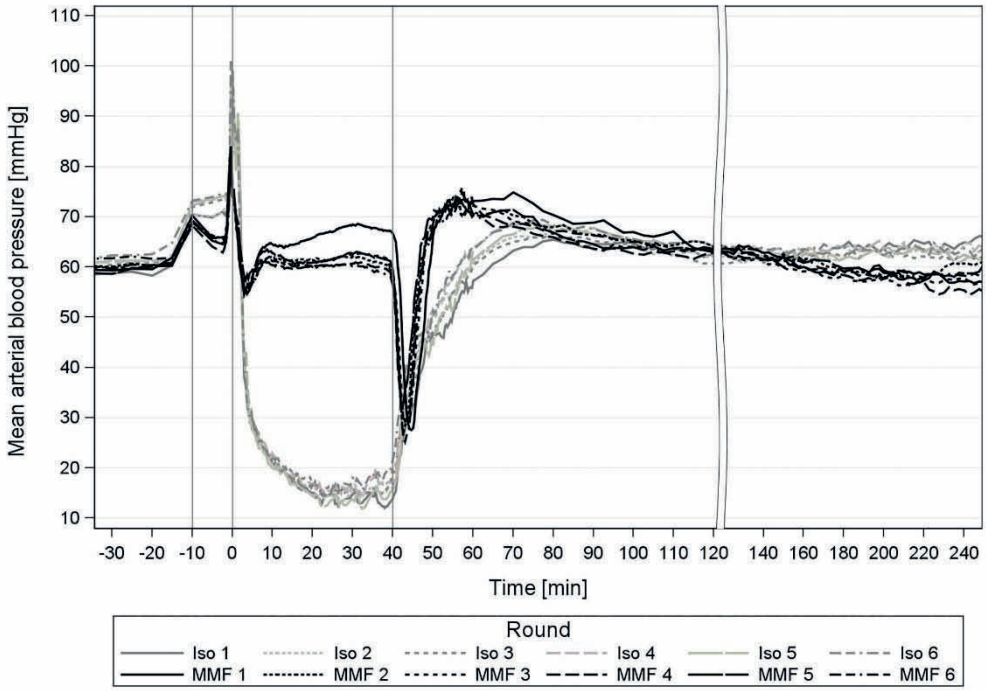

Fig 6. MAP during 6 times repeated anaesthesia with Iso and MMF in guinea pigs. MAP = Mean arterial blood pressure [mmHg]; isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) anaesthesia in 12 male guinea pigs; 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ antagonization at 40 min.

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GP because of the strong mucus production in the airways. Overall, the HR increase by up to 24 bpm (Table 9) is not disadvantageous for the GPs.

Although the repetitions of Iso anaesthesia did not show any significant influence on the cardiovascular parameters, the strong effects on BP occurred nevertheless. The combination of a very low BP, an only slightly increased HR and a highly reduced ReR and during Iso exposure, creates a serious risk for inadequate peripheral tissue oxygenation. In this context, the hypotension during Iso anaesthesia was suggested to be the cause for hearing loss in GPs [14]. Other tissues like the brain, may suffer equally from insufficient oxygen supply. The Iso anaesthesia duration after which an oxygen deficiency may lead to tissue damage in the GP, should be examined in further studies. The probability of this rises, however, with increasing anaesthesia duration, which is why a prolonged Iso anaesthesia (> 1 h) is not recommended.

Just as with the cardiovascular parameters, the temperature loss remained unaffected during the repetitions. However, as noticed with the single anaesthesia study, the BT decreased faster and more marked with Iso anaesthesia compared to MMF (Fig 8). The peripheral vasodilatation induced by Iso likely explains that effect [14].

In comparison to the single Iso and MMF anaesthesia [3], the GPs' BT values with repeated MMF and Iso anaesthesia were higher in the surgical phase (MMF: +0.52–0.71˚C, Iso: +0.24–

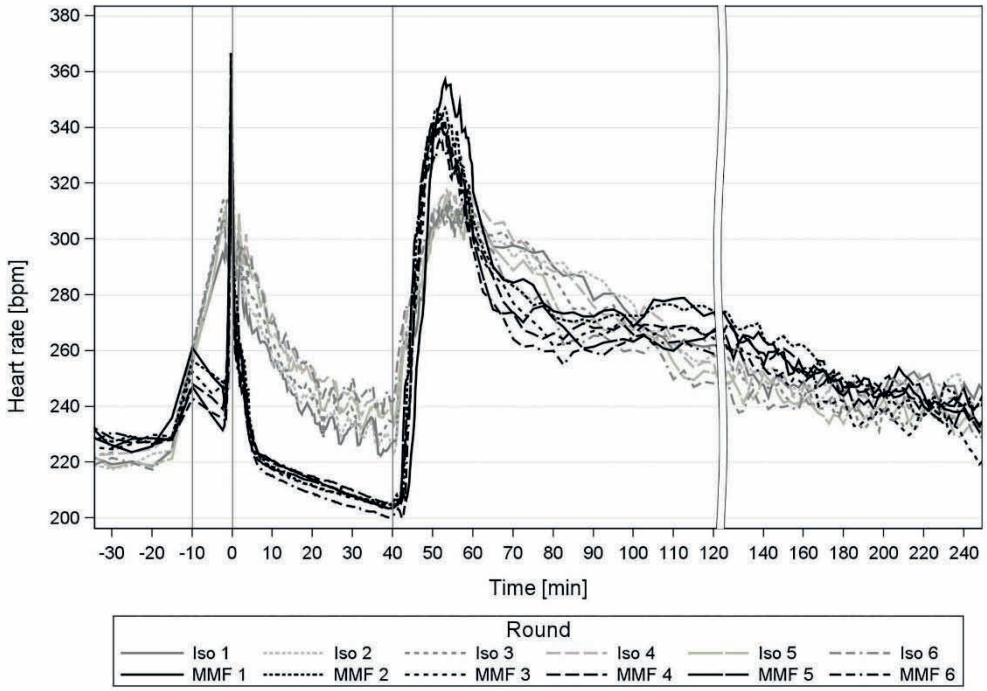

Fig 7. Heart rate during 6 times repeated anaesthesia with Iso and MMF in guinea pigs. Heart rate [bpm] during repeated isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) anaesthesia in 12 male guinea pigs; 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ antagonization at 40 min.

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0.48˚C) and in the wake-up phase throughout all rounds (MMF: 0.66–0.99˚C, Iso: 0.7–0.87˚C). These differences are probably caused by the animals' weight differences, such that comparatively heavier GPs (+97–145 g) were anaesthetized in this repeated anaesthesia study. As the GPs are no longer thermoregulatory competent after the loss of their RR, larger GPs probably retain more BT through a greater warmth storage capacity because of their more beneficial surface-to-volume-ratio.

3.3 Effects on respiratory rate

The repetitions of the anaesthesia with MMF and Iso did not lead to any relevant changes in the anaesthesia profiles, as seen with the highly uniform curves in Fig 4. There were slight differences between the rounds in the wake-up phases. These increases in ReR were associated with an earlier return to voluntary movement with MMF, (particularly pronounced in rounds 2 and 5). The Iso concentration was lowered towards the end of anaesthesia maintenance in round 1, to induce a smooth wake-up (all GPs still displayed the defined surgical tolerance reflex responses until 40 min). That caused the slightly increased ReR in round 1 and therefore this practice was discontinued in order not to falsify the ReR in subsequent rounds. However,

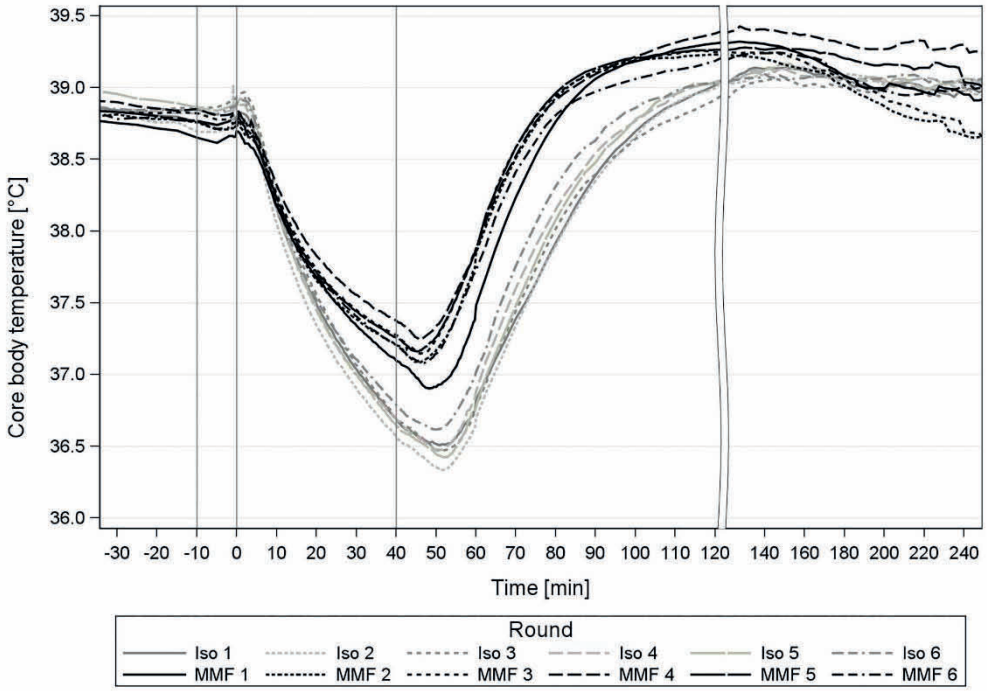

Fig 8. BT during 6 times repeated anaesthesia with Iso and MMF in guinea pigs. Core body temperature [°C] during repeated isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) anaesthesia in 12 male guinea pigs; 1st grey line ≙ premedication at -10min, 2nd line ≙ induction at 0min; 3rd line ≙ antagonization at 40 min.

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no influence of the anaesthesia repetitions on the ReR still meant pronounced respiratory depression for Iso with breathing impairment in half of the GPs and spontaneous respiratory arrests. Since intubation is challenging in GPs and entails the risk of transferring food into the lungs [15], reliable spontaneous breathing is essential during anaesthesia. Iso anaesthesia did not produce reliable breathing and must, therefore, be considered significantly more dangerous with regard to anaesthesia safety. In comparison, MMF anaesthesia only led to a slight hypoventilation and no respiratory irritation or arrest. Iso's respiratory influence may be partly compensated by careful observation throughout the anaesthesia, but the universal use of Iso is limited due to the high monitoring effort.

3.4 Effects on blood glucose

We expected a BG increase with MMF anaesthesia on the basis of the single administration study results [3]. However, the repetition of MMF anaesthesias yielded significantly higher values at 20 and 40 min in the rounds 2, 4 and 6 (Fig 9). Medetomidine is the BG influencing component in MMF, inhibits the insulin secretion in the β-cells in the pancreas and induces a higher hepatic glucose production and secretion.

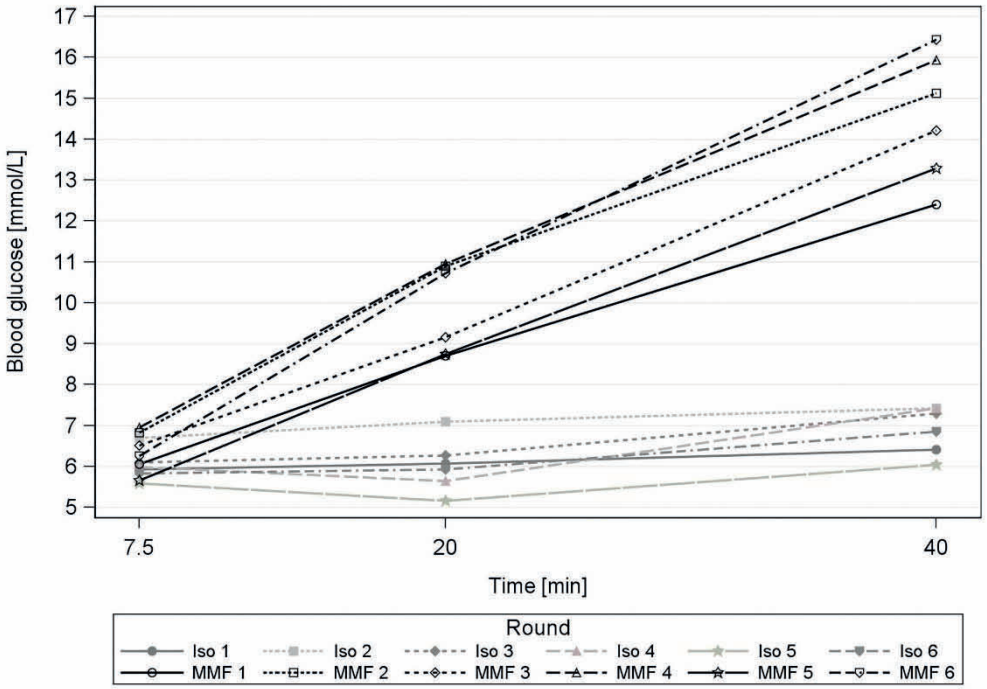

Fig 9. Blood glucose course during 6 times repeated anaesthesia with Iso and MMF in guinea pigs. Blood samples were taken at 7.5, 20 and 40 min during anaesthesia with isoflurane (Iso) and medetomidine-midazolam-fentanyl (MMF) in 12 male guinea pigs.

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Our study design dictated 2 days between rounds 1 and 2 but 3 days between rounds 2 and 3.

Apparently, with a MMF anaesthesia interim of only 2 days, there was still a remaining influence of the last medetomidine injection present, which together with the renewed dose, resulted in the increased BG values.

Since all anaesthesias share the same close value range at 7.5 min, that also matches the lower reference range (4.95–15.95mmol/L, [16]), those values can also be considered reference values for the preanaesthetic period. Admittedly, the BG development after the anaesthesia would have been interesting, however the blood sampling on the ear was attempted in awake training animals and they reacted with a high stress response that created stress artefacts in the hemodynamic parameters. Therefore, awake BG testing was omitted.

3.5 Effects on body weight

An influence of repeated anaesthesia on the BW was not seen, as the total and the BW increase between the rounds was similar in MMF and Iso. GPs without the anaesthesias might possibly have gained weight more rapidly, but that was not addressed in this study. As regular weighing

enables a fast and cheap option to evaluate the general health, especially in rodents, we strongly advise the inclusion of BW assessment into the standard protocol for repeated procedures.

3.6 Effects on the behaviour

In the course of the study, all GPs grew progressively more defensive to being captured. This could have been amplified by the discomfort of repeated blood sampling from the ear. Although the ear pricking was carried out in the unconscious state, the sensation of wound pain in the waking state is, nevertheless, possible. The GPs may have linked this pain to the process of being handled for anaesthesia. An alternative would be the implantation of an implantable glucose telemetry device (DSI HD-XG®, DSI, St. Paul, MN, USA) [1] for BG relevant studies. Especially the premedications in the later rounds were accompanied with strong flight reactions, which made the safe application of the total injection volume increasingly more difficult. Thus, some of the breathing problems during the later rounds might have been the result of an insufficient atropine injection volume. The eye squinting during the recovery after Iso exposure is a transient result of Iso's mucous membrane irritation and of the hypothermia in that phase.

A substantial amount of animal stress can be reduced with MMF anaesthesia by omitting the sodium chloride premedication, which was only done for comparative purposes in this study.

Under animal welfare criteria, the use of MMF and Iso for repeated anaesthesia is not optimal. The recurrent injections into the hind limbs for the MMF anaesthesia resulted in transient, firm swelling of the muscles at the injection sites, presumably haematomas, in some defensive animals. Histological examinations of the injection sites would have been helpful to assess the extent of the local effect, but they were outside of the scope of this study. The airway and mucous membrane irritation, as well as the need for pre-medication with Iso anaesthesia, were further disadvantageous. The injection disadvantages can be influenced to a certain extent, however, the irritation by Iso cannot be avoided. The adverse effect on the animal welfare of both anaesthetics is approximately equivalent and can be considered as mild, since the effects of both anaesthetic applications were clinically completely reversible.

Conclusion

Overall, the repetitions showed only minor influences on the physiological parameters of the GPs. With MMF, the induction phase was shortened and the MAP was lower from the 2nd repetition. In addition, the BG value increase was steeper, depending on the number of days between the MMF anaesthesias. With Iso, there was a slight increase in HR due to the injection of atropine. Thus, the repetitions altered the known effects of the anaesthetics on the physiological parameters only slightly, which is why repeated applications may be carried out with MMF and with Iso. At the same time, the disadvantages of the anaesthesia profiles remained unaltered. Iso repeatedly led to severe respiratory depression, airway irritation, and sometime even apnoea. It also induced a pronounced BP loss and required atropine pre-medication, which necessitates two handlings for the induction of anaesthesia. Therefore, with the focus on anaesthesia safety and well-being for the animal, the MMF anaesthesia is preferable in the GP, due to the more advantageous anaesthesia profile.

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Formal analysis: SS.

Funding acquisition: JH.

Investigation: SS.

Methodology: SS JH.

Project administration: SS.

Resources: JH BG.

Software: SS.

Supervision: JH ST BG.

Validation: JH BG SS.

Visualization: SS.

Writing – original draft: SS.

Writing – review & editing: SS BG.

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6 General discussion

6.1 Study aim

This study's aim was to test the influence of the 3 most commonly used anaesthetics in GPs on their physiological parameters, focusing on the anaesthesia relevant parameters HR, BP, BT and ReR. With the aid of implanted blood pressure radiotelemetry devices, the anaesthesia was monitored continuously from 2 hours before anaesthesia until at least 4 h after anaesthesia induction. In the first part of the study, the effect of one-time anaesthesia with MMF, Iso or KX was investigated. The second part looked at the effect of repeatedly administered Iso and MMF, for which each GP was anaesthetised 2 times per week over 3 consecutive weeks, resulting in a total of 6 anaesthesias. The examinations of the effects were revealing and significant effects on the physiological parameters could be demonstrated.

6.2 Discussion for publication 1

This is the first publication that addresses the critical 24 h after abdominal surgery in the GP. Many findings can not only be useful for radiotelemetry implantation but may also be applied for all other types of abdominal or major surgeries in GPs.

The implantation of the blood pressure catheter provides the most robust results, but has the disadvantage that the animals must be opened abdominally. Of course, other arterial implant sites are also conceivable for measuring peripheral arterial blood pressure (67,68). Implanting the blood pressure transduce into the carotid artery, avoids opening the abdominal cavity which is associated with a faster hypothermia, a bigger incision, the need to handle the intestine and generally a longer recovery. In our case, however, this approach was not feasible. The device body would need to be positioned subcutaneously on the animals' neck because of its large size. In this case, however, the length of the blood pressure catheter of 8 cm is not sufficient to guarantee sufficient movement margin during movement and for the size growth of the animal. Maintaining the sterility throughout this implantation approach would be challenging. Furthermore, a skin thinning over the device body, implanted on the neck, has been described (67). Therefore, and because the internal working group was practiced in the abdominal implantation approach, we decided to take the abdominal approach.

Although the general surgical approach is similar to rats, performing the surgery in GPs is much more complicated. Of course, any implantation should be strictly sterile, but in reality rats forgive small deviations from this claim. GPs don't. They require strict sterile conditions for the successful BP implantation. Furthermore, a GP's abdomen remains quite barrel shaped, resulting in difficult visibility and preparation of the abdominal aorta. Thus, reliable positioning of the aortic clamps is made more difficult in GPs. For the correct positioning of the clamps, the tissue around the v. cava and a. abdominalis must be dissected, but this has to be done in a tissue sparing way, since a too brisk approach can lead to muscle and nerve irritation. A GPs intestine is more voluminous, consumes valuable working space for the abdominal aortic catheterisation and requires gentle handling. From experience, PD Dr. Henke knew that if the intestinal convolutions were little manipulated during implantation, the GPs returned to normal feeding behaviour after the procedure. To create more working space, the intestines may be lifted from the abdominal cavity. However this accelerates the hypothermia and requires consistent tissue moistening. GPs have thinner and more fragile blood vessels than rats and therefore the *v. cava* and abdominal aorta can rupture easily. This occurred in 50% of Provan, et al. (67) GPs, leading to their death. Dissecting and clamping the abdominal aorta was challenging and caused bleeding in 3 cases, but they were nonfatal. All of these differences mean that GP implantation is longer, much more complex and riskier compared to the rat.

Other groups have also published their results of abdominal BP radiotelemetry device implantation in the GP. However, they described long-term survival rates between 0 % (67) and 64 % / 74 % (68). The latter group used Iso anaesthesia for their implantations. However, they did not provide information on a possible premedication or for which reason some animals died. In our second publication, we were able to confirm the already existing findings (11) that Iso leads to a strong respiratory depression in GPs and an increased risk of apnoea. In the investigation by Heide (11), the GPs also developed rattling breathing sounds after 30 min of Iso maintenance, wherefore she recommended the use of Iso only for anaesthesias up to 40 min. Additionally, we were able to show that the blood pressure during Iso exposure remains constantly low. With this unfavourable combination of physiological influences, a sufficient supply of oxygen to the tissues, especially the brain, may not be guaranteed during long anaesthesia. Our mean anaesthesia duration was on average 131 min.

For such a long anaesthesia (on the average 131 min), in which the GPs had to be maintained under anaesthesia without narcotic emergencies, Iso was too risky and MMF injection anaesthesia was chosen.

6.2.1 Analgesia

Precise descriptions of the analgesic protocol and the use of a multimodal approach (80) (combining analgesics with complementary mechanisms of action) are rare in the work with laboratory GPs. It has long been known that pain influences almost all organ systems (81) and may thereby impact study results. If an analgesic regimen is necessary, then it must be started early on and must be maintained for an adequate duration as well as on a consistently level. The choice of Iso as the main implantation anaesthetic of the other group (68) has already been discussed above, with regard to the influence on the physiological parameters. Viewed from analgesia points, Iso has no relevant analgesic component. In addition to Iso, this group used 50 mg/kg of tramadol s.c. and a single dose of 3 mg/kg carprofen during the surgery. The postoperative pain was treated with 25 mg/kg s.c. tramadol every 24 h for 2 days. Tramadol may be used for adjunctive treatment but is not analgesic enough as a sole agent (82) and also has a very short half-life time (no pharmacological data for GPs; 1.1 h in the mouse, 3.0 - 3.9 h in the rat (83)). The applied dose of carprofen was below the recommended dose of 4-5 mg/kg BW and day (49), NSAIDs only treat pain originating from inflammation and correct dosing of carprofen is challenging in the GP because of the high drug quantities in tablets (20 mg) and solutions (50 mg/mL).

With the analgesic possibilities available for GPs, we have aimed to implement an extensive, multimodal pain regime. During the implantation, the GPs were covered with fentanyl, metamizole and meloxicam. The abdominal incision line was infiltrated with lidocaine to stop muscle contractions when the skin was incised. This also blocks pain stimuli from being forwarded to the brain and can thereby prevent long term pain sensitization (84). Meloxicam is superior to carprofen in that 0.5 and 1.5 mg/mL oral suspensions are available (Vetidata 11.2016). Metamizole has a similarly short application interval (estimated in GPs every 4-6 h) compared to tramadol, but has comparable analgesic potency to opioids, rarely provokes relevant side effects and can be applied orally with a fast onset (85). With this analgesia regime, our GPs started increasing their BW after the second day after the operation instead of after one week (68).

6.2.2 Radiotelemetry technique

This technique is considered the gold standard for the collection of physiological signals in unrestrained, conscious animals (43). By using the radiotelemetry technique, we have been able to achieve previously unknown data results, including baseline and recovery parameters in calm, unrestrained GPs. The same animal could be closely monitored during repeated anaesthesia and the observation of cumulated anaesthesia effects was possible for the first time. The high sample rate of the physiological signals (500 Hz for BP) eliminated rounding errors or large gaps from graphs which was especially useful in the anaesthesia induction and wake-up phases. The usability of the implant depends decisively on its battery performance. It was sufficiently long that we did not have to exclude a GP because of signal loss from the data evaluation from the beginning of the implantation until after the end of the anaesthesia repetitions. Similarly, Hess, et al. [70] were able to perform measurements until the end of the battery life (< 8 months). This long possibility for data collection relativizes the high effort associated with the successful implantation of BP transducer in GPs. Of course, the radiotelemetry technique also has disadvantages, such as the very high installation price and the long transitional period until an implanted GP can be used for the first time in a study. In our study, with the habituation, surgery and recovery, it took 35 days until the first anaesthesia was performed.

6.2.3 Vitamin C

As mentioned in chapter 2.3, GPs are dependent on dietary intake to cover their vitamin C supply. Their food intake, and with that vitamin C coverage, may, however, be reduced due to illness, social stress or after surgery. Deficiency symptoms include loss of energy, weakness, wound healing disorders, strong or inner bleeding, rough hair coat and reduced appetite. Normally, the food for laboratory GPs contains an adequate vitamin C amount. Recommendations suggest daily dosages between 10-30 mg/kg (86). After surgery, GPs are expected to suspend their food and water consumption for varying durations of time. We were unsure about the need for vitamin C supplementation, since the animals had no clinical signs of under-supply and there were few published data about the use of vitamin C in combination with surgery. However, because we wanted to avoid losing GPs due to vitamin C under-supply, we began daily oral supplementation with 20 mg/kg vitamin C dissolved in water, starting 7 days before the implantation until 14 days thereafter (68). The results with the supplementation of vitamin C were compared to our own experiences

during our training implantations which were performed without vitamin C supplementation. With vitamin C, we found that the skin incisions healed faster and with lesser complications and that the GPs recovered faster overall. These observations are so far just clinical observations and need to be confirmed by an appropriate study design. However, up to this point, the oral supplementation was without side effects, vitamin C is easily accessible and in-expensive and the solution is easy to administer orally. Therefore, according to our experience, we can recommend a vitamin C substitute for those procedures which might reduce the food intake in GPs.

6.2.4 Stress

The behaviour of mice and rats cannot be applied to GPs (7). When stressed, GPs will not show "usual" stress symptoms, like running around or aggressive behaviour. Instead, they will respond with inconspicuous symptoms like "freezing" in a cage corner. Although clinically invisible, their physiological parameters will be altered. Examining the changes of the physiological parameters (BP, ECG, BT) with radiotelemetry is, therefore, superior to manual measuring because this is accompanied with handling stress. To illustrate this point, the effect of short, non-painful transport on the HR and BT in a healthy awake GP are pictured in figure 11. Before 0 min, the GP was removed from its home cage, weighed, placed in a single cage with the familiar home cage shelter, put on a trolley and transported over a distance of 15 m into the measuring room. This whole process lasted approx. 5 min. When the measurements began at 0 min, the GP was sitting in its cage without moving and it also remained mostly still throughout the 2 h. The HR and BT influence were therefore indistinguishable by means of checking the GPs behaviour.

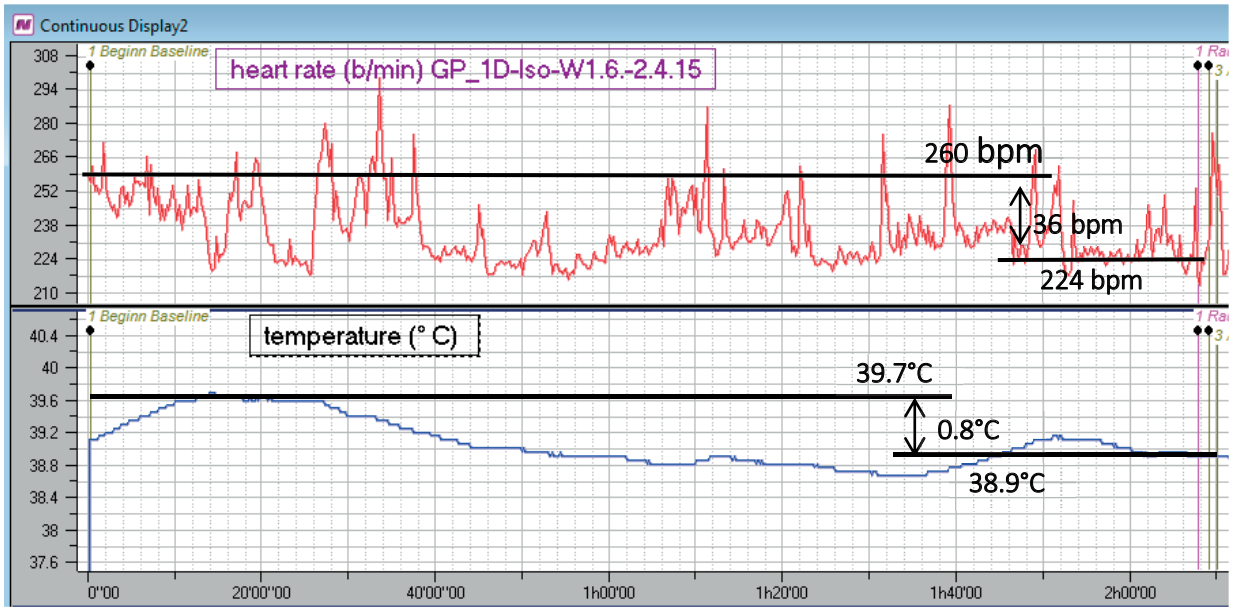

Fig 11: HR and BT directly after transport to the telemetry room (0 min) and the end of habituation time (2 h later).

This graph shows that after transport, GPs need an adaptation time to return to physiological resting values before values for use in a study can be measured. Since we could already show such physiological effects for a short, simple transport, and they were clinically invisible, we have tried in total to save the GPs as much stress as possible. Our focus was especially on a quiet environment and safe handling, performed by familiar staff. The quiet and secure handling was rewarded with lesser stress symptoms like freezing, hectic movements or loud squealing. Although this behaviour should be used in all animals, it is especially necessary in GPs, since they could otherwise suffer unseen under avoidable stress.

6.3 Discussion for publications 2 and 3

6.3.1 Baseline

In chapter 6.2.4, it has already been shown that stress (here transport stress) changes the physiology of GPs without clinically conspicuous behaviour being visible. Baseline measurements are therefore necessary to capture the possible day-to-day fluctuations. Ideally, these data sets should be acquired just before the start of the study measurements which was performed in a comparable study in rats (53). Although multiple animals were measured in the same room, induction of anaesthesia in one rat did not affect the other rats' physiological parameters. This measuring approach was not possible with GPs. Fig 12 (black frame) shows that the physiological parameters in this GP changed slightly when the measuring room was entered and anaesthesia was induced in another animal.

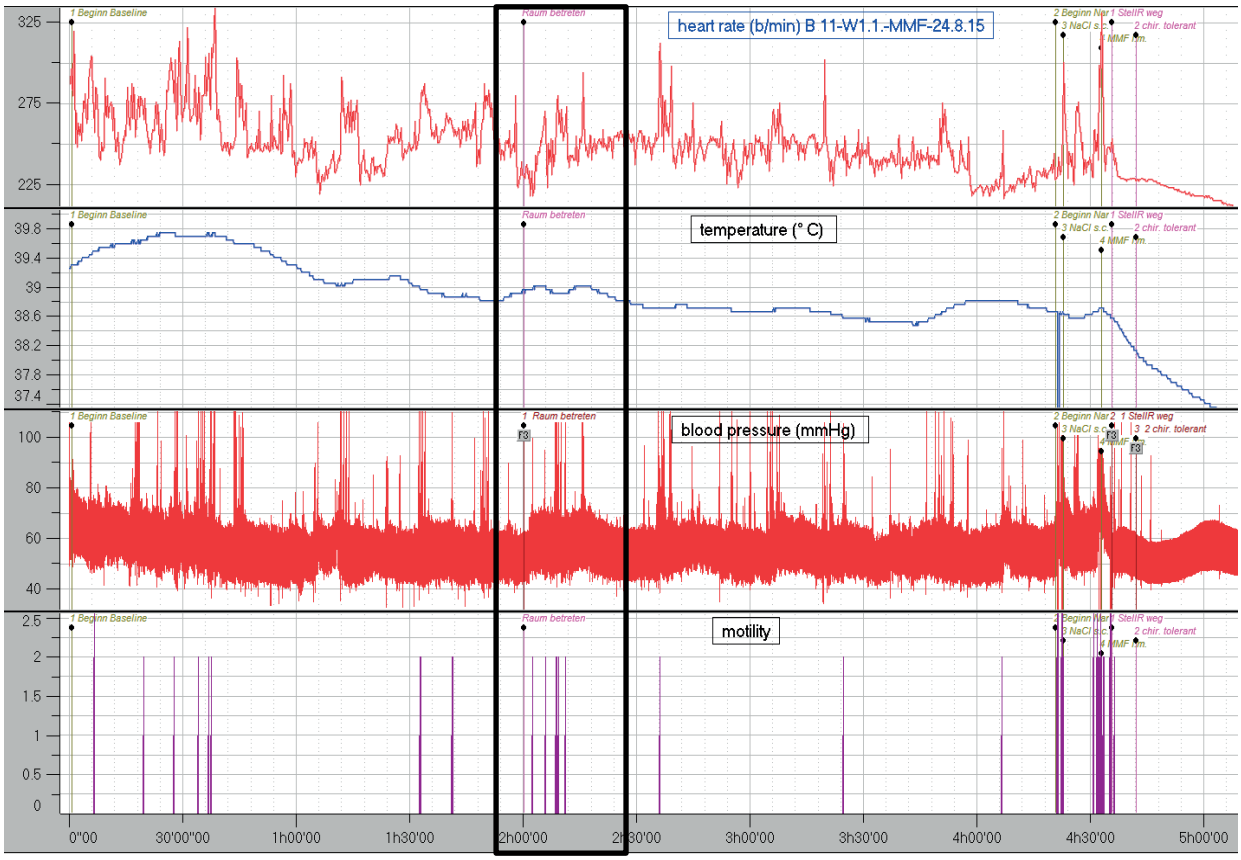

Fig 12: Impact (black frame) of the start of an anaesthesia in another animal in the same room (1st pink marker = entered the room) on the baseline values of this GP.

In addition, at the end of a 4 h acclimatisation period, the HR and the BT were slightly lower compared to the values at 2 h, as seen in the GP in Fig 12. According to this, baseline measurements just before the start of anaesthesia would not have been comparable between the GPs. Therefore, the baselines for all animals were determined prior to the first anaesthesia of the day.

The following Table 7 shows descriptive baseline values for BP, HR and BT prior to the anaesthesia with MMF, Iso or KX, averaged over 2 groups of GPs with 5 months between the groups. These values can be considered to be reference values for these parameters in the unstressed, conscious GP.

Parameter	Treatment	N	Baseline
SAP [mmHg]	MMF	11	66.9 ± 2.7
	Iso	13	66.3 ± 5.0
	KX	7	68.3 ± 3.4
DAP [mmHg]	MMF	11	48.1 ± 2.7
	Iso	13	48.4 ± 3.8
	KX	7	48.5 ± 4.7
MAP [mmHg]	MMF	11	57.6 ± 3.3
	Iso	13	56.9 ± 3.7
	KX	7	58.9 ± 3.2
HR [bpm]	MMF	11	245.8 ± 18.2
	Iso	13	247.0 ± 16.0
	KX	7	237.9 ± 11.1
BT [°C]	MMF	11	38.9 ± 0.2
	Iso	13	38.9 ± 0.4
	KX	7	39.0 ± 0.2

Table 7: Baseline values averaged over 15 min at the end of 2 h habituation in male GPs prior to anaesthesia with medetomidine-midazolam-fentanyl (MMF), isoflurane (Iso) and ketamine-xylazine (KX).

The BP is particularly interesting in the GP, because it is much lower compared to other lab animals and there are only a few measuring methods (mostly invasive ones) which provide robust results. Comparing our baseline BP values to those in the literature showed that our values were lower than those of DePasquale, et al. (72) (MAP 61-65 mmHg) and Hess, et al. (68) (MAP 63-68 mmHg) although their values were also measured by means of BP radiotelemetry. However, both publications did not describe after which time the baseline measurements were collected, and what kind of handling was carried out beforehand with the GPs. The higher baseline results in the other

two publications could, therefore, be due to the lack of an adequate habituation. These differences in the baselines may not appear to be large, but count when put in relation to the physiological BP level of GPs.

6.3.2 Medetomidine-midazolam-fentanyl

Publication 2 (78) investigated the impact of MMF anaesthesia on physiological parameters in the GP for the first time by means of radiotelemetry. The advantages of this measuring technique, already described in detail in chapter 2, make it the gold standard with presumably undistorted values. Prior to this study, there was no published information on BP, BG or the BT throughout all phases of MMF anaesthesia. The results of this study suggest that among the available anaesthesia options in the GP, MMF provides the most advantages in the GP. In chapter 2.1 the criteria of good adjustability, reliable effect, large safety margin, quick induction and recovery, stress-free application for both animal and anaesthetist and low impact on physiological parameters (HR, BP, respiratory rate and metabolism) were introduced. Looking at these criteria, MMF provided a fast and smooth induction and recovery and the anaesthesia could be terminated at any time or maintained by additional injections, as performed for the extension of anaesthesia for radiotelemetry transmitter implantation (77) (Fig 13). In an emergency the wake-up can be sped up by administering the antagonist i.m. or even i.v.. Injection of the MMF combination reliably led to anaesthesia. The fact that 2 GPs in the publication 2 did not show a surgical tolerance, was presumably attributable to the lower level of experience of the anaesthetist, since in the experiments for the 3rd publication all animals showed a surgical tolerance with MMF.

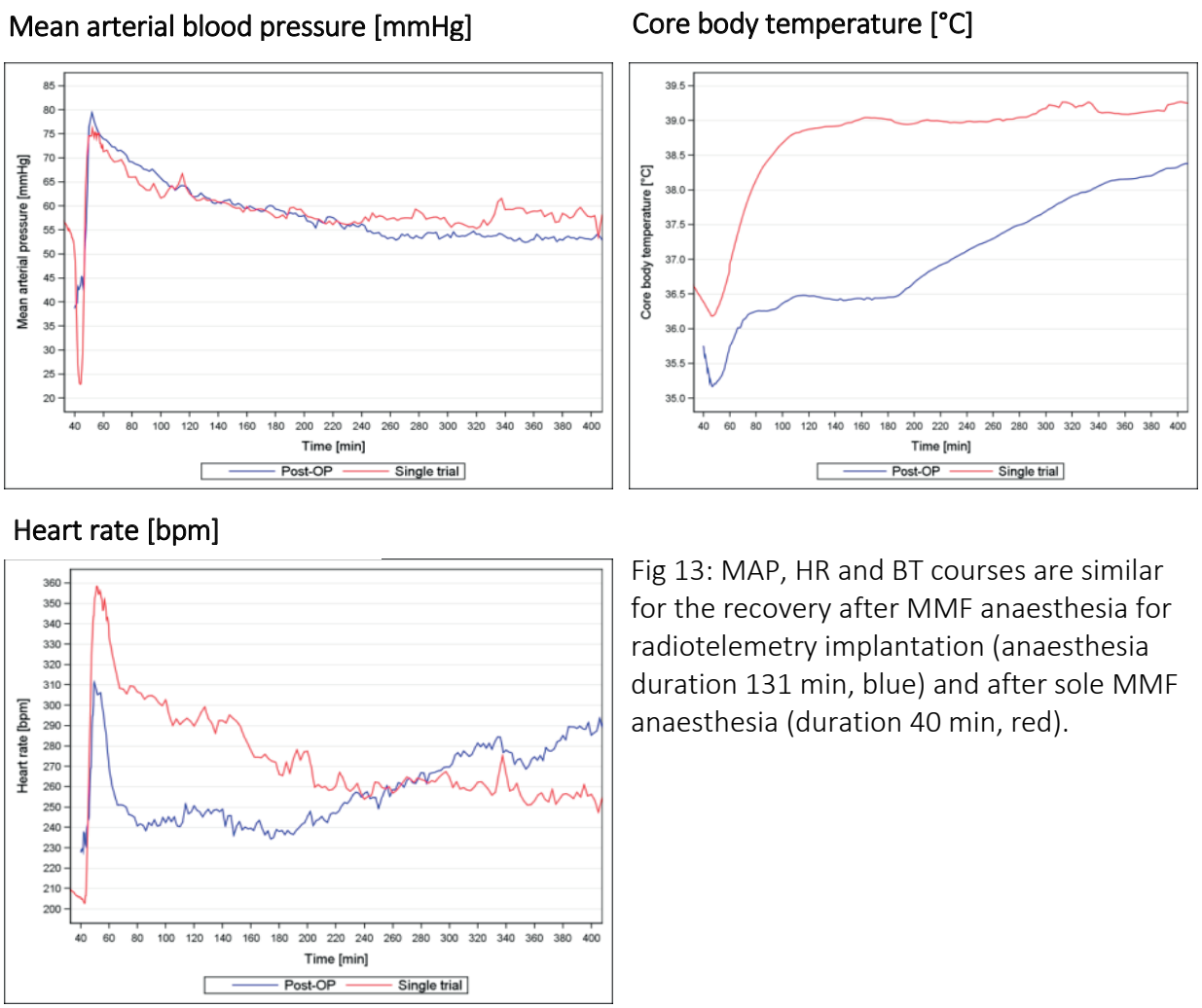

Fig 13: MAP, HR and BT courses are similar for the recovery after MMF anaesthesia for radiotelemetry implantation (anaesthesia duration 131 min, blue) and after sole MMF anaesthesia (duration 40 min, red).

In addition, the use of MMF is safe for the animal with a relatively large volume safety margin and there is no need for additional work safety measures, in comparison to working with Iso. It does not require expensive equipment and transporting the anaesthetised GP is comparatively easy, even though heat and oxygen supply must still be guaranteed. The induction of anaesthesia is comparably stress-reduced as MMF anaesthesia does not require premedication and the GPs gradually drift into unconsciousness. Furthermore, MMF anaesthesia caused the least adverse variations in the anaesthesia safety relevant parameters ReR, HR and BP. On the contrary, among the three tested agents, MMF was the anaesthetic that kept the BP closest to the physiological levels during anaesthesia. This also ensured a significantly better tissue oxygen supply compared to the low BP during Iso anaesthesia. Due to the strong reflex responses caused by MMF, the anaesthesia depth can be determined more clearly than with the slowed reflex responses with Iso. The provided analgesia which may be maintained for the recovery after painful procedures by partial antagonisation, is a unique advantage over KX and Iso.

Of course, the MMF anaesthesia also has its weaknesses. The one with the greatest impact for the GP is the occurring hypothermia. In MMF, however, this is only caused by the elimination of the central thermoregulatory capacity and not by the loss of temperature by peripheral vasodilatation, as with Iso. Although MMF induced the largest increase in BG, the values during anaesthesia were not considered to be dangerous for the animal. The rapid return to food intake and self-regulation in the other parameters suggests that the BG will return to normal after the end of anaesthesia. However, this needs to be confirmed in further studies.

Study designs in the laboratory environment sometimes require repeated anaesthesia in the GP but they are mostly just for immobilisation purposes. Therefore, the anaesthetic selected for repeated procedures should only have a small impact on physiological parameters. MMF met this ambition, as it only induced negligible changes in the anaesthesias after the 2nd repetition which were due to faster metabolic reactions (79). It was particularly striking that the GP typical strong BP fall after the antagonisation in the recovery phase could not be shortened by the repetitions. Only the duration after the maximal MAP depression until the return of the RR was reduced by the anaesthesia repetitions. Furthermore, the large variance of the individual BP levels during the anaesthesia maintenance was conspicuous. Such individual differences have not been presented previously in the GP and occurred despite the high standardisation level. These individual responses to the same anaesthesia illustrate the importance of individual monitoring of each anaesthesia. The studies further showed that in the case of repeated anaesthesia the BG levels increased more, if there were only 2 days instead of 3 days between the anaesthesia applications. The differences in BT between the single and the repeated MMF anaesthesia are associated with the different sizes of the GPs used during the study. The GPs that were anaesthetised for the single MMF anaesthesias, were smaller and therefore lost BT more quickly (study interim of 28 d, 123 g BW difference, Fig 14 green graph).

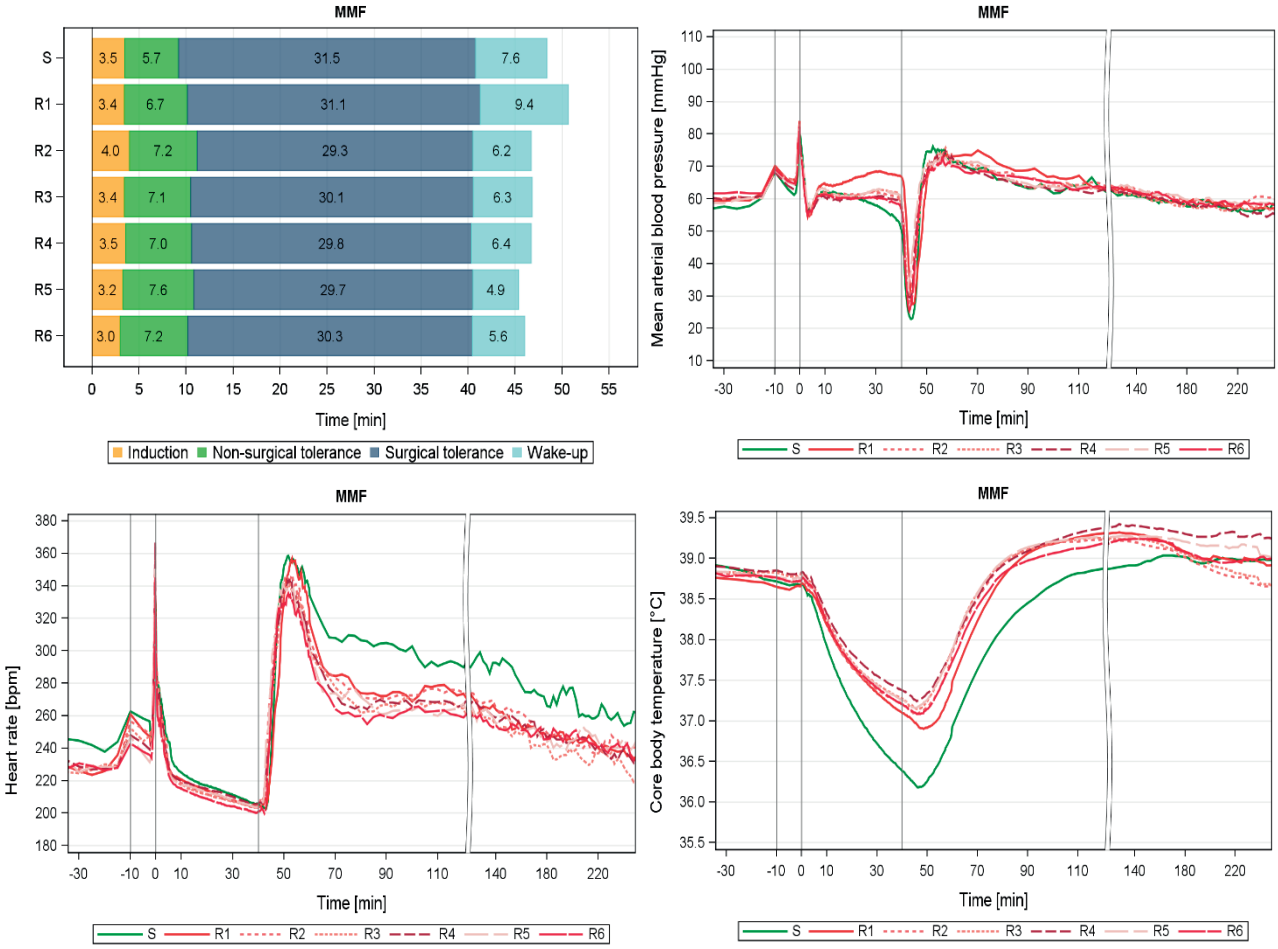

Fig 14: Comparison of the anaesthesia phase times and the MAP, HR and BT courses during single (S, green) and 6 times repeated (R, red) MMF anaesthesia in GP, grey lines: -10 min = premedication with atropine, 0 min = Iso exposure, 40 min = exposure stop.

This deficit required a longer thermoregulatory shivering in the recovery phase which was associated with an increased HR. The regular recurrence of the MMF combination also with older animals, proved the constant effect of MMF anaesthesia in GPs. The stress factor of anaesthesia plays an important role in GP anaesthesia at such short intervals. With this focus, MMF is preferable to Iso anaesthesia, since without pre-medication it only requires a one-time handling and an enables an undisturbed induction. Among the 3 investigated anaesthesias, MMF is the combination that can be recommended for all procedures. Restrictions arise only if several anaesthesias are necessary on the same day, since the antagonisation then interacts with the new MMF application.

6.3.3 Isoflurane

The effect of Iso anaesthesia on BP was never investigated previously using radiotelemetry in the GP. This technique is particularly useful for the examination of the strong BP depressing effects in the GP because of their already low physiological BP.

If one again compares the properties of Iso anaesthesia with the criteria for a good anaesthesia (chapter 2.1), Iso anaesthesia offers the quickest and easiest adjustability among the three anaesthetics. Because Iso is delivered by way of the ventilation, the GP will be anaesthetised as long as it's breathing which cannot be guaranteed for the injectable anaesthetics. The safety margin of the amount of anaesthetics, however, is very small for GPs, namely in the tenths of a % range and had to be adjusted many times during anaesthesias. Since Iso is also effective for humans, the use requires the compliance of working safety measures (use of a suction). The anaesthesia induction time was short and although the wake-up phase duration was longer than after MMF anaesthesia, the time until the GPs regained their RR was still tolerable. After Iso exposure stopped, the GPs were quickly able to adjust their physiological parameters back to normal ranges. However, the longer anaesthesia was maintained, and the older the animals become, the longer the recovery phase lasted with more frequent respiratory sounds. The induction of Iso anaesthesia is more stressful, compared to KX and MMF, as it requires a double handling due to the atropine premedication. This was mirrored by a HR increase during induction and the first part the maintenance (publication 2, p. 53, fig. 8) (11). Insertion into the whole body chamber and the irritation of the mucous membranes by the gas in the wake state also caused strong defense and excitatory reactions in the animals. As a result, they did not fall asleep as quietly as with MMF. Also the injection in the neck must function at the first, maximum at the second try, since otherwise the animals will not sit calmly on the arm anymore. This puts a performance pressure on the anaesthetist.

Compared to MMF, Iso induces massive changes in many physiological parameters. The HR and BG remain almost unchanged to the physiological level during Iso anaesthesia. However, Iso was the anaesthetic that induced the most undesirable changes during the anaesthesia maintenance concerning the BP, ReR and BT. BP and ReR dropped severely during Iso maintenance (respiratory emergency [11]) and this can only be handled for short anaesthesia because the animals return very quickly to their baseline levels after the stop of the Iso exposure.

As intubating GPs is very difficult and therefore not advisable, they need to breathe on their own during anaesthesia which is not ensured during Iso exposure. The breathing is restricted by a very low ReR and also by impaired ventilation due to mucous production which blocked the airways.

Atropine can reduce the mucous volume transiently, but even s.c. injection which provides the longest possible action duration, only reduced the mucous production for approx. 40 min of Iso anaesthesia (11). Even if a complete prevention of the mucous production could be reached (renewed or nasal (87) atropine administration, glycopyrrolate), the reduced ReR cannot be compensated by medication. Despite the exceptionally high attention to anaesthesia, resuscitation could not be prevented in some GPs during Iso exposure. Had this occurred during an invasive surgery, the resuscitation would have caused the GP to reawaken from Iso anaesthesia and the break in surgical sterility would have endangered the animal's survival chances.

The combination of a very low BP, a highly reduced ReR and only slightly increased HR during Iso maintenance creates a serious risk for inadequate peripheral tissue oxygenation. In this context, the hypotension during Iso anaesthesia was suggested to be the cause for hearing loss in GPs (88). It is questionable, if a GP's brain is sufficiently supplied with oxygen during long Iso anaesthesia. A possible neuronal damage is challenging to assess in the GP and, if present, remains mostly undetected. Therefore, Iso anaesthesia should be performed no longer than 40 min not only because of the increasing risk of respiratory impairment, but also because of the risk of tissue ischemia. Among the three anaesthetics, Iso led to the fastest and most pronounced BT drop. However, this can be counterbalanced by a good heat management and the short wake-up also enabled a quick return to an autonomous BT regulation. The rapid BT loss can therefore be dealt with, whereas the BP and respiratory depression are difficult to counteract. Additionally, the use of Iso comes with further using challenges. Transporting the animal while maintaining Iso anaesthesia requires continuous exposure to the gas and is, therefore, harder to achieve in comparison to injection anaesthesia. As Iso does not provide analgesia, procedures under Iso anaesthesia demand additional analgesia. Overall, the required level of attention and monitoring during Iso anaesthesia was considerably higher compared to MMF anaesthesia and difficult to maintain by one person during work intensive procedures. The impact of Iso is critical, but its use is tolerable for short and, above all, painless procedures like substance applications. Overall, Iso's anaesthesia profile has more negative effects on the physiological parameters in GPs in comparison to the MMF anaesthesia which is why Iso is not the first choice for GPs.

The repetition of Iso anaesthesia did not alter the profile of effects on the parameters measured (79) (Fig 15). The observed differences between the single and the repeated Iso anaesthesias are just like with MMF, associated with the different BW of the GPs.

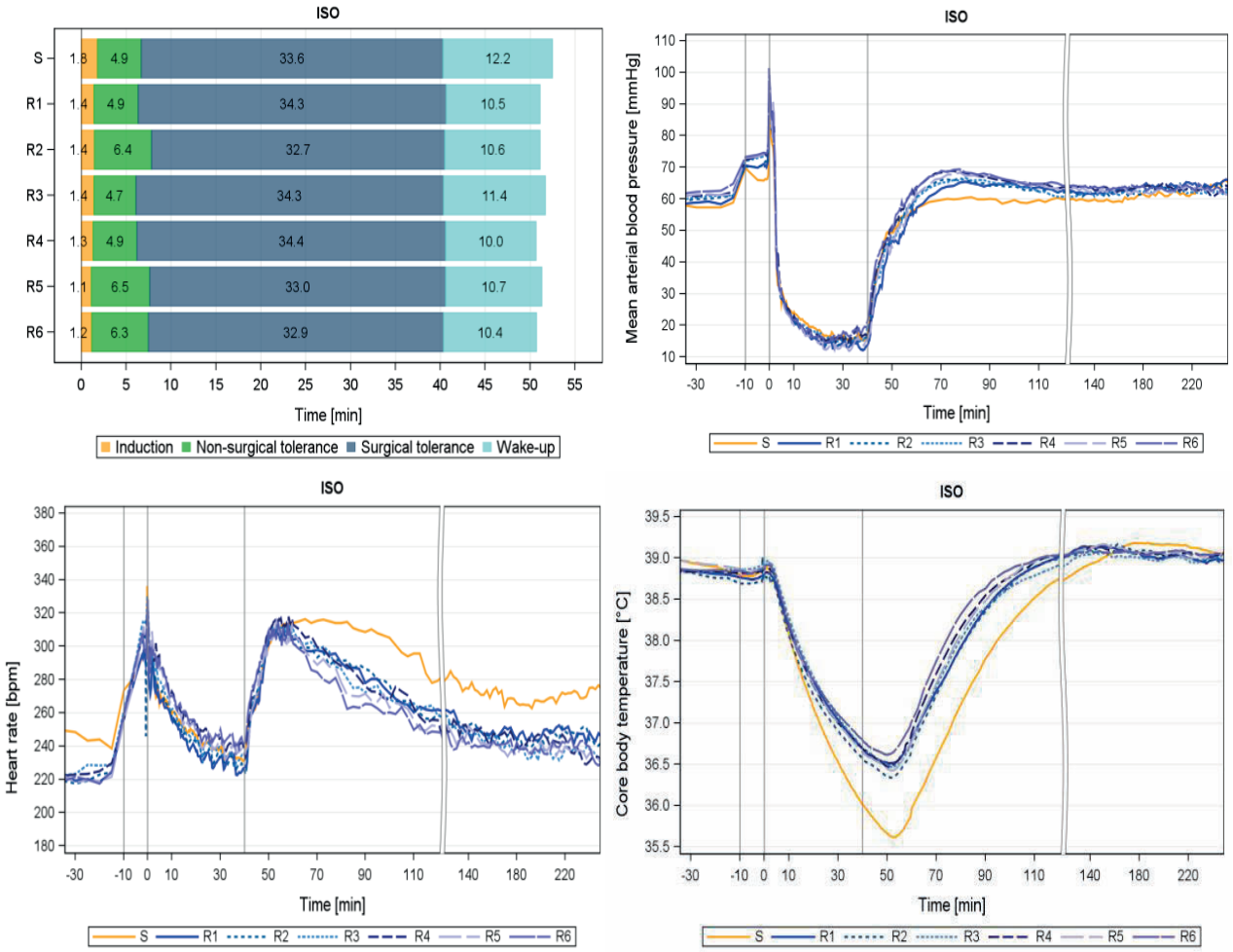

Fig 15: Comparison of the anaesthesia phase times and the MAP, HR and BT courses during single (S, orange) and 6 times repeated (R, blue) Iso anaesthesia in GP, grey lines: -10 min = premedication with atropine, 0 min = Iso exposure, 40 min = exposure stop.

A faster and more severe BT loss during single Iso anaesthesia was observed which was associated with a more intensive shivering that affected the HR in the recovery phase. The basal HR prior to the repeated anaesthesias was lower compared to the single anaesthesias which was also seen with MMF anaesthesia. Either the GPs in the repeated study had adapted to the study environment over time, or those animals in the single anaesthesia study had a higher sympathetic tone. The latter is further supported by the need of younger animals for a higher HR to compensate for their lesser cardiac output.

Despite the desirably small effects of the anaesthesia repetitions on the physiology, the adverse effects of Iso on the BP, ReR and BT still remained. In contrast to the younger GPs during the single

anaesthesia, the older and heavier animals during the repeated Iso anaesthesias became increasingly more susceptible to respiratory depression while they were exposed to Iso. Therefore, the older GPs could only be dosed for a minimally positive FWR and IG and the lower Iso concentration during anaesthesia maintenance led to correspondingly shorter recovery times. In the course of the Iso anaesthesia repetitions, the GPs showed increasingly more and stronger defensive reactions to the premedication. This increased the risk for incorrect atropine premedication and therefore for respiratory incidents. Performing repeated anaesthesia with Iso is possible but should be carefully weighed, even for short, non-painful procedures. However, for a study design requiring multiple anaesthesias per day, Iso is the only anaesthesia option because the antagonisation of the MMF anaesthesia would prevent a renewed MMF application in such a time frame.

6.3.4 Ketamine-xylazine

KX was used in GPs many times (see 2.5.9), but the measurements of the BP, HR and BT have never been performed with radiotelemetry before. KX requires with only 3 substances (KX and atipamezole) fewer components than MMF (total of 6 narcotics) does and just like with MMF the injection allows the anaesthetized animal to be moved easily. The time until the RR was lost was short and the ReR, BT and HR were only moderately affected during KX maintenance.

However, the large number of KX publications and the different component dosages reflect KX's still existing disadvantages. We had to abandon our preferred i.m. injection site in the hind limb because it led to muscle necrosis in rats (53) due to ketamine's low pH. Instead, we performed an altered i.p. injection (89) with the GPs' hind feet in contact with a solid surface (Fig 16) which led to fewer defence movements. Despite the more reliable induction of anaesthesia, only 7 out of 13 GPs reached a surgical tolerance which could have been because of failed injections into the intestine or because of different responses to the anaesthetic. However, the biggest drawback of KX anaesthesia was seen in the wake-up and recovery period. Despite partial antagonisation, the GPs were unable to right themselves for close to an hour. They developed a persistent catalepsy and lay on their backs in a crouched position without being able to turn around (publication 2, p. 55, Fig. 7). When the GPs finally returned to their RR, this state was also not stable. The GP rather fell back into a sedated state, so that a renewed turn on the back was not promptly answered with a position correction. When the FWR and IG were tested between the antagonisation and the return to the RR, the GPs responded with loud squealing and excessive reflex responses. The partial antagonisation with atipamezole produced a BP drop from which the GPs recovered only slowly. The omission of atipamezole would, however, be even more disadvantageous, since a wake-up duration of 182 min has been reported after unantagonised KX anaesthesia in rats (53). The BT did not fall as low as during MMF or Iso anaesthesia, but it decreased further after partial antagonisation and the hypothermia and a lack of food and water intake persisted long after the GPs had regained their RR. Hypothermia and hypoglycaemia have been named as the main causes for anaesthesia-related death in small mammals (9). With 2 of our GPs the BT

Fig 16: Intraperitoneal application in a GP.

dropped below 35°C and the GPs may have died without our external warming. Overall, our GPs probably survived the KX anaesthesia because none of them had a primary illness which would have raised the anaesthesia- and sedation-related risk considerably (9). Because of the uncertain anaesthesia induction and the long wake-up and recovery with disadvantageous effects on BP, BT and muscle tension development, KX anaesthesia should not be performed in the GP. MMF provides a better alternative for injection anaesthesia in the GP. However, if the combination is chosen for GP anaesthesia, close supervision is compulsory until the animal returns to normal behaviour and BT.

The disadvantageous effects of KX anaesthesia were even more severe, if the anaesthesia was repeated, something which was investigated in the rat (90). Of 6 rats, one died in the 6th repetition, in 25 % of the repetitions the animals remained surgically intolerant, 4 rats developed tissue necrosis after repeated KX i.m. injection and all animals lost BW with increasing number of KX repetitions. The results of the single KX anaesthesia made a similar course during KX repetitions in the GP very likely. Overall, there is no reason to consider performing repeated KX anaesthesia in GPs and it was therefore excluded from the repeated anaesthesia study.

7 Conclusion

GPs have been shown to be challenging anaesthesia patients. Until now, there have been no studies that have examined the effects of different anaesthesias on physiological function in the GP, as a means of understanding why they may be so difficult to anesthetise successfully. To address this, we have used an experimental approach (radiotelemetry) to provide high quality, continuous measurement of the parameters of interest. The implantation of the radiotelemetry transmitter into the GPs could be performed reliably with MMF anaesthesia and its use can serve as an example for abdominal surgeries with the GP in general. Although a large skin portion was warmed during the procedure, the GPs developed substantial hypothermia during the operation. Despite the invasive procedure, the animals were able to return their BP and BT to physiological values 8 h after the implantation and their return to BW gain was faster than in any similar study.

In the individual anaesthesia tests MMF anaesthesia caused the smallest deviations from the baseline, physiological values. Apart from the characteristic BP decrease after MMF antagonisation, the BP was stabile during anaesthesia and the short wake-up time enabled the quick compensation of hypothermia. Furthermore, MMF anaesthesia induced only a slight decrease in ReR and the BG increased during MMF anaesthesia. Iso led to short anaesthesia induction and wake-up times, but caused a strong decrease in BP and a severe ReR depression despite the premedication with atropine. During anaesthesia maintenance the Iso concentration had to be constantly adjusted and the balance between surgical tolerance and intolerable respiratory depression was difficult to maintain. The influence on the HR and the BG were negligible, but the BT dropped to the lowest values among the 3 anaesthetics. KX had a mild influence on the hemodynamic parameters during the anaesthesia maintenance, but the necessary partial antagonisation induced a BP drop which was only slowly compensated for. Substantial disadvantages of KX were seen in the very long weak-up phase, the unreliable achieving of a surgical tolerance and the long-lasting hypothermia.

The repetition of MMF and Iso anaesthesia revealed that their profiles were mainly unaltered, but the GPs developed increasingly more defense reactions against anaesthesia induction which made the induction of anaesthesia progressively more uncertain. During MMF anaesthesia maintenance, the GPs showed conspicuous BP variances. Because of the disadvantageous wake-up and recovery phase in the individual anaesthesia protocol, the effects of repeated KX anaesthesia were not tested at all. Thus, Iso and MMF can be recommended for repeated use.

Based on these results, Iso should only be used for short and non-painful anaesthesias and KX should not be used at all in GPs. MMF displayed the best overall profile and is recommended for all studies in GPs needing anaesthesia, except studies related to BG effects or those requiring multiple anaesthesias on the same day.

8 Lessons learned

Besides the investigated study results, I also gained more general learning successes, like how to approach an unknown, scientific topic and how to establish a new technique. As each person who is new to handling GPs, anaesthesia or to radiotelemetry will find themselves in a similar starting situation, I'd like to provide them with my main learning successes.

„Practice makes perfect"

Practising the whole process before the start of the study enabled me to prevent major flaws and changes while the study was already ongoing. I went through all processes before the start of my real project and applied the "Plan-Do-Check-Act" (The Deming Cycle).

"The only mistake you can make, is not asking for help" – Sandeep Jauhar

Being inexperienced in basically every field at the beginning of my project, it was essential to collect expertise. Learning from the experiences of others has spared me many mistakes and I always encountered a great willingness to share the already acquired knowledge.

Like any study, this also aimed at a maximum of standardization and reproducibility. Nevertheless, there was no possibility to completely rule out the learning effect. It may be reduced by careful preparation and practise but to some amount, learning, experience and therefore better results always occur over time. Therefore I am aware that the acquired results may be different (especially success rates for long-term survival after implantation or anaesthesia induction), when the experiments are performed by someone with another level of experience. Learning was probably the reason why only 11 of 13 GPs reached the surgical tolerance during the single MMF anaesthesia, whereas all GPs became surgically tolerant during the repeated application. However, I believe that this is a "general" reproducibility mistake which everyone encounters. Limiting it as much as possible by practicing and learning is essential but thereafter it just needs to be accepted.

9 Outlook

Further questions have emerged from the answers received and they are listed below for future research projects in the GP.

1. Atropine/Iso
   a. How long does one atropine injection in a GP last (with different dosing)?
   b. Could atropine also be administered over the nose?
   c. Can the breathing sounds be eliminated in the later phase of Iso exposure by applying an additional atropine dose?
   d. Would scopolamine or glycopyrrolate premedication provide a more beneficial effect on the breathing in the GP during Iso anaesthesia?
2. BG
   a. What are the BG values before and after MMF and KX anaesthesia in the GPs? (Maybe test them with the methods of Birck, et al.[7] or implantable BG systems by DSI)
3. KX modification
   a. Investigation of anaesthesia with S ketamine + xylazine.
   b. Investigation of anaesthesia with ketamine and dexmedetomidine.
4. MMF
   a. How long does one MMF injection induce anaesthesia?
   b. How long can the surgical tolerance/ the loss of righting be maintained?
   c. Comparison of question 4a & b in different small mammal species.
5. Does the high definition oscillometry produce reliable data in the GP?
6. ReR measured with plethysmography in the GP during different states of the animal (resting, person entering the room, animal moving, …).
7. Which reflex is lost when?
   a. Foot withdrawal reflex hind leg/ front leg;
   b. interdigital reflex hind leg/ front leg;
   c. inguinal reflex left/ right.
   d. Does the response change when the test is repeated? After which number of repetitions?

10 Enclosures

10.1 Physiological Parameters in GPs

Parameter	Measuring unit	Awake value
Mean arterial blood pressure	mmHg	53.2-62.1
Systolic arterial blood pressure	mmHg	61.3-72.0
Diastolic arterial blood pressure	mmHg	43.8-53.2
Heart rate	beats per min	219-263
Body temperature	°C	38.5-39.4

10.2 Dosage sheet for MMF anaesthesia in GPs

MMF (triple anaesthesia) in the guinea pig

Substance	Dosage [mg/kg BW]	Amount/1kg BW [mL]
Midazolam	1.0	0.2
Medetomidine	0.2	0.2
Fentanyl	0.025	0.5
Total	1.225 mg	0.9 mL

Substance	Amount /1kg BW [mL]	Amount /2kg BW [mL]	Amount /3kg BW [mL]	Amount /4kg BW [mL]	Amount /5kg BW [mL]
Midazolam	0.2	0.4	0.6	0.8	1.0
Medetomidine	0.2	0.4	0.6	0.8	1.0
Fentanyl	0.5	1.0	1.5	2.0	2.5
Total	0.9 mL	1.8 mL	2.7 mL	3.6 mL	4.5 mL

[1], BW = Body weight

Prepare the 3 substances in a sterile glass container and administer them in one syringe.
Administration route: i.m. into the thigh musculature, divide into 2 injections if the volume exceeds 0.5 mL

The following times are averaged [2]. Please re-evaluate the anaesthesia depth individually and regularly.

- Loss of righting reflex: after approx. 3.5 min
- Surgical tolerance: after approx. 10 min
- Surgical tolerance duration of minimally 40 min
- Extension of anaesthesia: + 1/3 of the initial dose.

1. Erhardt, W., J. Henke, and J. Haberstroh, *Anästhesie und Analgesie beim Klein-und Heimtier sowie bei Vögeln, Reptilien, Amphibien und Fischen*. 2004: Schattauer Verlag.
2. Schmitz, S., et al., *Comparison of Physiological Parameters and Anaesthesia Specific Observations during Isoflurane, Ketamine-Xylazine or Medetomidine-Midazolam-Fentanyl Anaesthesia in Male Guinea Pigs*. PLoS ONE, 2016. **11**(9): p. e0161258.

Body weight [g]	Dosage volume [mL]
250	0.23
275	0.25
300	**0.27**
325	0.29
350	0.32
375	0.34
400	**0.36**
425	0.38
450	0.41
475	0.43
500	**0.45**
525	0.47
550	0.5
575	0.52
600	**0.54**
625	0.56
650	0.59
675	0.6
700	**0.63**
725	0.65
750	0.68
775	0.7
800	**0.72**
825	0.74
850	0.77
875	0.79
900	**0.81**
925	0.83
950	0.86
975	0.88
1000	**0.9**

10.3 Dosage sheet for MMF antagonisation with AFN in GPs

AFN (triple anaesthesia antagonist) for guinea pigs

Substance	Dosage [mg/kg BW]	Amount /1kg BW [mL]
Atipamezol	1.0	0.2
Flumazenil	0.1	1.0
Naloxone	0.03	0.075
Total	1.13 mg	1.275 mL

Substance	Amount /1kg BW [mL]	Amount /2kg BW [mL]	Amount /3kg BW [mL]	Amount /4kg BW [mL]	Amount /5kg BW [mL]
Atipamezol	0.2	0.4	0.6	0.8	1.0
Flumazenil	1.0	2.0	3.0	4.0	5.0
Naloxone	0.075	0.15	0.225	0.3	0.375
Total	1.275 mL	2.55 mL	3.825 mL	5.1 mL	6.375 mL

[1], BW= Body weight

Prepare the 3 substances in a sterile glass container and administer them together.
Administration route: subcutaneous injection in a skin fold (e.g. the axillary region).

In average [2], the guinea pigs will return to their righting reflex after approx. 7.6 min after antagonization.
Please re-evaluate the anaesthesia depth individually and regularly.

Body weight [g]	Dosage VAA [mL]
250	0.32
275	0.35
300	**0.38**
325	0.41
350	0.45
375	0.48
400	**0.51**
425	0.54
450	0.57
475	0.61
500	**0.64**
525	0.67
550	0.7
575	0.73
600	**0.77**
625	0.8
650	0.83
675	0.86
700	**0.89**
725	0.92
750	0.96
775	0.99
800	**1.02**
825	1.05
850	1.08
875	1.12
900	**1.15**
925	1.18
950	1.21
975	1.24
1000	**1.28**

1. Erhardt, W., J. Henke, and J. Haberstroh, *Anästhesie und Analgesie beim Klein-und Heimtier sowie bei Vögeln, Reptilien, Amphibien und Fischen*. 2004: Schattauer Verlag.
2. Schmitz, S., et al., *Comparison of Physiological Parameters and Anaesthesia Specific Observations during Isoflurane, Ketamine-Xylazine or Medetomidine-Midazolam-Fentanyl Anaesthesia in Male Guinea Pigs*. PLoS ONE, 2016. **11**(9): p. e0161258.

10.4 Typical isoflurane anaesthesia with atropine premedication

Anaesthesia interval	Induction	Non- surgical tolerance	Surgical tolerance	Wake-up	Recovery
Definition	Isoflurane exposure to RR -	RR – to FWR/IR < ±	FWR/IR < ± to isoflurane stop	FWR/IR > ± to RR +++	From RR +++ →
Duration [min] (mean interval)	1.8 (0 – 1.8)	4.9 (1.8 – 6.7)	33.6 (6.7 – 40.3)	12.2 (40.3 – 52.5)	52.5 <
Exposure through	Whole body chamber	Nose cone	Nose cone	-	-
MAP [mmHg]	drop 80 to 62	fast drop 62 to 27	further drop 27 to 17, $\bar{x}$: 19	steep increase 17 to 52	slight increase 52 to physiological
HR [bpm]	Slight drop 327 to 295	Slight drop 295 to 268	further drop 268 to 235	steep increase 244 to 306	drop 306 to physiological (270)
ReR [min^{-1}]	Arrhythmic, panting	drop 92 to 77	further drop 77 to 30	increase 30 to 75	75 to physiological
BT [°C]	38.9	early drop 38.9 to 38.4	drop 38.4 to 36.0	further drop 36.0 to 35.6	increase 35.6 to 39.2
Isoflurane [%] (min-max)	$\bar{x}$: 4.4 (4.1 - 4.6)	Reduce individually, start from ~ 3.1	Reduce individually, end ~ 2.4	0	0
Righting reflex	+++ → -	-	-	- → +++	+++ (don't test)
Lid reflex	untestable	-	-	- → +++	+++
Ear reflex	untestable	-	-	- → +++	+++
Foot withdrawal reflex (FWR)	untestable	++/+ → ±	± or – (slow, weak)	≥ ± → +++	+++
Inguinal reflex (IR)	untestable	++/+ → ±	± or – (slow, weak)	≥ ± → +++	+++
Muscle tone hind legs	untestable	++/+ → ±	-	- → +++	+++
Excitation	+++ → -	-	-	- → <++	< ++ → -
Cleaning	+++	-	-	- → ++	+ or ++
Tear production / squinting	+++	-	-	< ++	< +

Chewing/ mouthing	-	-	-	- → < +++	< ++
Shivering	-	-	-	- → +++	+++ → -
Bulbus rotation	+++ → -	-	-	- → +++	+++
Defecation/ Urination	+/ +++	-	-	++/-	Not specific
Piloerection	-	-	-	- → < +	+++ → < -
Respiratory sounds	untestable	-	- → < +++	-	-

Symbols: - = no reaction/not present, ± = minimal reaction, + = mild reaction, ++ = delayed/reduced/weaker reaction, +++ = physiological reaction. RR= righting reflex, FWR = foot withdrawal reflex, IR = inguinal reflex, MAP = mean arterial pressure, HR = heart rate, ReR = respiratory rate, BT = core body temperature. Atropine premedication (0.04 mg/kg) in the GP.

(On the basis of the results from Publication 2, reflex definitions see there; parameter values are to be treated as reference values)

10.5 Typical MMF anaesthesia with AFN antagonisation

Anaesthesia interval	Induction	Non- surgical tolerance	Surgical tolerance	Wake-up	Recovery
Definition	MMF injection to RR -	RR – to FWR/IR < ±	FWR/IR < ± to AFN injection	FWR/IR > ± to RR +++	RR +++ →
Duration [min] (mean interval)	3.5 (0 – 3.5)	5.7 (3.5 – 9.2)	31.5 (9.2 – 40.6)	7.6 (40.6 – 48.2)	48.2 <
MAP [mmHg]	slight drop 75 to 56	Stable 56 to 62	slight drop 62 to 48	Abrupt U-shape 48 over 22 to 70	decrease 70 to physiological (57)
HR [bpm]	drop 328 to 249	slight drop 249 to 225	slight drop 225 to 205	steep increase 205 to 334	Slight increase → drop 334 to 358 to physiological (270)
ReR [min^{-1}]	slight drop 94 to 73	stable 73 to 67	stable 67 to 65	increase 65 to 89	89 to physiological
BT [°C]	early drop 38.7 to 38.5	slight drop 38.5 to 38.0	drop 38.0 to 36.4	further drop 36.4 to 36.2	increase 36.2 to physiological
Righting reflex	+++ → -	-	-	- → +++	+++
Lid reflex	+++ → < ±	-	-	- → +++	+++
Ear reflex	+++ → < +	+ → < -	± or -	- → +++	+++
Foot withdrawal reflex (FWR)	+++ → < ++ (only test if necessary)	++ → < - (sharp, quick)	± or – (sharp, quick)	≥ ± → +++ (only test if necessary)	+++ (don't test, only causes distress)
Inguinal reflex (IR)	+++ → < ++ (only test if necessary)	++ → < - (sharp, quick)	± or – (sharp, quick)	≥ ± → +++ (only test if necessary)	+++ (don't test, only causes distress)
Muscle tone hind legs	+++ → < +	++ → -	-	- → +++	+++
Excitation	< ++ → -	-	-	- → <+	< ++ → -
Chewing / mouthing	< +	-	-	- → < +++	< +++
Shivering	-	-	-	- → +++	+++ → -
Bulbus rotation	+++ → -	-	-	- → +++	+++
Piloerection	< +	-	-	- → < +	+++ → < -

Skin colour	pink → pink-red → pink	pink → pale pink	pale pink	pink-red → pink	pink

Symbols: - = no reaction/not present, ± = minimal reaction, + = mild reaction, ++ = delayed/reduced/weaker reaction, +++ = physiological reaction. RR= righting reflex, FWR = foot withdrawal reflex, IR = inguinal reflex, MAP = mean arterial pressure, HR = heart rate, ReR = respiratory rate, BT = core body temperature, AFN= atipamezole-flumazenil-naloxone antagonisation.

(On the basis of the results from Publication 2, reflex definitions see there; parameter values are to be treated as reference values)

10.6 Typical KX anaesthesia with atipamezole antagonisation

Anaesthesia Phase	Induction	Non- surgical tolerance	Surgical tolerance	Wake-up	Recovery
Definition	KX injection to righting R -	Righting R – to FWR/IR > ±	FWR/IR < ± to atipamezole injection	Atipamezole injection to righting R +++	From RR +++ →
Duration [min] (mean interval)	2.6 (0 – 2.6)	3.4 (2.6 – 6)	34.6 (6 – 40.6)	59.7 (40.6 – 100.3)	100.3 <
MAP [mmHg]	slight drop, 72 to 57	stable, 57 to 51	slight rise 51 to 60	U trend, 60 over 37 to 54	Stable Physiological (57)
HR [bpm]	slight drop 270 to 232	drop 232 to 207	slight drop, 207 to 197	Very slow increase 197 to 226	Ongoing slow increase to physiological (260)
RR [min^{-1}]	drop 94 to 56	stable 56	stable, 56	Slow increase 56 to 65	Slow increase to physiological
BT [°C]	38.7	early drop 38.7 to 38.2	drop 38.2 to 37.3	further drop 37.3 to 36.4	Very slow increase 36.4 to 38.3 (after 5.5 h)
Righting reflex	+++ → -	-	-	- → +++ (unreliably +++)	+++ (little movement)
Lid reflex	+++ → < -	-	-	- → +++	+++
Ear reflex	+++ → < ±	± → < -	± or -	- → +++	+++
Foot withdrawal reflex (FWR)	+++ → < + (only test if necessary)	< + → < -	± or -	≥ ± → +++	+++ (only test if necessary)
Inguinal reflex (IR)	+++ → < + (only test if necessary)	< + → < -	± or -	≥ ± → +++	+++ (only test if necessary)
Muscle tone hind legs	+++ → < +	< + → -	± or -	± or - → +++	+++
Excitation	< +++ → -	-	-	- → <+++	< +++ → -
Chewing / mouthing	< +	-	-	- → +++	< +++
Shivering	-	-	-	- → +++	+++ → -

Bulbus rotation	+++ → -	-	-	- → +++	+++
Piloerection	< +	-	< +++	< +++	+++ → < -
Sedation	-	-	-	<+++	+++ → <+
Skin colour	pink → pink-red → pink	pink → pale pink	pale pink	pink-red → pink	pink

Symbols: - = no reaction/not present, ± = minimal reaction, + = mild reaction, ++ = delayed/reduced/weaker reaction, +++ = physiological reaction, RR= righting reflex, FWR = foot withdrawal reflex, IR = inguinal reflex, MAP = mean arterial pressure, HR = heart rate, ReR = respiratory rate, BT = core body temperature.

(On the basis of the results from Publication 2, reflex definitions see there; parameter values are to be treated as reference values)

11 Summary

Anaesthesia in guinea pigs (GPs) has often been described as difficult and risky. Therefore, the included publications invested the effect of the mostly used anaesthesias MMF (medetomidine-midazolam-fentanyl), isoflurane (Iso) and ketamine-xylazine (KX) on physiological parameters in GPs. Throughout the anaesthesia, the GPs' cardiovascular parameters were monitored, using abdominally implanted radiotelemetry devices to obtain optimal data quality.

The surgical approach used for the implantation of the radiotelemetry transmitter is presented in detail in the first publication. For surgery, the GPs were anaesthetised with MMF (medetomidine-midazolam-fentanyl) and they were antagonised with AFN (atipamezole-flumazenil-nalxone) at the end of the surgery. For pain medication, the GPs were started on meloxicam (0.4 mg/kg) and metamizole (80 mg/kg) 30 min before the surgery. Metamizole was continued for 24 h after surgery and meloxicam for 2 more days. During the implantation, they were additionally covered with the fentanyl component in MMF. Prior to the surgery, enrofloxacin (10 mg/kg) was applied for antibiotic coverage which was continued for 2 more days. Using the implanted system, arterial blood pressure (BP), heart rate (HR) and core body temperature (BT) were measured throughout the first 24 h after the end of the implantation. The implantation approach led to the highest long-term survival rate reported to date, with 13 of 16 GPs (81 %) surviving. The GPs lost body weight (BW) until 2 d after surgery (-11.9 %, -53.6 g) but steadily increased their weight thereafter. The GPs had returned to physiological values in BP and BT at 8 h after abdominal surgery and at 24 h regarding HR. As GPs are stress-prone, recommendations for stress reduced handling were given for before, during and after the implantation. The findings on the effects of the implantation can be used as a model for other abdominal operations in the GP.

The second publication described the investigation of the effects of one-time Iso, MMF and KX anaesthesia on the physiological parameters using the 13 implanted GPs. Each animal was anesthetised once with MMF, Iso and KX at an interval of 7 d. The entire anaesthesia pass was recorded radiotelemetrically and supplemented by manual measurements of respiratory rate (ReR), reflexes and blood glucose (BG). One anaesthesia pass included 120 min acclimatization time, of which the last 15 min were averaged as individual baseline values. The GPs were then premedicated; with Iso with atropine and with MMF and KX with sodium chloride as placebo. Ten minutes

later, anaesthesia was initiated, for Iso anaesthesia using a pre-filled whole body chamber and for MMF and KX with intramuscular injections into the hind limbs. Anaesthesia was discontinued after 40 min by Iso supply stop, AFN antagonisation for MMF or partial antagonisation with atipamezole for KX anaesthesia. The MAP, HR, BT were measured continuously until at least 240 min after anaesthesia induction. Respiratory rate (ReR) was measured until at least 55 min and reflexes were tested until the GPs showed a positive righting reflex again. BG values were measured at 7.5, 20 and 40 min during anaesthesia.

With Iso use, all GPs reached a surgical tolerance, 11 did so with MMF anaesthesia and only 7 reached an operable state with KX. The induction, non-surgical tolerance and surgical tolerance phase durations did not differ considerably between the 3 anaesthetics. Following MMF and Iso there were short wake-up times (7.6 & 12.2 min), whereas it required 59.7 min until the GPs re-gained their RR after KX. MMF anaesthesia led to a marked transient MAP decrease after antagonisation, otherwise the MAP and the HR were only mildly altered. Iso exposure led to a marked hypotension during anaesthesia maintenance (approx. 20 mmHg) and the HR was only mildly increased at the beginning of the anaesthesia. KX caused mild deviations from the normal physiology for MAP and HR during maintenance. However, after partial antagonisation, the MAP dropped and the GPs recovered only slowly. The HR was also reduced and increased only gradually during the wake-up following KX anaesthesia.

All anaesthetics induced hypothermia, but the animals lost the most BT with Iso anaesthesia. Immediately after the end of the MMF and Iso anaesthesia, the GPs were able to quickly lift their BT back to the starting level through shivering. After KX anaesthesia only 3 of the 7 GPs had returned to 38.8°C after 5 h, the other 4 still had not reached pre-anaesthetic BT values after 8 h. Respiratory depression occurred with all 3 anaesthetics, with KX leading to a moderate (-52%) and Iso to a severe (-71%) hypoventilation. There was also a strong irritation of the mucous membranes of the respiratory tract through the respiratory gas. Subsequent mucosal secretion could only be alleviated by atropine pre-medication in the short term. BG increases were observed during KX (moderate) and strongly during MMF anaesthesia. The reflex responses varied considerably between the anaesthetics. They were strong and quick during MMF anaesthesia and slightly less so with KX use. Iso exposure led to weak and slow reflex responses. Overall, MMF was determined to be the anaesthesia of choice; Iso can only be advised for short and non-painful procedures and we advise against the use of KX anaesthesia in GPs.

After investigating the effects of single anaesthesia with Iso, MMF and KX, the third paper described the impact of repeated MMF and Iso anaesthesia. KX was not tested for anaesthesia repetition because of its highly unfavourable effects in the recovery phase. Twelve instrumented MS were anesthetised in 2 anaesthesia sets, 6 times over 3 weeks with either only Iso or only MMF. Each anaesthesia repetition was performed as described for the single anaesthesia.

All GPs reached a surgical tolerance and this could be maintained for the desired 40 min. Overall, the anaesthetic profiles of MMF and Iso did not change greatly with anaesthesia repetition.

During Iso exposure, the repeated atropine premedication caused the HR to increase, and this increase remained longer with progressing repetitions. During MMF the wake-up phase shortened from the 1st to the 2nd repetition and the MAP and the HR decreased from the first to all following anaesthesias. During the MMF maintenance, there was a large individual variation in the BP between the GPs, but the single animal always exhibited similar MAP values during all of the repetitions. At 40 min of the MMF anaesthesia, the BG had increased particularly strongly in those anaesthesias that were performed with an interval of 2 d.

The BT decrease and the BW increase were not altered. Both anaesthetics can therefore be used repeatedly in the GP with very little change in the anaesthesia profile compared to the single anaesthesias. The GPs developed increasingly stronger defensive reactions which were particularly pronounced with Iso. They reduced the reliability with which the injections of MMF and atropine could be performed and with that the induction of anaesthesia. Although repeated Iso anaesthesia led to lesser repetition-related effects, the highly disadvantageous effects of hypotension, mucous production and hypoventilation with Iso anaesthesia remained and were further worsened by the strong defensive reactions. MMF led to a much more beneficial anaesthesia with the only drawback of altering the BG and the occurring hypothermia. In conclusion, MMF is superior for both single and repeated anaesthesia use in the GP. Iso is only preferable to MMF, if multiple anaesthesias need to be performed on the same day.

12 Zusammenfassung

Die Anästhesie an Meerschweinchen (MS) wurde schon oft als schwierig und risikoreich beschrieben. Mit diesem Hintergrund wurden die drei häufigsten Narkosemittel, Medetomidin-Midazolam-Fentanyl (MMF), Isofluran (Iso) und Ketamin-Xylazin (KX), hinsichtlich ihres Einflusses auf die physiologischen Parameter im MS untersucht. Die Überwachung vor, während und nach den Anästhesien wurde durch einen abdominal implantierten Radiotelemetriesender durchgeführt.

In der ersten Publikation wurde die Implantation des Telemetriesenders detailliert dargestellt. Für den Eingriff wurden die MS mit MMF anästhesiert und die Narkose wurde am Ende des Eingriffes mit AFN (Atipamezol- Flumazenil-Naloxon) wieder aufgehoben. Die Analgesie bestand aus Meloxicam (0,4 mg/kg) und Metamizol (80 mg/kg), womit 30 Minuten vor dem Start der Operation begonnen wurde. Während der Anästhesie wurde die Analgesie zusätzlich über die Fentanyl Komponente des MMFs erhalten. Nach der Implantation wurde Metamizol für 24 Stunden und Meloxicam für 48 h fortgesetzt. Zur antibiotischen Versorgung erhielten die MS Enrofloxacin (10 mg/kg) vor dem Beginn der Operation und in den zwei darauffolgenden Tagen. Unter Verwendung des implantierten Senders wurden der arterielle Blutdruck (BD), die Herzfrequenz (HF) und die Kernkörpertemperatur (KT) in den ersten 24 h nach dem Ende der Implantation gemessen. Die durchgeführte Implantationsherangehensweise führte zu der bisher höchsten publizierten Langzeitüberlebensrate mit 13 von 16 Tieren (81%). Bis 2 Tage nach der Operation verloren die Tiere an Körpergewicht (-11,9 %, -53,6 g). Danach stieg ihr Gewicht jedoch stetig wieder an. Die MS waren nach 8 h nach ihrer abdominalen Operation zu physiologischen Werten in Blutdruck und Körperkerntemperatur und nach 24 h zu normalen HF zurückgekehrt. Aufgrund der hohen Stressanfälligkeit von MS, wurden Empfehlungen für stressarmes Handling, für vor, während und nach der Implantation gegeben. Die Erkenntnisse über die Auswirkungen der Implantation können modelhaft auf andere Bauchoperationen im MS angewendet werden.

Die zweite Veröffentlichung beschrieb die Untersuchung der Wirkungen von einmaligen Iso, MMF und KX Anästhesien auf die physiologischen Parameter unter Verwendung der 13 implantierten MS. Jedes Tier wurde einmalig mit MMF, Iso und KX im Abstand von 7 d anästhesiert. Der gesamte Anästhesiedurchgang wurde radiotelemetrisch aufgezeichnet und durch manuelle Erhebungen für Atemfrequenz (AF), Reflexe und Blutglukose (BG) ergänzt. Ein Anästhesiedurchgang beinhaltete 120 min Akklimatisierungszeit, wovon die letzten 15 min als individuelle Baselinewerte gemittelt wurden. Danach wurden die MS prämediziert; bei Iso mit Atropin und bei MMF und KX mit

Natriumchlorid als Placebo. Zehn min später wurde die Anästhesie eingeleitet mit einer vorgefluteten Ganzkörperkammer für die Iso Anästhesie und für MMF und KX mit intramuskulären Injektionen in die Hintergliedmaßen. Die Anästhesie wurde für nach 40 min aufgehoben durch Iso-zufuhrstopp, AFN Antagonisierung oder Teilantagonisierung mit Atipamezol. Der BD, die HF, und die KT wurden kontinuierlich bis mindestens 240 min nach Anästhesieeinleitung gemessen. Die AF wurde bis mindestens 55 min gemessen, und die Reflexe wurden bis zum Erreichen eines positiven Stellreflexes getestet. Bei 7,5, 20 und 40 min während der Anästhesie wurden BG Werte erhoben.

Mit der Anwendung von Iso erreichten alle MS eine chirurgische Toleranz, mit der MMF-Anästhesie waren es 11 MS und nur 7 erreichten einen operablen Zustand mit KX. Die Induktion, nichtchirurgische Toleranz und chirurgische Toleranzphasendauer unterschieden sich nicht erheblich zwischen den 3 Anästhetika. Auf die Narkosen mit MMF und Iso folgten kurze Weckzeiten (7,6 & 12,2 min), während es 59,7 min benötigte, bis die MS ihren RR nach KX wiedererlangten. Die MMF-Anästhesie führte zu einer kurzen, deutlichen BD-Abnahme nach Antagonisierung, ansonsten wurden der BD und der HF nur geringfügig verändert. Die Iso-Exposition führte zu einer ausgeprägten Hypotonie während der Anästhesieerhaltung (ca. 20 mmHg) und die HF war zu Beginn der Anästhesie nur leicht erhöht. KX verursachte nur milde Abweichungen von der normalen Physiologie für BD und HF während der Anästhesieerhaltung. Nach der Teilantagonisierung fiel der BD die HF und die KT jedoch ab und die MS erholten sich nur langsam.
Alle Anästhetika induzierten eine Hypothermie, aber unter der Iso Narkose verloren die MS am schnellsten und am meisten Körpertemperatur. Direkt nach dem Ende der MMF- und Iso-Narkose konnten sie ihre KT über Kältezittern schnell wieder auf das Ausgangslevel heben. Nach der KX-Anästhesie waren jedoch nur 3 der 7 GPs auf 38,8 ° C KT zurückgekehrt, die anderen 4 hatten auch nach 8 h noch nicht wieder ihre Ausgangskörperkerntemperatur erreicht. Eine Atemdepression trat bei allen 3 Narkosemitteln auf, am ausgeprägtesten jedoch unter der Iso-Narkose (-71%). Dort trat zusätzlich eine starke Reizung der Schleimhäute der Atemwege durch das Atemgas auf. Die nachfolgende Schleimsekretion konnte durch die Atropinprämedikation nur kurzfristig gelindert werden. Der BG-Spiegel stieg während der Anästhesie mit KX (mäßig) und während der MMF-Anästhesie stark an. Die Reflexantworten variierten erheblich zwischen den Anästhetika. Sie waren stark und schnell während der MMF Anästhesie und etwas weniger deutlich ausgeprägt unter der KX-Anwendung. Die Iso-Exposition führte hingegen zu schwachen und langsamen Reflexantworten. Insgesamt ist MMF die Anästhesie der Wahl beim MS; Iso sollte nur für kurze und nicht

schmerzhafte Verfahren beraten werden und wir empfehlen die Verwendung von KX Anästhesie bei GPs.

Nach der Untersuchung der Einzelanästhesieffekte mit Iso, MMF und KX, beschrieb die dritte Publikation die Auswirkungen der wiederholten MMF- und Iso-Anästhesie. Aufgrund der deutlichen Nachtteile der KX-Narkose in der Aufwachphase wurde KX von der Wiederholungsstudie ausgeschlossen. Zwölf instrumentierte MS wurden in 2 Anästhesiesets jeweils 6 mal über 3 Wochen mit entweder nur Iso oder nur MMF anästhesiert. Der Aufbau der einzelnen Anästhesiedurchgänge war derselbe wie bei in Einzelanästhesien beschrieben. Alle GPs erreichten eine chirurgische Toleranz, und diese konnte für die gewünschten 40 min aufrechterhalten werden. Insgesamt änderten sich die Anästhesieprofile von MMF und Iso nur sehr gering im Verlauf der Anästhesiewiederholung. Durch die wiederholte Atropinprämedikation nahm die HF zu und dieser Anstieg blieb mit fortschreitenden Wiederholungen immer länger bestehen. Bei der MMF Anästhesie verkürzte sich die Aufwachzeit vor allem von der 1. auf die 2. Wiederholung. Der BP und die HF nahmen nach der ersten Wiederholung für die nachfolgenden Anästhesien ab. Während der MMF-Anästhesieerhaltung zeigten die MS große individuelle Variationen im BD. Jedes einzelne Tier blieb jedoch während allen Wiederholungen auf seinem eigenen Niveau. Die BG war bei den MMF-Anästhesien zwischen denen nur 2 Tage lagen 40 min nach der Anästhesieeinleitung besonders hoch. Die KT-Abnahme trat sowohl bei MMF, als auch bei Iso während der Anästhesie auf und die Körpergewichtsentwicklung veränderte sich durch die Wiederholungen nicht.

Sowohl Iso als auch MMF können nach diesen Ergebnissen daher wiederholt in MS eingesetzt werden. Trotzdem entwickelten die MS zunehmend stärkere Abwehrreaktionen gegen die Anästhesieeinleitung, die bei Iso besonders ausgeprägt waren. Sie reduzierten die Zuverlässigkeit, mit der die Injektionen von MMF und Atropin durchgeführt werden konnten und damit die Induktion der Anästhesie. Die Iso-Anästhesie führte zwar zu geringeren Wiederholungswirkungen im Vergleich zu MMF, das Anästhesieprofil mit der starken Atemdepression und Schleimproduktion und der starken Hypotension bestand jedoch weiterhin. Insgesamt sind die Effekte der Wiederholungen bei der MMF Anästhesie weitgehend vernachlässigbar und das Anästhesieprofil ist wesentlich vorteilhafter für die MS. Daher ist MMF für die einfache und wiederholte Anästhesie im MS das Anästhetikum der Wahl, es sei denn am selben Tag müssen mehrere Anästhesien durchgeführt werden müssen. In dem Fall ist Iso vorzuziehen.

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14 Table list

15 Figure list

16 Abbreviations

AFN	atipamezole-flumazenil-naloxone
BG	blood glucose
bpm	beats per minute
br/m	breaths per minute
BT	core body temperature
ECG	Electrocardiogram
Fig	figure
GP	guinea pig
HR	heart rate
i.m.	intramuscular
i.p.	intraperitoneal
Iso	isoflurane
KX	ketamine-xylazine
MAP	mean arterial blood pressure
min	minute
MMF	medetomidine-midazolam-fentanyl
ReR	respiratory rate
RR	righting reflex
s.c.	subcutaneous

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