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Expert information

from the GV-SOLAS Committee for Anaesthesia in collaboration with Working Group 4 in the TVT

Pain management for laboratory animals

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Introduction

This recommendation is intended for licence applicants, animal welfare officers, and authorities. Its purpose is to serve as a guide to humane work practices and the standardization of procedures used today. The values indicated in the tables are recommendations, from which procedures may diverge where necessitated by experimental conditions, provided approval for this has been obtained. It is the responsibility of each and every one to keep up to date about current standards and the most recent developments in pain management for laboratory animals.

Special instructions

The revised expert information of 2019/2020 addresses mammalian species for which pain management is often used in animal experiments. For pain management in other animal species and especially in birds, reptiles, amphibians, cephalopods and fish, specific specialist literature and expert knowledge must be consulted. For pain management in zebrafish during experiments, recommendations are currently being worked out in a FELASA Working Group - http://www.felasa.eu/working-groups/working-groups-present/pain-management-in-zebrafish/

The recommendations and doses are based on the latest literature (as of 2020) and on the knowledge and experience of specialists.

Feedback may be sent to the committee for anaesthesia anaesthesie@gv-solas.de.

1. Pain, suffering and harm

By establishing the terms pain, suffering, and harm in its Animal Welfare Act, German law defines the relevance of animal welfare for handling animals and performing surgical procedures on them (§1 TSchG; “No one may cause an animal pain, suffering or harm without good reason”). Effective pain management is an essential of the refinements of animal experiments and an important part of experimental planning.

1.1. Pain and nociception

Pain is defined by the International Association for the Study of Pain (IASP 1994; Jensen & Gebhart 2008) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”. A further explanatory note states: “Pain is always subjective! “.

Nociception is the neuronal process of recognition and processing (“coding”) of noxious stimuli. Note: consequences of coding may be autonomic (e.g., increased blood pressure) or behavioural (motor withdrawal reflex or more complex avoidance behaviour). Pain is not necessarily implicated. Nociception can occur during anaesthesia and is reflected in autonomic signs. Pain however is a conscious experience (IASP 1994; Sneddon 2017).

1.2. Suffering

Suffering is a mental state based on the failure to meet an individual‘s basic needs (Gärtner 2002).

The varying needs of the different species and individuals make it impossible to give a universal definition of suffering. From a legal point of view, the extent of the suffering and the time factor also play a role: on the one hand, the concept of “suffering” does not require a prolonged or lasting impairment of well-being. On the other hand, it implies a relevant condition; suffering also means more than plain discomfort, listlessness, or a mere temporary state of stress (BGH, German Federal Court of Justice, 1987). It is also important to note that suffering can be addressed as a subjective sensation and can also occur as a consequence of pain (Hackbarth & Lückert 2000).

1.3. Harm

Harm experienced by individuals is linked to pain and suffering, with time being a decisive factor. Harm occurs when an animal’s condition is changed for the worse. Harm can be a cause, side effect or consequence of pain and suffering. However, pain and suffering can also precede, accompany or succeed harm (Hackbarth & Lückert 2000; Schiwy 2000).

From an animal welfare point of view, analgesic therapy covers only part of the stresses to which laboratory animals are exposed as painless impairments (e.g., anxiety or stress) play an important role in their suffering.

2. Development of pain

The pain pathway should be outlined in order to understand the pathogenesis of pain, but also to understand the effect of pain-relieving therapies. A review article by Marchand offers a detailed description of the physiological mechanisms of pain (Marchand 2008). A simplified summary is presented here.

2.1. Physiological pain

Pain is extremely important for survival and has the fundamental function of preserving the integrity of the body. This adaptive, functional pain is usually described a physiological nociceptive pain.

A noxa or trauma usually causes an inflammatory reaction, thus initiating a pathophysiological process in the affected tissue. This process is accompanied by a release of mediators (e.g. prostaglandins, histamine, bradykinin, leukotrienes, nitrogen monoxide and other cytokines, among others) that trigger a sensitization of the nociceptors. This leads to primary hyperalgesia, i.e., elevated pain sensitivity, resulting from a reduced threshold for pain induced by a damaging stimulus. This stimulus is converted to electrical activity at the peripheral nociceptor (transduction) and transmitted to the spinal cord via myelinated Aδ fibres and non-myelinated C fibres (transmission). With their high conduction velocity, myelinated fibres serve mainly to transmit impulses for the perception of primary pain that is readily localized. Primary pain leads to avoidance and a motor response that works as a means of protection. The slow-conducting C fibres transmit impulses that convey dull, often burning pain that is difficult to localize. This kind of pain, which can be long-lasting even if the initial stimulus has stopped, is also called secondary pain.

In the central nervous system, beginning in the dorsal horn of the spinal cord, a **modulation** of impulse transmission can occur as a result of excitatory and inhibitory transmitters of interneurons. Various neurotransmitters are responsible for the synaptic transmission of information in the spinal cord. Synaptic transmission within the spinal cord and to higher centres can be reduced by a variety of endogenous mechanisms and substances. For example, endogenous opioids can reduce the release of excitatory neurotransmitters. In addition, **descending modulation** has a lasting effect on the spinal cord. Modulating nerve pathways from higher regions of the brain (mainly noradrenergic and serotonergic) make it possible to reduce or increase the ascending information. The processing of pain stimuli involves several brain structures and a diverse network of neurons with complex connections, which is generally referred to as the “pain matrix”. After transduction, transmission and modulation, it is only **perception** that represents the actual conscious, subjective and emotional experience of pain. That is what is commonly understood as “pain“.

In general, physiological pain is correlated with the intensity and duration of the causal stimulus and subsides in healthy tissue as the stimulus fades.

2.2. Pathological pain

Unlike physiological pain, pathological pain is a sensation that extends beyond the warning function and is experienced separately as suffering. This pain has no function and is referred to as “maladaptive”; it outlasts the causal trigger and may also be present without any stimulus.

Repeated harmful stimuli – including stimuli triggered in relation to surgery or trauma – lead to changes in the characteristics of the dorsal horn neurons, causing a steady increase in neuronal activity. This is known as ***wind-up*** phenomenon, which primarily occurs following activation of the glutamate NMDA receptor and is one of the fundamental mechanisms of action of central sensitization (Woolf 2011).

A typical feature of pathological pain is the joint occurrence of key characteristics, such as **allodynia** (a change in the pain threshold so that non-painful stimuli are perceived as painful), and **secondary hyperalgesia** (where increased sensitivity to pain extended to adjacent, non-traumatized areas). These are also described as pain memory.

Chronic pain: The *International Association for the Study of Pain* defines chronic pain in humans as a pain that lasts longer than three months or keeps recurring. Pain can persist without any identifiable harm or continue once the healing phase is over.

Neuropathic pain: any lesion (traumatic or surgical) or disease of the somatosensory nervous system, e.g., resulting from a tumour or osteoarthritis, has the potential to cause neuropathic pain. A peculiarity of this type of pain is its continuation after the cause of the pain has disappeared and the contingent effect of conventional analgesic therapies. A prime example is phantom pain following the amputation of a limb.

3. Pathophysiological effects of pain

For German law, the relevance of pain management in laboratory animals is evident from §1[1] and §7a, (2) 4 of the German Animal Welfare Act (TierSchG)[2] and §17 of the Ordinance on Laboratory Animal Welfare (TierSchVersV).

On the basis that the nociceptive system of all vertebrates is very similar to that of humans (Gebhardt 1994; Van Hooff et al. 1995; Flecknell 1996), it must always be assumed that every vertebrate, regardless of age and species, feels pain after an experimental procedure that compromises its physical integrity (Erhardt 1992; Pascoe 1992; Henke et al. 1999). Therefore, any animal experiment that is associated with postoperative or other pain requires adequate pain management.

Some of the pathophysiological effects of pain on the various organ systems are listed in the following examples (Jage 1997; Larsen 1998; Henke and Erhardt 2001; Jirkof 2017).

Nervous system

- Allodynia
- Hyperalgesia
- Untreated acute pain can lead to chronic, often neuropathic pain

[1] No one may cause an animal pain, suffering or harm without good reason.

[2] Pain, suffering, or harm may only be inflicted on the animals when unavoidable to attain the purpose of the experiment. In particular, they may not be inflicted in order to save work, time or costs.

Endocrine system

- Increased secretion: catecholamines, corticoids, glucagon, growth hormone, ACTH, ADH
- Decreased secretion: insulin, testosterone
- Consequences:
  - Impaired mobilization
  - Lethargy
  - Muscular atrophy
  - Prolonged convalescence
- Fluid retention, oliguria, electrolyte disorder
- High metabolic rate with increased oxygen consumption
- Effects on the organ systems (see below)

Neuroendocrine system

- Beta-endorphin level increase
- Blocking of NMDA channels

Sympathetic nervous system

- Increase in activity, release of catecholamines
- Effects of a prolonged stimulation of the sympathetic nervous system:
  - Decreased tissue circulation with increasing tissue acidosis, which results in amplification of pain
  - Risk of hypoxia in organs with poor circulation (heart, brain, colon, lung)
  - Gastrointestinal atony to the point of a paralytic ileus
  - Activation of the renin-angiotensin system with poor renal perfusion
  - thus, increasing peripheral vasoconstriction
  - Increased platelet aggregation
  - Increased release of noradrenaline in peripheral nerve endings and resulting amplification of pain

Immune system

- Immunodeficiency (increased susceptibility to infections), impaired wound healing
- Immunosuppression
  - Inhibition of the mitotic rate and locomotion of T cells
  - Inhibition of lymphokine production
  - Inhibition of phagocytosis

- Decrease in
  - Interleukin release
  - Cell immunity
  - Tumour immunity
  - Host defence status
  - Generation of antibodies

Changes in blood count

- Depletion of spleen and skin vessels
- Lymphopenia
- Eosinopenia
- Neutrophilia

Respiratory system

(particularly affected after thoracic and abdominal surgery)

- Reduced tidal volume and vital capacity, tachypnoea
- Consequences:
  - Atelectasis with impaired pulmonary gas exchange, and as a result
  - predisposition to infections, pneumonia
  - respiratory and metabolic acidosis

Cardiovascular system

(Effects are due to act **Genitourinary system** ivation of the sympathetic nervous system)

- Tachycardia, peripheral vasoconstriction with increased blood vessel resistance
- Increased heart contractility with increased myocardial O2 consumption
- Increase in blood pressure

Gastrointestinal system

(Cause: excitation of peritoneal nociceptors, increased activity of the sympathetic nervous system, ischemia)

- Consequences:
  - Gastrointestinal atony to the point of a paralytic ileus with nausea, vomiting, colon distension and increased abdominal pressure
  - Elevated diaphragm with restrictive pulmonary function
  - Irritation of visceral nociceptors
  - Impaired visceral circulation with ischemia, resulting in amplification of pain symptoms
  - Reduced food and water intake (hypoglycaemia, dehydration)

Genitourinary system

- Reduced motility in the entire urinary tract
- Urinary retention

Musculature

- Spasms, tremor, cramps
- Longer term: impaired mobilization, lethargy, muscular atrophy

Bone healing

- Reduced activity lessens the mechanical stimulation that is crucial to bone healing and may delay healing processes.

Behaviour

- Depression, disturbed circadian rhythm, reduced behavioural repertoire, increased aggressiveness
- Intensive licking of body parts to the point of self-mutilation
- Reduced or altered grooming activity (particularly small rodents)
- Reduced food and water intake

To summarize, pain has pathophysiological effects that can bias experimental results in a non-quantifiable manner. This bias can be reduced by administering analgesics (Jirkof 2017; Peterson et al. 2017).

4. Identification and quantification of pain

Assessing pain in animals is difficult because animals are not able to express themselves verbally. It is impossible to investigate their emotional state directly. It is always only possible to draw indirect conclusions on the basis of clinical symptoms, physiological changes or behavioural changes.

In the recent past, major advances have been made in the development and validation of methods for the identification and quantification of pain in different animal species. Aside from classical bioindicators (clinical symptoms, physiological changes and appearance) there are also various ethological indicators for pain and diminished wellbeing that have been introduced for different animal species. They are based on pain-induced changes (reduction or stimulation) of typical behaviour (Tansley et al. 2019). The indicators should be tested for their sensitivity (the ability to identify animals in pain correctly) and their specificity (the ability to identify those that are not suffering pain) (Golledge & Jirkof 2016).

In rodents, pain induces e.g., abnormal movements, such as *back arching* or *flinching* (Roughan & Flecknell 2004; Roughan et al. 2004), a changed facial expression, i.e., *grimaces* (Langford et al. 2010) and certain vocalizations in the ultrasound range. Normal species-specific behaviour patterns such as movement (Leach et al. 2010), burrowing, (Jirkof et al. 2013b) or nest-building behaviour (Jirkof et al. 2013a) are suppressed. Turner et al. and also

Tappe et al. each published a review of pain assessment in rodents that may be useful both clinically and in experiments (Tappe-Theodor et al. 2019; Turner et al. 2019).

Score sheets can help in formalizing and standardizing the assessment and monitoring of pain and even recommended when they are not required by law. Monitoring tools of this kind make it easier to implement refinement measures, such as pain management, and are an important tool for improving the welfare of animals used in research. The score sheets should include several meaningful and robust parameters, such as species-specific pain symptoms, model-specific clinical symptoms that measure the specific effect of experimental manipulation on the system or the organ, and general indicators of animal wellbeing. Appropriate monitoring frequencies must also be defined (see also Expert Information *Belastungsbeurteilung im Tierversuch* [Assessment of stress in animal experiments] GV-SOLAS).

Pain is a dynamic process and can vary chronologically in its severity because pain is influenced by the duration of effect of the analgesics administered, the concomitant occurrence of inflammation and other factors. The pain should therefore be regularly and frequently assessed during the expected peaks of pain, e.g., in the first hours following an operation or in the later stage of painful, progressive diseases. These times are dependent on the species and the nature of the experimental procedure.

It may be necessary to adjust therapy to ensure the welfare of the animal. The response to a therapy is an important instrument in the assessment of pain. Measures resulting from this assessment, such as increasing the analgesic dose, administering additional analgesics or terminating the experiment, should be determined in advance.

The legal provisions laid down in the Animal Welfare Act and the Ordinance on Laboratory Animal Welfare, as well as the articles of Directive 2010/63/EU, stipulate that applications for approval to conduct experiments must classify and weight the anticipated pain for a laboratory animal according to severity and any pain that occurs must be kept to an absolute minimum through adequate analgesic medication (§5 TierSchG[3], §7 1a TierSchG[4], §8 TierSchG[5], §17 TierSchVersV[6], Art. 4 (3) Directive 2010/63/EU[7]). A need for action to quantify the pain is thus given in many respects.

In view of species-specific and individual differences in the perception of pain, as well as the efficacy of analgesics, pain management strategies must be accompanied by a suitable plan for the assessment and monitoring of pain. Researchers should search the literature for new knowledge on the identification and quantification of pain in the animal species in question and use suitable score sheets.

[3] A procedure associated with pain may not be performed in a vertebrate without anaesthesia.

[4] Animal experiments with regard to a) the pain, suffering and harm to which the animals are to be exposed.

[5] Approval…compliance with…requirements for pain relief and anaesthesia of animals

[6] Pain relief and anaesthesia

[7] Member States ensure that – unless deemed inappropriate – procedures are conducted under general anaesthesia or local anaesthesia and that analgesia or another suitable method is used to ensure pain, suffering and anxiety are reduced to a minimum.

5. Methods for treating pain

Pain is usually managed by pharmacological means, typically with the using of non-opioid and/or opioids analgesics, depending on the nature and intensity of the pain. Non-pharmacological measures should also be used, see also section 5.4.

Pain management, i.e. the choice of the appropriate analgesics, routes and intervals of administration and also other reducing measures, must be planned in advance according to the animal species and their specific requirements. The timing of the analgesia must be considered, i.e. the start and duration of pain management must be planned and implemented according to the development of the pain and the time when it arises. For more information on perioperative analgesia in the context of anaesthesia, see section 5.3.

The effect of pain management must be monitored. It can be documented using score sheets in conjunction with action and endpoints. In many experiments, it makes sense to plan a rescue analgesia in case the animals are not free from pain with the planned pain management regimen. The effect of the additional analgesic on the experimental results must be considered.

5.1. Systemic analgesia

The following may be used **for example** as systemically acting analgesics:

1. Opioids
    - Buprenorphine, piritramide, pethidine, butorphanol, tramadol, fentanyl, methadone, morphine, oxymorphone, hydromorphone, nalbuphine
2. Non-Opioids

2.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

- Carprofen, meloxicam, flunixin meglumine, ibuprofen, ketoprofen, etodolac, diclofenac, meclofenamic acid, phenylbutazone, piroxicam, tepoxaline, tolfenamic acid

2.2. Non-acidic antipyretic analgesics (antipyretics “)

- Metamizole, paracetamol, acetylsalicylic acid

3. Phencyclidine
    - Ketamine, tiletamine
4. Others
    - Pregabalin, amantidine, gabapentin, grapiprant (EP4 receptor antagonist), nortriptyline

Routes of administration

Intravenous: Intravenous injection offers a safe route of administration ensuring a quick onset of action (exception: delayed onset of action e.g. with buprenorphine). A catheter can be used for continuous administration (continuous drip infusion, CRI) of short-acting analgesics. This

type of administration is not common in small laboratory animals because the small size of the animals clearly limits the feasibility of this method.

Intramuscular: muscles are covered by fascia, the muscle can accommodate the injection of a fluid to a limited extent. Even with very low quantities of fluid, therefore, the tissue pressure increases sharply, which can prove very painful. In addition, there is a risk of the injection damaging a nerve either directly or indirectly by triggering so-called compartment syndrome. For these reasons, only small volumes can be administered by intramuscular injection. When alternatives are feasible, this route of administration should be avoided in small laboratory animals.

Subcutaneous, intraperitoneal: Intraperitoneal and subcutaneous injection are the most common routes of administration in small rodents. Besides repeated injection of analgesics continuous administration of analgesics can be achieved with, subcutaneously implanted, osmotic, or battery-driven mini-pumps which represent an option for postoperative pain management. Depot forms of analgesics (such as buprenorphine or carprofen, liposomal or polymer) developed especially for rodents to provide continuous dosing (for up to 72 hours) are currently not available in the EU (as of 2020).

Percutaneous: Percutaneous administration of analgesics involves the use of patches, i.e. with fentanyl and buprenorphine. This type of administration should only be used after careful consideration due to its special characteristics:

- Dosing is difficult because these patches are designed for humans and are geared to the composition of skin layers, body size and weight of animals, especially small laboratory animals
- Pain and anaesthesia alter cutaneous blood flow, which can lead to changes in absorption and hence lower plasma concentrations of active substance
- The onset of action and efficacy of analgesics vary considerably from one species to another and one individual to another
- If administered early on (pre-emptively), respiratory depression induced by general anaesthesia might be intensified
- May only be applied to hairless skin

Oral: in small rodents, it may make sense to continue pain management after initial parenteral administration with oral dosing, because this ensures uninterrupted analgesia with minimal stress for the laboratory animal.

The reduced bioavailability caused by metabolism before it reaches the systemic circulation (first-pass effect) must be borne in mind.

Aside from direct administration (e.g. by means of a pipette) it is also possible to administer analgesics with flavoured gelatine, nut nougat cream, baby food, feed pellets, condensed milk, (sweetened) drinking water (orally) or liquid vehicles (e.g. Syrspend®) and gels (e.g. MediGel® Sucralose). Some analgesics in human medicine are available as a sweet syrup for children.

It must be borne in mind here that

- The use of palatable carrier substances can be problematic, depending on the experimental design, when it comes to the standardization and validity of the results. In the run-up to the experiment therefore, it is necessary to consider whether the use of such non-standardized feed supplements is acceptable.
- Furthermore, the hygiene requirements must be agreed with the animal facility management (see also Expert Information Stellungnahme aus dem Ausschuss für Ernährung zum Einsatz von nicht standardisierten Futtermitteln bei Versuchstieren [Statement from the Committee on Nutrition for the use of non-standardized feed in laboratory animals], GV-SOLAS 2012) and the pharmaceuticals law for the preparation and inclusion of mixtures before use must be met. In the case of sweetened drinking water, it the sweetness may lead to an increase in water intake. In extreme cases, overdosing of the animals is possible. In addition, sweetened drinking water offers a particularly good breeding ground for bacteria and should therefore be changed regularly (every 1-2 days).
- The chemical and physical properties of water (pH, pKa, hardness, etc.) differ from one region to another and can lead to variable interactions between water and medication.
- With medicines that are sensitive to light, dark or wrapped bottles should be used.
- In the case of a bitter drugs, such as tramadol, the medication should already be added to the water one day before the procedure, so that the animals become accustomed to the taste. Side effects cannot be excluded during the period, especially with opioids. It is important to be aware of this and monitor the animals accordingly. Nevertheless, achieving adequate pre-emptive analgesia generally outweighs the risk of side effects.

While direct oral administration is fully controllable, amount of oral intake and timing is variable and difficult to predict when it comes to mixtures especially during the resting phase (rodents: light phase). Therefore, additional subcutaneous injections should be given if the pain is severe. In all cases, the regular and adequate intake of the feed or water with added analgesic should be checked.

Transmucosal (nasal, oral, rectal, conjunctival): Medication is administered directly via the nasal, oral, rectal, or conjunctival mucosa by applying it to the surface of the mucous membrane, which ensures that a pharmaceutically effective quantity of analgesic is delivered through transmucosal absorption.

5.2. Local analgesia

Local anaesthetics may be administered as part of a multimodal anaesthesia/analgesia or as a supplement to parenteral therapy.

Performing procedures under local anaesthesia alone is associated with considerable stress for the animals. For this reason, local anaesthesia is almost always combined with general anaesthesia.

An exception is the administration of creams or patches containing a local anaesthetic to numb the skin (e.g., for vessels catheterisation).

Frequently used local anaesthetics are lidocaine and bupivacaine, but also ropivacaine, procaine, tetracaine and mepivacaine.

The following are used as local analgesic procedures:

- Surface anaesthesia: skin, cornea, mucous membranes (all species)
- Infiltration anaesthesia: skin (all species)
- Conductive anaesthesia: e.g., plexus block (from rat size upwards)
- Central anaesthesia: e.g., epidural anaesthesia: puncture of the epidural space and instillation of local anaesthetics, morphine, ketamine or alpha-2 agonists (from rat size upwards)

Particular caution is required with small laboratory animals because of the risk of overdosing.

In general, the use of local anaesthetics in combination with vasoconstrictive agents (e.g. adrenaline, noradrenaline) on the extremities must be avoided. The vasoconstrictive effects can lead to disturbances of blood flow to circulatory with corresponding complications in the region of the terminal vessels.

5.3. Preventive analgesia: aspects specific to laboratory animals

In the case of surgical or painful procedures, preventive analgesia that includes a) pre-emptive, b) intraoperative and c) postoperative analgesia must be planned, to effectively cover the chronological development of pain and the estimated intensity. This is regarded as an integral part of balanced anaesthesia and includes the postoperative phase.

Preventive analgesia is intended to prevent the wind-up phenomenon and the development of secondary hyperalgesia. For this reason, this has a positive effect on the perception of pain following the return to consciousness; in addition, postoperative recovery may also be improved (Clark 2014). It should be used in all experiments with recovery whenever possible.

a) Pre-emptive analgesia: the analgesic should already be effective before the painful procedure and, in this sense, may be administered before the procedure as a part of sedative premedication (= analgosedation). Aside from opioid analgesics (e.g. fentanyl, morphine, buprenorphine), so-called adjuvant analgesics - sedatives with an analgesic effect, such as alpha-2 agonists (e.g. xylazine, medetomidine) or dissociative anaesthetics (e.g. ketamine, esketamine) - may also be used. In addition, analgosedation has the positive synergistic effect of reducing the need for anaesthetics to maintain an adequate anaesthesia for surgical procedures.

   If a partial μ-opioid receptor agonist such as buprenorphine is used in premedication, the effect of a full μ-opioid receptor agonist, such as fentanyl, is reduced as a result of the higher receptor affinity of buprenorphine.

b) Intraoperative analgesia: The optimum intraoperative analgesia is achieved through the use of a balanced anaesthesia in which various anaesthetics and analgesics are combined using different forms of administration. Synergistic and complementary effects of the administered drugs are exploited here to achieve the main components of anaesthesia, namely hypnosis, muscle relaxation and intraoperative analgesia, and thus reduce the adverse effects of anaesthesia as well as peri-anaesthetic mortality. Ketamine

plays an important role with its dual effect as an anaesthetic with analgesic properties; with relatively low, sub-anaesthetic dosing, it reduces or prevents central sensitization with hyperalgesia and/or allodynia for up to several hours. Postoperative pain is reduced (Qian et al. 1996; Richebe et al. 2005). The use of balanced anaesthesia is the gold standard for painful procedures in experiments with recovery.

The necessary intraoperative analgesia can also be achieved by means of mono-anaesthesia (where only an injectable or inhalable hypnotic is used), if the anaesthesia is deep enough to eliminate cortical perception. This should be monitored using clinical and/or instrumental, species-specific methods. At the spinal level, however, there is overexcitation of the intersynaptic neurons ("wind-up") and spread of hyperactivity and nociceptive activity to other CNS segments. When the animal wakes up, the pain will be intense, making it more difficult to control. For this reason, for painful procedures, mono-anaesthesia should only be used for terminal experiments (non-recovery) with short anaesthesia times. Since the depth of anaesthesia is dose-dependent, a correspondingly sufficient depth over a longer period of time can lead to severe respiratory and circulatory depression, especially with injectable mono-anaesthetics (e.g., propofol, alfaxalone, pentobarbital and etomidate) because of the narrow therapeutic index; this can have a relevant influence on the scientific data obtained and is associated with a high attrition rate due to deaths.

The possibility of an opioid-induced respiratory depression must be considered when administering sedatives and/or hypnotics concurrently or over the course of anaesthesia. Preference should therefore be given to the use of controllable inhalation anaesthetics (isoflurane, sevoflurane) or intravenous anaesthetics such as propofol to induce and maintain general anaesthesia. For larger animals, intubation and ventilation options should be available, especially during longer periods of anaesthesia.

Regardless of whether inhalation or injection anaesthesia is used, the supply of oxygen is obligatory because general anaesthesia can lead to more or less severe respiratory depression with consequent hypoxia. This can at least be reduced by increasing the inspiratory oxygen concentration. In the event of marked hypoventilation with severe hypercapnia, oxygenation and CO_2 expiration can only be guaranteed through assisted or controlled ventilation.

c) Postoperative analgesia: Depending on the intensity of the pain and the model, NSAIDs and opioids may be administered. Thanks to the high receptor affinity of buprenorphine it can be used to antagonize the sedative and respiratory depressive effect of fentanyl, while ensuring continued analgesia is maintained at the same time. However, if a pure opioid antagonist such as naloxone is used to antagonize fentanyl, e.g. in the case of fully antagonizable anaesthesia with midazolam, medetomidine and fentanyl, this also cancels out the analgesic effect of fentanyl and endogenous opioids. An appropriate analgesic must therefore be administered in good time (before antagonization).

Further information on the anaesthesia of rodents and rabbits in animal experiments can be found under **Recommendations on anaesthesia methodologies for animal experimentation in rodents and rabbits** of the GV-SOLAS website.

5.4. Accompanying measures

Since pain is modulated by stress, a stress-free environment and stress-free handling of the animal, i.e. generally anything that promotes the wellbeing of the animal is very important.

Non-pharmacological measures

- Acclimatization, handling, and training reduce stress (Conour et al. 2006; Obernier & Baldwin 2006).
- Health status: only animals that are fit for experiments may be sued for research (Annex III EU Directive 2010/68) (COUNCIL 2010)
- Atraumatic surgical methods, i.e., mastery of the relevant surgical approach (tissue-conserving surgery, short operation, tension-free sutures, little drainage), use of minimally invasive procedures
- Asepsis (University NC 2019)
- Adequate perioperative monitoring and care (fluid therapy, positioning, eye cream)
- Active pre-, intra- and postoperative thermal management and oxygen supply
- Keeping the animals in familiar groups and/or in familiar surroundings as far as possible. In the case of small rodents, a change of cage immediately after a surgical procedure should be avoided. The understandable thought of wanting to do something good for an animal that has just undergone surgery by putting it in a fresh, clean cage means in practice that the animal is subject to increased stress in the waking phase because it cannot wake up in its familiar surroundings with the familiar smells. Small rodents should therefore always wake up in the old cage they are familiar with.
- Soft flooring and/or bedding, accustomed nest, removal of cage fittings on which the animal could injure itself, etc.
- Feeding: wet feed and fluid in gel form on the floor for rodents, palatable feed in small portions to cover the increased calorie requirements
- Measures/food to support gut activity especially in rabbits
- Urinary bladder control (should be emptied towards the end of anaesthesia)

Pharmacological measures (adjuvants)

Pharmakon		Use		Indication
Scopolamine + metamizole (Buscopan compositum®)		**Rabbit**	0.1-0.2 ml/kg i.v., i.m., s.c.	Spastic colic, gastroenteritis, tympani (ruminant), spasms in the urogenital area
		Dog, cat	Dog single dose of 0.1 ml/kg i.v., i.m.	
		Sheep, pig	single dose of 0.1 ml/kg i.v., i.m.	
Perphenazine		**Rodent**	5 mg/kg s.c. duration of action usually several days (Pachtner 1998) Rat Perphenazine enanthate (Decentan-Depot©, Merck, Darmstadt) for i.m. injection at 5 mg/kg BW in 1% solution in medium-chain triglycerides (Miglyol 812©, Caesar & Loretz, Hilden) (total injection volume: 0.2 ml). First i.m. injection one day before the operation; further injections at intervals of 3 days over a prolonged period.	Prevention of self-mutilation, phantom pain
Steroidal anti-inflammatory drugs	Prednisolone	**Rodent**	1-2 mg/kg every 24 h i.v., s.c.	in inflammatory oedematous processes Not in combination with NSAIDs
		Rabbit	1-2 mg/kg every 24 h i.v., s.c.	
		Dog, cat	Dog 0.5-1 mg/kg every 12-24 h, then every 48 h i.v., i.m., p.o. Cat 2.2 mg/kg every 12-24 h, then every 48 h i.v., i.m., p.o.	
		Sheep, pig	Ruminant, pig 0.5 mg/kg i.m.	
	Dexamethasone	**Rodent**	0.2 mg/kg every 24 h i.v., s.c.	
		Rabbit	0.2 mg/kg every 24 h i.v., s.c.	
		Dog, cat	Dog 0,1-0,2 mg/kg every 12-24h i.v., i.m., p.o. anti-inflammatory anti-allergic: 0.1 - 0.5 mg/kg i.m. or i.v. cerebral and spinal cord oedema post trauma/discopathy/tumour: initially 2-3 mg/kg i.v., then taper to 0.2 mg/kg per day Cat 0.1-0.2 mg/kg every 12-24h i.v., i.m., p.o.	
		Sheep, pig	Ruminant, pig 0.06 mg/kg i.v., i.m.	

5.5. Analgesia in foetuses and neonates: thoughts and suggestions

Prenatal and neonatal rodents, pigs / minipigs, rabbits and sheep are frequently used as experimental models in biomedical research.

Knowledge of species-specific, age-dependent developmental stages is important not only for the selection of suitable animal models, but also for predicting potential differences in the pharmacology and pathophysiology of pain since the developmental state at birth is highly species-specific. Altricial animals (e.g., rats, mice, rabbits), which remain in the nest for a long time, are more similar to humans than precocial animals (e.g., guinea pigs, mini-pigs, sheep). Mice and rats are considered neonates until the 10th day after birth (PN10); minipigs until the age of two weeks.

Physiology and pathophysiology of pain

In humans, it is often reported that the ability of the foetus to feel pain develops in the last trimester, when the cortex becomes functional and the thalamocortical tract develops. This occurs after the 24th week of gestation. However, recent findings (Derbyshire & Bockmann 2020) suggest that nociception may develop as early as the 12th week of gestation.

It is not known when developing animals acquire the ability to feel pain. The reflex-like withdrawal of the limb in response to a noxious stimulus can already be observed in rodents in the late stage of gestation, for example in the rat foetus from day 17 (E17, for “embryonic day 17”) of gestation (Narayanan et al., 1971). Behavioural responses to the injection of an irritant substance (formalin) into the paw can be observed in the rat foetus from E19. Furthermore, this response correlates with expression of the c-fos protein, an indicator of neuronal activity, in the spinal cord from E20 onwards (Research 2003).

At the time of birth, neural substrates for the perception of noxious stimuli are present in the peripheral nervous system and spinal cord of rat neonates, but the corresponding sensory systems are not yet mature and undergo significant restructuring during the first weeks after birth. From the very first day after birth, young rats already show behavioural agitation and avoidance reactions to noxious, thermal and mechanical stimuli. From the age of 3 weeks, the response of rat pups to analgesics is similar to that of an adult rat. This period corresponds to the maturation of the descending supraspinal inhibitory system (Barr & Rossi 1992, Fujinaga et al. 2000).

Exposing neonates to pain early on already could affect the development of the central and peripheral nervous system and influence both behaviour and pain tolerance in adulthood (Page 2004). Studies in rats before they reach weaning age have shown that various characteristics of the developing nervous system may serve to enhance sensory and pain-associated responses in very young rats (Fitzgerald 1985; Reynolds & Fitzgerald 1995).

Analgesia

Based on the aforementioned physiological and pathophysiological arguments, all neonates should receive adequate pain therapy if they undergo procedures that are likely to be painful. In the case of studies which are conducted in foetuses or which involve foetuses, the wellbeing of both the mother and of the foetus must be taken into account accordingly.

Unfortunately, there are various challenges in the practical observance of these requirements that make it difficult to ensure safe, effective, and monitored pain management for the foetus and neonate:

- The small body size of neonates and foetuses, especially in rodents, limits the possible administration routes and volumes. If possible, therefore, oral administration should be considered (if necessary, also via breast milk), followed by rectal and finally systemic administration.
- There are significant physiological differences between neonates and adults, such as the higher permeability of the blood-brain barrier, the proportionally higher water content in the total body mass, the fewer mature and hence fully functional liver enzymes, or the lower serum albumin concentration. These differences have an influence on the pharmacodynamics and pharmacokinetics of analgesics and anaesthetics. Moreover, neonates are inherently more susceptible to cardiovascular and respiratory depression, which makes the line between effective, safe dosing and overdose much narrower than in the adult animal.
- Knowledge of pharmacokinetics and pharmacodynamics in the target species is very limited. Therefore, it is difficult to establish satisfactory guidelines regarding dosage, treatment interval and frequency and to ensure that the treatment is effective.
- The difficulty of identifying and recognizing pain in neonates; pain-related behaviours are much more general and non-specific in neonates than in adult animals. Moreover, in the case of rodents, neonates are usually in the nest or hidden by the mother, which makes observation and diagnosis much more difficult. In humans, pain in neonates is associated with general hyperactivity, among other things.
- Repeated interactions cause stress both in the young and in the mother animal, which can lead to neglect of the young by the mother or cannibalism, and last but not least also can negatively impact the maturation of the neuroendocrine and immunological systems (Page 2004).

As regards the main classes of analgesics in use, namely NSAIDs, opioids and local anaesthetics, certain adjustments must be made to the physiology of neonates before their use.

Prostaglandins play a major role in the development of neonates. Since the use of NSAIDs leads to a reduction in the concentration of various prostaglandins through the inhibition of cyclooxygenase activity, the development of the animals can expect to be affected when NSAIDs are used (Finkel 2007).

Opioids ensure effective analgesia with respect to thermal, inflammatory and mechanical pain in new-born rodents from PN1 onwards (McLaughlin & Dewey 1994; Barr 1999). Numerous reference pages of the IACUC list buprenorphine as the preferred choice for the analgesic treatment of new-born mice or rats using opioids. This is because the unusual receptor affinities of buprenorphine mean that it is safer to use (Kongstorp et al. 2020) and exerts a less marked respiratory depressive effect. However, there is a lack of robust data on doses and treatment intervals in these target species. The use of depot preparations that release buprenorphine over a prolonged period could ensure a lasting, continuous and sufficient degree of analgesia, while avoiding the stress that is inevitably associated with regular handling in the case of repeated dosing; but here too, according to the current level of knowledge, there is a lack of robust evidence.

Local anaesthesia should be a standard part of multimodal pain management in neonates. The dose of local anaesthetic should be reduced to 50% of the dose per kg of bodyweight typically used in adults (Morton 2004). The small size of animal neonates means that, in all probability, instillation or infiltration are the only feasible forms of administration.

According to current knowledge and based on pathophysiological evidence, pregnant animals or neonates used in studies that may cause noteworthy pain must be given appropriate analgesic treatment. Opioids and local anaesthetics can be a useful for this purpose. There is an urgent need for research that satisfactorily plugs existing gaps in knowledge in the areas of pharmacokinetics, efficacy, and safety, and generates innovative solutions for the use of analgesics in neonates.

Adequate anaesthesia for neonates and foetuses is beyond the scope of the contents in this recommendation.

6. Influence of analgesics on results of animal experiments

The examples listed in section 3 show how untreated pain affects organs, physiology and behaviour. Pain resulting from surgical trauma and painful diseases are complex in nature and are not easy to predict or control. Abstaining from pain management to reduce the side effects of analgesics is a double-edged sword and only justified in a few cases (Jirkof 2017; Peterson et al. 2017). Basically, it can be said that, for ethical and scientific reasons, analgesics must be used in cases of suspected or actually identifiable pain, as well as during and after painful procedures. An optimum analgesia protocol should reliably relieve pain and have minimal side effects that could compromise animal welfare. In addition, it should have a controllable effect on the specific scientific read out. Rigorous selection of the best analgesia protocol for a given research question is therefore essential and is best done with the help of an expert in veterinary analgesia. In view of the many aspects that need to be considered, e.g., animal species, nature of the pain, the “question” or “objective of the research project”, it is difficult to make a general statement on the suitability of certain substances for a specific question or a certain experiment. An extensive literature search on the efficacy and side effects of certain substances is therefore an important part of the experimental design.

The possible side effects of treatment for pain or of failure to treat it should be known to everyone who conducts experiments. However, with careful selection of analgesia protocols, the effects of analgesia can be controlled and standardized to a certain extent. If information on side effects of new analgesia protocols is lacking in certain experiments, it may be advisable to involve an analgesia control group. Publications based on such applied approaches can provide the scientific community with valuable information. It must be borne in mind that full reporting on analgesic measures in scientific publications is an important prerequisite for reproducible animal experiments (Carbone & Austin 2016).

Therefore, only general information on frequently used substance classes is given below.

Opioid analgesics

Opioids are effective analgesics that exert their pharmacological action by binding to and activating specific opioid receptors, which are widely distributed in the nervous system and the

gastrointestinal tract. The pain-relieving effect of opioid analgesics is induced by means of two mechanisms: inhibition of pain transmission and suppression of the affective discrimination of pain. As a result of the abundance and differences in receptor properties, the side effects of opioids vary widely and include constipation, respiratory depression, nausea and urinary retention, as well as addiction, tolerance and hyperalgesia (Aronson 2010; Sehgal et al. 2011; Williams et al. 2013).

A much-discussed topic is the immunomodulating effect of some opioids. Immunomodulation refers to substances that can alter the immune function by influencing the generation, function and maturation of immune cells through various mechanisms. These mechanisms have been described both in humans and in laboratory animals. While tramadol and buprenorphine have been reported to have little or no effect, fentanyl and morphine appear to significantly reduce immune system activity (Sacerdote 2008; Al-Hashimi et al. 2013). These effects should be considered if the immune response is of interest in experiments.

The effect of opioids on carcinogenesis, which has been demonstrated in animal experiments, must be taken into account when planning pain treatment in oncological models. For example, tramadol appears to inhibit the proliferation, migration and invasion of breast cancer cells in mice (Xia et al. 2016), whereas fentanyl inhibits tumour growth and cell invasion in colon cancer (Zhang et al. 2015).

Although the side effects listed above may only be relevant to specific scientific questions, other side effects of opioids may affect the general condition of an animal and are therefore of interest for many areas of research. Opioid-induced respiratory depression and other opioid-related respiratory reactions are known side effects of opioid treatment and are caused by the activation of opioid receptors expressed in the respiratory centres of the brain stem (van der Schier et al. 2014). These effects can cause complications during anaesthesia and the following recovery phase. Reduction in food intake and bodyweight gain are known side effects of opioids such as buprenorphine in rodents (Bomzon 2006; Jirkof et al. 2015). Opioids cause nausea and vomiting in humans. Pica behaviour (also known as allotriophagy or geophagy) in rodents, especially rats, corresponds to the symptom of vomiting in other species. It involves eating non-nutritive substances, mostly litter or nesting material (Takeda et al. 1993; Clark et al. 1997).

NSAIDs and coxibs

Non-steroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of organic acids which may have analgesic, antipyretic, anti-inflammatory and/or antiplatelet effects, depending on the active substance. The primary mechanism of action is the inhibition of cyclooxygenase (COX) activity followed by inhibition of the synthesis of prostaglandins, which play an important role as mediators of inflammation. Two enzymes are involved: COX1, which is expressed in many cells and tissues, is important for normal homeostasis, while COX2 is induced mainly and selectively by inflammatory cytokines at the site of inflammation. Selective COX2 inhibitors, also known as coxibs, are assumed to cause fewer side effects and, in particular, less gastrointestinal toxicity. However, treatment with coxibs may carry an increased risk for cardiovascular side effects. Most cardiovascular and gastrointestinal side effects (particularly intestinal ulcers with melaena) (Strub et al. 1982) and the renal toxicity of some NSAIDs and coxibs are mainly associated with chronic use (Aronson 2010; Mathiesen et al. 2014).

Although the administration of NSAIDs before a surgical procedure can have beneficial effects, such as reducing inflammation at the site of surgery and alleviating pain after the procedure, NSAIDs cannot be unreservedly recommended for pre-surgical analgesia in view of their potential effect on platelet function and the resulting risk of perioperative bleeding (Mathiesen et al. 2014). Excessive perioperative bleeding can lead to longer surgery and anaesthesia times and an increased risk of complications. However, not all NSAIDs irreversibly inhibit platelet function. Platelets are susceptible to COX1 inhibition, so perioperative administration of coxibs may be a safer alternative to conventional NSAIDs in surgical procedures where increased bleeding may be a problem (Wickerts et al. 2011).

Several studies have shown that NSAIDs, coxibs and aspirin help in the prevention of cancer (Umar et al. 2016). In particular, the inhibitory effect of meloxicam on primary tumour growth has been demonstrated in a mouse model with hepatocellular or ovarian carcinoma (Xin et al. 2007) and reported to have an antimetastatic effect in an orthotopic model of osteosarcoma (Husmann et al. 2015).

Inflammation that involves prostaglandin synthesis by COX2 is an essential step in fracture healing. Data from animal studies suggest that NSAIDs and coxibs may delay fracture healing. However, results from clinical trials in humans on the effect of COX2 inhibition on fracture healing are inconclusive and suggest that effects are reversible and depend on duration and dosage (Wickerts et al. 2011; Pountos et al. 2012).

Non-acidic antipyretic analgesics

Paracetamol (acetaminophen), acetylsalicylic acid (aspirin) and metamizole are so-called non-acidic antipyretic analgesics and are commonly used to treat mild to moderate acute pain and fever. Antipyretics and NSAIDs share a mechanism of action: inhibition of COX1 and COX2. Unlike NSAIDs, antipyretics inhibit prostaglandin synthesis primarily in the central nervous system and only to a minor extent in the periphery. This central effect is also the basis of their antipyretic action. Details concerning paracetamol's mechanism of analgesic action remain to be clarified (Aronson 2010). The side effects of acetylsalicylic acid include damage to the gastrointestinal mucosa, inhibition of platelet aggregation and, at high doses, an increased bleeding propensity and hepatotoxicity. Unlike acetylsalicylic acid, paracetamol does not have a major effect on platelets or inflammation and may therefore be the preferred non-opioid analgesic when surgical bleeding is a problem (Smith 2011). It has no effect on the gastric mucosa and therefore cannot therefore lead to the stomach ulcers caused by many COX inhibitors. At high doses or when hepatic activity is impaired, the potential lethal hepatotoxicity of the metabolite N-acetyl-p-benzoquinonimine should be considered as an adverse effect.

Metamizole is recommended for the treatment of mild to moderate pain, especially abdominal pain and exerts a spasmolytic effect. Generally, metamizole has low potential for side effects. Known adverse reactions are gastrointestinal effects, vasodilatation with hypotension when administered rapidly by intravenous injection and, in rare cases during chronic administration of metamizole in humans, severe agranulocytosis. There are no known reports of agranulocytosis associated with metamizole administration in laboratory animals (Jasiecka et al. 2014).

7. Dosage tables

Dosages must be adapted to the respective clinical situation and requirements of the experiment.

The recommendations and dosages given here are based on the current literature, state of the art and expert knowledge. The information in these tables are examples and lay no claim to be an exhaustive list.

Side effects and influences on the results of the experiments must be individually researched in the current literature.

7.1. Dosage table mouse

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	5-20	s.c.	12 h	(Ingrao et al. 2013; Foley et al. 2019)
	10-25	p.o. in DW	continuous	(Cho et al. 2019)
Flunixin meglumine	5	s.c.	12 h	(Arras et al. 2007)
Meloxicam	2-5	s.c.	6-12 h	Duration and analgesia subject to debate (Ingrao et al. 2013; Chen et al. 2016; Foley et al. 2019)
	10-20	p.o.	12 h	
Paracetamol	200	s.c.	2-4 h	Depending on model
	3.5 mg/ml drinking water	p.o. in DW	continuous	Suitable e.g. paediatric syrup, sweet flavour (Fleischmann et al. 2017)
Buprenorphine	0.1	s.c.	4-6 h	Pre-emptively 20-30 min before the start of the intervention To be re-dosed depending on the intensity of pain and the model
	1	p.o. in DW	continuous	In the light phase, s.c. injections should additionally be given in the case of severe pain. Dosage and dosing interval as above. (Jirkof et al. 2019)
Buprenorphine + meloxicam	0.1 + 5	s.c.	≤12 h	

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Buprenorphine + carprofen	0.1 + 5	s.c.	≤12 h	(Parker et al. 2011b)
Tramadol	0.1-1 mg/ml drinking water	p.o. in DW	continuous	Dosage in drinking water depending on model Pre-emptive for invasive procedures s.c. injection or tramadol recommended (Evangelista-Vaz et al. 2018; Jirkof et al. 2019)
	25	s.c.	approx. 2 h	e.g. 10 min. before start of procedure
Tramadol + paracetamol	1 mg tramadol + 3.5 mg paracetamol in 1 ml drinking water	p.o. in DW	continuous	(Jirkof et al. 2018)
Bupivacaine	1-2 (max. 8)	Infiltration anaesthesia of tissue before/after an incision Splash application directly on wound or mucosa	Onset of effect after approx. 15 min. Duration of effect approx. 4-8 h	Bupivacaine 0.5%; 50 µl Bupivacaine should be diluted to 0.25% to obtain a larger volume (Source: UC Denver)
Lidocaine	2-4 (max. 7)	Infiltration anaesthesia of skin before/after an incision Splash application directly on wound or mucosa	Onset of effect after approx. 5-10 min. Duration of effect approx. 30 min	Lidocaine (1-2%); lidocaine 0.5 / 1% 50 µl Lidocaine should be diluted to 0.5% to obtain a larger volume (Source: UC Denver)
Combinations of lidocaine and bupivacaine	10 lidocaine and 2-5 bupivacaine	Infiltration anaesthesia of skin before/after an incision Splash application directly on wound or mucosa	Onset of effect after approx. 5-10 min. Duration of effect approx. 1 h	diluted with NaCl (50:50)
Ropivacaine	1-2 (max. 8)	s.c., tissue (infiltration, splash)	Onset of effect after approx. 15 min. Duration of effect approx. 4-8 h	Ropivacaine (0.2% Naropin®); ropivacaine 0.5%; 50 µl Ropivacaine is not diluted beforehand here (Source: UC Denver)
Metamizole	1.25 mg/ml drinking water	p.o. in DW	continuous	Note: bitter taste with some commercially available metamizole products; sweetening may be useful (Kumstel et al. 2020)

7.2. Dosage table rat

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	2-5	s.c.	12-24 h	Effect is subject of debate (Waite et al. 2015; Foley et al. 2019)
Flunixin - meglumine	1-2.5	s.c.	12 h	
Ibuprofen	20	p.o.	12 h	max. 72 h
	0.2 mg/ml	p.o. in DW	continuous	max. 72 h
Ketoprofen	5	s.c.		(Flecknell et al. 1999)
Meloxicam	1-2	s.c., p.o.	12 h	Duration of effect (up to 24 h) is subject to debate
Paracetamol	200-300	p.o.		(Ince et al. 2015; Foley et al. 2019)
	2-4.5 mg/ml in drinking water	p.o. in DW	continuous	
Buprenorphine	0.05	s.c.	4-6 h	(Hestehave et al. 2017)
	1 drop (0.3 mg/ml)	p.o.	4-6 h	
	0.5	p.o. in DW	continuous	6-9 mg/l drinking water
Butorphanol	2	s.c.	4 h	
Fentanyl	0.03	i.v.	2 h	Ohtsuka et al. 2007)
Morphine	2.5 – 5	s.c., i.m.	4 h	(Hestehave et al. 2019)
Pethidine	15	i.m.		(Lascelles et al. 1995)
Piritramide	0.3	s.c.		
Tramadol	30	s.c.	continuous	(Taylor et al. 2016)
Tramadol	0.5 g/l in drinking water	p.o. in DW		(Taylor et al. 2016)
Bupivacaine	5	s.c.	4 -6 h	
Lidocaine	10	s.c., tissue (infiltration, splash)	20-40 min	
Ropivacaine	2	s.c., tissue (infiltration, splash)		(Charlet et al. 2011)
Ketamine	4	i.p., i.m.	30-60 min	(Nadeson et al. 2002)
Metamizole	100-250	s.c., p.o.	6 h	(Ince et al. 2015)
Grapiprant	1-133	p.o.	4 h	
Gabapentin	55	p.o.	4 h	(Vollmer et al. 1986; Radulovic et al. 1995)
	100	i.p.	12 h	(Ma et al. 2011)

7.3. Dosage table rabbit

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	4-5	i.v., s.c., p.o.	24 h	Effect is subject of debate (Wenger 2012; Hedenqvist et al. 2016; Benato et al. 2019)
Meloxicam Fentanyl	0,3-1,5	s.c., p.o.	24 h	(Turner et al. 2006a; Turner et al. 2006b; Fredholm et al. 2013)
	1-10 µg/kg	i.v.	2-4 h	
Tramadol	Patch 3 µg/kg	transcutaneous	Administration 24 h before procedure, duration of effect up to 72 h	Do not use depilation cream (Foley et al. 2001) Plasma concentration and effect are subject to debate (Souza et al. 2008; Udegbunam et al. 2015)
	10-20	p.o.	12 h	
Bupivacain	5	i.v., sc.		Lokale Infiltration, loko-regionale Analgesie, Infiltrationskatheter
Bupivacaine Lidocaine	0,5-2	s.c., tissue (infiltration, splash)	4-6 h	Local infiltration, locoregional analgesia, infiltration catheter Local infiltration, locoregional analgesia, infiltration catheter (Schnellbacher et al. 2017)
	2-4	s.c., tissue (infiltration, splash)	2 h	
Prilocain + Lidocain (EMLA Creme®)	Drip 100 µg/kg/min	i.v.	48 h	(Keating et al. 2012)
Prilocaine + lidocaine (EMLA Creme®)	1 mm thick	transcutaneous	20 min beforehand	(Keating et al. 2012)

7.4. Dosage table primates *Macaca spp* (M) and *Callitrix jaccus* (Cj)

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	M: 2	p.o., s.c., i.m.	12 h	
Flunixin meglumine	M: 2	i.m.	12 h	
Ketoprofen	M: 2	i.m., i.v., s.c.	24 h	
Ketorolac	M: 15-30 mg/animal initially; then 10-15 mg/animal		8 h	

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Meloxicam	M: 0.2	p.o., s.c., i.v.	24 h	
Paracetamol	M: 6 10	p.o. i.v.	6 h	
Buprenorphine	M: 5-20 µg/kg Cj: 5-10 µg/kg	s.c., i.m., i.v.	6 h	
Butorphanol	M: 0.05 Cj: 0.02	s.c.	4-6 h	Sedative, may induce respiratory depression
Fentanyl	M: 3-20 µg/kg	i.v., i.m.	60 min	Sedative, may induce respiratory depression
	Drip periop: 7-10 µg/kg/h i.v.	i.v.	as needed	
	Patch: 25 µg/5-10 kg, 50 µg/10 kg animal	transcutaneous	Administration at least 12 h before procedure; 48-72 h	Make sure the animal does not remove it
Morphine	M: 0,5-2	s.c., i.m.	6 h	
Tramadol	M: 2-4	p.o	12 h	
Bupivacaine	up to 4	s.c., tissue (infiltration, splash)	4 h	
Lidocaine	up to 2	s.c., tissue (infiltration, splash)	2 h	

7.5. Dosage table dog

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	4	s.c., i.v., p.o.	24 h	May be administered in divided doses
Cimicoxib	2	p.o.	24 h	Use for up to 90 days
Meloxicam	First dose 0.2; further doses 0.1	s.c., p.o., possibly also i.v.	24 h	
Robenacoxib	1-2	s.c., p.o.	24 h	On empty stomach
Buprenorphine	0.01-0.03	i.m., i.v.	4-6 h	
Butorphanol	0.2-0.4	s.c., i.m., i.v., p.o.	1-2 h	Antitussive effect
Fentanyl	Bolus: 1-10 µg/kg	i.v.	approx. 30 min	Short-lasting action makes it useful as continuous drip
	Drip periop: 10-36 µg/kg/h	i.v.		

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
	Patch 3 µg/kg/h	transcutaneous	Administer at least 12 h before procedure; duration of effect up to 72h	No skin depot; apply new patch 12 h in advance
Methadone	0.1-0.5, up to 1.0	s.c., i.m., i.v.	3-4 h	
Morphine	0.1-0.5, up to 1.0	s.c., i.m., i.v.	3-4 h	Administer slowly i.v. in view of histamine release
Tramadol	1-5	i.v., p.o.	8 h	Administer slowly i.v. Effect and duration of effect are subject to debate
Bupivacaine	up to 2 per 24 h	s.c., tissue (infiltration, splash)	4-6 h	Do not administer i.v.
Lidocaine	max. 8	s.c., i.m., i.v. tissue	30-120 min	
Ropivacaine	up to 5 per 24 h	s.c., tissue (infiltration, splash)	4-6 h	Epidural/spinal less motor blockade
Ketamine	Bolus: 1-5	s.c., i.m., i.v.	20-40 min	Dissociative effect markedly longer
	Drip periop: 10-30 µg/kg/min Drip postop: 2-5 µg/kg/min	i.v.	as needed	
Metamizole	20-50	s.c., i.m., p.o.	4 h	
Grapiprant	2	p.o.	24 h	Chronic and/or neuropathic pain
Gabapentin	5-15	p.o.	8-12 h	Chronic and/or neuropathic pain
Amantadine	2-3	p.o.	24 h	Chronic and/or neuropathic pain

7.6. Dosage table pig

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Acetylsalicylic acid	10	p.o.	4-6 h	
Carprofen	4	s.c., p.o., i.v.	24 h	
Flunixin meglumine	2.2	i.m.	24 h	Single administration recommended; second administration possible
Ketoprofen	3	i.m.		Single administration
Meloxicam	0.4	i.m., p.o., i.v.	24 h	
Phenylbutazone	10	p.o.	12 h	

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Buprenorphine	0.01-0.05	s.c., i.m., i.v.	4-6 h	
Butorphanol	0.2	s.c., i.m., i.v.	2-4 h	
Fentanyl	5 µg/kg	i.v.	20-30 min	Intraoperative in combination with anaesthetics; dose must be adjusted according to substance combination
	Drip periop: 10-50 µg/kg/h	i.v.	Continuous drip	
	Drip postop: 3-10 µg/kg/h	i.v.	Continuous drip	
	Patch 3-4 µg/kg/h	transcutaneous	Administer at least 12 h before procedure; duration of effect up to 72h	No skin depot; apply new patch 12 h in advance (Osorio Lujan et al. 2017)
Methadone	0.3	i.v., i.m.	4 h	
Morphine	0.2	i.v., i.m.	4 h	Administer slowly i.v.
Pethidine	2	i.m., i.v.	2 h	Maximum 1g/animal
Piritramide	0.1-0.5	s.c., i.v.	2-3 h	
Sufentanyl	Bolus 5 µg/kg	i.v.	5-10 min	Intraoperative in combination with anaesthetics Dose must be adjusted according to substance combination
	Drip 5-10 µg/kg/h	i.v.		
Tramadol	5	p.o.	6-8 h	Effect and duration of effect are subject to debate
Bupivacaine	1-2	s.c., tissue (infiltration, splash)	4-6 h	Do not administer i.v.
Lidocaine	2-4	s.c., tissue (infiltration, splash)	30-60 min	
Ropivacaine	1-2	s.c., tissue (infiltration, splash)	4-6 h	
Ketamine	Bolus 2-5	i.m., i.v.	20-40 min	as adjuvant therapy Dose must be adjusted according to substance combination
	Drip possible 0.6 mg/kg/h	i.v.	as needed	
Metamizole	20-50	i.v., i.m., p.o.	4-6 h	Administer slowly i.v.

7.7. Dosage table cat

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	4	s.c., i.v., p.o.	24 h	May also be given in divided doses and administered 2x/day
Meloxicam	First dose 0.1; further doses 0.05	s.c., p.o., possibly also i.v.	24 h	
Robenacoxib	1-2	s.c., p.o.	24 h	On empty stomach, for a maximum of 6 days
Buprenorphine	0.01-0.02	i.m., i.v., oral transmucosal	4-6 h	
Butorphanol	0.2	s.c., i.m., i.v.	1-2 h	For visceral pain
Fentanyl	Bolus: 1-5 µg/kg	i.v.	approx. 30 min	
	Drip periop: 6-10 µg/kg/h Drip postop: 2-15 µg/kg/h	i.v.	As needed	
	Patch 3 µg/kg/h	transcutaneous	Administer at least 12 h before procedure; duration of effect up to 72h	Skin depot; new patch does not need to be applied in advance
Methadone	0.1-0.4, up to 0.8	s.c., i.m., i.v.	3-4 h	
Morphine	0.1-0.4, up to 0.8	s.c., i.m., i.v.	3-4 h	
Tramadol	1-4 (i.v. 2)	i.v., p.o.	8 h	
Bupivacaine	up to 2 per 24 h	s.c., tissue	4-6 h	Do not administer i.v.
Lidocaine	max. 4-6 / day	s.c., i.m., i.v. tissue	30-120 min	
Ropivacaine	up to 2 per 24 h	s.c., tissue	4-6 h	Epidural/spinal less motor blockade
Ketamine	Bolus: 1-6	s.c., i.m., i.v.	20-40 min	Dissociative effect markedly longer
	Drip periop: 10-30 µg/kg/min Drip postop: 2-5-µg/kg/min	i.v.	as needed	
Metamizole	20-50	s.c., i.m., p.o.	6 h	Oral administration in awake cats may lead to marked salivation
Gabapentin	5-15	p.o.	8-12 h	Chronic and/or neuropathic pain

7.8. Dosage table guinea pig (GP), chinchilla (CHI)

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Acetylsalicylic acid	GP: 50 - 100	p.o.	once	High dose is ototoxic (Neiger-Aeschbacher 2002)
Carprofen	4	s.c.	12-24 h	(Neiger-Aeschbacher 2002) (Oliver et al. 2017)
Flunixin meglumine	GP: 3-5 CHI: 1-3	s.c.	12-24 h	(Fehr et al. 2014)
Meloxicam	0.2 initially, then 0,1 Dose increments up to 0.6 possible	p.o.	24 h	
Paracetamol	GP: 60-120	p.o, i.p.	24 h	Approved for GP in Germany in 2018
	1-2 mg/ml drinking water	p.o. in DW	continuous	
Buprenorphine	0.05-0.1	s.c., i.p.	6-12 h	(Mueller 2018)
Butorphanol	GP: 0.5-1	s.c.	4-6 h	
Metamizole	100-200	p.o.	every 4-6 h	

7.9. Dosage table ferret

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Carprofen	4	s.c., i.v., p.o.	24 h	May also be given in divided doses and administered 2x/day
Meloxicam	0.2	s.c., p.o.	24 h	
Buprenorphine	0.01-0.05	i.m., i.v.	4-6 h	
Butorphanol	0.1-0.4	s.c., i.m., i.v.	1-2 h	
Fentanyl	Bolus: 1-5 µg/kg	i.v.	approx. 30 min	
	Drip: 6-20 µg/kg/h	i.v.	as needed	Short-lasting action makes it useful as continuous drip
Methadone	0.1-0.5	s.c., i.m.	3-4 h	
Morphine	0.1-0.6	s.c., i.m.	3-4 h	
Bupivacaine	max. 2 / day	s.c., i.m., tissue	4-6 h	Do not administer i.v.
Lidocaine	max. 4-6 / day	s.c., i.m., i.v. tissue	30-120 min	
Ketamine	Bolus: 2-10, up to 20	s.c., i.m., i.v.	20-40 min	Duration of effect is dose-dependent, avoid i.m. injection in small animals

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Ketamine	Drip periop: 10-30-µg/kg/min Drip postop: 2-5 µg/kg/min	i.v.	as needed	
Metamizole	20-50	s.c., i.m., p.o.	4-6 h	

7.10. Dosage table sheep and goat

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Acetylsalicylic acid	50-100	p.o.	6-12 h	
Carprofen	1.4 - 4	s.c., i.v.	24 h	
Flunixin meglumine	2,2	i.v., i.m.	12-24 h	Maximum 5 days
Ketoprofen	3	i.m., i.v.	24 h	Maximum 3 days
Meloxicam	0.5	s.c., i.v., i.m., p.o.	24 h	Maximum 3 days
Phenylbutazone	10-20 initially, then 2.5-5	p.o.	24 h	
Tolfenamic acid	2	i.m.	24 h	
Buprenorphine	1-10 µg/kg	s.c., i.m., i.v.	4-6 h	
Butorphanol	0.05-0.2	s.c., i.m., i.v.	2-3 h	With benzodiazepine as premedication
Methadone	0.2-0.5	i.m., i.v.	2-4 h	
Fentanyl	Bolus 5 µg/kg	i.v.	20-30 min	Intraoperative in combination with anaesthetics Dose must be adjusted according to substance combination
	Drip 10-20 µg/kg/h	i.v.	Continuous drip	
Morphine	0.2-0.5	i.m., i.v.	2-4 h	Administer slowly i.v.
Pethidine	2	i.m., i.v.	2 h	
Bupivacaine	1–2	s.c., tissue	4-6 h	
Lidocaine	2-4	s.c., i.m., i.v. tissue	30-60 min	
Ketamine	2-5 up to 10	i.m., i.v.	20-40 min	As adjuvant therapy Dose must be adjusted according to substance combination
	Drip 0.3-0.6 mg/kg/h	i.v.	DTI	
Metamizole	20-50	i.m., p.o., i.v.	4-6 h	

7.11. Dosage table hamster

Substance	Dose (in mg/kg, unless otherwise stated)	Route of administration	Dosing interval	Remarks
Acetylsalicylic acid	240	p.o.	24 h	(Neiger-Aeschbacher 2002)
Carprofen	5	s.c.	24 h	
Meloxicam	0.5	p.o.	24 h	
Flunixin meglumine	2.5	s.c.	24 h	(Emmerich & Hein 2018)
Paracetamol	200	p.o., i.p.	24 h	(Neiger-Aeschbacher 2002)
	1-2 mg/ml drinking water	p.o., drinking water	continuous	
Buprenorphine	0.05-0.1	s.c., i.p.	6-12 h	
Butorphanol	1-2	s.c.	4-6 h	

8. Recommendations for analgesia

The information on the magnitude of pain and suggestions on the duration of therapy are based on the general knowledge and the experience of specialists. The latest findings from the scientific literature (as of 2019) have been included where the relevant publications are available.

The recommendations refer to the conditions in known and established models with an optimum experimental procedure course. The focus here is on postoperative analgesia. Intraoperative analgesia is only addressed in selected cases. In principle, good intraoperative analgesia should be multimodal, mechanistic and balanced. This objective is pursued through the combined use of opiates, ketamine, local anaesthetics and corresponding adjuvants, such as alpha-2 agonists.

The models, procedures and experimental circumstances are often subject to modifications, refinements and many other factors (e.g. genetic modification, the experience of the person conducting the experiment, experimental treatment, multiple interventions), which can alter the expected course and magnitude of pain and stress. This must be taken into account when establishing pain management protocols and the criteria for discontinuing the experiment (humane endpoints). Clinical symptoms should be considered in the monitoring and appropriate measures provided for on the basis of the specific conditions of the experiment (e.g. rescue analgesia; shortening or prolonging the period of pain management, gradual tapering off the pain therapy and discontinuation of the experiment).

8.1. Head

Viscerocranium

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Oral cavity	mostly oral	Stomatitis, gingivitis Procedures on the dental apparatus Jaw bones: implants, partial resections, tumour models Tumours growing on the root of the tongue	Minor in dog, moderate to severe in cat Minor, pulpitis moderate Minor if sufficient stability, moderate to severe in the event of instability and damage to the alveolar nerve Suffering and harm resulting from reduced food intake	3-5 days periop; longer as needed, if clinical symptoms present	Dog Pig Rodent	Local anaesthesia during procedure Anti-inflammatory drugs (steroidal and non-steroidal) Opioids often required in addition if there is irritation of the cranial nerves
Mandibular joint	several	Implants, resections Tooth extractions	Depending on chewing load, myofascial syndrome of masticatory muscles, symptoms with convergence to other structures supplied by the trigeminal nerve moderate to severe Suffering and harm due to reduced food intake	3-5 days	Pig Dog Rodent	Local anaesthesia during procedure NSAIDs Possible use of muscle relaxant Additional opioids only occasionally necessary
Paranasal, frontal sinuses		Tumour induction, formation	similar to headaches minor to moderate	as needed, if clinical symptoms present	Mouse Rat	Local anaesthesia during procedure Metamizole, paracetamol up to opioids
Inner and middle ear, external auditory canal		Bulla osteotomy	Structures very sensitive to pain (alveolar facial and trigeminal nerves); exposure to severe pain	3-5 days	all	Multimodal: metamizole, paracetamol, NSAIDs with opioids, local anaesthesia

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Eye, eye socket		Ophthalmological models	Irritations of cornea or the optic nerve extremely painful	3-5 days	All	Anti-inflammatory drugs (steroidal and non-steroidal), local anaesthesia Opioids as needed Possibly tarsorrhaphy (e.g., lid flaps)
Horn		e.g., dehorning	well innervated	1-3 days	Ruminants	Long-acting regional anaesthesia, NSAIDs

Nervous system

Neurocranium

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Neurocranium	Trepanation drill holes	Stereotactic surgery (*head mount*) Probe implantation Lens implantation (*cranial window*)	painful upon access and post-op. irritation (instability of implants, infections) Periosteum and meninges are very sensitive to pain	1-5 days p.op.	all Mouse Rat	Local anaesthetic combined with opiates and NSAIDs Aseptic technique required
	Minimal trepanation	Inoculations of tissue, cells or infectious substances	directly p.op. minor	2 days		Local anaesthetic in combination with NSAIDs

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
		Tumour implantation	increase in intracranial pressure painful (tumour growth, oedema) radiation to facial and cervical region	long-term therapy for existing space-occupying lesions		Local anaesthetic in combination with opioids or anti-inflammatory drugs Treat any oedema (mannitol infusion)
			epileptiform seizures may intensify suffering!	Endpoints!		Additionally: anticonvulsants, sedatives
		Kainate model	epileptiform seizures	24 h	Mouse	Additionally: anticonvulsants, sedatives (midazolam, diazepam)
		Traumatic brain injury Global brain trauma	painful with increasing intracranial pressure	3 days	Mouse Rat	Opioids and/or anti-inflammatory drugs Treat oedema (mannitol infusion) As a rule *non-recovery*
		Meningitis	severe pain		Mouse	Buprenorphine

Peripheral nervous system

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
		Neuropathic pain 1. *Sciatic nerve lesion*	Mechanical allodynia (increased sensitivity to touch)		Mouse Rat	Tricyclic antidepressants, e.g. nortriptyline 5 mg/kg intraperitoneal 2x daily (effect only noticeable after about 2 weeks) (Yalcin et al. 2014) Anticonvulsants, e.g. gabapentin, pregabalin
		2. *Nerve spared models* (lesion of peroneal and tibial nerves) (Sural nerves are spared)	increased sensitivity to touch in the lateral skin area of the paw which is innervated by the sural nerve		Mouse Rat	Tricyclic antidepressants Anticonvulsants
		3. Spinal cord injury	neuropathic pain occurs in more than 60% of all patients with spinal cord injuries		Mouse Rat	Tricyclic antidepressants, e.g. nortriptyline 5 mg/kg intraperitoneal 2x daily (effect only noticeable after about 2 weeks) Anticonvulsants, e.g. Serotonin and noradrenaline reuptake inhibitors, e.g. duloxetine
		Experimental autoimmune encephalomyelitis (EAE)	CNS and PNS are affected in the chronic phase, thermal hyperalgesia and/or mechanical allodynia occur, depending on the mouse model used		Mouse	Pregabalin (Wang et al. 2017)

Spine

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Nerve root; also, spinal disc	Dorsolateral, dorsal, ventral, ventral laparoscopy, thoracoscopy	experimental radiculitis, stenosis surgery and experimental inflammation or degeneration of spinal discs irritation possible after any spinal surgery	radicular pain: often distally radiating, sensitivity disturbance pain often of a stabbing, pulling nature, usually also with expressions of pain cervical spine: pain in head, neck and shoulder area due to projection (Kerr, 1961) autonomic dysregulation possible symptom dysfunction pain severe to moderate	2-3 days	all	Anti-inflammatory drugs (steroidal and non-steroidal) and opioids Muscle relaxation (midazolam, diazepam) Treat any oedema (mannitol infusion) Urinary bladder control
small vertebral joints end plates of vertebral bodies also, disc	dorsal, dorsolateral dorsolateral ventral	Implants, stiffening Procedures on the disc or the spinal cord Laminectomy	non-radicular pain: dull, deep-seated, difficult to localize spasm of deep paravertebral muscles (Bogduk, 1983) moderate to severe	3-5 days	all	NSAIDs Additional opioids only occasionally necessary

8.2. Musculoskeletal system

Extremities

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Bones	several	Fracture models	stable: minor to moderate unstable: severe	2-3 days Long-term therapy or *non-recovery*	all	Locoregional anaesthesia, tramadol, other opioids (buprenorphine)
Bone marrow		Injection Bone marrow biopsy		2 days	Rodents Large animals	Under anaesthesia, NSAIDs
Muscle, fascia	Several	Access to skeletal system, arthrotomy, trauma models	myofascial pain: dysfunction and local algesia, also distally radiated pain autonomic dysregulation possible moderate to severe	3-5 days	All	NSAIDs, metamizole (short-acting) Additional opioids only occasionally necessary Anti-inflammatory therapy often not compatible with experimental model, resulting in particular exposure to pain
Joint	several	Orthopaedic models Cartilage regeneration	frequently myogenic and arthrogenic pain moderate	3-5 days	all	Multimodal: local anaesthesia, NSAIDs, opioids
Paw Toe end organ		Injections: formalin test, CFA to induce inflammation Immunizations, burns, incl. chemical burns, contusions	In most cases markedly painful Inflammation Mechanical allodynia	at least 5 days	Mouse Rat	NSAIDs Metamizole, pregabalin

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Transplantation		Rear extremity	very painful neuropathic pain	up to 5 days	Rat	Regional anaesthesia: nerves Splash, NSAIDs and opioids Ketamine (especially pre-emptively) Try therapy with gabapentin Perphenazine (Decentan®) for 3-5 days
Tumour models			Tumour growth: • Bone and soft-tissue infiltration • Compression and infiltration of nerves, blood and lymph vessels • Oedema with poor circulation • Tumour necrosis of the skin, ulceration and secondary infection Treatment-related: • Radiotherapy: fibrosis, neuropathy, radiation-induced osteomyelitis • Chemotherapy: inflammation, neuropathy, bone and periosteal pain, soft-tissue pain, radicular pain, functional deficits and irritation directly tumour-related 60-90%, therapy-related approx. 5% exposure to pain (Twycross, Fairfield 1982) 37% painful already in early stage (human)! Pain prevalence (Zech et al. 1988, Bonica 1985): 60% soft tissue, 75-80% bone tumours in most cases severe pain (Bonica, 1990)	Long-term therapy	Mouse Rat	Steroidal and non-steroidal anti-inflammatory drugs, opioids also necessary in later stages

8.3. Respiratory tract

Trachea

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Trachea		Stent implantation	12-24 h	1-2 days		Local anaesthesia and NSAIDs

Lung

Tissue / organ	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Partial resection	Thoracotomy thoracic opening		severe (thoracotomy)	3-8 days	All	Local anaesthesia (e.g. intercostal) + opioid + possibly NSAIDs and/or possibly metamizole
Transplantation Allotransplantation	Thoracotomy		severe (thoracotomy) in the case of transplant failure	3-8 days longer according to symptoms	Mouse Rat Pig Dog	Local anaesthesia (e.g. intercostal) + opioid + possibly NSAIDs and/or possibly metamizole
Ventilation			none		All	usually only possible in the anaesthetized or heavily sedated animal (analgo-sedation: opioid/NSAIDs)
Pneumonia			none		All	
Pneumonia/pleuritis			very painful	total duration of experiment	Rabbit	NSAIDs

8.4. Cardiovascular system

Heart surgery

Model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Heart surgery with open thorax (chronic open chest models)	Lateral thoracotomy Intercostal access	Myocardial infarction caused by complete or partial occlusion of coronary vessels (ligature, ameroid constrictors) Stenosis of large vessels (pulmonary artery, aorta) Testing of surgical techniques, substances, or implants (surgical robots, laser devices, cardiac valve replacement) Surgery for chronic instrumentation for intrathoracic measurements (telemetry electrodes on the heart, indwelling catheter) Gene therapy experiments	Severe pain for 1-2 days Moderate pain for up to 5 days after surgery Analgesia indispensable Additional exposure to pain variable / depending on model Pain assessment and management according to clinical findings	Up to 3-5 days, depending on clinical findings	Pig Calf Sheep Dog Rabbit Rat Mouse	Multimodal analgesia recommended: Opioids, NSAIDs, antipyretics, local analgesia, adjuvants Intraoperative and postoperative conductive anaesthesia of intercostal nerves recommended

Model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Heart surgery with open thorax (chronic open chest models)	Sternotomy	Myocardial infarction caused by complete or partial occlusion of coronary vessels (ligature, ameroid constrictors) Stenosis of large vessels (pulmonary artery, aorta) Testing of surgical techniques, substances or implants (surgical robots, laser devices, cardiac valve replacement) Surgery for chronic instrumentation for intrathoracic measurements (telemetry electrodes on the heart, indwelling catheter) Gene therapy experiments	Severe pain for 2-4 days, followed by prolonged moderate pain Analgesia indispensable Sternotomy is not recommended in chronic experiments because of the invasiveness of the procedure and the severity of the consequences. Additional exposure to pain variable / depending on model Pain assessment and management according to clinical findings	until pain symptoms disappear, at least 7 days	Pig Calf Sheep Dog Rabbit Rat Mouse	Multimodal analgesia: Opioids, NSAIDs, antipyretics, local analgesia, adjuvants Intraoperative and postoperative conductive anaesthesia of intercostal nerves recommended
Heart surgery WITHOUT opening the thorax.	Thoracoscopy	Pericardial surgery	Moderate pain for 1-3 days minor pain for 1 to 3 days; in exceptional cases (larger tissue lesions) moderate pain also possible for 1-2 days	1-3 days	Dog Sheep Pig	Opioids, NSAIDs, antipyretics Local analgesia
Heart surgery WITHOUT opening the thorax.	Femoral artery/vein Carotid artery Jugular vein	Cardiac catheterization, coronary vessel dilatation with and without substance administration Stent implantation, arteriosclerosis induction with catheters, microembolization, myocardial infarction	Minor pain for 1-3 days in exceptional cases (larger tissue lesions) moderate pain is also possible for 1-2 days	1-2 days	Pig Sheep Dog Rabbit	Opioids, NSAIDs, antipyretics Local analgesia

Model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Allotransplantation heart / abdomen	Donor: thoracotomy Recipient: laparotomy	Allotransplantation between different rat strains or transgenic mouse strains	Donor: no pain therapy necessary Recipient: moderate pain exposure for 1-3 days as a result of laparotomy and implantation or foreign organ Prolonged pain possible as a result of secondary reactions, e.g., rejection, thrombosis etc.	2-5 days	Rat Mouse	Opioids, NSAIDs, antipyretics Local analgesia
Induction of pathological changes in the heart due to toxic substances	Oral, par-enteral	Oral or parenteral administration of substances that cause pathological changes (inflammation, calcification, microinfarction) in the heart and/or blood vessels, e.g., inflammatory mediators, antigens, antibiotics, carcinogens, coagulants, detergents, electrolytes	Exposure to pain varies depending on the administered substance and dose: evaluation based on established data and clinical examination. The research project may rule out pain therapy.	Depending on the clinical course	Rabbit Rat Mouse	Opioids NSAIDs, antipyretics
Secondary damage to the heart and blood vessels	several	Previous surgery in other organs (kidney, brain) Hypertension models Genetic modifications Inducible transgenic systems	Heavily dependent on the nature of the prior modification: Evaluation based on known data and clinical examination	Depending on the clinical course	Rabbit Rat Mouse	Opioids NSAIDs, antipyretics

Blood vessel surgery

Model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Instrumentation in peripheral blood vessels and tissues	Femoral artery Carotid artery, abdominal artery Laparotomy Hypoderm, connective tissue Muscle	Implantation of telemetry systems for measuring ECG and/or blood pressure Indwelling catheter in peripheral blood vessels	Depending on the type of access and the extent of tissue lesion Monkey, dog, pig: minor pain for 1-3 days Rat: minor to moderate pain for 3-5 days mouse: moderate to severe pain for 3-5 days.	Monkey, dog, pig: 1-3 days Mouse, rat: 3-5 days	Monkey Dog Pig Mouse Rat	Opioids NSAIDs
Occlusion of peripheral blood vessels	Femoral artery Blood vessels in the ear	Ischaemia due to ligature of the femoral artery or blood vessels in the ear	Minor to moderate pain for 1-3 days; surgery-induced pain only	1-3 days	Rabbit Rat Mouse	Opioids NSAIDs Caution: In guinea pigs, gerbils, chinchillas and some mouse and rat strains, occlusion of the carotid or femoral artery leads to ischaemia in the region supplied by these arteries (limb necrosis)
Stroke MCAO model	Middle cerebral artery	Induction of stroke (focal ischaemia) by cauterization of or catheterization of the middle cerebral artery	Extent of pain dependent on localization and size of infarct Evaluation based on known data and clinical examination	Depending on the clinical course	Mouse Rat Rabbit	Paracetamol, NSAIDs and possibly opioids
	Carotid artery	Global ischaemia of the brain resulting from transient occlusion of both carotid arteries	Extent of pain dependent on localization and size of infarct Evaluation based on known data and clinical examination	Depending on the clinical course	Mouse Rat Rabbit	Paracetamol, NSAIDs and possibly opioids

Model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Subarachnoid haemorrhage (SAH)	Cisterna magna	Induction cerebral haemorrhage by: - direct injection of blood into the cisterna magna - bleeding due to anastomosis of subclavian artery	Minor to moderate pain for 1-3 days Pain due to surgery and possibly central (such as severe headache in humans) Evaluation in animal difficult Evaluation based on known data and clinical examination	1-3 days	Rabbit	NSAIDs, opioids (e.g. fentanyl patch)
Induction of arteriosclerosis or inflammation in peripheral blood vessels	Carotid artery Femoral artery	Arteriosclerosis of carotid artery	Minor to moderate pain for 1-3 days	1-3 days	Rabbit Rat	Opioids NSAIDs A single administration before recovery from anaesthesia is often sufficient
Transplantation of peripheral blood vessels, implantation of vascular prostheses and coronary stents	Carotid artery Jugular vein Vena cava Abdominal aorta	Allotransplantation of carotid artery between different transgenic mouse strains Auto transplantation of blood vessels (artery, vein); prosthesis testing in large abdominal vessels	Minor to moderate pain for 1-3 days	1-3 days	Mouse Rat Sheep Pig Dog	Opioids NSAIDs
Growth of peripheral blood vessels	Skin	Skinfold chamber: transparent chamber integrated in the skin	Minor to moderate pain for 1-3 days	1-3 days after implantation	Mouse Hamster	Opioids NSAIDs

8.5. Digestive tract

Oesophagus

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Oesophagus	non-invasive	Stent implantation	none with intact organ		Mouse	If necessary NSAIDs
		Acidification	Minor to moderate depending on tissue damage		Mouse	NSAIDs

Gastrointestinal tract

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Laparotomy	Ventromedian in linea alba Paramedian Paracostal	Sham operation Surgery of abdominal organs (pain treatment, see there) Alzet pumps, telemetry transponder in abdomen	Minor to moderate pain for several days	1-3 days	all	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Laparoscopy	several	Surgical training Organ removal Implantations	Minor pain as it requires only minor surgical incisions	1-2 days	Pig Sheep	NSAIDs, metamizole, paracetamol

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Stomach	Ventromedian or paramedian laparotomy	Gastric resection (total/partial) Gastric fistula (cannula, port) Pouches (Heidenhain, Pavlov) Stomach ulcers Pyloroplasty	Minor to moderate pain for 2 to 3 days resulting from laparotomy Stomach pain due to severe distension, pulling as a result of positional changes in the peritoneum or inflammatory changes, possibly over a prolonged period Inflammatory pain may be caused e.g., by bacterial infections, ischaemic conditions, the release of mediators.	2-3 days in case of complications until symptoms subside	Pig Ruminants Mouse	Locoregional anaesthesia, NSAIDs (opioids)
Rumen		Rumen fistula Abdominal opening		3 days	Ruminants	Paravertebral block, linear infiltration with local anaesthetics, NSAID
Intestine	Ventromedian or paramedian laparotomy	Pouch, fistula (e.g. duodenum: excretory pancreas function) Artificial anus Colon resection Colon transplantation Mucosa transplantation Intestinal bypass Balloon dilatation of colon (colic model) Ileus models	Minor to moderate pain for 2-3 days resulting from laparotomy Severe pain with colic symptoms due to spasmodic muscular contractions or severe distension of the colon for the duration of symptoms. Pain-induced autonomic symptoms may also occur (vomiting, colonic inertia).	2-3 days in cases of colic until symptoms subside	Mouse Rat Rabbit Pig Dog	NSAIDs, metamizole, spasmolytic agents according to symptoms, Opioids if pain is severe CAUTION: colonic inertia

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Intestine	Sepsis	Caecal ligation and faecal slurry injection, LPS injection			Mouse Rat Pig (non-recovery)	Opioids
	Inflammation	e.g., colitis			Mouse Rat	At the present time, it is unclear which form of analgesia is effective. Possible negative interactions with analgesics are known (Blennerhassett et al. 2017)

Liver

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Gall bladder	Laparotomy	Cholecystectomy	Laparotomy: moderate pain	2-3 days	Mouse Pig Dog	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
	Laparoscopy	Cholecystectomy	Laparoscopy: minor to moderate pain; Somatic pain resulting from irritation (low pH) of the peritoneum by insufflated CO_2	1-2 days	Mouse Pig Dog	Metamizole, NSAIDs Opioids if necessary
Biliary tract	Laparotomy	Cannulation to obtain bile Injections (e.g., in major duodenal papilla)	Laparotomy: moderate pain	2-3 days	All	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Biliary tract	Laparoscopy	Cannulation to obtain bile Injections (e.g., in major duodenal papilla)	Laparoscopy: minor to moderate pain; Somatic pain resulting from irritation (low pH) of the peritoneum by insufflated CO_2	1-2 days	All	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Gallstones	without	Therapeutic experiments (e.g., laser)	Not painful as long as there is no - inflammation or - obstruction of the biliary tract: then severe pain	According to symptoms	Mouse Pig	Metamizole NSAIDs Spasmolytics if necessary Opioids if necessary
Liver biopsy	Percutaneous		Post-operatively hardly any pain		All	Under general anaesthesia; preventive analgesia is sufficient
Transfection of liver cells	Laparotomy	Hydrodynamic injection into portal vein	Laparotomy: moderate pain; Minor pain from transient swelling of the liver	2-3 days	Pig Mouse	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Ischaemia (partial ischaemia lobes of the liver, with or without reperfusion)	Laparotomy	Induction by occlusion of blood vessels: - partial - total	Laparotomy: moderate pain	2-3 days	Mouse Rat Pig	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Hepatectomy	Laparotomy		Laparotomy: moderate pain	2-3 days	All	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Transplantation (recipient)	Laparotomy	Orthotopic, heterotopic Autologous, syngeneic Allogeneic, xenogeneic	Laparotomy: moderate pain with complications, severe pain is possible	2-3 days; or longer according to symptoms	Mouse Rat Pig Dog	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Tumour implantation	Laparotomy	Implantation of tumour cells, tissues or fragments in the liver	Laparotomy: moderate pain Tumour: depending on specific characteristics of the tumour (e.g. growth, metastasis, etc.) Pain ranging from none to severe	Laparotomy: 2-3 days, Tumour: according to symptoms	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Hepatosis	Laparotomy	Biliary obstruction	Laparotomy: moderate pain Adaptation processes in early stasis phase cause hardly any pain. Except for individual mouse strains or GM lines in which morbidity, signs of pain and mortality have been observed.	2-3 days With signs of pain longer, according to clinical course	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Liver cirrhosis, liver necrosis	Injection i.v., i.p.	Hepatotoxins e.g., D-galactosamine	Not painful		Mouse Rat	
	Oral, injection i.p.	Carbon tetrachloride	Not painful		Mouse Rat	
	Diet	high-fat - low choline - low protein	Not painful		Mouse Rat Rabbit	
Liver cirrhosis, liver necrosis Hepatitis		Spontaneous, phenotype, secondary disease	Not painful		Mouse	
Liver cirrhosis, liver necrosis	Injection i.v., i.p.	Hepatotoxins e.g., D-galactosamine	Not painful		Mouse Rat	

Pancreas

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Pancreatectomy	Laparotomy	Complete/partial	Minor to moderate pain (laparotomy)	2-3 days	Rat Pig Dog	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Transplantation (recipient)	Laparotomy		Minor to moderate pain (laparotomy) If the transplant fails, severe pain is possible	2-3 days longer according to symptoms	Mouse Rat Pig Dog	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Pancreatic duct	Laparotomy	Cannulation to obtain pancreas secretion Injection (e.g. for gene therapy)	Minor to moderate pain (laparotomy)	2-3 days	Rat Pig Dog Mouse	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Tumour implantation	Laparotomy	Implantation of tumour cells/fragments	Minor to moderate pain (laparotomy) Depending on the nature of the tumour growth, pain may be severe	2-3 days or longer according to symptoms	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Acute, oedematous pancreatitis	Injection i.p., (i.v.)	Cerulein	Minor pain		Mouse Rat	Metamizole The influence of different analgesics on inflammation of the pancreas is still a subject of debate (Stumpf et al. 2016)
Chronic pancreatitis	Injection i.p.	Repeated cerulein over several weeks Repeated L-arginine	pain can be severe	Throughout the experiment according to symptoms	Mouse Rat	Metamizole, opioids

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Acute necrotizing pancreatitis	Injection i.p.	L-arginine	Minor pain		Mouse Rat	Metamizole
	Laparotomy	Retrograde injection of sodium taurocholate into the choledochopancreatic duct Occlusion of the choledochopancreatic duct Duodenal stenosis	Severe pain, severe course	Throughout the experiment	Rat	Opioids, metamizole

Spleen

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Splenectomy	Laparotomy	Splenectomy	Minor to moderate pain (laparotomy)	1-3 days	all	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Immunization	Laparotomy	Injection under the splenic capsule, implantation of foil with antigens under the splenic capsule	Minor to moderate pain (laparotomy)	1-3 days	Mouse Rabbit	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids

8.6. Genitourinary tract

Urinary tract

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Laparotomy	Ventromedian in linea alba Flank for retroperitoneal access	Sham operation	Minor to moderate pain for several days Slightly less painful for animals than for humans due to different exposure and tension of the abdominal wall	1-3 days	All	Local anaesthesia, NSAIDs, metamizole, paracetamol Opioids
Kidney	Ventromedian Retroperitoneal (bilateral surgery) Flank (unilateral)	nephrectomy (sub)total, unilateral, bilateral, transplantation, hydronephrosis model Implantation of tissue under the renal capsule Ischaemia (reperfusion model)	Minor to moderate pain Pain is caused by capsular tension due to swelling of the kidneys (pain receptors in organ capsule). A gradual stretching of the capsule causes only minor pain due to receptor adaptation.	At least 3 days, if there are no complications	Mouse Rat Pig	Locoregional anaesthesia (linear infiltration, epidural) Opioids (oxymorphone) metamizole, paracetamol NSAIDs (Caution! Renal function)
Bladder	ventromedian	Bladder resection (total/partial) augmentation, Stents	Minor pain Moderate to severe pain if leakage occurs with the development of peritonitis	At least 3 days, if there are no complications otherwise until symptoms subside (particularly in the case of cystitis	Mouse Rat Rabbit Pig	Locoregional anaesthesia (linear infiltration, epidural) Metamizole, paracetamol Opioids (oxymorphone)
		Models for voiding dysfunction (hyperreflexia - mustard oil; areflexia - flaccid bladder paralysis, spinal cord and nerve lesions)	Minor to severe pain depending on the model and on postoperative complications			
		Inflammation models (instillation of mustard oil)	Moderate to severe pain depending on mustard oil concentration (induction of severe haemorrhagic cystitis if concentration is too high)	Until symptoms subside		

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
		Infection models	moderate to severe pain		Mouse Rat	NSAIDs, metamizole, paracetamol, spasmolytics Opioids (oxymorphone)
Ureter	Ventromedian, if necessary retroperitoneal (bilateral surgery) Flank (unilateral)	Stent Ureteral occlusion (total/partial) for hydronephrosis or reflux nephropathy models	Minor pain with slow development of hydronephrosis Moderate to severe pain with rapid development of stasis caused by colicky pain due to a sudden stretching of the ureter	At least 3 days, depending on the postoperative course	Rat Rabbit Pig	Locoregional anaesthesia (linear infiltration, epidural) Metamizole, paracetamol Opioids (oxymorphone)
Urethra	Perianal, ventromedian	Urethrostomy (perianal, ventromedian) catheterization	Moderate pain (epithelium)	Until symptoms subside	Rat Pig Dog	Locoregional anaesthesia (linear infiltration, epidural) Metamizole, paracetamol Opioids (oxymorphone)

Genital tract

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Ovary	Ventromedian Flank	Ovariectomy, e.g., osteoporosis models Tumour implantation	Minor to moderate pain Pain due to pulling on the peritoneum	Up to 3 days if there are no complications Long-term treatment for pain resulting from tumour growth	Mouse Rat Guinea pig Sheep	Locoregional anaesthesia (linear infiltration, epidural), NSAIDs Metamizole, paracetamol Opioids

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Uterus	Ventromedian Flank	Foetal operations	Great risk of abortion, increased by use of abdominal press due to postoperative pain	1-3 days 3-5 days	Mouse Rat Rabbit Sheep	Locoregional anaesthesia (linear infiltration, epidural), NSAIDs Metamizole, paracetamol Opioids Spasmolytic agent
Prostate	Ventromedian, with pelvic osteotomy if necessary	Prostatectomy	Moderate to severe pain depending on the type of access; pelvic osteotomy should only be performed in exceptional cases if there is good reason, because it is very painful and often followed by postoperative complications	2-3 days At least 5 days in the case of pelvic osteotomy		Locoregional anaesthesia (linear infiltration, epidural), NSAIDs Metamizole, paracetamol Opioids
		Cancer models Infection models	Pain occurs as a result of pulling on the capsule	Long-term treatment for pain resulting from tumour growth		NSAIDs and/or opioids
Testicle	Scrotal	Orchiectomy	Minor Pain is increased as a result of postoperative swelling	1 day if there are no complications and no marked swelling	All	Local anaesthesia NSAIDs
External genitalia (labia, scrotum)			Moderate to severe pain as the density of nociceptors region in this region is particularly high; increased pain due to postoperative swelling	Until symptoms subside		NSAIDs (detumescent effect) Opioids if necessary

8.7. Skin

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Allotransplantation of tissue in the skin or subcutaneous tissue	Skin Subcutaneous tissue	Implantation of cardiac tissue in the subcutaneous tissue Transplantation of skin (human) on immunodeficient mice Allotransplantation of e.g., tracheal tissue in the subcutaneous tissue	Minor to moderate pain	At least 1 day	Mouse Rat Pig	NSAIDs, paracetamol, metamizole, opioids Ketamine
		Alzet pumps s.c.	Minor pain	4-8 h	Mouse Rat	NSAIDs, paracetamol, metamizole once
Tumour implantation	Skin Subcutaneous tissue	Injection of tumour cells into/under the skin Implantation of organoids, tumour fragments (e.g. into mammary fat pad)	Not painful immediately after injection Minor Pain may occur as a result of tumour growth	none max. 1 day	Mouse Rat	Paracetamol Metamizole
Burn	Skin	Surface wounds	Extent of pain depends on the size of the lesion; large lesions lead to allodynia and hyperalgesia Pain particularly severe during manipulation, such as change of dressing; short-acting general anaesthesia may be useful	Depending on the extent of pain, longer period possible		NSAIDs, paracetamol, opioids Ketamine
Wound healing	Skin	Simple incision Skin punches (open wound model)	Minor	A few hours to 3 days depending on extent of trauma		Paracetamol Metamizole

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Tattooing	Skin	Identification	Minor pain for a short time	None	Rodent Rabbit Dog Cat Primates	Anaesthesia only possibly local EMLA® Creme (bear in mind the time for it to take effect!)
Intradermal administration	Skin / epidermis	Immunization – if necessary, with Freund's adjuvant	Moderate	1 day	All	Injection under anaesthesia, if necessary NSAIDs, opioids (Kolstad et al. 2012)
Transplantation	Skin	Flap transplantation in defect models	Moderate pain	3-5 days	All	NSAIDs, paracetamol, metamizole, opioids Ketamine drip infusion

8.8. Surgery involving reproduction techniques, genetic modification and breeding of small rodents

Surgery, biopsies, marking

Tissue / organ / model	Access	Examples	Exposure to pain (degree and duration)	Duration of therapy	Animal species	Proposed treatment
Vasectomy	Mini-laparotomy	Production of infertile males (for pseudopregnant foster mothers)	Minor to moderate pain for 1-2 days	1-2 days	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol
Epididymectomy, vasectomy	Scrotum	Production of infertile males (for pseudopregnant foster mothers)	Minor to moderate pain for 1 day	1 day	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol

Tissue / organ / model	**Access**	**Examples**	**Exposure to pain (degree and duration)**	**Duration of therapy**	**Animal species**	**Proposed treatment**
Embryo transfer	Flank	hygienic sanitation; embryo transfer after cryopreservation, pronuclear injection, blastocyst injection, *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI)	Moderate pain for 1-2 days	1-2 days	Mouse Rat	Local anaesthesia, NSAIDs, metamizole, paracetamol (Parker et al. 2011a; Schlapp et al. 2015)
Implantation of ovaries	Flank	Preservation of mouse lines by transplantation of ovaries	Moderate pain for 1-2 days	1-2 days	Mouse Rat t	Local anaesthesia, NSAIDs, metamizole, paracetamol
Biopsy for extraction of DNA	Tail tip ≤ 2 mm, first time at age < 4 weeks Ear	Cell extraction for genotyping by means of PCR: amputation of tail tip, ear notching, ear punching	Minor pain		Mouse Rat	No analgesia necessary
Biopsy for extraction of DNA	Tail tip > 2 mm; age > 4 weeks Repeated amputation of the tail tip	sampling for Southern Blot Repetition of tail-tip biopsy for new PCR	Minor pain for ≤ 12 hours	12 h	Mouse Rat	Under anaesthesia NSAIDs, metamizole, paracetamol
Marking	ear punching, ear notching, application of ear tags, tail tattooing	Identification	Minor pain	None	Mouse Rat	No analgesia necessary
	Insertion of transponder in mice and rats		Depending on the size of the transponder in relation to the size of the animal, pain may be minor to moderate and can last for up to 1 day.	4-8 h		NSAIDs, metamizole, paracetamol

8.9. Pain in tumour models and cancers

In view of their diversity, each model must be individually assessed, and the duration and nature of the therapy adapted. The criteria for discontinuation (endpoints) are crucial here for animal welfare.

8.10. Legal framework for the use of medicines

The following paragraphs refer to the regulations applicable in the jurisdiction of the Federal Republic of Germany at the time of publication.

The handling of - i.e., the prescription and use of - and the permissible transportation routes for medicinal products (MPs) are regulated in Germany's Medicinal Products Act (AMG) and the ordinances based thereon (in this case the Ordinance on the Prescription of Medicines (AMVV) and the Ordinance on Veterinary Dispensaries (TÄHAV).

The German Medicinal Products Act provides guidance "for security in the marketing of medicines and in particular for the quality, efficacy and safety of medicines in the interest of ensuring a proper supply of medicines to humans and animals" (§1 German Medicinal Products Act). It consists of 18 sections which regulate, among other things, the definition, manufacture, marketing authorization and sale, prescription requirements, distribution channels, pharmacovigilance, monitoring, collection and evaluation of drug risks and liability for serious adverse events.

The marketing of narcotics is regulated in Germany by the Federal Narcotics Act (BtMG) and related ordinances.

The provisions of the Ordinance on Veterinary Dispensaries (TÄHAV) apply to the purchase, preparation, testing, storage and sale of medicinal products by veterinarians and by pharmacies of veterinary educational establishments. The TÄHAV also regulates the prescription and use of medicinal products by veterinarians.

The legal provisions of the respective federal states that govern the handling of medicinal products and narcotics must be observed and complied with.

For further information and access to legal texts and ordinances:

- BfArM = Federal Institute for Drugs and Medical Devices; Medicinal Products Act (AMG) and Narcotic Products Act (BtMG), Ordinances, Federal Opium Agency and many others
- BMEL = Federal Ministry of Food and Agriculture; Ordinance on Veterinary Dispensaries (TÄHAV)

The term "proprietary medicinal product" or "finished medicinal product" is defined as a medicinal product or medical device manufactured in advance and, subject to authorization, and placed on the market in a package intended for supply to the consumer.

Only medicines authorized for use in humans and/or animals (medical grade) should be used, bearing in mind the redesignation cascade. Due attention must also be paid to the careful handling of veterinary medicinal products, particularly narcotics.

The properties and indications of the analgesics mentioned in this document mean that these substances clearly fall within the definition of medicinal products as laid down in §2 of the German Medicinal Products Act, making the handling and use of these products subject to the provisions of the AMG. This more or less rules out, for example, the possibility of obtaining relevant substances as chemicals and using them in animals. It should also be noted that, quite apart from the formal restrictions, such a procedure is also urgently discouraged for reasons of quality assurance and animal welfare. Moreover, most of the analgesics mentioned are listed in Appendix 1 of the AMVV, meaning they are only available on prescription. If they are also listed in Appendix III of the Federal Narcotics Act (BtMG), then they are subject to other restrictions in the handling and marketing of these products.

The above-mentioned regulations can make it difficult for persons engaged in animal research to obtain and administer medicinal products (MPs) for use in animal experiments in a way that is consistent with the law, since unfortunately the AMG does not take into account the use of MPs in animal experiments. There are no clear regulations in this respect. If the persons concerned are licensed veterinarians, they may source MPs from their personal veterinary pharmacy or a public pharmacy. The sourced products may need to be redesignated for use in laboratory animals (§ 56a AMG). But even dispensing to other persons is only allowed under the AMG for animals undergoing treatment by a veterinarian, where lawful treatment pursuant to §12 TÄHAV involves certain measures that can hardly be presented in the approval procedure for animal experiments. There are differences of opinion on this issue between the various federal states and competent authorities there, and it is recommended that agreement be reached in advance with the relevant competent authority on what is permissible and legal when it comes to sourcing active substances for use in animal experiments.

Paradoxically, sourcing is simplified for all those substances which additionally fall under the BtMG, since a provision has been established in §3 BtMG that allows participation in the narcotics trade for scientific purposes. The Federal Opium Agency at the Federal Institute for Drugs and Medical Devices issues licences to this effect (www.bfarm.de).

Chemical products that require the production of a ready-to-use formulation in the laboratory pose an increased risk in their use. In addition to a potential risk for personnel, errors or carelessness during production (e.g. when it comes to the concentration, solvent, solubility, especially precipitation or excessively high temperature, contamination, etc.) can give rise to considerable uncertainties regarding their safe use, up to and including the risk to animals.

The “bench” formulation of preparations (drops, gels, pastes [Abelson et al. 2012] etc.) and the dilution of ready-to-use drugs (§13 AMG) is considered an offence against the law on medicinal products; AMG and TÄHAV must be observed! It is recommended that the competent authority be consulted about the legal requirements for the production of a medicinal product by means of mixing procedures.

Abbreviations

ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
AMG	German Medicinal Products Act
AMVV	German Ordinance on Prescription-Only Medicinal Products
ASA	Acetylsalicylic acid
CFA	Complete Freund's adjuvant
CNS	Central nervous system
ECG	Electrocardiogram
ICSI	Intracytoplasmic sperm injection
IVF	*in-vitro* fertilisation
LPS	Lipopolysaccharide
MCAO	Middle cerebral artery occlusion
MP	Medicinal product
NMDA	N-methyl-D-aspartate
p.o.	*per os* (orally; also by gavage)
p.o. in DW	Orally in drinking water
PCR	Polymerase chain reaction
PNS	Peripheral nervous system
SAB	Subarachnoid haemorrhage
TÄHAV	German Ordinance on Veterinary Dispensaries
TierSchG	German Animal Welfare Act

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