Taking samples
What samples?

- blood / plasma / serum
- body fluids: liquor, tears, urine, stomach/gut contents, stool,
- cells: smears, scratches, suspensions, lavages, biopsies
- tissues / organs (fixation, perfusion)
- whole animals
Metabolic cages

Abb. 16-3: Stoffwechselkäfig für kleine Nagetiere zum getrennten Sammeln von Urin und Kot.
Blood withdrawal
Points to consider:

• volume to be withdrawn
• frequency of withdrawal
• site and method of withdrawal
• personal skills and experience
• (kind of) anesthesia
• species, age, gender, body condition and health status
Puncture of the facial vein

- take the animal by the scruff
- puncture with an animal lancet dorsocaudal from the mandible
- collect blood
- stopp sqeezing the vessel
- compress the puncture site
Superficial Temporal Vein Collection

- A = Length of eye
- B = Width of eye

Sebaceous gland
Puncture of the retro-orbital sinus

- only under anesthesia!!!!!
- squeeze the vessels of the neck by scruffing the animal
- introduce the capillary at the inner angle of the eye and move it towards the ear of the opposite side
- under soft pressure and slight rotations penetrate the tissue
- collect the blood
- loose the grip around the neck

Each eye can be used twice (maximum), with a 2-week-break in between
Puncture of the retro-orbital sinus
Puncture of the retro-orbital sinus

only under anesthesia!!!
Puncture of the lateral tail vein

- same procedure as i.v. application
- puncture with a needle
- inzision should be avoided
- can lead to granuloma in rats

cutting the tip of the tail is only allowed in animals younger than 3 weeks
Puncture of the lateral tail vein
Cardiac puncture

• terminal procedure
• under anesthesia

Two methods:
1. open chest: under visual control
2. closed chest: through the lateral wall of the thorax; on elbow-level, vertical to the body axis
Cardiac puncture

Only under anesthesia and only as a terminal procedure
NO waking up again!!!
Further sites for blood withdrawal

- Vena saphena
  on the outside of the lower leg
  squeeze the vessel above the knee joint
  flat insertion of the needle
- Vena sublingualis (rat)
Vena saphena
Ausschuss für Tierschutzbeauftragte in der GV-SOLAS und Arbeitskreis 4 in der TVT

Empfehlung zur Blutentnahme bei Versuchstieren, insbesondere kleinen Versuchstieren


Die Empfehlung gliedert sich in zwei Abschnitte. Der erste Abschnitt erörtert die grundsätzlichen und technischen Fragen der Blutentnahme, der zweite Abschnitt enthält Tabellen über geeignete Methoden der Blutentnahme sowie -volumina.

Stand: Mai 2009
Amounts of blood to be withdrawn

Tab. 1: Durchschnittliches Blutvolumen bei verschiedenen Tierarten

<table>
<thead>
<tr>
<th>Spezies</th>
<th>Gesamtvolumen</th>
<th>Hämatokrit (%)</th>
<th>Geschätztes absolutes Blutvolumen bei angegebenem Gewicht (ml)</th>
<th>Anwendungsbeispiel Blutentnahme² (ml) bei angegebenem Körpergewicht</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/kg KGW</td>
<td>% KGW</td>
<td></td>
<td>einmalig³ (max. 10 %)</td>
</tr>
<tr>
<td>Maus (25 g)</td>
<td>74 (70 - 80)</td>
<td>6,6</td>
<td>42 (33 - 50)</td>
<td>1,7</td>
</tr>
<tr>
<td>Ratte (300 g)</td>
<td>64 (50 - 70)</td>
<td>6,4</td>
<td>46 (40 - 61)</td>
<td>19</td>
</tr>
<tr>
<td>Meerschweinchen (400 g)</td>
<td>75 (65 - 90)</td>
<td>7,5</td>
<td>44 (37 - 50)</td>
<td>30</td>
</tr>
<tr>
<td>Goldhamster (100 g)</td>
<td>78 (65 - 80)</td>
<td>7,8</td>
<td>51 (39 - 59)</td>
<td>7,8</td>
</tr>
<tr>
<td>Mongolische Rennmaus (100 g)</td>
<td>67 (60 - 85)</td>
<td>6,7</td>
<td>48 (40 - 52)</td>
<td>6,7</td>
</tr>
<tr>
<td>Frettchen (800 g)</td>
<td>75 (60 – 80)</td>
<td>7,5</td>
<td>45 (38 - 54)</td>
<td>60</td>
</tr>
<tr>
<td>Kaninchen (3,2 kg)</td>
<td>56 (45 - 70)</td>
<td>5,6</td>
<td>41 (31 - 50)</td>
<td>180</td>
</tr>
<tr>
<td>Hund (15 kg)</td>
<td>86 (79 - 90)</td>
<td>7,5</td>
<td>50 (42 - 58)</td>
<td>1130</td>
</tr>
<tr>
<td>Katze (3 kg)</td>
<td>56 (47 - 66)</td>
<td>5,6</td>
<td>38 (30 - 45)</td>
<td>168</td>
</tr>
<tr>
<td>Huhn (1,1 kg)</td>
<td>65 (60 - 90)</td>
<td>6,5</td>
<td>34 (25 - 45)</td>
<td>71</td>
</tr>
<tr>
<td>Marmoset (350 g)</td>
<td>70 (58 – 82)</td>
<td>7,1</td>
<td>45 (37 - 52)</td>
<td>25</td>
</tr>
<tr>
<td>Rhesusaffe (9 kg)</td>
<td>54 (44 - 67)</td>
<td>5,4</td>
<td>41 (33 - 50)</td>
<td>480</td>
</tr>
<tr>
<td>Minipig (20 kg)</td>
<td>65 (61 - 68)</td>
<td>6,5</td>
<td>39 (30 - 50)</td>
<td>1300</td>
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<tr>
<td>Schaf (50 kg)</td>
<td>66 (55 - 80)</td>
<td>6,6</td>
<td>32 (26 - 37)</td>
<td>3300</td>
</tr>
<tr>
<td>Ziege (40 kg)</td>
<td>70 (57 - 90)</td>
<td>7,0</td>
<td>33 (26 - 37)</td>
<td>2800</td>
</tr>
<tr>
<td>Rind (400 kg)</td>
<td>57 (52 - 61)</td>
<td>5,7</td>
<td>40 (33 - 50)</td>
<td>22800</td>
</tr>
<tr>
<td>Pferd (300 kg)</td>
<td>75 (56 – 118)</td>
<td>7,5</td>
<td>33 (26 - 42)</td>
<td>22500</td>
</tr>
</tbody>
</table>

¹ Unterschiede bezüglich Rasse, Stamm und Alter sind möglich; der prozentuale Anteil ist bei fettleibigen Tieren geringer als bei normalgewichtigen.
² Diese Abnahmemengen gelten für gesunde, erwachsene Tiere. Möglicherweise tolerieren Tiere nach experimentellen Manipulationen oder kranke, alte oder gestresste Tiere nicht die gleichen Blutentnahmervolumina.
³ sich anschließende Erholungsphase von mindestens 2 – 3 Wochen
⁴ tägliche Blutabnahmengen über max. 2 Wochen, anschließende Erholungsphase von mindestens 2 – 3 Wochen
⁵ in Narkose; ca. 50 % der Blutmenge (empirischer Wert)
## Sites for blood withdrawal

### Tab. 2: Lokalisationen für Blutentnahmen

<table>
<thead>
<tr>
<th></th>
<th>Maus</th>
<th>Ratte</th>
<th>Hamster</th>
<th>Rennmaus</th>
<th>Meerschweinchen</th>
<th>Kaninchen</th>
<th>Hund, Katze</th>
<th>Huhn</th>
<th>Schwein</th>
<th>Schaf, Ziege</th>
<th>Rind, Pferd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohrvene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohrarterie</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrobulbärer Venenplexus(^1)</td>
<td>G</td>
<td>(G)</td>
<td>G</td>
<td>K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. facialis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamm (Huhn)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. jugularis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
<td>(E)(^1)</td>
<td>G</td>
<td>(E)(^1)</td>
<td>K, G</td>
<td>K, G</td>
</tr>
<tr>
<td>Venenwinkel</td>
<td>G(^1)</td>
<td>G(^1)</td>
<td>G(^1)</td>
<td>G(^1)</td>
<td>G(^1)</td>
<td></td>
<td></td>
<td>K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. cephalica antebrachii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K, G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. saphena</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K, G</td>
<td>K, G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwanzvene</td>
<td>K, G</td>
<td>K, G</td>
<td>K</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td></td>
<td>K (Rind)</td>
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</tr>
<tr>
<td>V. cava(^2)</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>K, G</td>
<td>G(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. sublingualis</td>
<td>K</td>
<td>K</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flügelvene (V. ulnaris)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta, große Baucharterien(^4)</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herz(^6)</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
</tbody>
</table>

**Legende:**
- K = geeignet für kleine Blutmengen; G = geeignet für größere Blutmengen; ( ) = bedingt geeignet; E = geeignet zum Entbluten

\(^1\) nur in Narkose; \(^2\) in Narkose bei eröffnetem Bauchhöhle bzw. eröffnetem Brustkorb (außer beim Schwein); \(^3\) V. cava cranialis bzw. V. brachiocephalica; 
\(^4\) in Narkose bei eröffnetem Bauchhöhle bzw. eröffnetem Brustkorb; \(^5\) nur final in Narkose
Species-specific information

<table>
<thead>
<tr>
<th>Entnahmemenge und -häufigkeit</th>
<th>Entnahmeort</th>
<th>Besondere Erfordernisse</th>
<th>Geschätzter Belastungsgrad und Dauer</th>
</tr>
</thead>
</table>
| 1. Kleine Blutmenge (0,02 - 0,04 ml) | a) Schwanzvene  
b) Amputation der Schwanzspitze  
c) Retrobulbär  
d) V. facialis  
e) V. saphena | b) ausschließlich bis zum Absetzalter  
c) Narkose, (z. B. Isofluran), Glaskapillare mit 0,8 mm Außendurchmesser, Heparinisierung unnötig | a) gering, < 1 Tag  
b-e) gering – mäßig, < 1 Tag |
| 2. Einmalige maximale Blutmenge (s. Tab. 1) | a) Retrobulbär  
b) Schwanzvene  
c) Venenwinkel  
d) V. facialis | a) wie unter 1c) beschrieben  
b) Kanüle: 24 - 26 G  
c) Narkose; Kanüle: 23 - 25 G | gering - mäßig, < 1 Tag |
| 3. Wiederholte Entnahme (s. Tab. 1) | Mehrfachabnahme innerhalb von 24 h: Schwanzvenen  
Tägliche bzw. wöchentliche Abnahme:  
a) Schwanzvenen  
b) V. facialis  
c) V. saphena  
d) Retrobulbär | Bei allen Entnahmarten alternierende Entnahmestellen wählen.  
d) Narkose, pro Auge maximal zwei Abnahmen im Abstand von 14 Tagen | gering - mäßig, < 1 Tag |
| 4. Finale Entnahme (Entbluten) | a) Kardial  
b) Aorta, V. cava  
c) Dekapitation | a, b) Narkose  
c) auch ohne Narkose möglich | a-c) gering |
Pathophysiology of blood withdrawal

up to 10 % of the total blood volume:
compensatory reactions: vasoconstriction and increase in heart rate; no hemodynamic effects

15 to 20 % of the total blood volume:
in spite of compensatory reactions: decrease in blood pressure, tachycardia, tachypnoe

20 bis 30 % of the total blood volume:
hypovolemia (standard-shock-model in experimental research)

> 30 % of the total blood volume:
decompensation
Taking organs
Plate 4  Organs of the ventral neck, ventral view, deep layer (organa colli ventralis, norma ventralis, stratum profundum).

A  lymphonodi mandibulares
B  glandula sublingualis
C  glandula mandibularis (submandibularis)
D  glandula parotis et lymphonodus cervicalis superficialis
E  glandula thyreoidea et parathyreoidea
F  esophagus
G  trachea

1  musculus sternohyoideus et sternothyreoides (resectus)
2  musculus digastricus, venter rostralis
3  musculus mylohyoideus
4  musculus masseter et ductus parotideus
5  mandibula
6  musculus sternomastoideus
7  musculus omohyoideus
8  musculus pectoralis major
9  vena jugularis externa
Abdominal cavity
Abdominal cavity
Abdominal cavity
Genitals of a male rodent
Genitals of a female rodent
Anesthesia and Analgesia
Legal provisions
§ 5 Animal Welfare Law

Vertebrates: no painfull intervention without anesthesia
Anesthesia has to be performed by a veterinarian
(exception: local anesthesia)
...
No anesthesia is required,
1. if there would be no anesthesia in the same procedure
   in a human being or if the intervention is less painfull
   compared to the negative effect of the anesthesia itself
2. if a veterinarian decides, that in this particular case
   anesthesia is not applicable

Is no anesthesia required, all opportunities have to be
exhausted, to minimize pain and suffering of the animals
§ 17 Decree on laboratory animal welfare

(1) When performing experiments in vertebrates and cephalopodes one has to make sure to reduce pain and suffering to the least possible extend by applying pain killing substances and using least painful methods.

(2) Experiments with vertebrates and cephalopodes are only allowed under anesthesia or local analgesia unless

1. the procedure causes less pain than the anesthesia itself
2. the aim of the experiment excludes anesthesia and does not lead to severe injuries
§ 17 Decree on laboratory animal welfare

Anesthesia is only to be performed by a person fulfilling the requirements of §7 Abs.1 Satz3 TSchG (required knowledge and skills) and of §16 Abs.1 Satz2 TSchVersV (person admitted to perform surgery)

If the anesthesia is performed for teaching purposes other people are allowed to perform it under supervision of such a person mentioned above
§ 17 Decree on laboratory animal welfare

(3) Is to expect, that in an anesthetized animal pain will occur when the animal wakes up, the animal has to be treated in time with analgesics or pain relieving methods.

This does not have to be the case, when it is ethically justified and scientifically well founded that applying these methods or substances is incompatible with the aim of the experiment.

In vertebrates and cephalopodes it is not allowed to use or apply substances, that hinder or impede the expression of pain!!
§ 16 Decree on laboratory animal welfare
Requirements concerning expert knowledge

... animal experiments including surgery are only allowed to be performed by ...
1. people with completed studies (degree) of veterinary or human medicine
2. people with completed studies in natural science (degree) plus the adequate knowledge and skills (verifiable)

The authority can approve and admit exceptions if the proof of the required knowledge and skills has been given otherwise
General remarks concerning anesthesia

Principles of anesthesia:
• hypnosis (unconsciousness, dormancy)
• analgesia
• relaxation

is reached by certain substances causing a reversible intoxication of the central nervous system

Anesthesia has to protect each individual animal from unnecessary suffering and distress
General remarks concerning anesthesia

local anesthesia / regional anesthesia
blockade of afferent (sensory) neurons
no transmission of pain
no loss of consciousness
in animals usually problem of acceptance

general anesthesia
reversible blockade of the CNS
1. injectable anesthesia
2. inhalational anesthesia
General remarks concerning anesthesia

depend on the kind of (surgical) intervention weight is shifted on either hypnosis, analgesia and / or muscle relaxation (example: local anesthesia)

perfect anesthesia is usually not achievable with one single substance

↓

combination of substances:

• synergistic effects (less dosing, less side effects)

• supplement of missing properties

• antagonism (contrary properties neutralise each other; convulsive / anti-convulsive)
The perfect anesthetic substance...

• would lead to the desired state of hypnosis, analgesia and relaxation without any side effects
• would allow a fast induction without excitations
• would reach the state of surgical tolerance quickly
• would allow quick and smooth recovery
• would have no irritating properties
• would be cheap, stable, non explosiv and non-flamable
• would not require specific equipment

... is non-existent!
Choice of suitable methods of anesthesia

Selection criteria:

- condition / status of the patient / laboratory animal
- goal of anesthesia / planned intervention
- equipment
- personal skills
- costs
Premedication

Sedative or sedative-analgetic premedications can be indicated:

- to calm down the animals and thereby prevent fear and movements of defense during the induction phase
- to exclude potential pain in the presurgical phase
- as a supplement to local or regional anesthesia to control / restrict spontaneous movements
- to reduce the necessary dosage of general anesthetics
- to prepare a smooth recovery
Stages of anesthesia

I. stage of analgesia:
   reduced sense of pain, cloudiness of consciousness

II. stage of excitation:
   elevated restlessness, unconsciousness, movements of defense, possibly vomiting, quiet surrounding necessary!

III. stage of tolerance:
   reduced or missing reflexes, reduced muscle tonus, surgery possible
   1. light (hypnosis)
   2. medium (tolerance)  
   3. deep (depression)

IV. stage of paralysis (after overdose):
   cessation of breathing and break-down of the cardiovascular system without adequate measurements: ➔ death
### Stages of anesthesia

<table>
<thead>
<tr>
<th>Anästhesie Stadien</th>
<th>Atmung</th>
<th>Herz-Kreislauf</th>
<th>Pupillengröße</th>
<th>3. Augenlid, Bulbusstellung, Bulbusbewegung (BB)</th>
<th>Pupillenreflex</th>
<th>Lid- und Kornealreflex</th>
<th>Pharynx-/Larynxreflexe</th>
<th>Muskeltonus</th>
<th>Reaktion auf chirurg. Reiz</th>
<th>Tränenfluss</th>
</tr>
</thead>
<tbody>
<tr>
<td>wach</td>
<td>konstitutionsabhängig</td>
<td>Pa = HF = Pa ↑↓ HF ↑↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Analgesiestadium I</td>
<td>verschieden, hechelnd</td>
<td>Pa = HF (↑)</td>
<td>Pa ↑↓ HF ↑↑↑</td>
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<tr>
<td>Exzitationsstadium II</td>
<td>unregelmäßig</td>
<td>Pa ↑↓ HF ↑↑↑</td>
<td>Pa ↑↓ HF ↑↑</td>
<td>geringer NHV</td>
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<tr>
<td>Hypnose-Stadium III</td>
<td>regelmäßig</td>
<td>Pa ↓↓ HF ↓↓</td>
<td>Pa ↑↓ HF ↑↑</td>
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<tr>
<td>Chir. Toleranz-Stadium III 2</td>
<td>regelmäßig, flach</td>
<td>Pa ↓↓ HF ↓↓</td>
<td>Pa ↓↓ HF = (↑)</td>
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<td>Stadium der Depression III 3</td>
<td>stoßweise</td>
<td>Pa ↓↓ HF ↓↓</td>
<td>Pa ↓↓ HF ↓↓</td>
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<tr>
<td>Asphyxietadium IV</td>
<td>fehlt</td>
<td>Pa ↓↓ HF ↓↓</td>
<td>Pa ↓↓ HF ↓↓</td>
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</tr>
</tbody>
</table>
Stages of anesthesia

Reflexes to estimate depth of anesthesia:

- (righting reflex)
- eye lid closing reflex
- pupillary reflex / light reflex
- swallowing reflex
- tonus of the jaw muscles
- toe pinch reflex (in rodents!!!!)

strong species-specific differences!
Injectable anesthetics

None of the injectable anesthetics alone - in a therapeutic dosage - fulfills all the requirements of a good general anesthesia.

differentiation into:

- hypnotics
- analgesics
Ketamin / Xylazin

dosage in mg/kg body weight:

<table>
<thead>
<tr>
<th></th>
<th>mouse</th>
<th>rat</th>
<th>rabbit</th>
<th>guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamin</td>
<td>80 - 120</td>
<td>80 - 100</td>
<td>40 - 50</td>
<td>40</td>
</tr>
<tr>
<td>Xylazin</td>
<td>10 - 15</td>
<td>5 - 10</td>
<td>4 - 6</td>
<td>5</td>
</tr>
</tbody>
</table>
Inhalational anesthetics

Nitrous oxide (laughing gas) and liquid substances with a low boiling point, whose inhaled vapors evoke anesthetic conditions:

- Diethyl-Ether (strongly irritating, today obsolete)
- Methoxyflurane (today obsolete, toxic to the kidneys)
- Halothane
- Isoflurane
- Enflurane
- Sevoflurane
- Desflurane
Inhalational anesthetics

Differences in:

- spectrum of effects
- strength of effects
- accumulation
- elimination
- metabolism
- effects on the cardiovascular system, respiratory system, liver, kidneys
Inhalational anesthetics

- are absorbed by the respiratory system exclusively
- absorption and elimination depend on $\lambda$, the blood-gas-solubility coefficient
- the smaller $\lambda$, the faster absorption and elimination
- very little metabolism in the body
- chemically stable and non-flammable
- compatible with other substances
- gentle application in whole-body chambers possible without restraint

Routes of application:
- inhalation chamber
- inhalation mask
- intubation
Inhalational anesthetics

Isoflurane:
- strong relaxation
- no analgesia
- $\lambda$ 1,4 (very good to regulate / manage)
- depressive on the respiratory system
- less cardiotoxic than other inhalational anesthetics
- strong reduction of the blood pressure

$\text{N}_2\text{O}$ (laughing gas)
- $\lambda$ 0,47
- only in combination with other anesthetics
- $\gg$ 80 Vol% would be necessary if used as a single substance (and thereby induce hypoxia)
- hardly any side effects
## Comparison

<table>
<thead>
<tr>
<th>Injectable anesthesia</th>
<th>Inhalational anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ketamin / Xylazin</strong></td>
<td><strong>Isoflurane</strong></td>
</tr>
<tr>
<td>• hard to control</td>
<td>• easy to control</td>
</tr>
<tr>
<td>• difficult to give multiple doses</td>
<td>• fast recovery</td>
</tr>
<tr>
<td>• long hangover with hypothermia</td>
<td>• low blood pressure</td>
</tr>
<tr>
<td>• instable blood pressure</td>
<td>• quick recovery of blood pressure</td>
</tr>
<tr>
<td>• cheap</td>
<td>• expensive</td>
</tr>
<tr>
<td>• easy to administer</td>
<td>• technically more demanding</td>
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</table>
Key data concerning anesthesia in mice:

- high metabolic rates lead to a high relative $O_2$-consumption (3500 ml $O_2$/kg/body weight/hour ↔ 155 ml in an elephant)
- mean art. blood pressure is about 90 mm Hg
- about 530 heartbeats/minute
- in relation to the body weight mice have a very large body surface and are therefore prone to hypothermia
- tidal volume (in all species) is about 10 ml/kg body weight, about 0,3 ml in a 30g mouse
- under anesthesia about 140-150 breaths/minute
- eyes stay open, danger of drying up, protection necessary!!!!! (Bepanthen, artificial tears etc.)
Possible complications during anesthesia:

- **Respiratory and cardiac depression**
  for example in animals with precursory problems, airway obstruction, depressiv action of certain anesthetics
  → oxygen supply, ventilation

- **Hypothermia**
  missing muscle activity, cold surfaces, disturbance of thermoregulation through anesthetics, loss of body warmth through open body cavities
  → shear and shave as little as possible, heat pads, red light, temperature control!!
Possible complications during anesthesia:

- **Dehydration**
  diuretic action of some anesthetics, blood loss, evaporation through open body cavities, breathing
  → substitution of liquids (body-warm!!!)
Prophylaxis of critical incidents during anesthesia

- preanesthetic examination / applicability of anesthesia
- exact weighing
- possibilities to warm the animals
- monitoring
- substitution of oxygen
- ventilation
- i.v. access
- substitution of fluids
(minimal) monitoring during anesthesia

- body temperature
- reflexes
- mucosa
  - rosy: ok
  - red: hypercapnia
  - pale: anemia, peripheral vasoconstriction
  - blue: cyanosis, hypoxia
- frequency of breathing
- heart beats
- time of capillary refilling (< 2 sec.)
- urination
Monitoring systems

- esophagus tube
  body temperature, respiratory rate
- capnography (in exhalation air, animal intubated)
  $O_2 / CO_2$-concentration, concentration of anesthetic gas, tidal volume
- pulsoxymeter
  heart rate
  $O_2$-saturation
- ECG
Monitoring systems

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Pain therapy

- pain is an unpleasant sensory and emotional experience with actual or potential tissue damage
- in animals it is usually associated in a complex together with fear and distress.
- reduction of one of the three components can reduce the other ones also

Inflicting pain upon an animal during a procedure or a treatment obligates to take all possible measurements to alleviate this pain
Pain therapy

Pain has a lot of physical consequences:

Cardiac circuit:
stimulation of the sympathetic nervous system (Adrenaline, Noradrenaline) and thereby tachycardia, vasoconstriction (cold extremities), increase in blood pressure

Breathing:
painfull breathing leads to hypoventilation and hypoxia; longlasting hypoventilation can lead to damage of the lung and predisposition for pneumonia
Pain therapy

Pain has a lot of physical consequences:

**Gastro-intestinal-tract:**
diminished appetite, reduced motility, delayed emptying of the stomach, salivation, vomiting

**Hormones:**
release of β-endorphins (intrinsic alleviation of pain)
increase in catecholamines (cardiac circuit)
release of ADH (antidiuretic hormone) stimulates water resorption in the kidneys and leads to imbalances in the homeostasis of body fluids
Pain therapy

Pain has a lot of physical consequences:

Nerves and muscles:
twitches, cramps, hyperesthesia, paralysis

In addition to all this pain can lead to depression, aggression, states of excitement and automutilation
**Pain therapy**

Intensity of pain depends on the body region

- **severe pain** occurs in wounds around the thoracic region, cranial abdomen, eyes, nasal cavity, perineum

- **moderate pain** occurs in wounds around the caudal abdomen and the larger joints

- **mild pain** occurs with traumata or surgeries along the extremities and the body surface
Pain therapy

specific pain symptoms can be:

dog: hardening of ventral and/or dorsal muscles, restlessness or no movements at all, yowling, whining, aggression, apathy, diminished appetite

cat: hardening of ventral and/or dorsal muscles, flight, holing up, hiss, growl, panting, diminished appetite

rabbit: inactivity, apathy, fear of contact, allotriophagy, diminished appetite, in acute pain sharp screams

rodents: decreased activity, untended coat, dirty eyes, piloerection, stiff and atactic way of walking, screams, allotriophagy, diminished appetite, attacked by cage mates
The "mouse grimace scale"

www.nc3rs.org.uk

National center for the replacement, refinement and reduction of animals in research
There is no anatomic or physiologic proof or reason to believe that perception of pain would be less developed in mammals and other animals compared to human beings.

IMPORTANT: newborns can perceive pain as well.

How much sense of pain we admit a certain species, is often depending on the sympathy we have for that particular species (ethical ranking).

- **Level of emotions**
- primates: similarity to humans
- cat and dog: cuddling animals
- small pets: stroke animals
- farm animals: "food"
Goals of pain relief

- avoid suffering according to the animal welfare law
- normalize the general condition
- prevent neurogenic shock
- ease the handling of the animal by reducing pain-dissress

Avoidance of fear and distress can elevate the pain threshold remarkably!

Preoperative conditioning minimizes fear and distress. Calm, secure, and friendly handling creates trust.
Analgesics

application of analgesics best still during anesthesia, before the recovery (if necessary together with a sedative)

• strong pain killer: act at their receptors in the CNS (opioids; can prolong the sleeping phase remarkably, may be depressiv on the respiratory system) Tramadol, Buprenorphin
Analgesics

application of analgesics best still during anesthesia, before the recovery (if necessary together with a sedative)

- weak pain killer (group of NSAIDs)
  a) substances with a clear central analgetic and antipyretic action, only mildly antiinflammatory (Aspirin, Paracetamol, Metamizol)
  b) substances acting in the periphery, strong anti-inflammatory, mild antipyretic action (Phenylbutazon, Indomethacin, Diclofenac)