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АССОЦИАЦИЯ СПЕЦИАЛИСТОВ
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Recognition of pain, suffering and distress of laboratory rodents and the means for their relief

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Reasons for the recognition and reduction of pain and distress

❖**Legislation**

Directive 2010/63EU, Article 14 (Anaesthesia), #1.

*"*Procedures that involve serious injuries that may cause severe pain ***shall not be carried out without anaesthesia***."

❖**Ethics and moral reasons**

Animal experimentation may be painful and provoking distress, and that is inhumane. The humanity of the experimental procedure or a kind of animal research becomes increases while the pain and distress is decreased more and more.

❖**Affects the results of the experiments**

- GLP OECD (ENV/MC/CHEM(98)17), 5.2. Biological test-systems

✓ «…At the experimental starting date of a study, test systems should be *free of any disease or condition that might interfere with the purpose or conduct of the study*.

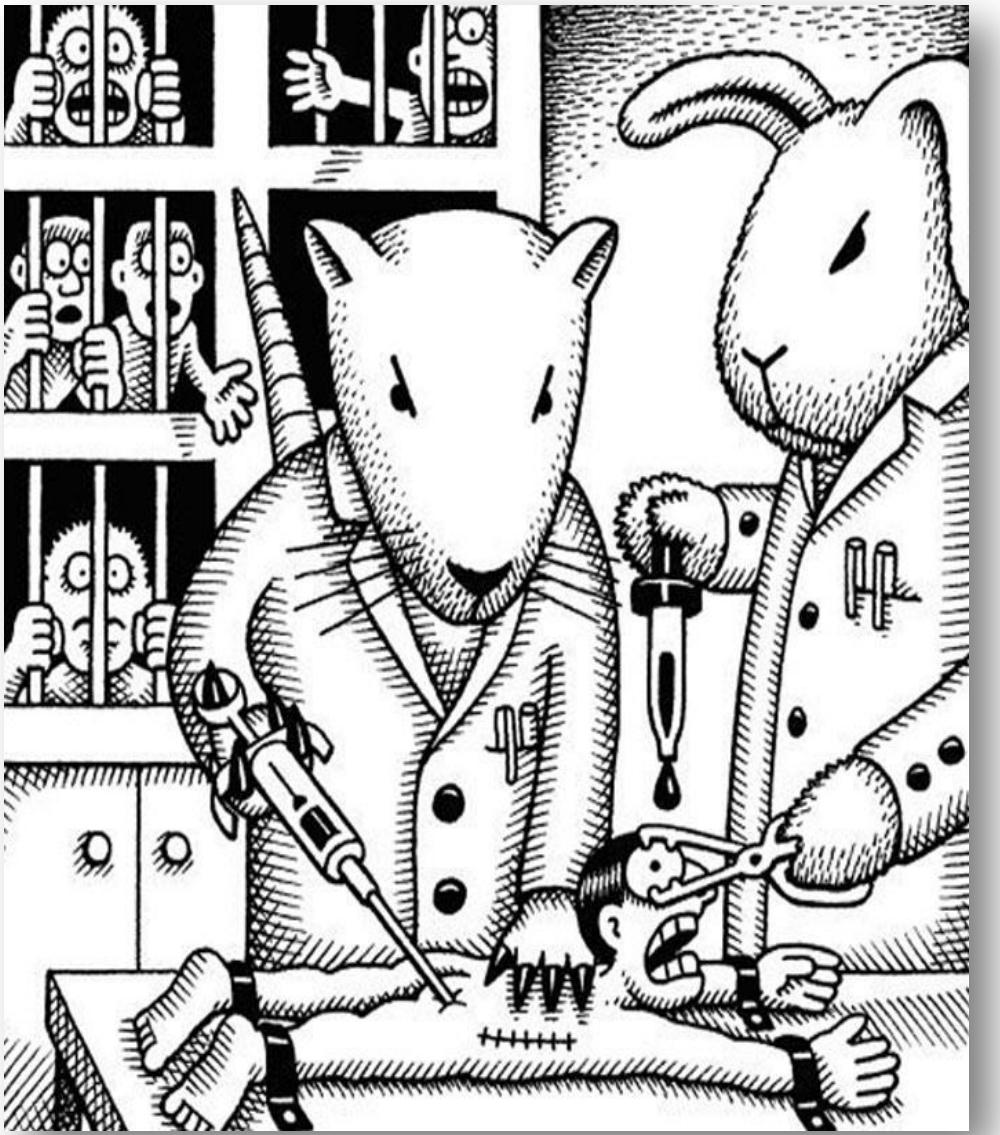
✓ Test systems that become diseased or injured during the course of a study should be *isolated and treated, if necessary to maintain the integrity of the study*.

✓ Any diagnosis and treatment of any disease before or during a study *should be recorded*»

The sources of pain and suffering for the laboratory animals in the animal facility

❑**Experimental procedures:**
✓Surgical operations
✓Invasive procedures

The need of pain relief
•Before the procedure
•During
•After the procedure



Drugs for the pain alleviation

Sedative treatment **-** drugs, ***causing a state of rest and low anxiety reducing pain sensitivity*** while maintaining consciousness, reflexes, control over yourself and the environment while

Analgetics **-** pecifically affecting the central nervous system, ***decreasing or eliminating pain.*** Analgesia is the dominant effect not accompanied (in therapeutic doses) with loosing of consciousness or severe motor disfunctions

Anesthetics **-** causes reversible inhibition of nociception at the central nervous system level ***provoking sleep, loss of consciousness and memory (amnesia), skeletal muscle relaxation, lowering or disabling some reflexes, and the pain sensitivity (the general anesthesia***) disappears in many cases

Local anesthetics **- *inhibits the nociception at any body region*** with complete preservation of consciousness, topical application

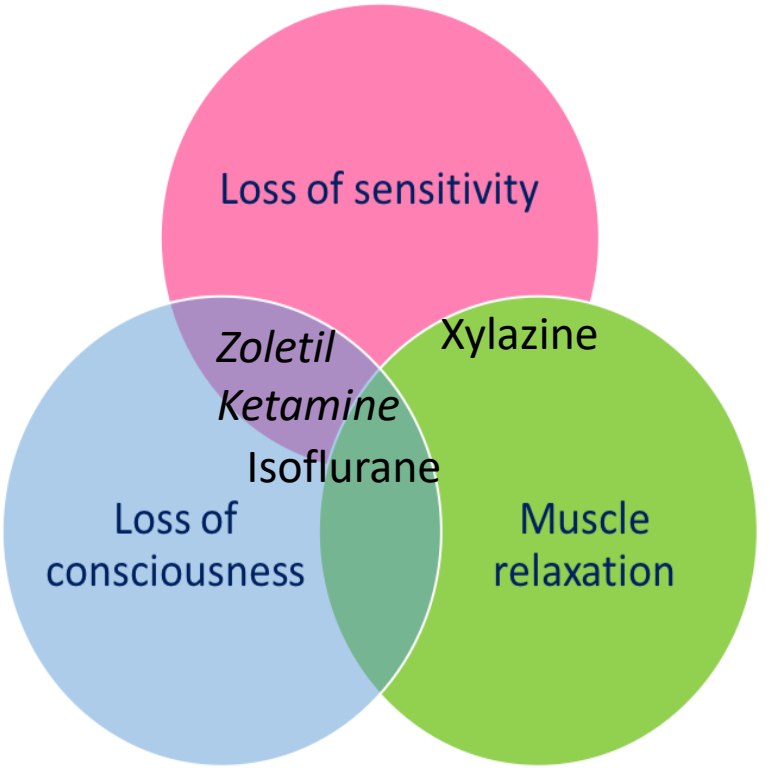
- cream (mainly for mucous membranes)
- anesthetic solutions (lidocaine, tetracaine)
- local cooling (by spraying ethyl chloride)
- injection of local anesthetics (novocaine, lidocaine, trimekain)

The triad of anesthesia

Directive 2010/63EU, Article 14 (Anaesthesia), #3

3. Member States shall ensure that animals are not given any drug to stop or restrict their showing pain without an adequate level of anaesthesia or analgesia.

In these cases, a scientific justification shall be provided, accompanied by the details of the anaesthetic or analgesic regimen.



- ✓ The proper pain management possible in combination of one or more anesthetics possessing analgesic effect.
- ✓ The optimal dose and combination of drugs is chosen depending on the type of procedure and taking into account the individual characteristics of the animal.

Injections: administration of anesthetic agents

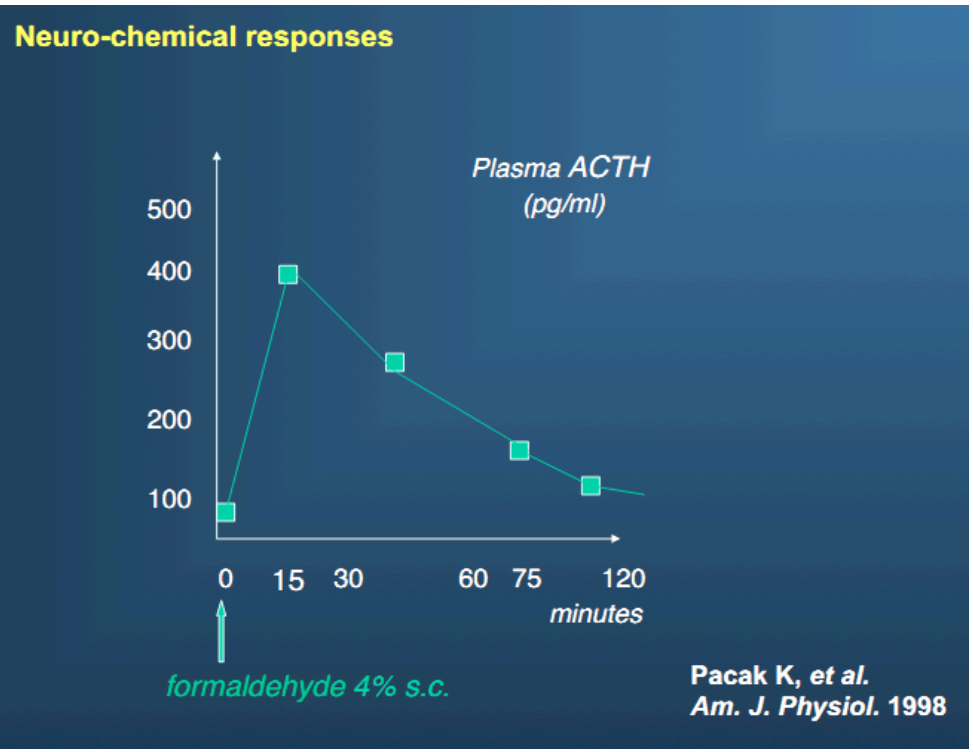
✓***Long-term procedures, surgical operations,***
✓***Long recovery***

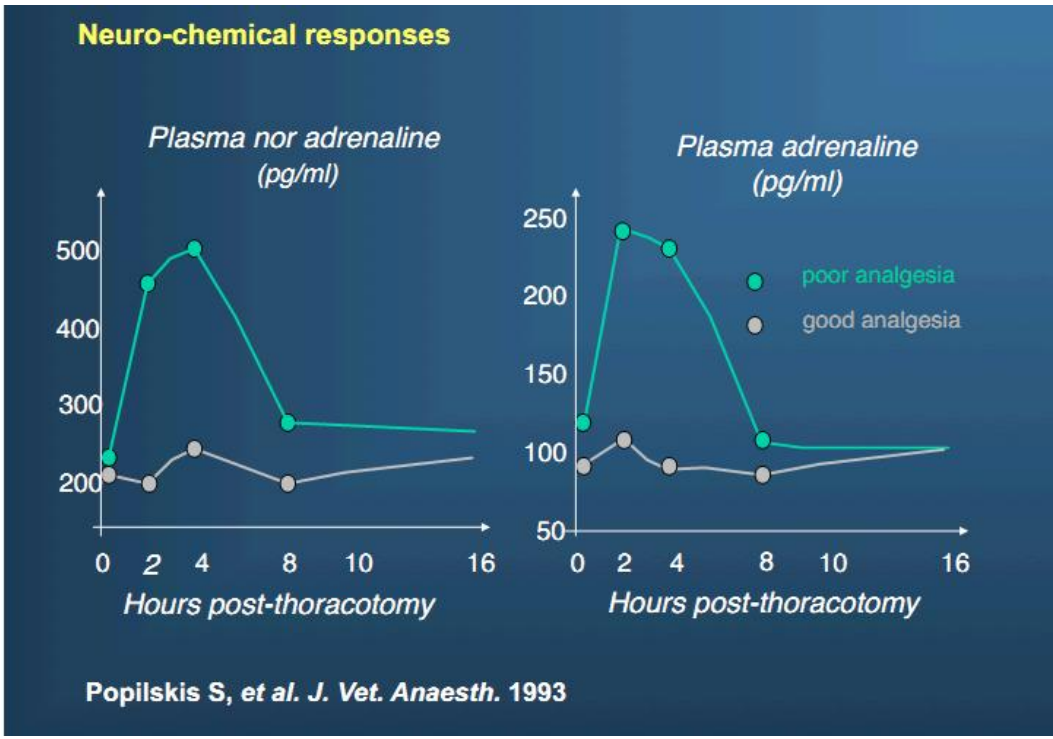
Zoletil: Anesthesia, analgesia

Ketamine: anesthesia, myorelaxation, sedation, analgesia

Xyla: analgesia, sedation, muscle relaxation

Pain after surgery

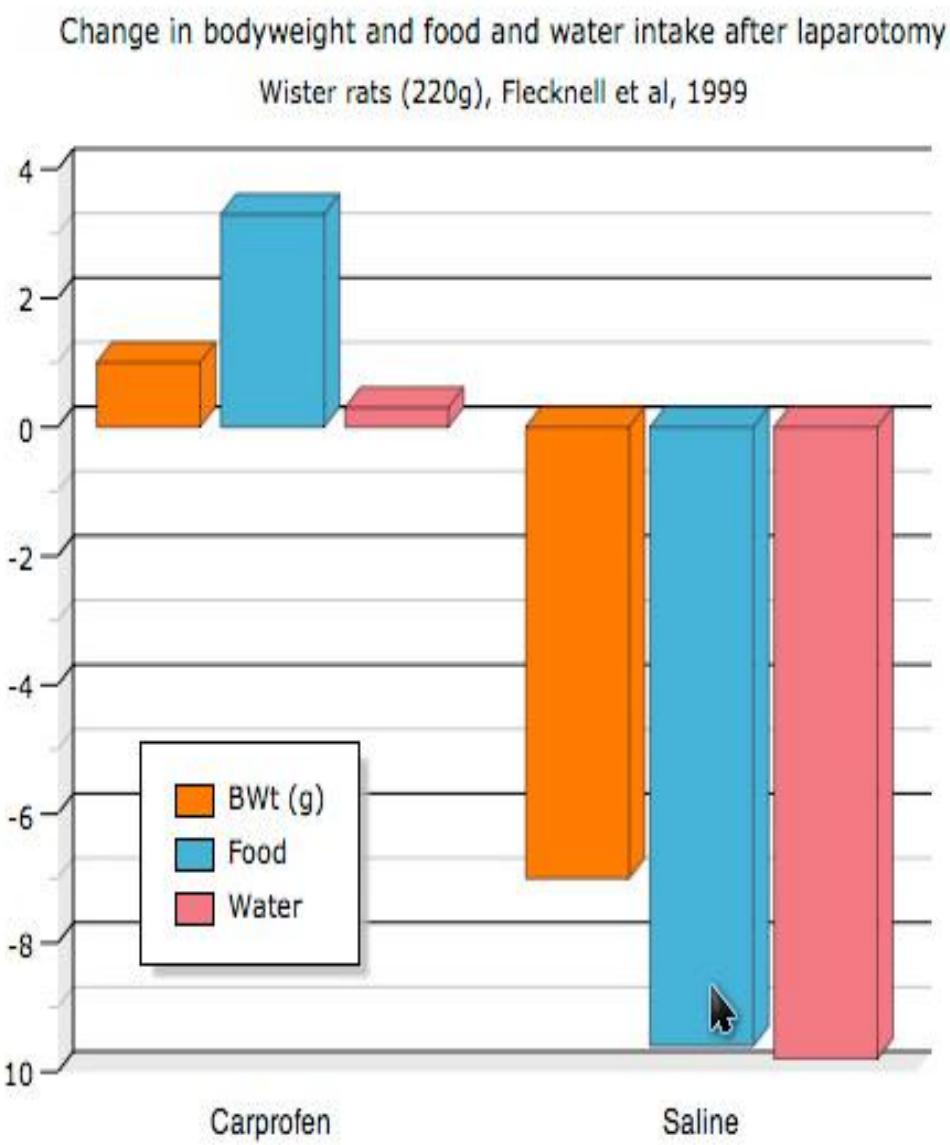




Increase of levels of ACTH, nor adrenaline, adrenaline because of the activation of sympathetic nervous system:

- The blood pressure growth - ***hypertension***
- The increased activity of the myocardium provoke ***cardiac abnormalities***
- Hypoxia symptoms - ***disorders of the respiratory system***
- Metabolic acidosis - ***inflammation***
- Causes ***complications after anesthesia***
- Increases ***pain***
- Causes ***sleep*** abnormalities

Pain after surgery



Inactivation of the *parasympathetic nervous system:*

Animals who underwent surgery and did not receive analgesics **lost more weight** than those receiving pain relief. Most of the body weight loss was caused by a **reduction in food and water consumption**.

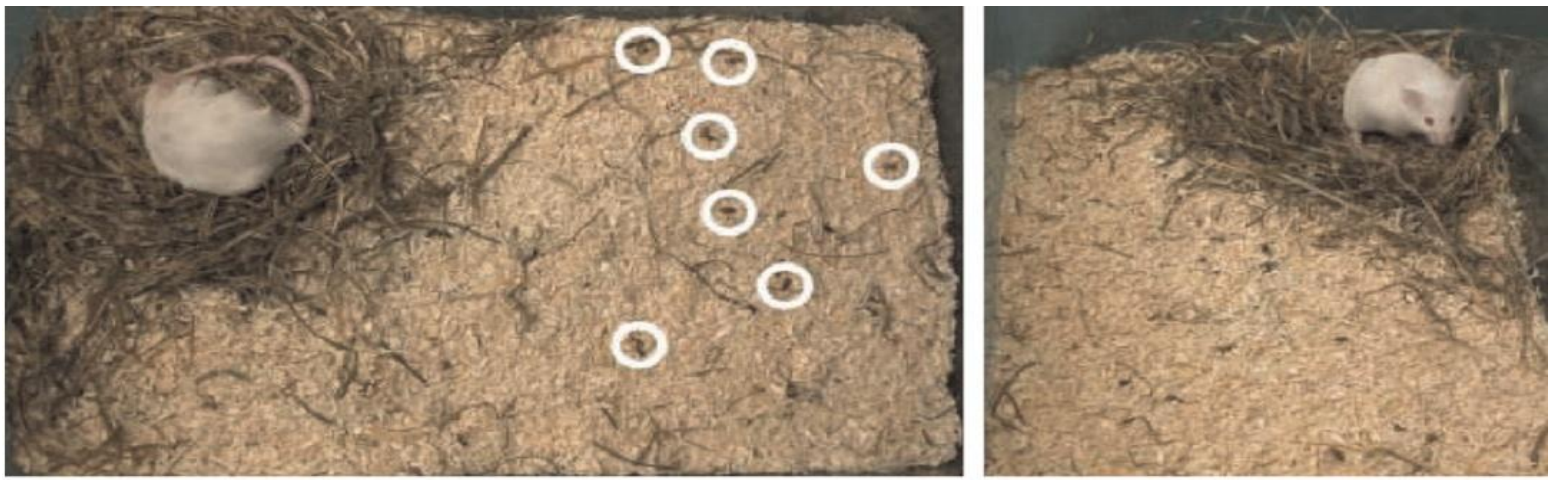
Measuring these effects is **quick and easy**, and allows those individuals who are not recovering to be identified.

The problem with this approach is that **it does not allow assessment of the animals as soon as they begin to experience pain.** This means that additional pain relief is only given long after it is needed.

Ideally, we should be **using a pain assessment method that allows rapid assessment**, so more analgesics can be given at the time they are needed.

Pain after surgery

Changes in behaviour



+ analgesia

- analgesia

Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability.Margarete Arras. *BMC Veterinary Research*20073:16

Post-operative analgesia

During the pre- and post-operative period

	Carprofen	Meloxicam	Buprenorphine	Morphine
Mouse	10 mg/kg s.c.	5 mg/kg s.c.	0.1 mg/kg s.c.	2 - 5 mg/kg s.c.
Rat	5 mg/kg s.c.	1 - 2 mg/kg s.c. or 4 mg/kg per os	0.01 - 0.05 mg/kg s.c.	2 - 5 mg/kg s.c.
Rabbit	4 mg/kg s.c.	0.2 – 0.4 mg/kg s.c., 0.2 - 0.6mg/kg per os	0.01 - 0.05 mg/kg s.c	2 - 5 mg/kg s.c. or i.m
Guinea Pig	2 - 5 mg/kg s.c.	?	0.05 mg/kg s.c.	2 - 5 mg/kg s.c. or i.m
Dog	4 mg/kg i.v., s.c. or per os	0.2 mg/kg s.c. or 0.1 mg/kg per os	0.01 - 0.02 mg/kg s.c., i.m. or i.v.	0.3 - 1.0 mg/kg s.c. or i.m.
Cat	2 - 4 mg/kg i.v. or s.c.	0.3 mg/kg s.c. or 0.1 mg/kg per os	0.01 - 0.02 mg/kg s.c., i.m. or i.v.	0.1 - 0.2 mg/kg s.c.
Rhesus Macaque	4 mg/kg s.c.	0.1 mg/kg s/c or per os	0.005 - 0.01 mg/kg s.c., i.m. or i.v.	1 - 2 mg/kg s.c. or i.m.
Sheep	1.5 - 2 mg/kg s.c. or i.v.	?	0.005 - 0.01 mg/kg i.m. or i.v.	0.2 - 0.5 mg/kg i.m.

The necessity of pain relief

Directive 2010/63EU, Article 14 (Anaesthesia), #2

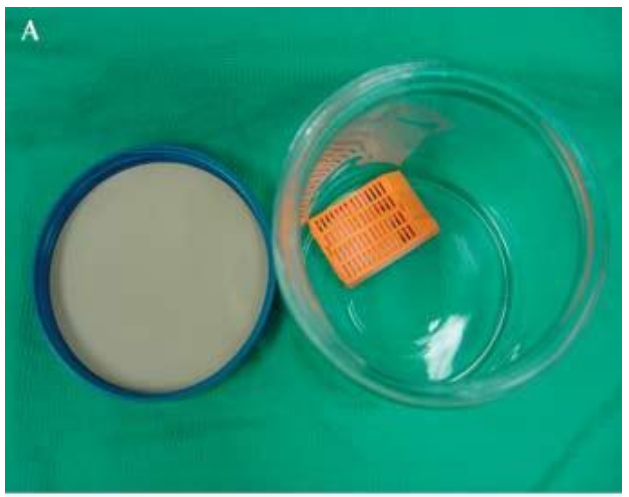
"…Member States shall ensure that, unless it is inappropriate, procedures are carried out under general or local anaesthesia, and that analgesia or another appropriate method is used to ensure that pain, suffering and distress are kept to a minimum.
Procedures that involve serious injuries that may cause severe pain shall not be carried out without anaesthesia…"

- ❑ **Painful procedures should be performed under general or local anaesthesia**
- ❑ **Stressful procedures should be performed under anaesthesia or sedation**
- ❑ **The choice of drugs should fit the type and length of the procedure and the type and degree of pain expected**

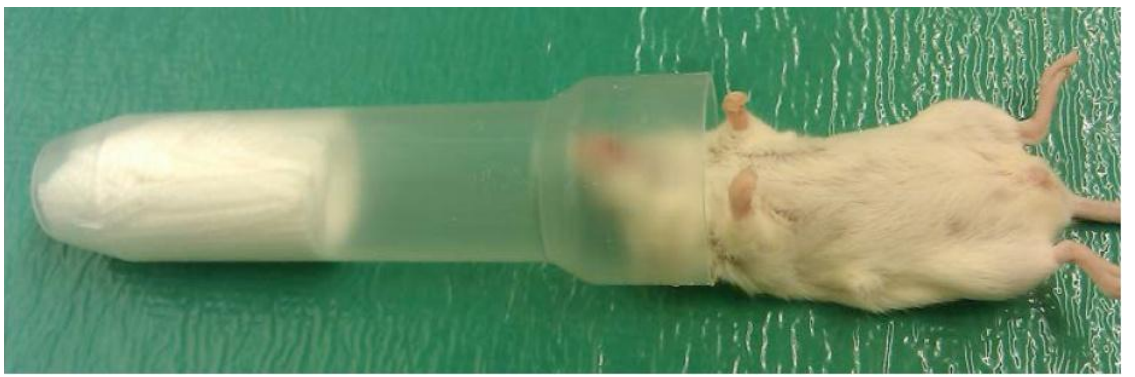
Administration of anesthetics or sedatives BEFORE immobilizing the animal!

Open-drop method of anesthetic administration

Short-term, not painful and non-invasive procedures (restraining)



- ✓Poor analgesia
- ✓Difficult to dose
- ✓Too fast recovery
- ✓Not safe



Ensure the air access!!!

Inhalational method of anesthetic administration

Flow of anesthetic through the inhaler equipped with a device for flow measurement and exhaust device

Isoflurane, Halotane, Sevoflurane

- ✓**Easy-managed**
- ✓**Fast recovery**
- ✓**Isoflurane in low dose may cause hyperalgesy!**

For surgery, and other painful invasive procedures it needs additional analgesia!

The sources of pain and suffering for the laboratory animals in the animal facility

Experimental procedures:

✓Surgical operations
✓Blood tissues withdrawal other sampling
✓Administration of (toxic) substances
✓Restraining
✓Wrong handling of animals
✓Isolation, food and water deprivation

Suffering during hours/days/weeks, sometimes-months

Transportation:

✓Inadequate size and shape of containers
✓Food and / or water deprivation
✓Duration
✓Noise, smells, sounds, etc.

Macro environment:

✓noise, vibration, bright light, overheating or hypothermia, humidity, etc.

Husbandry and care:

✓Food and water deprivation, isolation, irregular cage changes, mother separation etc.

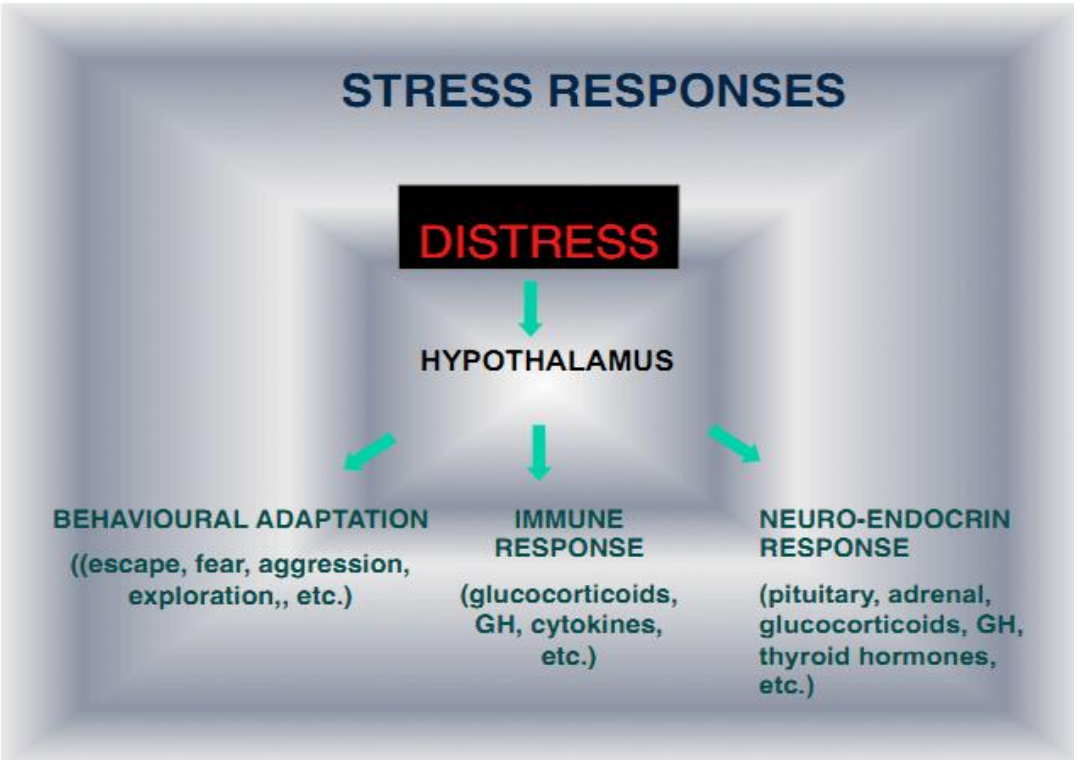
Microenvironment :

Overcrowded cages, resources competition, aggressor in cage, etc.

Suffering during all animal's life in the facility

Distress

The animal puts ***substantial efforts (both unconsciously and consciously) in the adaptive response, but those efforts are unsuccessful***. Other biological processes are affected, with negative collateral consequences



Distress is a state that affects significantly the welfare of an animal.
The animal must devote substantial effort or resources to adapt to stimuli from the environment
It may or may not be associated with pain.
Many biological processes are involved

https://www.youtube.com/watch?v=Um4rGCt8p7I https://youtu.be/ojPtKnCB1pA

P1070309.MP4

Physiological abnormalities:
increased lymphocytosis, the hyperactivation of the nervous and endocrine systems of the body, releasing of hormones, stress-reactions, exhaustion of the stress-resistance

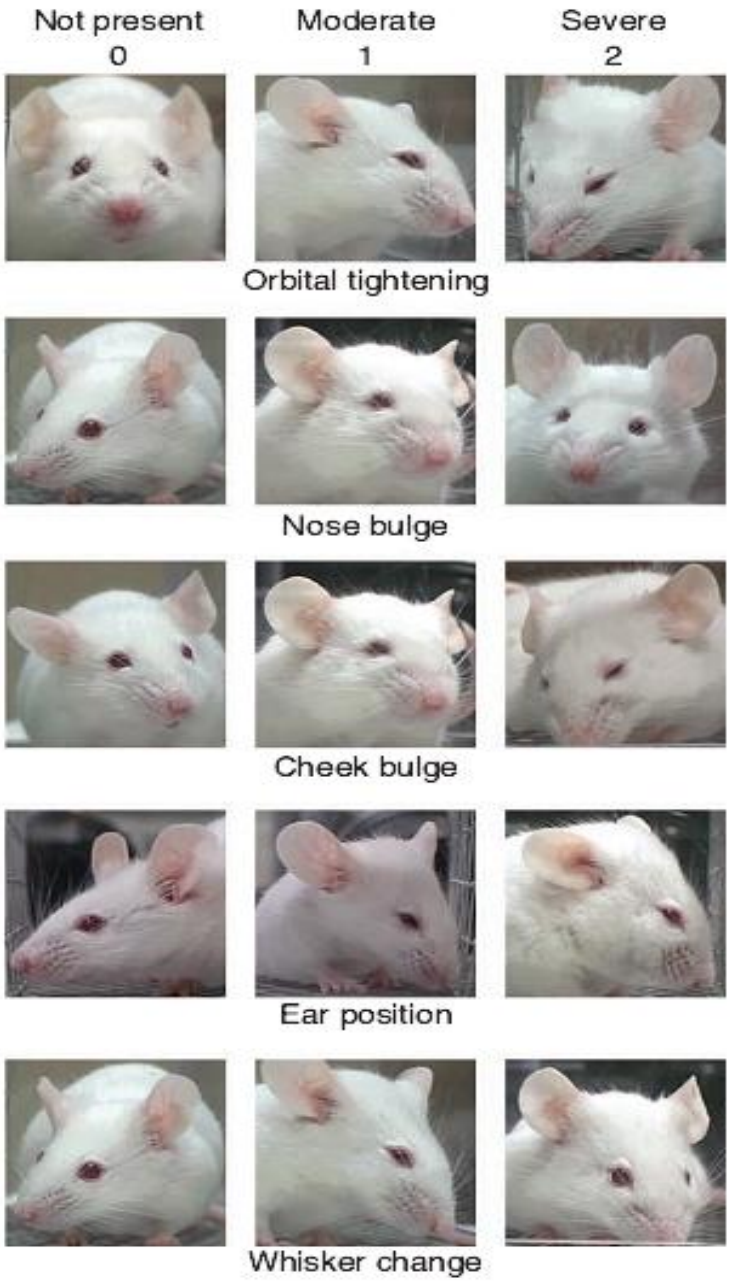
Behavioural abnormalities: changes in locomotor and exploratory reactions, stereotyped behavior, avoidance behavior, overt manifestations of fear, anxiety, pain symptoms

The animal condition selection criteria

❑**Appearance**
❑**Behaviour:** ✓*Spontaneous* ✓*Provoked* ✓*During the experiment or everyday care*
❑**Organism functioning**
❑**Environment**
❑**Instrumentally measured parameters**
❑**Experimental procedure parameters**

Mouse grimace scale

Pain intensity is defined as the sum of scores for each symptom



Coding of facial expressions of pain in the laboratory mouse. NATURE METHODS| ADVANCE ONLINE PUBLICATION. PUBLISHED ONLINE 9 MAY 2010

The scale of the of signs of pain and distress severity

Mild	Moderate	Substantial
Reduced weight gain	Weight loss of up to 20%	Weight loss greater than 25%
Food and water consumption 40-75% of normal for 72 h	Food and water consumption less than 40% of normal for 72 h	Food and water consumption less than 40% for 7 days, or anorexia (total inappetence) for 72 h
Partial piloerection	Staring coat – marked piloerection	Staring coat – marked piloerection – with other signs of dehydration such as skin tenting
Subdued but responsive, animal shows normal provoked patterns of behaviour	Subdued animal shows subdued behaviour patterns even when provoked	Unresponsive to extraneous activity and provocation
Interacts with peers	Little peer interaction	
Hunched transiently especially after dosing	Hunched intermittently	Hunched persistently ('frozen')
Transient vocalization	Intermittent – vocalization when provoked	'Distressed' – vocalization unprovoked
Oculo-nasal discharge transient (typically signs of chromorhino-dacryorrhoea in rodents)	Oculo-nasal discharge persistent	Oculo-nasal discharge – persistent and copious
Normal respiration	Intermittent abnormal breathing pattern	Laboured respiration
Transient tremors	Intermittent tremors	Persistent tremors
No convulsions	Intermittent convulsions	Persistent convulsions
No prostration	Transient prostration (less than 1 h)	Prolonged prostration (more than 1 h)
No self-mutilation	No self-mutilation	Self-mutilation

According to “Pain and distress in laboratory rodents and Lagomorphs”. Report of the FELASA Working Group 1992

Decision making algorithm

Sign	Score	Actions
Weight loss		
5-10%	**1**	Review frequency of monitoring
11-15%	**2**	Consider supplementary care
16-20%	**3**	Consider supplementary care
20%+	**4**	**Humane Euthanasia (HEP)**

Example of score sheet for the severity assessment

Cage № 1, mice №№ 1-5	Дата	01.06	02.06	03.06	04.06	05-25.06
Appearance						Continuing observations…
Body weight decreasing		0	0	1 (mouse № 1, 2)	1 (mouse № 1)	…
Bad coat condition		0	1 (mouse № 1)	1 (mouse № 1)	1 (mouse № 1)	…
Body functions						…
Breathing changes		0	0	0	1 (mouse № 1)	…
Environment						…
Diarrhea		0	1 (mouse № 1)	1 (mouse № 1, 2)	1 (mouse № 1,2)	…
Blood in diarrhea		0	0	0	0	…
Behaviour						…
Reaction to handling		1 (mouse № 1)	1 (mouse № 1)	1 (mouse № 1)	1 (mouse № 1)	…
Aggression		0	0	0	0	…
Abnormal gait		0	0	0	1	…
Abnormal posture		0	0	0	0	…
Reluctance to move		0	0	0	1 (mouse № 1)	…
Procedure-specific indications						…
Tumor size		0	0	0	0	…
Ulceration of tumor		0	1 (mouse #3)			…
Tumor impending movement		0	0	0	0	…
Total score		**1**	**3**	**4**	**6**	…
Decisions		**Increase the health check – 2 times per day**	**HEP – mouse # 3 Change the bedding (mouse #1)**	**Consultation to vet**	**HEP (mouse№ 1)**	…

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ООО «НИИ МИТОИНЖЕНЕРИИ МГУ»

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Instruction for minimizing pain and distress in laboratory animals

1. Receive adequate training before starting a procedure: be able to identify the normal behavior the signs of pain and distress.
2. Determine the minimum period, necessary to obtain adequate experimental data during which the animals will experience pain.
3. Choose the anesthetic/analgesic drug that is suitable to the animal species choice and the tasks of the experiment
4. Develop a score sheet for revealing the state of the animal for your particular research procedure, you may also use simple measures such as food and water consumption and body weight changes.
5. Make the pilot study to ensure the right dosage of drugs.
6. Prepare a program of the interventions to eliminate or reduce pain.
7. Perform surgical procedures using aseptic technique to prevent infection. Apply the post-surgical analgesia and other appropriate techniques.
8. Use score sheets to identify abnormalities in animal welfare, and evaluate signs of pain and distress at intervals appropriate to their state (the worse the more frequent). Trace all abnormalities in animals welfare, alleviate pain if necessary, adjust drugs. Write down all the prescriptions. Keep up to date with developments in this area, and review your protocols for animal care regularly.
9. Use painkillers if necessary, and if the animal condition worsens adjust your therapy or perform humane euthanasia.
10. If the planned intervention is terminal, kill the animal before it starts feeling pain!
11. ***Experiments, which results may be distorted by analgesia, could be done without anesthesia only if there is a scientific justification approved by the bioethics commission***

Use of drugs for anesthesia and analgesia

Procedures	Aim of use	Moment of provision	Types of drug
Invasive and painful experimental procedures, non-experimental injuring of animals	Elimination and / or reduction of pain	Before, during, and after the procedure (if necessary)	Local General anaesthetics Sedatives Analgesics
Immobilization for imaging procedures (MRI, CT)	Prevention of stress and injury of animals	Administration of anesthetics or sedatives BEFORE the animal's immobilisation!	Sedatives General anaesthetics
Blood sampling, catheterization etc.	To ensure the safety of stressful and traumatic procedures		Local General anaesthetics Sedatives