Session 2.2
Pain, welfare and analgesia

Workshop of Experts: Definition, Recognition, Assessment, and Alleviation of Animal Distress in the Laboratory

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Summary
Distress in animal research is difficult to address and often overlooked, despite mandates in the U.S. to minimise animal distress. Consequently, The Humane Society of the U.S. held an expert workshop on animal distress, including whether creation of an operational definition of distress is possible. Discussion topics included what an operational definition of distress should encompass; prevention, causes, and measurement of distress; and regulation of distress. Participants agreed that creating a practical definition of distress is challenging, but that crafting a description of what might constitute animal distress, supported by specific examples, is possible. Workshop findings and future efforts on distress are discussed here.

Keywords: distress, animal welfare, refinement

Introduction

Distress in animal research is a major concern in regards animal welfare and quality of scientific results (Carstens and Moberg, 2000; Robertson, 2002), as well as public support of animal research (Aldhous et al., 1999; HSUS, 2001). Despite these issues and the fact that legislation in most countries requires minimisation of animal pain and distress, distress tends to be largely overlooked in the animal research laboratory. The main reasons for this lack of attention likely include the challenges of recognising, assessing and alleviating animal distress. Regardless of these challenges, animal distress can no longer be disregarded and must be made an urgent priority. As a result, The Humane Society of the United States (HSUS), through its Pain and Distress Campaign, has sought to increase attention to distress, particularly non-pain induced distress, by scientists, animal ethics and oversight committees, policymakers and the public. As part of this effort, The HSUS held a workshop of invited participants to discuss the state of knowledge of animal distress and whether it is possible to define distress in operational terms and to measure distress for application to animal research. The results of the workshop and future efforts to increase attention to distress will be discussed here.

Legislative requirements regarding distress in animal research in the United States

Animal Welfare Act

The Animal Welfare Act (AWA) and the Health Research Extension Act (HREA) are the two main laws that govern animal research in the United States. The AWA is enforced by the Animal Care division of the United States Department of Agriculture (USDA). The AWA pertains to warm-blooded animals *other than* purpose-bred mice, rats and birds, which make up an estimated 85-90% of warm-blooded animals used in animal research.

While the use of anaesthetics, analgesics, and tranquilising drugs was mentioned in the 1970 AWA amendments, it wasn't until 1985 that the AWA emphasised animal pain and distress. The 1985 amendments specify that

- Pain and distress are to be minimised;

- Anaesthetics, analgesics, and tranquilising drugs are to be used, unless there is scientific justification otherwise;
- Alternatives to procedures that cause pain and distress are to be considered by the investigator; and
- Each institution registered with the USDA must form at least one Institutional Animal Care and Use Committee (IACUC) to review animal use protocols and oversee the institution's animal care and use program.

Despite the fact that the term "distress" is used throughout the regulations, the USDA has never officially defined what is encompassed in its concept of distress. USDA Animal Care Policy #11 (Painful Procedures) defines "painful procedure" as "any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied" (U.S. Department of Agriculture, 1997). The USDA policy defines "painful procedure" but not "distressful procedure", even though distress can be pain induced or non-pain induced. The absence of a regulatory definition of "distress" means that there is a lack of adequate guidance on distress. This could hamper or discourage institutions from expending the same effort in tackling distress as in addressing pain.

Health Research Extension Act

The Health Research Extension Act (HREA), passed in 1985, mandated that the National Institutes of Health (NIH) upgrade its requirements for animal research oversight. The HREA applies to all research facilities that receive federal funds from the NIH or its parent agency, the Public Health Service (PHS). The provisions of the HREA were implemented through the PHS "Policy on the Humane Care and Use of Laboratory Animals", which is enforced by the NIH's Office of Laboratory Animal Welfare (OLAW) and applies to all vertebrate species, thereby partly compensating for the exclusion of birds, mice, and rats from the AWA.

PHS Policy, like the AWA, emphasises avoidance or minimisation of pain and distress and calls for the formation of an Institutional Animal Care and Use Committee to review animal protocols and oversee the institution's animal care and use program. The "U.S. Government Principles for the Utilisation and Care of Vertebrate Animals Used in Testing, Research and Training" are also incorporated into PHS Policy. Three of the nine government principles directly address animal distress and pain, demonstrating the importance of these issues. Finally, PHS Policy calls upon research facilities to follow the provisions in the publication the "Guide for the Care and Use of Laboratory Animals" (often referred to as The Guide). The Guide recommends consideration of alternatives for all animal-based research, emphasises the importance of minimising distress, and offers examples of procedures that have the potential to cause distress and pain, such as physical restraint, multiple major survival surgery, food or fluid restriction, and the use of abnormal environmental conditions (Institute for Laboratory Animal Research, 1996). The Guide, however, does not clarify what is encompassed in its concept of distress, despite the fact that the term is used throughout the text.

Recent regulatory efforts

On July 10, 2000, the USDA published a call for public comment regarding whether the term "distress" should be defined and, if so, what the elements of the definition should be; and whether the current system used by research institutions to classify and report pain and distress should be improved. The published call for comments emphasised the advantages of these efforts, including helping research facilities to recognise and minimise animal distress in accordance with the AWA.

There were over 2,800 comments submitted in response to USDA's call for public comments, approximately 2,150 of which were from the research community. The HSUS examined all of the comments submitted by the research community and found that even though a majority of these respondents opposed the adoption of a definition of distress, 97% supported a definition developed by the National Research Council (NRC)[1] should the USDA decide to adopt a definition. In other words, although the research community doesn't want the USDA to define distress or otherwise expand its authority over research practices (a standard response when enhancements of the AWA are considered), they nearly all agree on the definition that should be used if the USDA does decide to move forward.

As of March 2006, the USDA has taken no official action on defining the term distress or changing the pain and distress categorisation system. The HSUS, however, believes that the adoption of a definition of distress and a new pain and distress categorisation system will increase attention to distress, will provide guidance on tackling distress, and will prompt needed data collection and research on distress.

Distress workshop convened by The HSUS

The HSUS held a workshop to discuss the state of knowledge of animal distress and whether it is possible to define distress in operational terms and to measure distress for application to animal research. The workshop was held February 11-13, 2004 in Baltimore, Maryland. Seventeen international participants were invited from diverse disciplines, representing the fields of laboratory animal medicine, animal physiology, animal behaviour and applied ethology, animal welfare, ethics and philosophy, regulation of animal research, and animal protection.

Workshop design, key objectives and discussion topics

The workshop was designed in order to meet pre-determined objectives. The key elements of the workshop design included:

1. Bringing together stakeholders from a range of disciplines;
2. Focusing on a manageable number of specific topics;
3. Examining and attempting to integrate a range of animal welfare-related scientific findings, including production,

[1] The definition of distress published by the National Research Council (Institute for Laboratory Animal Research 1992): "an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress, and shows maladaptive behaviors."

behaviour, physiology, and veterinary health;
4. Identifying areas of consensus and determining how to communicate findings to a relevant audience;
5. Identifying barriers to consensus; and
6. Clarifying requirements for future efforts on specific issues of definition, measurement, and alleviation of laboratory animal distress.

A number of efforts were made in order to stimulate discussion at the workshop, such as advanced preparation of briefing papers by some participants, consideration of specific questions and sharing of answers with all participants, and brief presentations by some participants at the workshop. Importantly, the workshop was structured to allow for lengthy discussion of specifically designated topics; points of contention and agreement were recorded.

The key objectives of the workshop were to:
1. Present the current state of knowledge on the definition of distress and science of distress recognition, measurement, and alleviation from a range of disciplines;
2. Reach a working agreement regarding an operational definition of distress, signs of distress, and modes of measurement;
3. Clarify requirements for future efforts on the definition, measurement, and alleviation of laboratory animal distress; and
4. Publish the workshop proceedings via print or electronic media.

With these objectives in mind, the topics posed for discussion at the workshop included:
- determination of what should be incorporated into the concept of distress;
- the reasoning behind incorporating these factors into the concept of distress;
- causes of distress and how this information can ultimately be used for prevention; measurement, scoring and validation of distress;
- alleviation of distress;
- incorporating an understanding of distress into regulation,
- finally but importantly, practical application of information to the laboratory.

Areas of general agreement: universal

General points of agreement were recorded, discussed and finalised at the workshop. Some of the points will be discussed briefly here, but additional points and more in-depth discussion of these issues will be provided in a future published executive summary of the workshop authored by workshop participants.

A general point that all participants agreed upon is that distress is of most concern when it is more than momentary or slight, and those are cases that should be priority in terms of attention. Extent of distress can also vary according to duration and intensity of the stimulus and in the context in which it occurs. An animal's distressful experience may represent states similar to (but not necessarily exactly the same as) negative human states, such as anxiety, boredom, hunger and malaise, for example. Animals may also experience states of distress that we simply aren't able to identify as of yet.

The terms "pain and distress" are often seen in conjunction, particularly in regulations and legislation, but it must be emphasised that distress is not necessarily related to, nor a consequence of, pain. For example, an animal may be anxious to the point of distress, but anxiety is not caused by pain. Distress is also difficult to define and has different meanings in different contexts. For example, distress can mean one thing to regulators, another to the public, and yet another to scientists working with animals in research. The experts agreed that in order to deal with the difficulties of defining distress and the issue of different contexts, a broad working description could be created and accompanied by specific examples of what would cause different levels of distress (such as mild, moderate and severe).

Areas of general agreement: causation, recognition and assessment of distress

There was lengthy and highly productive discussion of causation, recognition and assessment of distress. As with other topics, general areas of agreement were found. There was unanimous agreement that distress can arise from various sources, such as those related to the experiment, housing and husbandry, as well as genetic factors.

In attempting to determine when an animal could be experiencing distress, the idea of using two broad questions was discussed (Dawkins, 2004):
1. Is the animal healthy?
2. Does the animal have what s/he wants?

There are a number of resources that address how to determine the health of an animal (such as courses provided at www.researchtraining.org); therefore the means of answering that question will not be discussed here. In terms of considering whether an animal has what s/he "wants", in general, "wants" are considered resources or behaviours that are important to the animals (Dawkins, 2004), without which the animal may experience unpleasant psychological states and may consequently develop or behave abnormally. The importance an animal places on gaining a resource, or removing itself from a particular situation, provides a basis on which to determine how distressed the animal might be without this resource.

Both physiological and behavioural measures can provide information about an animal's health and psychological state. However, there is no single measure that is sufficient to indicate distress in any given situation; therefore, a selection of appropriate measures should be considered when assessing distress. The type of measures used to assess distress are often complex and the interpretation of results may be guided by looking at the species evolutionary history. Ongoing daily experience, familiarity with individual animals, knowledge of species-typical behaviour, and high quality diagnostic skills also play an important role in recognising distress. Experienced clinical observation and divergences from the animal's normal state were also identified as key to identifying potential acute distress.

Aside from addressing distress, there was a brief discussion surrounding how to improve well-being so that the animals have positive experiences. This brought up a third question that could be asked, namely, how can we determine what resources the animal requires in order to perform behaviours and social interactions that provide positive experiences? It was agreed that those working with animals in research should maximise opportunities for animals to perform behaviours that are important to them.

Areas of general agreement: Recommendations for oversight

The participants recognised that oversight is an important factor in addressing distress in animal research. Three main recommendations were made in regards to oversight: a team approach must be taken, there should be post-approval protocol monitoring, areas of needed research should be determined, and guidance from professional bodies is currently lacking, yet needed.

Areas of agreement: development of resources for oversight bodies and researchers

If there is going to be increased attention to, recognition of, and prevention of distress, the research community urgently needs additional resources. Some resources that should be taken into consideration include a journal that focuses on refinement; increased information in current journals regarding how pain and distress were minimised, such as how humane endpoints were determined; identification of research priorities related to distress by those qualified to do so; earmarked funding for the set priorities; and, finally, development of good practice guidelines, written by experts.

Future efforts to increase attention to animal distress

Several products have been developed from this workshop. An executive summary of the workshop authored by participants has been submitted to a laboratory animal science journal for publication. Furthermore, The HSUS is coordinating publication of a technical book on distress and pain entitled "Recognition and Alleviation of Pain and Distress in Animal Research", authored by international experts and to be published by Humane Society Press in 2006. The intended audience of the publication encompasses laboratory animal veterinarians, scientists, members of Institutional Animal Care and Use Committees (or comparable bodies outside of the United States), caretakers, and other laboratory personnel, as well as regulatory authorities and policymakers.

The purpose of the book is to promote animal well-being in terms of operational consideration and management of animal distress and pain, draw attention to the latest developments in recognition and alleviation of pain and distress in animal research and testing, and to discuss related policy issues. A number of topics concerning pain and distress in animal research and testing will be included, such as philosophical aspects and concepts; international regulatory definitions of distress; causes and measurement of distress; distress in the context of pain; understanding stress, distress and suffering; animal illness and distress; animal emotions, psychological well-being and distress; the role of clinical veterinary medicine in assessment and treatment of distress, and resolving animal distress (with specific examples of good practice provided). Practical application to the animal research laboratory is what will make this publication on pain and distress unique.

Conclusion

Although there has been little work on laboratory animal distress at a regulatory level in the United States for some years, this workshop demonstrated that the issue of distress is neither dormant nor dominated by dissenting scientific voices. The workshop participants, all experts in their respective scientific fields, reached important general agreement on the contexts in which distress might arise and the types of negative experiences that might constitute distress. The lively debate produced a range of proposals and practical solutions for moving forward the discussion about defining distress, as well as methods for recognition, assessment, alleviation and prevention of distress. The forthcoming book, developed directly as a consequence of the workshop, will assist those making difficult operational and ethical decisions in the laboratory to make informed, comprehensive judgments on animal distress. It is hoped that this workshop and its associated publications will provide further stimulus toward attention to animal distress, to benefit both animals and science.

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Use of Analgesics in Experiments

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Summary
Refinement in animal experiments means to diminish pain, suffering and harm. The use of analgesics in experiments is applied refinement and an essential part of good veterinary care.
It was the task of this report to give a comprehensive overview about the topic of pain treatment in laboratory animals. This report is based to the manual of the working group for anaesthesia and analgesia of the German Society for Laboratory Animal Science (GV-SOLAS) published in the year 2002. By the extent of this report it is only possible to give proposals by example for pain relief in different species used in the diverse kinds of experiments.

Keywords: analgesics, pain relief, postoperative care

Introduction

Profound knowledge is needed to rule out the most effective pain management protocol by use of general anaesthesia, regional anaesthesia and systemic analgesics. This has to take into account the side effects of drugs used to physiological parameters, behaviour and pathophysiological patterns.

The concept of the cited recommendations is explained, which provides easy access to the necessary information to investigators.

This paper should help researchers to plan the animal experiments with concern for an adequate pain treatment protocol, which provides a maximum of benefit for the animals as well as optimal conditions for a high scientific output.

Representatives of the authorities working in the field of animal welfare giving license or controlling animal experiments should find very easily guidelines and diverse alternatives for pain relief in animals.

The paper should be also a formula for all people caring for animal welfare.

The influence of pain to animal experiments – why alleviate pain? (Pathophysiological aspects)

There is a relevance of pain therapy for laboratory animals first by ethical aspects and second due to most national animal welfare acts. Therefore, adequate pain therapy needs to be carried out in any animal experiment where post-operative pain occurs.

Furthermore, we have to point out that there is an effect of pain to the results of animal experiments. The laboratory animal is not a passive measuring instrument in the experiment.

Pain provokes pathophysiological effects which can influence experimental results, more than the use of pain killers *per se*. Long duration and intensity of pain can cause death.

Pain has pathophysiological effects on different organ systems as listed below (Jage, 1997; Larsen, 2002; Henke and Erhart, 2001).

Endocrine System
- Increased secretion: catecholamines, corticoids, glucagon, growth hormones, ACTH, ADH
- Decreased secretion: insulin, testosterone
- Effect: adynamia in mobilisation, floppiness, amyotrophia, extended anastasis
- Fluid retention, oliguria, disbalance of electrolytes
- High metabolic rate with increased oxygen consumption
- Effects on organ systems

Neuroendocrine system
- Increased β-endorphin index
- Blockade of NMDA-channels

Sympathetic nervous system
- Increased activity, release of catecholamine during excess stimulation of the sympathetic nervous system
- Decreased tissue perfusion with increased tissue acidosis, resulting in hyperalgesia
- Risk of hypoxia in poorly perfused organs (heart, brain, intestine, lung)
- Atonia of the gastrointestinal tract, risk of paralytic ileus
- Activation of the renin-angiotensin-aldosterone system during a compromised renal perfusion
- Thereby inducing peripheral vasoconstriction
- Increasing platelet aggregation
- Increasing release of noradrenalin from peripheral nerves causing hyperalgesia

Immune system
- Immune suppression (susceptibility to infection)
- Impaired wound healing

Tab. 1: Synopsis of the effects and side effects of the most known agents

	Active ingredient	Trade name	Application area	Side effects	Comment
Opioids	Piritramide	Dipidolor	Strong pain of all kinds	Long-time treatment (>3 day) because of constipation	Titrate with strength monitoring of the respiration
	Fentanyl-plaster	Durogesic	Evenly strength analgesics about 3-5 d by dogs, rabbits, pigs, ruminant animals	Rodent, immediate analgesics, fever, respiratory depression	Beginning therapy hole for 6-12h, good bandage needed
	Tramadol	Tramal	Weaker pain, continuous drop infusion (CDI)	Strong pain	Very short effect, possibly CDI
	Pethidine	Dolantin	Weaker pain, on bowel, A-bile and pancreas duct due to spasmolysis	Long-time application	Very acute effect
	Buprenorphine	Temegesic	Medium- string pain, for part ial antagonism of effects of μ-agonists	A-bile and pancreas duct examination, constipation by application >5d	In low doses excitatory, in high doses sedation, allotriophagy, CAVE by preoperative. administration
	Butorphanol	Morphasol	Temporary therapy low - medium pain	Long-time treatment, strong pain	Excellent antitussive
Antipyretics	Acetylsalicyl acid	Aspirin, ASA	Low inflammatory pain, accessorily an antithrombotic effect, fever	Bleeding risk, GIT average	CAVE not using it pre op. because of bleeding risk
	Metamizole	Vetalgin, Novalgin	Low-medium pain, especially in abdomen, spasmolysis, long-time treatment just as CDI	Long-time treatment	Good in combination with opioids CAVE very slow i.v.
NSAIDs	Carprofen	Rimadyl	All kinds of inflammatory pain, especially by operation, also for acute and chronic inflammation of the musculoskeletal system	Gastrointestinal disorder	Good effects, when you use it for the insult, long range treatment, good effects in combination with opioids
	Flunixin-Meglumine	Finadyne	Acute and chronic inflammatory pain, endotoxin shock		Nephrotoxic
	Ketoprofen	Romefen	See above, addition on eyes		
	Meloxicam	Metacam	Pain states all kind		Good effects, when you applicate it for the insult, long range applicable, good effects in combination with opioids
	Phenylbutazone	Tomanol, Phenylarthrit	Anti-inflammatory, especially the musculoskeletal system, contingent ruminant animal		Many side effects, not good for routine use
	Tolfenamic acid	Tolfedine	Acute and chronic inflammatory pain, fever loweing		Especially good for cats

- Inhibition of the mitotic rate and locomotion of T-cells
- Inhibition of the lymphokine production, inhibition of phagocytosis
- Decrease in interleukin release, cell immunity, tumor immunity, host defence, production of antibodies

Blood
- Depletion in the spleen and skin: Lymphopenia, eosinopenia, neutrophilia

Respiratory system
- Especially affected after thoracic and abdominal intervention
- Reduced tidal volume and vital capacity, laboured breathing
- Symptoms: atelectasis with disturbances of the pulmonary gas exchange, therefore resulting in the development of infection, pneumonia
- Respiratory and metabolic acidosis

Cardiovascular system
- Occurs after the activation of the sympathetic system: tachycardia, peripheral vasoconstriction with increased vessel resistance, increased heart contractility and myocardial O_2-consumption and hypertension.

Gastrointestinal system
Cause: Excitation of peritoneal nerves, increased sympathetic activity and ischemia
- Symptoms: gastrointestinal atonia, risk of paralytic ileus, sickness, vomiting, distension of the intestine and higher abdominal pressure, reduced diaphragm movement with pulmonary dysfunction
- Excitation of visceral nerves
- Disturbance in visceral perfusion causing ischemia, thereby aggravating pain, reduced food and water intake (hypoglycaemia, dehydration)

Urogenital system
- Decreased motility in the urinary tract
- Urine retention

Musculature
- Spasms, tremor, convulsions
- For longer periods: adynamia, floppiness, amyotrophia

Behaviour
- Depression, hyperagressiveness, self-mutilation
- reduced grooming activity (especially in rodents)

→ Behavioural changes induced by pain in animals (particularly small ones) are a good indication for the evaluation of severity

Systemic analgesics (for p. op. supply)

Advisable are (see tab.1):
- Opioids (act predominantly central):
Buprenorphin, Piritramid, Pethidin, Butorphanol, Tramadol and Fentanyl-Patch.
- Antipyretics (act central and peripheral):
Acetylsalicylic acid, Metamizol.
- NSAIDs (act predominantly peripheral):
Carprofen, Etodolac, Flunixin-Meglumin, Ketoprofen, Meclofenamin acid, Meloxicam, Niflumin acid, Phenylbutazon (ruminant), Piroxicam, Tepoxalin, Tolfenamin acid, Vedaprofen.

Application in form of: Plaster, suppositories, SC, PO, IV, CID, osmotic mini pumps. It is not advisable to use uncritically human analgesics like Diclofenac and Paracetamol in experimental animals (toxic, to short a half-life period).

The following particulars about the dosage table are based on appropriate literature and the experience of the authors (see tab. 2 and 3). The dosage must be adapted to the respective clinical situation and the aim of the experiment. Frequent side effects will be found in the information letters of the product, but most apparent side effects in a routine use will be found in the following table. The acute appearance of side effects is often based on a to rapid i.v. injection. Acute side effects are close to a certainty if intravenous injections are performed too rapidly. The dosage plans are given exemplary for guinea pig, chinchilla, mouse, hamster and dog. For recommendations for other species see the recommendation of GV-SOLAS (2002).

Tab. 2: Dosage table for the guinea pig, chinchilla, mouse and hamster

Substance	Dose (mg/kg)	Administration	Interval of administration	Notes
Buprenorphine	0.05-0.1	s.c./i.p.	8h	not hamsters
Butorphanol	1.0-5.0	s.c.	4-6h	not hamsters
Flunixin-Meglumine	3.0-5.0 (mouse)	s.c.	12h	
ASA	120.0-300.0	p.o.	24h	not hamsters, guinea pigs
Metamizole	80.0(guinea pig) (*) supplementary stress see Thoracoscope, Laparoscope – 2 drops/animal) 200.0 (mouse) 100.0 (hamster) (0.5-2 drops of 1:4 diluted solution/animal)	p.o.	every 6h	
Carprofen	4.0-5.0	s.c.	24h	

Tab. 3: Dosage table for the dog

Substance	Dose (mg/kg)	Administration	Interval of application	Notes
Buprenorphine	0.01-0.02	i.v., i.m., s.c.	8-12h	
Butorphanol	0.1-0.5-1.0	i.v., i.m., s.c.	1-2h	
Fentanyl-Patch	5-10kg: 25 mg/h 10-20kg: 50 mg/h 20-30kg: 75 mg/h from 30kg:100 mg/h	transdermal	48-72h	CAVE oral intake
Pethidine	2.0-6.0	i.m., s.c.	1-2h	spasmolytic at musculature
Piritramide	0.1 0.2 0.1 (-0.3)/h	i.v. s.c. i.v.	1-2h 2h DTI	
Tramadol	1 (-3)/h	i.v.	DTI	
ASS	25.0 10.0	p.o. slowly i.v.	6-8h	
Metamizole	20.0-50.0	slowly i.v.	4h	spasmolytic at musculature
Carprofen	4.0 or 2.0	i.v., i.m., p.o., s.c.	24h 12h	
Flunixin-Meglumine	0.5-1.0	i.v., i.m., s.c., p.o.	24h	over max. 3d at endotoxin-shock every 12h
Ketoprofen	1.0-2.0 from 2.d: 0.5-1.0	i.v., i.m., s.c., p.o.	24h	over 3-5d
Meloxicam	0.1	p.o., s.c., i.v.	24h	
Phenylbutazone	5.0-10.0 20.0-60.0/d	slowly i.v., i.m., p.o.	24h	then reduce, max. 800/animal/d
Piroxicam	0.3	p.o.	48h	
Tolfenamic acid	4.0	s.c., p.o.	24h	max. 3d

Recommendation for analgesic methods in experimental procedures

The cited report gives also recommendations for the alleviation of pain in selected experimental standard procedures. These procedures are listed in a topographical synopsis. For examples see tables 4 to 7.

Pain dispensary

In general: For postoperative analgesia it is enough for the routine use of an experimental unit to have one substance from the different substance groups (opioides, antipyretics, NSAIDs, local analgesics, analgesic anaesthetics). It will be impossible to work without opioide substances. With these analgesics you can treat nearly all species – for example:

Stocking up of postoperative analgesics:

Examples: Buprenorphin, Metamizole, Carprofen (standing in for NSAIDs of the new generation like also Meloxicam, Flunixin-Meglumine, Tepoxalin), Bupivacaine, Ketamine, Methadone (for the after-effect in the postoperative phase). Other substances could be needed after experiment planning.

In my experience you can treat nearly 95% of all studies just with Buprenorphin, Metamizol or Carprofen (or in combination) to get enough analgesic effect (tab. 8).

Dosage instructions

For all animal species and all used substances it is recommended to make a dosage instruction (tab. 9).

Remark to legal regulations

Veterinary bodies in the European Union and in Germany are confronted with a lot of legal regulations to deal with opioide pharmaceutical products.

Conclusions

Analgesic treatment should be the standard in all not insignificantly painful experimental procedures. It was pointed out that less stressful and painful protocols lead to better and more valid results. Indeed, the use of analgesics is not recommended in all experiments because effects and side-effects of the drug may interfere with the expected results. But painful procedures without any analgesic treatment should be limited to the absolutely necessary exception.

The data presented should be a base for an intensive discussion by all persons involved in designing better animal experiments.

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Tab. 4: Recommendations for the alleviation of pain in selected experimental procedures

Facial cranium						
Tissue/Organ	**Route**	**Example**	**Extent of pain stress (intensity and duration of pain)**	**Duration of analgesia**	**Animal species**	**Suggestions for therapy**
Oral cavity	oral	Stomatitis, Gingivitis; intervention in teeth; jaw-bone: implants, partial resection	dog low-grade, cat moderately till high-grade low-grade, Pulpitis moderately at adequate stability low, instability and at injuries of *N. alveolaris* moderately till high-grade disease and damage by anorexia	3-5d	dog, pig	antiphlogistics (steroidal and non-steroidal) at harms of cranial nerves often opioids necessary
Maxillary articulation	various	implants, resection	dependent on the masticating exposure myofacial syndrome of masticating muscle symptoms with convergence to other structures in the service area of *N. trigeminus* moderately till high-grade disease and harms by anorexia	3-5d	dog, pig	NSAIDs take care of muscle relaxation sometimes opioids necessary
Paranasal sinus, (frontal) sinus		tumour development	headache similar low-grade till moderately		mouse, rat	opioids
Eye, Eye socket		all ophthalmologic models	irritations of cornea or of N. opticus, raised intraoculare pressure extrem painful	3-5d	all	antiphlogistics (steroidal and non-steroidal cornea local glaucoma therapy if required opioids
Inner, middle ear		bulla osteotomy	very sensitive structures (*N.facialis, N. trigeminus*)	3-5d	all	NSAIDs most with opioids
External auditory canal			only if the duration of injury is very short you can do without analgesic			
Horn		amputation	highly innervated	1-3d	sheep	Effective: local anaesthesia, NSAIDs

Tab. 5: Recommendations for the alleviation of pain in selected experimental procedures

Neurocranium						
Tissue/Organ	**Route**	**Approach**	**Degree of pain (intensity and duration)**	**Duration of analgesia**	**Animal species**	**Suggestions for therapy**
Neurocranium	trepanation, bore hole	stereotactic operation probe implantation inoculation of tissue, cells or infectious substances tumour implantation	painful at aditus and irritations p. op. (instability of implants, infections) periosteum and meninges are very pain sensitive direct p. op. low at increase of intracranial pressure painful (tumour growth, oedema), emission to facial and cervical region epileptic seizure could increase the disease	1-3d p. op. 0-1d at existing tumour permanent therapy no-go criteria	all mouse rat	opioids in combination with steroidal antiphlogistics if low: Metamizole, NSAIDs treat the oedema (Mannit-infusion) anticonvulsants, sedatives
		general brain trauma	at increase of intra cranial pressure painful	3d	mouse rat	treat the oedema (Mannit-infusion) Metamizol NSAIDs

**Tab. 6: Recommendations for the alleviation of pain in selected experimental procedures.
Pain in neoplasia.**

Lesions of the tissues (Stumpf, 1993)	**Examples**	**Extent of pain load (intensity and duration of pain)**	**Duration of treatment**	**Com. used species**	**Recommendations**
Tumor growth: Bone- and soft tissue infiltration Compression and infiltration of nerve-, blood- and lymph vessels Edema with impaired perfusion Skin: necrosis, ulceration and secondary infection **Caused by therapy:** Radiation: - fibrosis, neuropathy, - radiation osteomyelitis, - Chemotherapy: - inflammation, neuropathy	Cancer models	**Non-uniform:** Bony and periosteal pain, soft tissue pain, radicular pain, loss of function, irritations Primary by cancer 60-90%, by therapy about 5% of pain load (Twycross and Fairfield, 1982) 37% already in an early stage painful (human)! **Pain prevalence:** (Zech et al., 1988; Bonica, 1985) 60% soft tissue-, 75-80% bone cancer usually severe (Bonica, 1990)	Longtime-therapy	Mice, rats	steroidale and not steroidale antiphlogistics, Metamizol, in advanced stage need of complementary opioides use of local anaesthetic techniques! endpoints!

Tab. 7: Recommendations for the alleviation of pain in selected experimental procedures

Transgenic technology						
Model	**Aditus**	**Example**	**Intensity and duration of pain**	**Duration of the analgetic therapy**	**Frequent animal species**	**Suggestions for therapy**
Vasectomy	Abdomen, ventral	Creation of infertile males for preparation of pseudogravid wet nurses	Low till median pain stress for 1 day	1 day	Mouse, rat	NSADIs, Metamizol, Opioids
Epidydectomy	Skrotum	Creation of infertil males for preparation of pseudogravid wet nurses	Low till median pain stress for 1 day	1 day	Mouse, rat	NSADIs, Metamizol, Opioids
Embryo-transfer	Abdomen, dorsolateral	Hygienic clean up; transmission of embryos after cryoconservation, prenucleusinjection, blastcystinjection, *in vitro* fertilisation (IVF), intracyto-plasmatic spermatozoainjection (ICSI)	Low till median pain stress for 1 day	1 day	Mouse, rat	NSADIs, Metamizol, Opioids
Implantation of ovary	Abdomen, dorsolateral	Preservation of mouselines through transplantation of ovaries	Low till median pain stress for 1 day	1 day	Mouse, rat	NSADIs, Metamizol, Opioids
Biopsy to get DNA	Tag, auricle, hair follicle, oral cavity, terminal bowel		Low pain tension under 1 day, no analgesia needed		Mouse, rat	
Mark	Ear crenation\ punching\ mark, transponder, tattoo	Identification	Low pain tension under 1 day, no analgesia needed		Mouse, rat	

Tab. 8: Main indication and -contraindication to use Buprenorphin, Carprofen and Metamizol

	Buprenorphin	**Carprofen**	**Metamizol**
to recommend	Middling till strong achestate each kind	Each inflammation ache also with injury and operation, especial in prevention	Not by inflammation, particularly use by cramps and spasm of the hollow entrails
inadvisable	Exploration of GIT-motality and bile secretion	Exploration in context of inflammatory	Animals who are to often been stressed by (i.m.) application and cats, because they could salivate to much

Tab. 9: Pain therapy with Buprenorphin, Carprofen and Metamizol

	Buprenorphin	Carprofen	Metamizol
Goat	0.006 i.v/ i.m./ s.c. 12h	4 i.v/ i.m. /s.c/ p.o. 12-24h	20-50 i.v/ i.m. 4h
Pig	0.05-0.1 i.v/ i.m./ s.c. 8-12 h	4 i.v/ i.m./ s.c./ p.o. 12-24h	20-50 i.v/ i.m. 4h
Dog	0.01-0.02 i.v/ i.m./ s.c. 8-12 h	4 i.v/ i.m./ s.c./ p.o. 12-24h	20-50 i.v/ i.m. 4h
Cat	0.005-0.01 i.v/ i.m./ s.c. 8-12 h	4 i.v/ i.m./ s.c./ p.o. 24h	20-50 i.v/ i.m. 4h
Rabbit	0.0075 i.v/ i.m./ s.c. 6-12 h	2-4 i.v/ i.m./ s.c./ p.o. 12-24h	20-50 i.v/ i.m. 4h 3-5 drops p.o 4h
Guinea pig	0.05- 0.5 s.c. 6-12h	4 i.m./ s.c. 12h	80 (1/2-2 drops/animal)p.o. 4h
Rat	0.05- 0.5 s.c. 6-12h	5 i.v/ s.c.. 12h	110 (2 drops/animal) p.o. 4h
Gerbil		4 s.c. 24h	100 (1-2 drops/animal) p.o. 4h
Hamster		4 s.c. 24h	100 (1-2 drops/animal) p.o. 4h
Mouse	0.05- 0.1, s.c. 6-8h	5 i.v./ s.c. 12h	200 (1-2 drops/animal) p.o. 4h

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