Harm Benefit analysis
Bergen, Norway, 2014

Identifying the harms and anticipating the benefits of animal research: a pain researcher’s perspective

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The Guide

... studies that have the potential for unrelieved pain or distress, there are special considerations for IACUC review. Specifically, the Guide indicates that “the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns.” This seems to indicate that for studies involving the potential for pain and distress, the IACUC should conduct a “harm/benefit” analysis. What does AAALAC expect with regard to Committee evaluation of these kinds of studies?

Page 27, Guide for the Care and Use of Laboratory Animals (NRC 2011)

‘To do this we need to identify pain in animals, and painful procedures’
The ARRIVE Guidelines
Animal Research: Reporting of *In Vivo* Experiments

The guidelines are intended to:

• Improve reporting of research using animals.
• Guide authors as to the essential information to include in a manuscript, and not be absolutely prescriptive.
• Be flexible to accommodate reporting a wide range of research areas and experimental protocols.
• **Promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts.**
• Improve the communication of the research findings to the broader scientific community.
• Study design
  • For each experiment, give brief details of the study design including:
    • a. The number of experimental and control groups.
    • b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when).
    • c. The experimental unit (e.g. a single animal, group or cage of animals).
    • A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.

• Experimental procedures
  • For each experiment and each experimental group, including controls, provide precise details of all procedures carried out.
  • For example:
    • a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia are used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).
    • b. When (e.g. time of day).
    • c. Where (e.g. home cage, laboratory, water maze).
    • d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).

• Experimental animals
  • a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).
  • b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
Text books: One recipe fits all ???

PK & PD of ketamine and xylazine with aging

• **Housing and husbandry**
  • Provide details of:
  • a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).
  • b. Husbandry conditions (e.g. breeding program, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).
  • c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment (Grids ? Distinction between disease (inflammation) and pain.

• **Sample size**
  • a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.
  • b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.
  • c. Indicate the number of independent replications of each experiment, if relevant.

• **Allocating animals to experimental groups**
  • a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.
  • b. Describe the order in which the animals in the different experimental groups were treated and assessed.

And others......... Neupsig Meeting Toronto 2013 (article review / meta-analysis)
Welfare

Absence of hunger, thirst, pain, stress, disease, ...

No induction of unnecessary pain

Accept painful procedures if they are necessary ....

... the research has to be very well done (knowledge about the models), experimental knowhow is required (training on animal techniques and experimental procedures (equipment) and integrity is required
Public’s view on pain

Do they have a knowledgeable understanding or an anthropomorphistic projection of animals models of pain? (‘welfarists’, ‘utilitarists’, abolitionists, …)

IACUCs in Sweden and other countries

My own IACUC…
Other aspects

What about animal models of disease in which pain is not the primary focus of the study, and where pain may occur (ex. abdominal masses)?

What about cases that would deserve more investigation? Concomitant lesions and pathologies...

Researchers often focus on one process, not the whole animal, and there is usually no budget for investigations ‘outside’ of the ‘project’.
‘Pain’ and evolution

‘...activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state.’ Wall 1999

Importance of the neocortex

C fiber nociceptors, the most prevalent type in mammals and responsible for excruciating pain in humans, are rare in teleosts and absent in elasmobranchs

JD Rose, R Arlinghaus, SJ Cooke, BK Diggles, W Sawynok, ED Stevens, CDL Wynne. Can fish really feel pain? Fish and Fisheries, 2012
Common nociceptive responses such as injury detection and escape are shared in genetically diverse species, however…

… in higher order species, pain perception activates brain areas associated with emotions

The USDA guideline on pain in animals states that if one has reason to believe that a stimulus would be painful to humans, it should also be regarded as painful to animals.
Hargreaves’ test is not useful for the evaluation of nociception following a surgical laparotomy in *Xenopus leavis* frogs.
Pain pathways in mammals

Activation of different brain regions in humans affected by neuropathic pain


- Orbitofrontal cortex
- Cingular cortex
- Medial thalamus

Altered spontaneous and evoked activity 

Altered sodium channels 

Transmitter release 

Glu: glutamate; 5-HT: serotonin 

Ca channel function 

GABA 

PAG 

RVM 

PB area 

Spinal neuron 

Frontal cortex 

Limbic system 

Sensory 

Posterior thalamus 

Sensory cortex 

Affective 

Opioids 

Antidepressants 

Sensory pain fibre 

Altered spontaneous and evoked activity 

Spinal neuron 

Summary
Spared nerve injury model

Mechanical sensitivity (allodynia)

Many behavioral tests used

von Frey filaments
EE decreases, and an environmental restriction increases, **mechanical sensitivity**

![Graph showing withdrawal threshold (g) over time for different conditions: Sham EE, Sham RE, SNI EE, SNI RE.](image)
Epigenetics
DNA methylation

Neuropathic animals and pain-related peptides

Persistent post-surgical pain in humans

Post amputation pain: 30 - 50 %
Post mastectomy pain: 10 - 30 %
Radical prostatectomy: 30 %
Thoracotomy: 20 –50 %
Colectomy: 30 %
Hysterectomy and caesarean section: 5 - 32 %
Joint replacement: 10 %
...

Chronic pain can originate from damage to nerves or neurons in:

- the peripheral nervous system (trauma, surgery)
- the viscera (surgery, lesions, tumors)
- the musculoskeletal system
- the articulations (overuse, age, cancer, …)

-and the CNS ???
no pain receptors in the CNS
(stroke, hemorrhages, …
Parkinson’s disease, multiple sclerosis, …)

Example 1
Coronal section of a rat brain showing the hematoma produced in the right cerebral hemisphere (collagenase model)

Behavioral and histopathological studies

Central Pain Following a Collagenase-Induced Hematoma in the Basal Ganglia and Thalamus Can Be Reversed with Gabapentin

GABAPENTIN REVERSES BILATERAL ALLODYNIA IN A RODENT MODEL OF CENTRAL PAIN INDUCED BY AN INTRATHALAMIC HEMORRHAGE

What to conclude?

To identify harms, one needs to:

Be knowledgeable about pain (pathways and mechanisms), analgesia and anesthesia and the animal models used (even when the research focus is not pain)

Have the know-how (procedures and equipment), stress the importance of training and supervision

and the benefits?

Scientific review of proposals? Is it justified in the research protocol?

One need to have integrity (students, young researchers and professors, start ups, CROs, … are sometimes telling only part of the story)
Benefits: Experiments need to translate from animals to humans

Lessons learned from substance P
Why SP did not work in humans?

Pharmacology: often no pharmacokinetics when evaluating treatment of disease. What dose to give? (morphine 4mg/kg = CNS depression)

AM Edwards et al., Nature 2011
Collaboration with Dr Eve Langelier, mechanical engineer
University of Sherbrooke, Québec
What is tendinosis?
Be **knowledgeable** about pain and the animal models used

NO

Have the **know-how** (procedures and equipment),

**YES** for the physical and mathematical aspects

and the benefits?

Scientific review of proposals? Is it justified in the research protocol?

**YES**

**Solution: collaboration**

Was discussed with the vet, which suggested to find a collaborator with pain research experience.

Need to present concerns to the researcher and discuss issues
Questions?

Tendinosis pain?
Compare to tendinitis

Knee cartilage degeneration?

Paw irritation with running? Test with von Frey filaments
Analysis of pain peptides in the spinal cord by LC/MS/MS

Substance P (MW: 1347.65)
Sequence: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met-NH2
Tendon of runners is much weaker
No pain with tendinosis (level of exercise not sufficient to cause pain)
No osteoarthritis
Pain with von Frey filaments (skin paw irritation)
Pain peptides are increased in the spinal cord

Conclusion 6 weeks of running is sufficient to study use the model for tendinosis
What to conclude?

To identify harms, one needs to:
Be knowledgeable about the animal models used

Have the know-how (procedures and equipment), stress the importance of training and supervision
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