Optimized surgical techniques and postoperative care

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Division of Surgical Research
Optimized surgical techniques and postoperative care

**Surgery**
- Optimized anaesthesia
- Pre-emptive analgesia
- Anti-infective prophylaxis
- Experienced personnel
- Surgical skills *per se*

**Postoperative care**
- Pain alleviation *in laboratory mice*
  - Pain killers

**Supportive measures**
- warmth, fluid, housing conditions, …

**Humane endpoints**
The laboratory mouse

... represents probably \( \geq 70\% \) of laboratory animals used in biomedical research and testing
... thousands of genetically modified lines/models available

Examples for the need of surgery in research
Organ transplantation
- liver
- lung
- stem cells
Open chest surgery
- myocardial infarction
Stroke
Physiology
...
Agenda: Pain alleviation in laboratory mice

Problems
- detection of pain in mice
- treating pain in mice

Example: Use of a minor surgery model for assessing (1) post-surgical pain and (2) the efficacy of the pain relief regimen

Read-out:
- Clinical investigation: cage-side observations, body weight, food consumption
- Telemetry: Heart rate, heart rate variability
- Natural behaviors: Nest building, burrowing

Where are we now?

Next steps
Detection of postoperative pain in mice

Examples (video recordings):
No effect of pain killers visible by cage-side observations of typical clinical signs
Pain treatment

Figure 2: The reported use of analgesics in papers published between 2000 and 2002, according to the procedure applied

Claire A. Richardson and Paul A. Flecknell; ATLA 33, p. 119-127, 2005
Detecting ongoing pain by its effects on …

- body weight?
- food, water?
- spontaneous behavior?
- experimental or GM induced changes?
- Clinical symptoms; appearance?
Clinical signs

Appearance
- fur
  - rough, soiled, hair loss, piloerection
- body
  - weight loss
  - decreased food intake
  - sunken abdomen
  - hunched posture
  - sunken eyes
  - dehydration

Behavior
- aggressive
  - biting response; automutilation
- apathy
  - unresponsive; isolation from the cage mates
- increased activity
  - restlessness; sudden running movements
- prolonged times for sleeping or resting

Constraint
= fear, distress, suffering, pain, disease, …
Food intake after surgery

Decrease of **food consumption** after surgery
Alteration of body weight, water intake, wheel running activity

Side-effects

**Example (picture):**
Pica behaviour in the rat from Buprenorphine (Opioid) → *high dosage?*
Detecting ongoing pain by its effects on …

- body weight?
- food, water?
- appearance?
- behavior?
- experimental or GM induced changes?
- pain killers
Obstacles in pain relief regimens

Table 3: Suggested analgesic dose rates for laboratory rats and mice

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Suggested analgesic dose rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.01–0.05mg/kg s.c., 6–12 hourly</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>2.0mg/kg s.c., 4 hourly</td>
</tr>
<tr>
<td>Morphine</td>
<td>2–5mg/kg s.c., 4 hourly</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Poorly effective</th>
<th>Effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pethidine</td>
<td>10–20mg/kg i.m., s.c., 2–3 hourly</td>
<td>10–20mg/kg i.m., s.c., 2–3 hourly</td>
</tr>
<tr>
<td>Carprofen</td>
<td>5mg/kg s.c., ? daily</td>
<td>10mg/kg s.c., ? daily</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1–2mg/kg s.c. or 4mg/kg per os ? daily</td>
<td>5mg/kg s.c. ? daily</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>5mg/kg s.c. ? daily</td>
<td>10mg/kg s.c. ? daily</td>
</tr>
</tbody>
</table>

Dose rates are based largely on uncontrolled clinical trials and a limited range of procedures, and so are likely to be subject to revision. Whenever possible, a pain scoring scheme should be used, so that the dose rates can be adjusted according to an animal’s response.

i.m. = intramuscular injection; per os = by mouth; s.c. = subcutaneous injection.

Table from Claire A. Richardson & Paul A. Flecknell, ATLA 33, p. 119-127, 2005
## Pain alleviation in mice

<table>
<thead>
<tr>
<th>Opioid</th>
<th>NSAID</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Burpenorphine</td>
<td>e.g.</td>
</tr>
<tr>
<td></td>
<td>Carprofen, Meloxicam,</td>
</tr>
<tr>
<td></td>
<td>Metamizol, …</td>
</tr>
</tbody>
</table>

- Opioid: 0.1 mg/kg BW, s.c., 2/die
- NSAID: 5 mg/kg BW, s.c., 1-2/die?
Problems of pain alleviation

Difficulties in detecting pain in the mouse
Side-effects of analgesics → sometimes masking symptoms
No control of success → efficacy of pain treatment remains questionable
Regimens (agents, dosages, application intervals) mostly rely not on systematic studies or scientific investigations

Knowledge about efficient pain treatment in mice:
→ Evidence-based regimens
   = agents, dosages and intervals
→ Animal-friendly application routes
Agenda: Pain alleviation in laboratory mice

Problems
- detection of pain in mice
- treating pain in mice

Example: Use of a minor surgery model for assessing (1) postsurgical pain and (2) the efficacy of the pain relief regimen
  - Read-out:
    - Clinical investigation: cage-side observations, body weight, food consumption
    - Telemetry: Heart rate, heart rate variability
    - Natural behaviors: Nest building, burrowing

Where are we now?

Next steps
Recovery of mice after minor surgery with and without pain killers (NSAID: Carprofen, Flunixin)

Telemetric measurements → preliminary implantation of transmitters
Change of heart rate

Δ heart rate [bpm]

-30 -20 -10 0 10 20 30 40 50 60 70 80

[day] 0 1 2 3

- s.c. injection (vehicle/analgesic)
- Vasectomy

- without pain therapy (n=8)
- with Carprofen (n=8)
- with Flunixin (n=8)

* p≤0.001
* p=0.003

Control groups (anesthesia + pain therapy only) not shown

**Vasectomy**

**Time domain analysis of heart rate variability: IBI**

- s.c. Injection (vehicle/analgesic)

Control groups (anesthesia + pain therapy only) not shown


\[ \Delta \text{Interbeat interval [ms]} \]

- without pain therapy (n=8)
- with Carprofen (n=8)
- with Flunixin (n=8)

\[ p \leq 0.004 \]
Time domain analysis of heart rate variability: SDANN

\[
\Delta \text{SDANN} [\text{ms}]
\]

- without pain therapy (n=8)
- with Carprofen (n=8)
- with Flunixin (n=8)

\* \(p \leq 0.009\)

Control groups (anesthesia + pain therapy only) not shown

Body weight and food consumption

**Body weight**

- Without pain therapy
- With Carprofen 5 mg/kg, 2/die, 24 h
- With Flunixin 5 mg/kg, 2/die, 24 h

**Food intake**

- Without pain therapy
- With Carprofen 5 mg/kg, 2/die, 24 h
- With Flunixin 5 mg/kg, 2/die, 24 h

(n=8)

*p* ≤ 0.007

Control groups (anesthesia + pain therapy only) not shown

Recovery of mice after minor surgery with and without pain killers (NSAID: Carprofen, Flunixin)

Attenuation of aberrations in heart rate and heart rate variability with analgesia

Inhibition of the reduction in body weight (~2%) and food consumption (~20%) with analgesia

Clinical investigation: no difference in outer appearance, posture, spontaneous behaviors

Arras et al. 2007
Recovery of mice after minor surgery with and without pain killers (NSAID: Carprofen, Flunixin)

Arras et al. 2007
Burrowing in rodents: a sensitive method for detecting behavioral dysfunction

Robert M J Deacon

- mice, rats, hamsters, gerbils
- home cage based
- read out: aberration of natural, highly motivated behavior
Burrowing

Species-specific, spontaneously occurring behaviour
High motivation to dig burrows persists during domestication
Deacon et al. 2001 > neurodegenerative diseases
Useful as indicator for impaired wellbeing???
Hypothesis: Abberration of a natural behavior is an indicator of pain, constraint, or impaired well-being

Aim: Changes in burrowing behavior as indicator of pain after minor surgery

Pre-study burrowing test
Time of testing (circadian rhythm), Setup (apparatus), Sex bias, Strain bias C57BL/6J, DBA/2J, 129Sv/Ev-IFNabRtmAgt

Study design
32 male & 32 female adult C57BL/6J
### Burrowing test: study design

<table>
<thead>
<tr>
<th>acclimatisation</th>
<th>baseline measurement</th>
<th>experimental measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>individual housing with burrowing apparatus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **n = 16**
- **surgery + anaesthesia**
- **surgery + anaesthesia + analgesia**
- **anaesthesia**
- **anaesthesia + analgesia**

<table>
<thead>
<tr>
<th>day 0</th>
<th>day 1</th>
<th>day 2</th>
<th>day 3</th>
<th>day 4</th>
<th>day 5</th>
<th>day 6</th>
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- **video recording**

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Jirkof et al. 2010
Burrowing test: study design

Surgical intervention

- Injection of analgesia
  (+/- carprofen, 5 mg/kg BW, s.c.)
- transport
  (5 min)
- inhalation anaesthesia
  (sevoflurane, 15 min)
- +/- one side minor laparotomy
- recovery on warming mat
  (at least 15 min)
Experimental Setup. Burrowing test apparatus (A), shelter (B) and nesting material (C) in home cage.

Jirkof et al. 2010
Burrowing test: analysis

Before & after treatment
Video recording
Analyses: software based, ObserverXT (Noldus)
Burrowing parameter:
- latency to burrow
- duration of burrowing
- weights of removed pellets after 2h

www.noldus.com
Results: burrowing performance

Almost all (98 %) healthy mice burrowed during baseline measurement
Example: infrared video recording of burrowing.

All parameters (latency to burrow, duration of burrowing, weight of removed pellets) responded to the different experimental treatments.
Results: burrowing performance
Results: baseline vs experimental

Increase in latency & duration of burrowing after treatment

$\Delta$ latency to burrow

$\Delta$ duration of burrowing
Results: baseline vs experimental

$\Delta$ weight of removed pellets after 2h

-160 -140 -120 -100 -80 -60 -40 -20 0 (gram)

surgery + anaesthesia  
surgery + anaesthesia + analgesia  
anesthesia  
anesthesia + analgesia
Results: group comparisons

Time to effect: Kaplan-Meier analysis

Procedural control treatments did not differ in latency

Jirkof et al. 2010
Results: group comparisons

Surgery without pain treatment causes significant longer latencies

Jirkof et al. 2010
Burrowing test: summary & conclusion

- Easy to perform in the animals home cage under routine laboratory conditions
- Burrowing parameters react sensitive on treatments
- Results allow to discriminate between groups of mice that are treated with a pain killer or not before surgery
- Results imply a balance between the motivation to burrow and an individuals level of constraint experienced

In conclusion, postsurgical impairment, mainly attributable to pain, can be assessed on the basis of the burrowing test
Where are we now?

Currently: new approaches and methodologies for the assessment of pain and constraint in mice

- Conditioned place preference → Frank Porreca [King et al. 2009]
- Facial expression (mouse grimace scale) → Jeff Mogil [Langford et al. 2010]
- General changes in behaviors (rearing, climbing, etc.) → van Loo et al. 2007, Roughan et al. 2009
- Aberrant behaviors (e.g. twitching, flinching, …) → Paul Flecknell [Roughan et al. 2009]
- Drug self administration → Vera Baumans [Pham et al. 2010]
- Alterations of natural behaviors → Jirkof et al. 2010
- Sympathetic reaction (telemetry) → Arras et al. 2007
- Ultrasound vocalisations?

Feasibility, validity, benefits, limitations in routine laboratory conditions
Reliability of specific tests for estimating the efficacy of pain relief regimens
Long-term goals

Utilization of new tests for verifying pain relief regimens which are in use to-date

Establish efficient, evidence-based modern pain relief regimens

- Sustained-release drugs
  - Foley et al. 2011
- Administration of analgesia via drinking water, food, or mini-pumps
- Multimodal analgesia protocols
- ...

Is there a market for analgesics or other drugs for use in mice???
Thank you for your attention

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