Damned if you do - damned if you don't

The potential confounding effects of painful conditions *and* pain relief on experimental readouts

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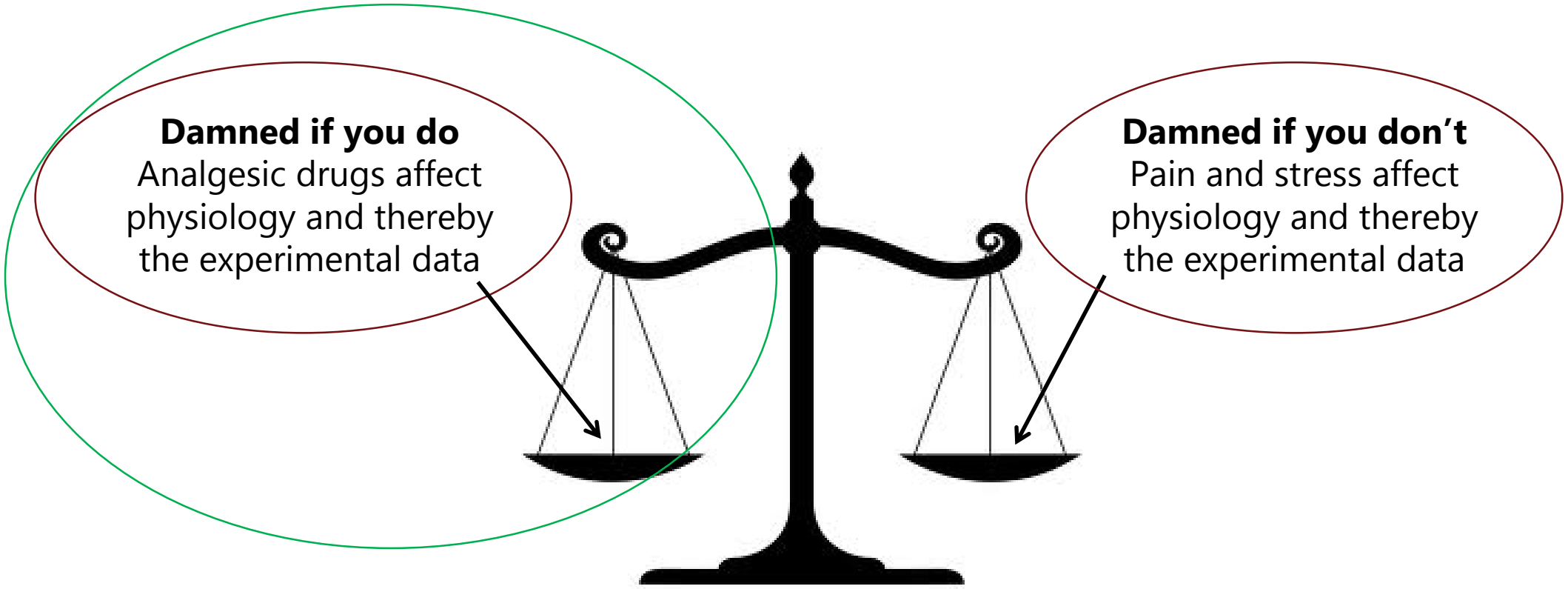
The problem with painful experimental procedures - I

In other words, animals are sentient beings and painful experimental procedures may cause suffering…

"The question is not, Can they reason?, nor Can they talk? but, Can they suffer? Why should the law refuse its protection to any sensitive being?"
Jeremy Bentham

The problem with painful experimental procedures - II

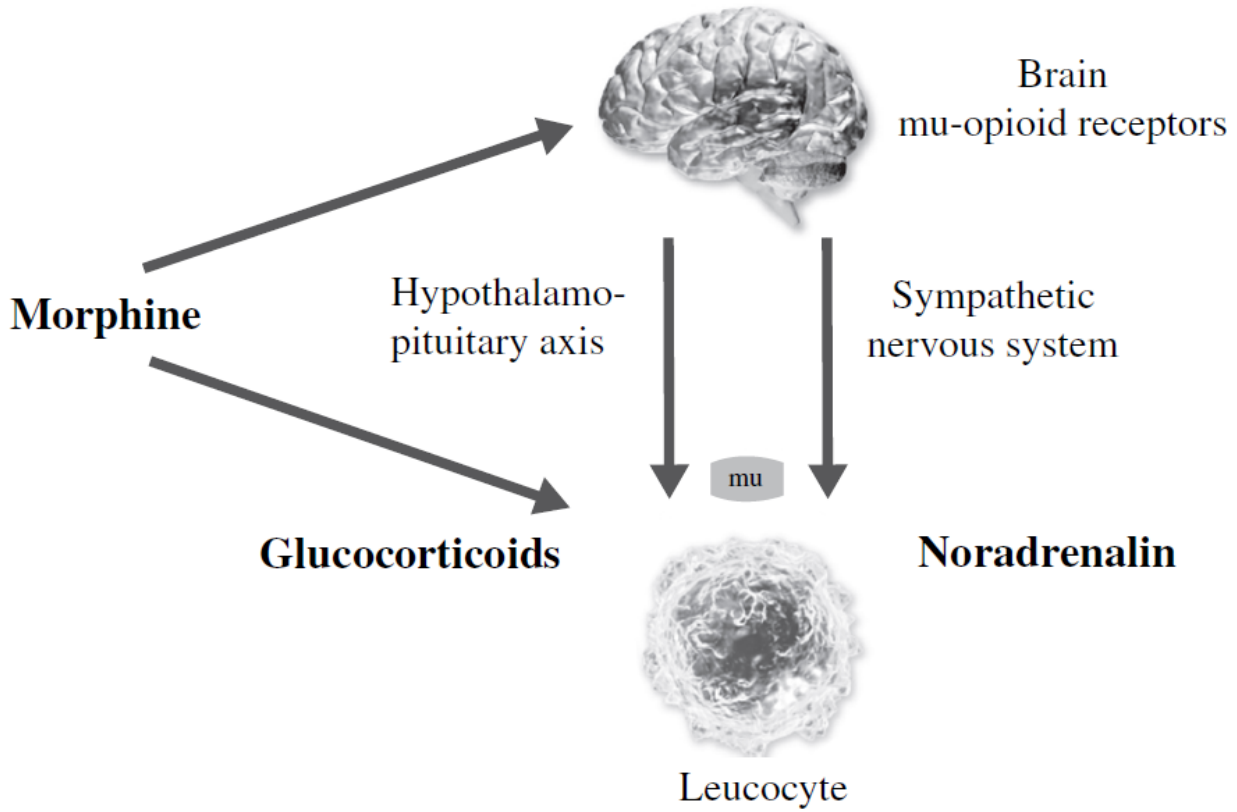


Effect of analgesic treatment on experiments

Indeed, analgesic drugs will affect several systems in the organism, and consequently the experimental data

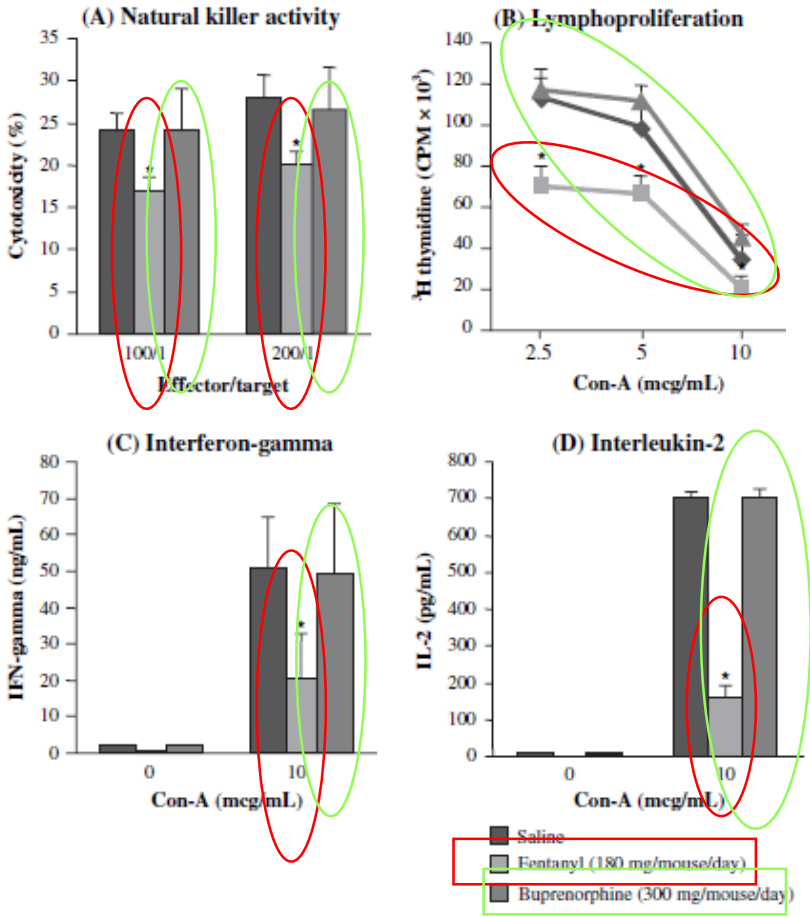
- However, this is not necessarily always the case
- and we don't really know until we have investigated it
- Let's take a look at some examples

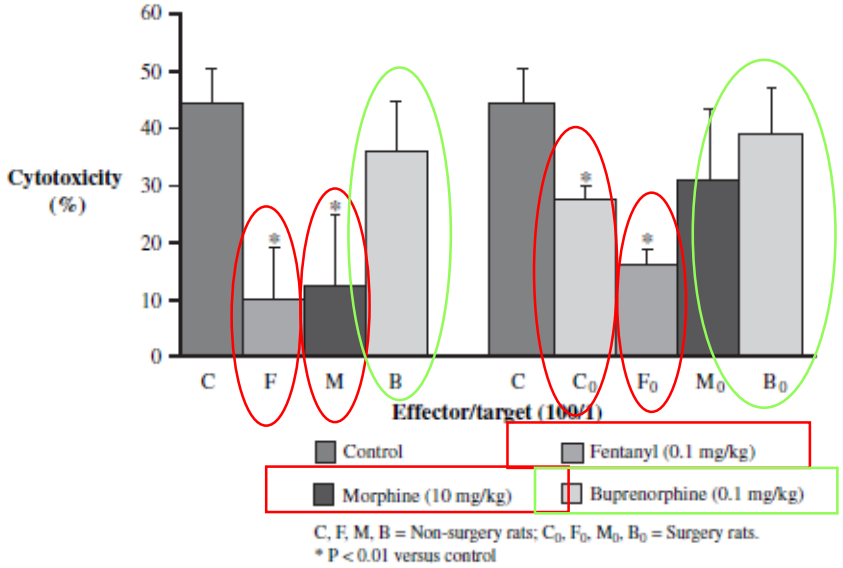
Effect of opioids on the immune system



Sacerdote P, Palliative Medicine vol. 20, 2006

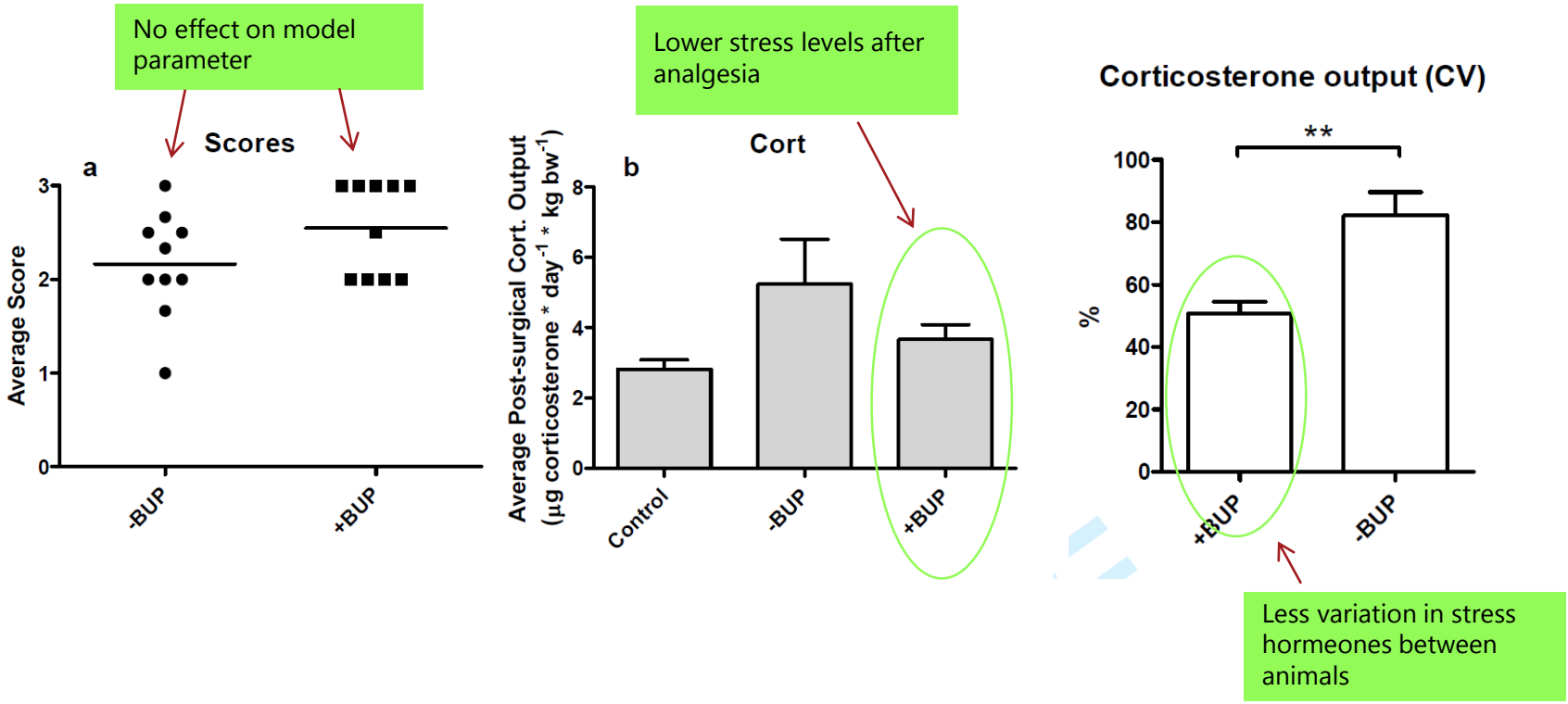
Effects of opioids on immune parameters





Sacerdote P, Palliative Medicine vol. 20, 2006

Effects of buprenorphine analgesia on a rat stroke model



Kalliokoski, Abelson et al, In Vivo vol. 24 2010

...and mouse ditto

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Original Research

Effects of Buprenorphine and Meloxicam Analgesia on Induced Cerebral Ischemia in C57BL/6 Male Mice

Kirsten R Jacobsen,[1,*] Natasha Fauerby,[2] Zindy Raida,[3] Otto Kalliokoski,[1] Jann Hau,[1] Flemming F Johansen,[2] and Klas SP Abelson[1]

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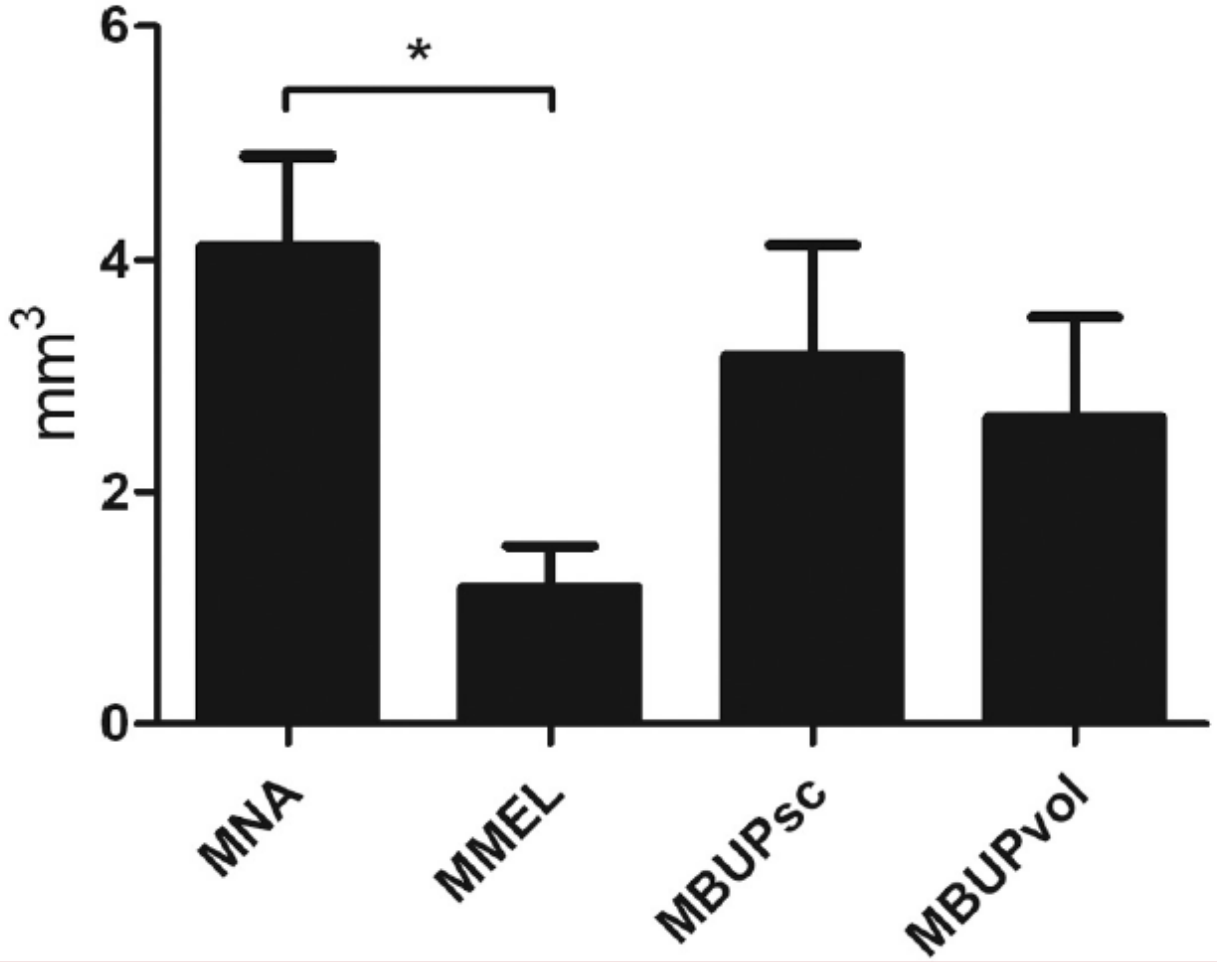
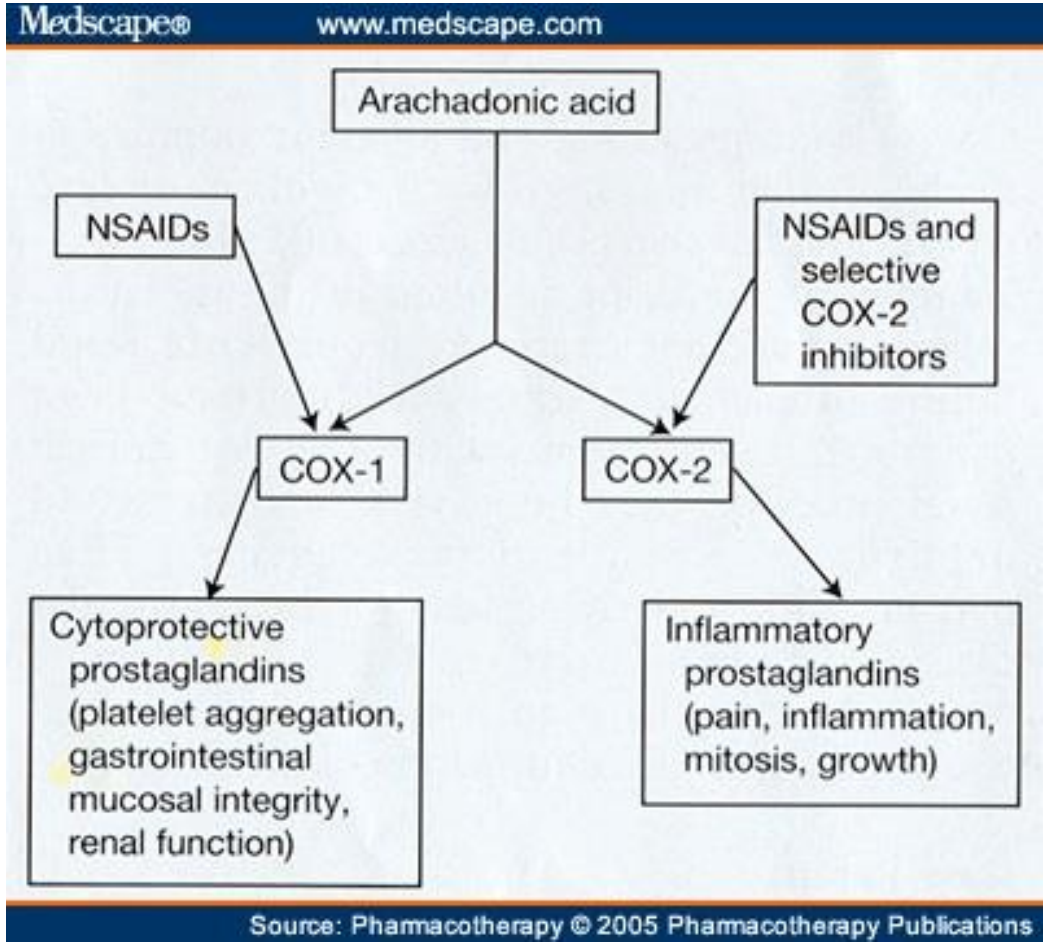
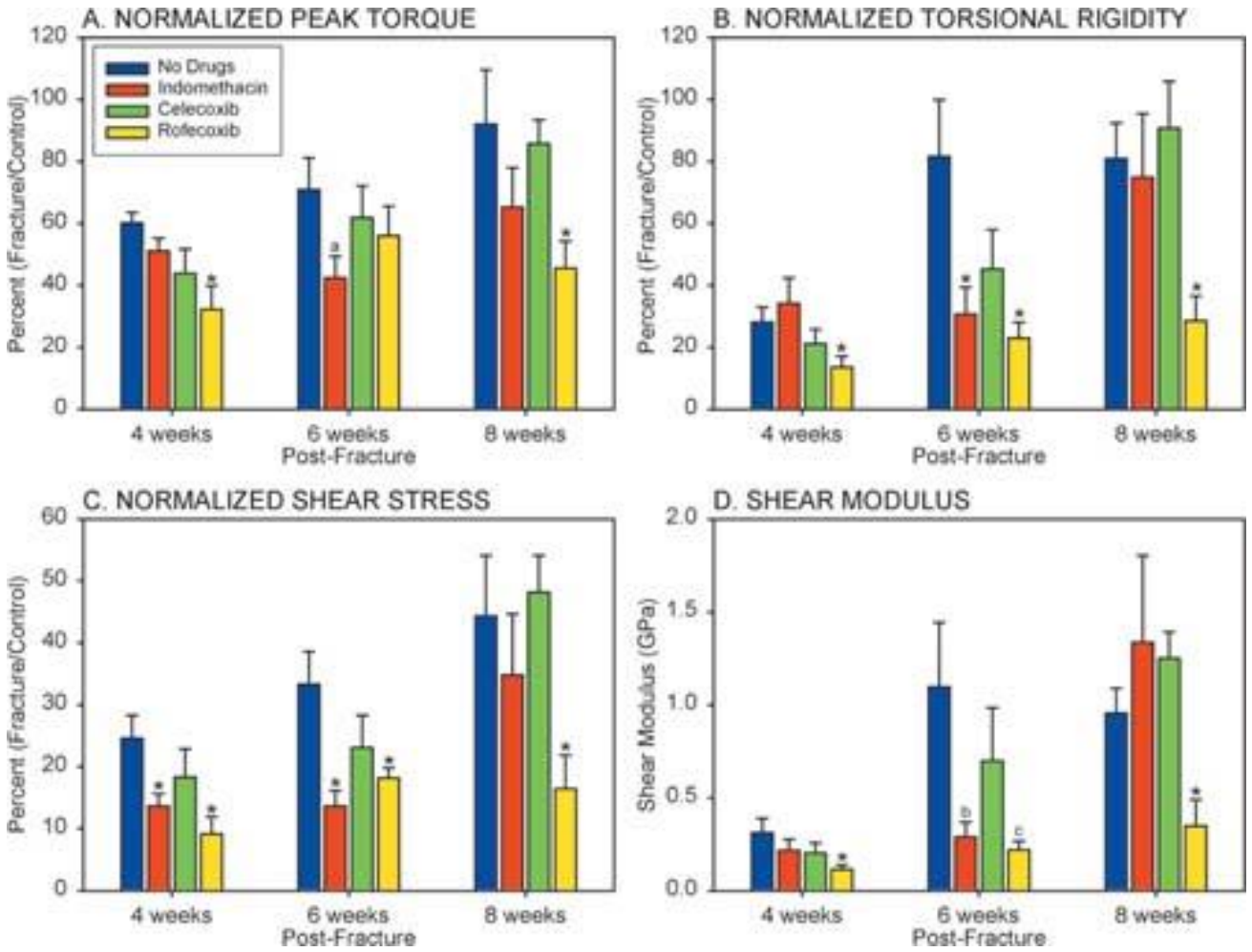


Effect of NSAIDs on bone healing



Effect of NSAIDs on bone healing

Cyclo-Oxygenase 2 Function Is Essential for Bone Fracture Healing



Journal of Bone and Mineral Research
Volume 17, Issue 6, pages 963-976, 1 JUN 2002 DOI: 10.1359/jbmr.2002.17.6.963
http://onlinelibrary.wiley.com/doi/10.1359/jbmr.2002.17.6.963/full#fig2

Effect of NSAIDs on bone healing

"Animal and *in vitro* studies present so conflicting data that even studies with identical parameters have opposing results."

Pountos *et al.,* The Scientific World Journal, Vol. 2012

Table 2: Animal studies: agents and model used in relation to the presented effect.

Impaired bone healing
(1) Aspirin [84]
(2) Celecoxib [101, 102]
(3) Diclofenac [97–99]
(4) Etodolac [104, 105]
(5) Ibuprofen [88, 94–96, 127]
(6) Indomethacin [78–91, 119, 127]
(7) Ketoprofen [77]
(8) Ketorolac [74–76, 107]
(9) Meloxicam [85, 103]
(10) Naproxen [92, 93]
(11) Parecoxib [74]
(12) Rofecoxib [92, 94, 95, 108, 109]
(13) Tenoxicam [99, 100]
(14) Valdecoxib [107]
No effect
(1) Celecoxib [80, 111, 119, 120]
(2) Diclofenac [123]
(3) Etoricoxib [110]
(4) Ibuprofen [111, 121, 122]
(5) Indomethacin [81, 111, 114–118]
(6) Ketoprofen [112, 113]
(7) Ketorolac [110, 111]
(8) Meloxicam [113]
(9) Nimesulid [124]
(10) Rofecoxib [111, 123, 125]
Short term has no effect
(1) Diclofenac [98, 106]
(2) Ketoprofen [126]
(3) Ketorolac [78]
(4) Parecoxib [106]
(5) Rofecoxib [85, 108]
(6) Valdecoxib [106]
Model used
(i) Rats [74, 77, 78, 80, 81, 83, 84, 89–91, 93, 94, 97, 98, 100–107, 110, 114, 116, 118, 121–124, 126]
(ii) Mouse [109, 111, 120]
(iii) Rabbit [75, 76, 79, 82, 85–88, 92, 95, 96, 99, 108, 112, 115, 119, 125]
(iv) Dog [117]
(v) Goats [113]

What about pain models?

- Can we treat against pain where pain is a part of the model?

- We should at least, if possible, treat against avoidable and unnecessary pain
  - Post-operative pain
  - When relevant pain is not tested

- **Says who?**
- Our morality says we should
- Our legislation says we should *(EU Directive 2010/63/EU, Article 15 §2)*
- Our scientific data may say we should

Refinement of pain models

Three fundamental questions that are the basis of our research

1. Is there any avoidable element of pain in animal pain models (pain that is not related to the relevant test parameters)?
2. If so, can these elements of pain be avoided, and what analgesic regimen should be applied to give adequate pain relief while having no unwanted effect on the model?
3. Are there other means of improving technical aspects of the model that may enhance the welfare of the animals without adverse effect on the model?

Neuropathic pain models

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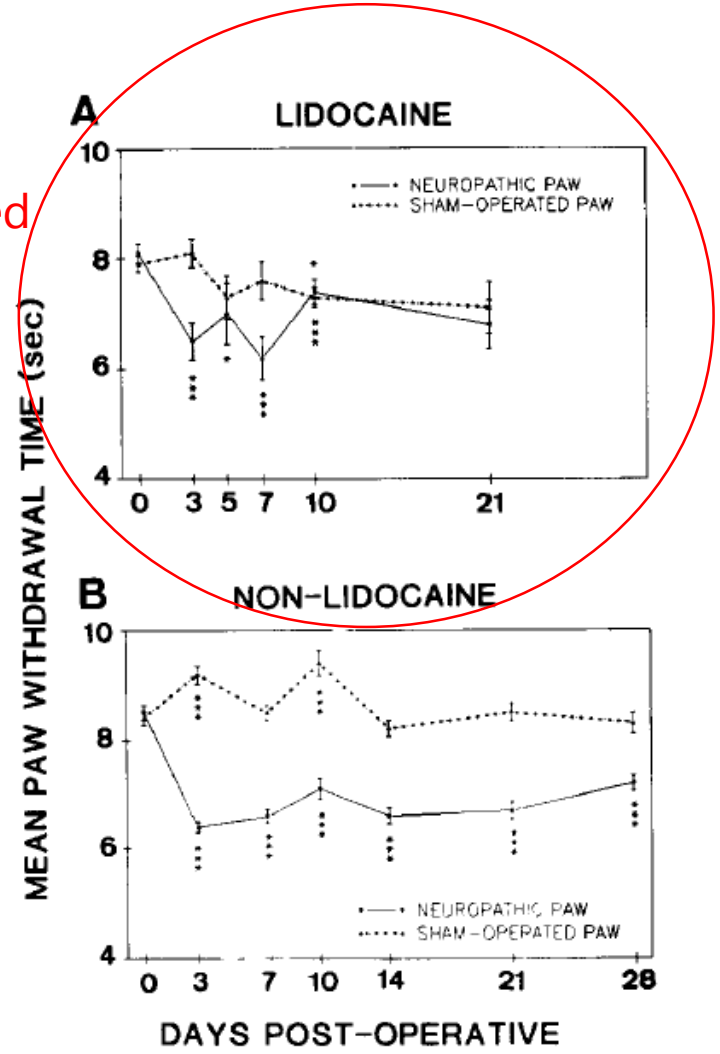
Effects of perioperative local anaesthetics on neuropathic pain models

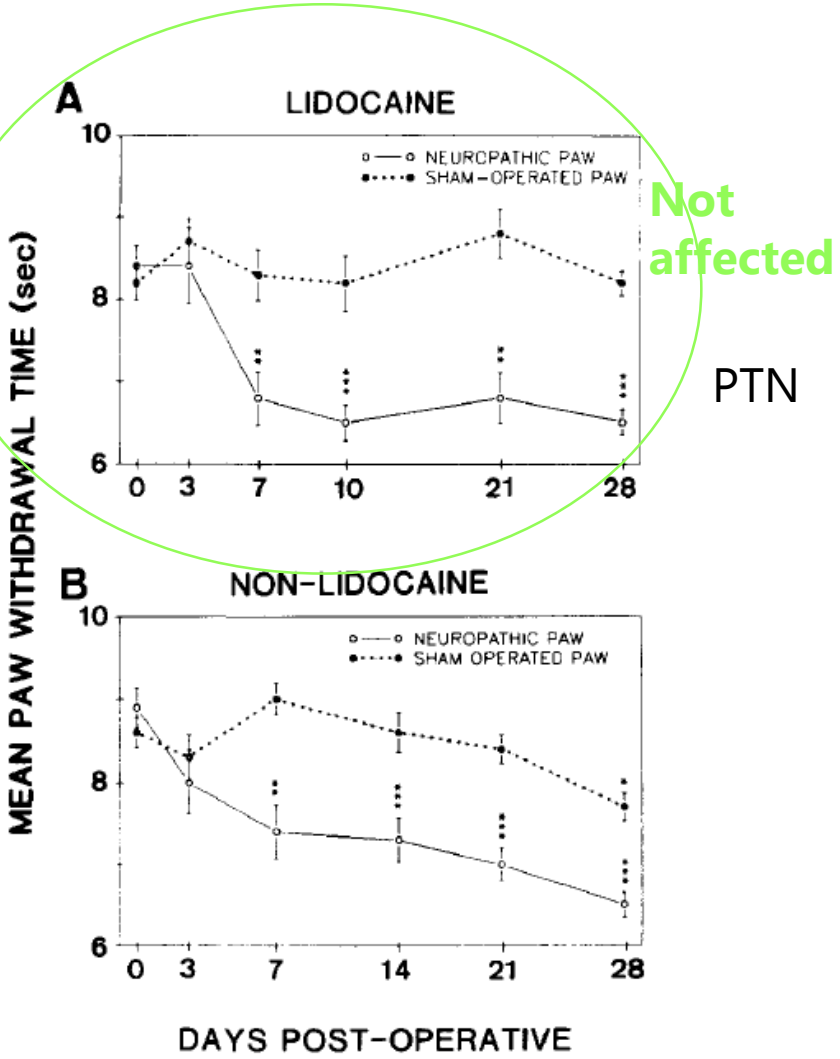
Affected

PCN



Not affected

PTN



Dougherty et al, Brain Res. 570, 1992

Neuropathic pain

PLOS ONE

RESEARCH ARTICLE

Is there a reasonable excuse for not providing post-operative analgesia when using animal models of peripheral neuropathic pain for research purposes?

Sara Hestehave[1,2]*, Gordon Munro[2,3], Rie Christensen[2], Tina Brønnum Pedersen[4], Lars Arvastson[5], Philip Hougaard[5], Klas S. P. Abelson[1]

https://doi.org/10.1371/journal.pone.0188113

Table 1. Treatment- and non-operated control-groups.

Group	Analgesic/dose	Administration	Treatment timing [1]	Abbreviation	Coverage / hours	N	N, end
A	Control (minimally handled and not operated)	-	-	Control	-	18	18
B	Control + von Frey tested (not operated)	-	-	Control + VF	-	14	14
C	Vehicle (saline)	s.c.	During anesthesia, prior to incision	Vehicle, s.c.	-	30	30
D	Buprenorphine, 0.1 mg/kg	s.c.	During anesthesia, prior to incision	Bup. s.c. 8h	8	30	30
E	Buprenorphine, 0.1 mg/kg	s.c.	During anesthesia, prior to incision + every 8 h	Bup. s.c. 72h	72	12	12
F	Buprenorphine, 1.0 mg/kg	Nutella/p.o.	45min prior to anesthesia	Bup. p.o. 8h	8	12	12
G	Buprenorphine, 1.0 mg/kg	Nutella/p.o.	45min prior to anesthesia + every 8 h	Bup. p.o. 72h	72	14	13*
H	Carprofen, 5.0 mg/kg	s.c.	During anesthesia, prior to incision	Carprofen, 24h	24	12	12
I	Carprofen, 5.0 mg/kg	s.c.	During anesthesia, prior to incision + every 24h	Carprofen, 72h	72	12	11**
J	Lidocaine 10 mg/Bupivacaine 2.5 mg, 1:1	Local	During anesthesia, post incision.	Lido/bupi	2–3	12	12
K	**High dose combination:** Carprofen, 5 mg/kg. Buprenorphine 0.1 mg/kg. Lidocaine10 mg/ bupivacaine2.5 mg– 1:1	s.c./local	During anesthesia, prior to incision + local post incision.	High combination	72	18	12*, **
L	**Low dose combination:** Carprofen, 5mg/kg. Buprenorphine 0.05 mg/kg. Lidocaine10 mg/ bupivacaine2.5 mg– 1:1	s.c./Local	During anesthesia, prior to incision + local post incision.	Low combination	72	12	12

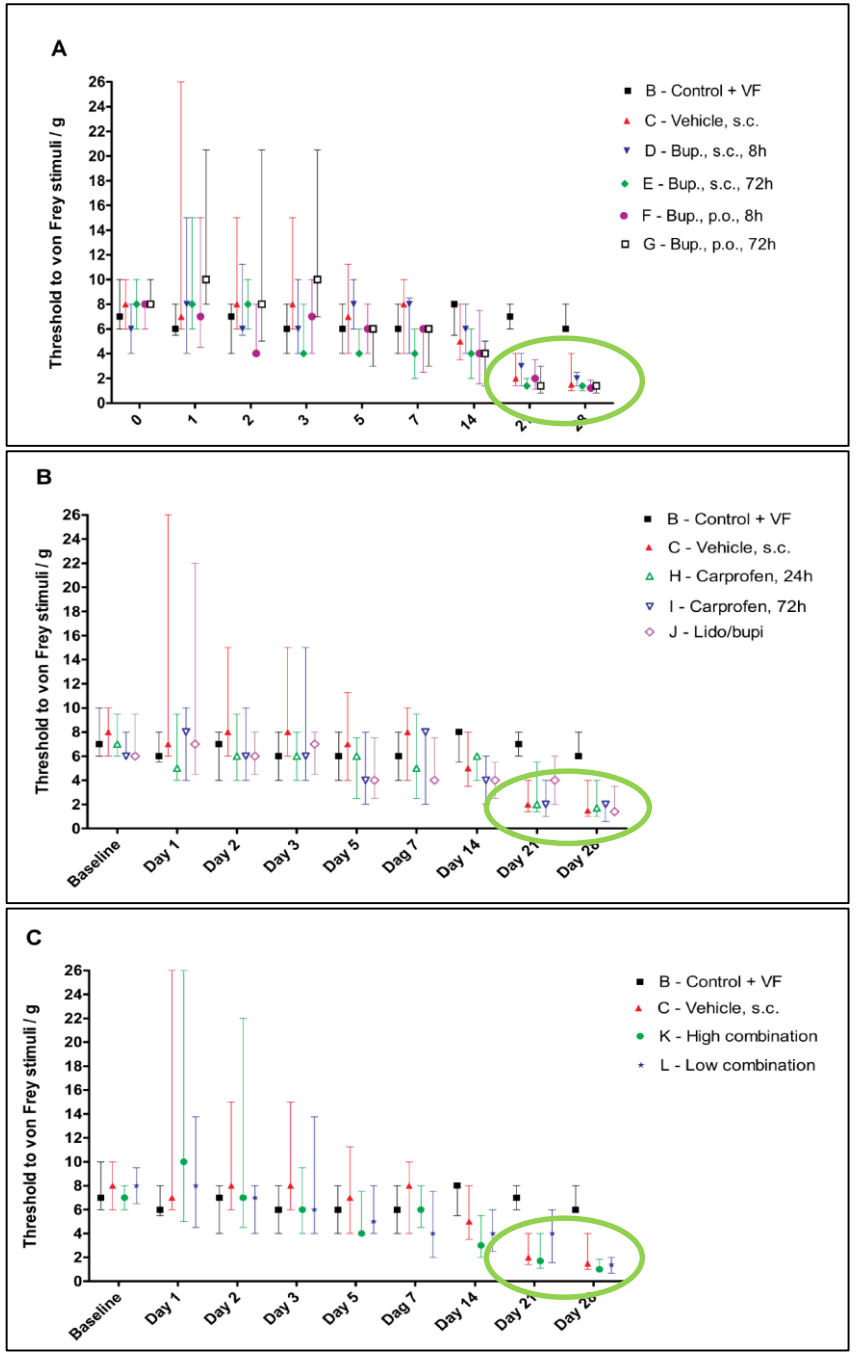
https://doi.org/10.1371/journal.pone.0188113

Is there a reasonable excuse?

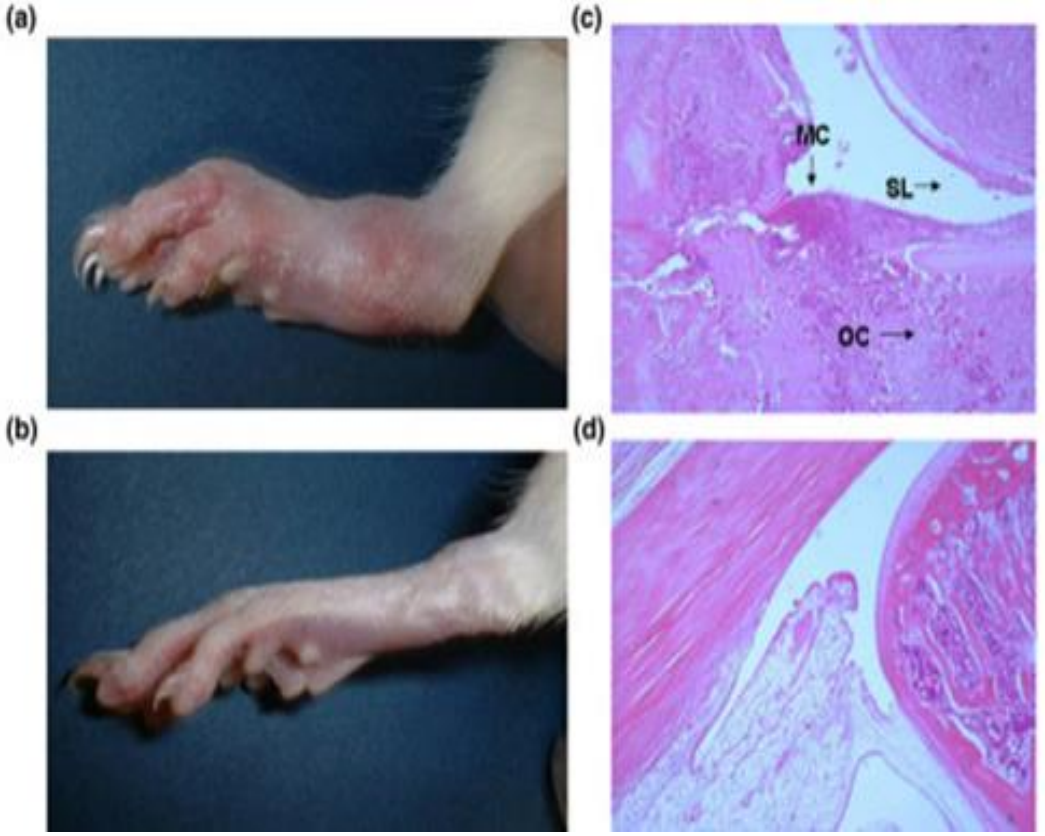
- Regardless of which peri-and/or operative analgesic treatment applied – the desired phenotype (circled in green) was always achievable

- However, the possible effect on specific pathophysiological mechanism or effect of drug candidates were not investigated

- Nevertheless, analgesia should not be withheld due to *suspicion* of adverse effect on experimental read-outs – any such suspicion should be confirmed!



Chronic inflammatory/arthritic pain



Omoto et al. Arthritis Research and therapy
vol 7, 2005

Chronic inflammatory/arthritic pain

in vivo *36*: 635-642 (2022)
doi:10.21873/invivo.12747

Antidepressant Fluoxetine Does Not Appear to Interfere With Key Translational Parameters in the Rat Adjuvant-induced Arthritis Model

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[2]Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Where should we aim our refinement?

Scandinavian Journal of Laboratory Animal Science

2020, Volume 46, Number 5
ISSN 2002-0112

Original scientific article

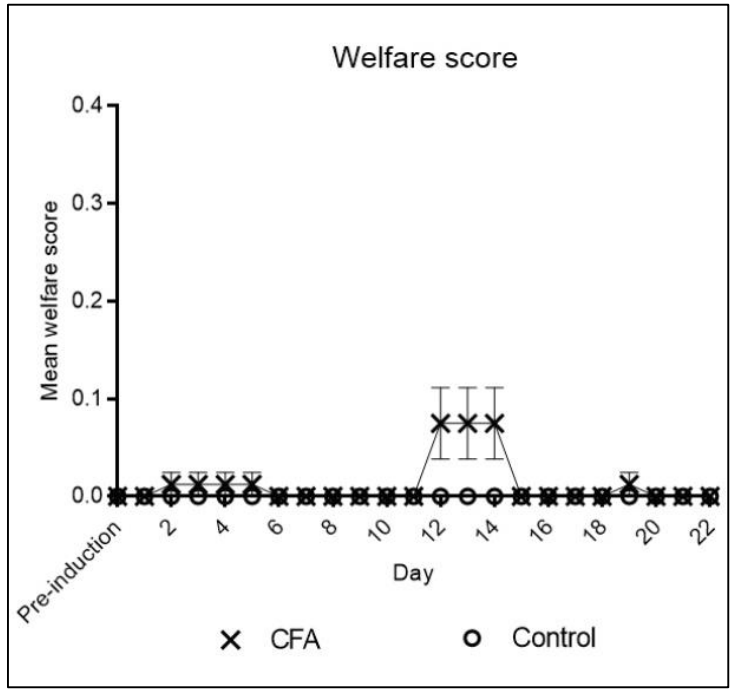
The adjuvant-induced rat model of monoarthritis: welfare implications and possible refinement strategies

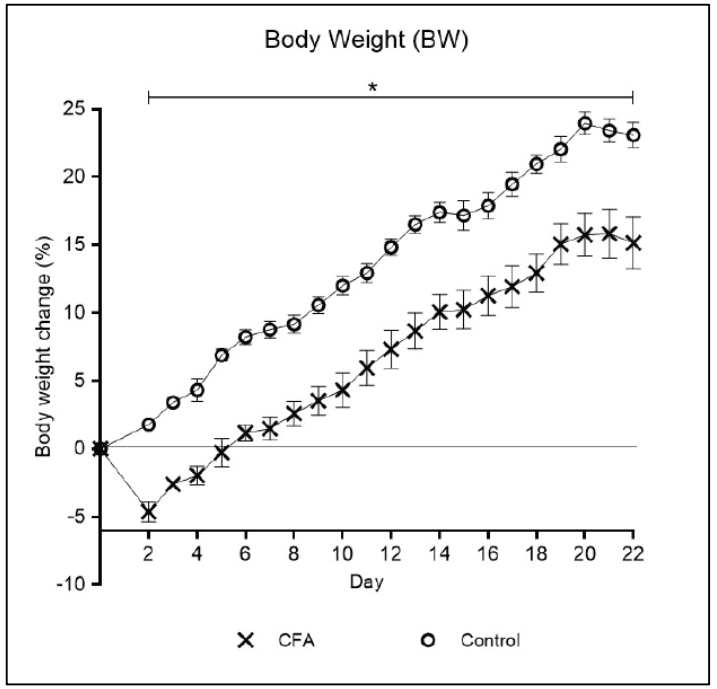
*By *Mie S. Berke and Klas S.P. Abelson*

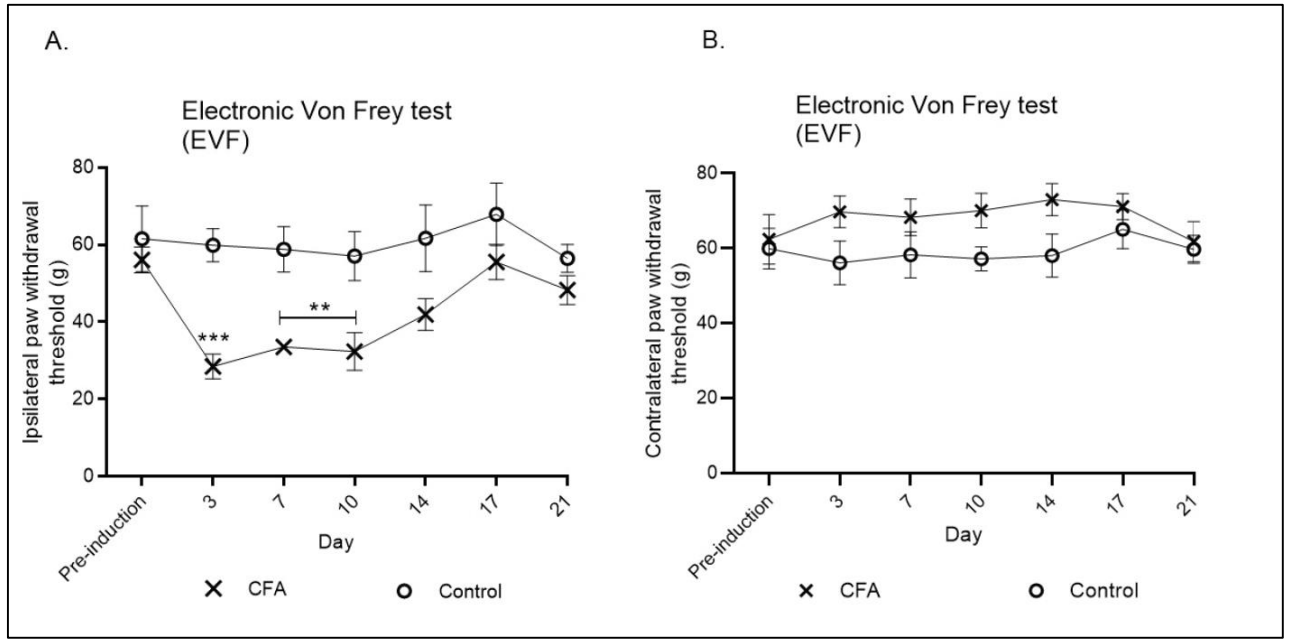
Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen

https://doi.org/10.23675/sjlas.v46i1.1046

- Overall welfare is not severely affected (not close to predefined humane endpoints)
- But animals are indeed negatively affected, with body weight loss and significant pain
- In particular during the early phase of the experiment







Follow up-studies

- Screening for relevant analgesic regimens

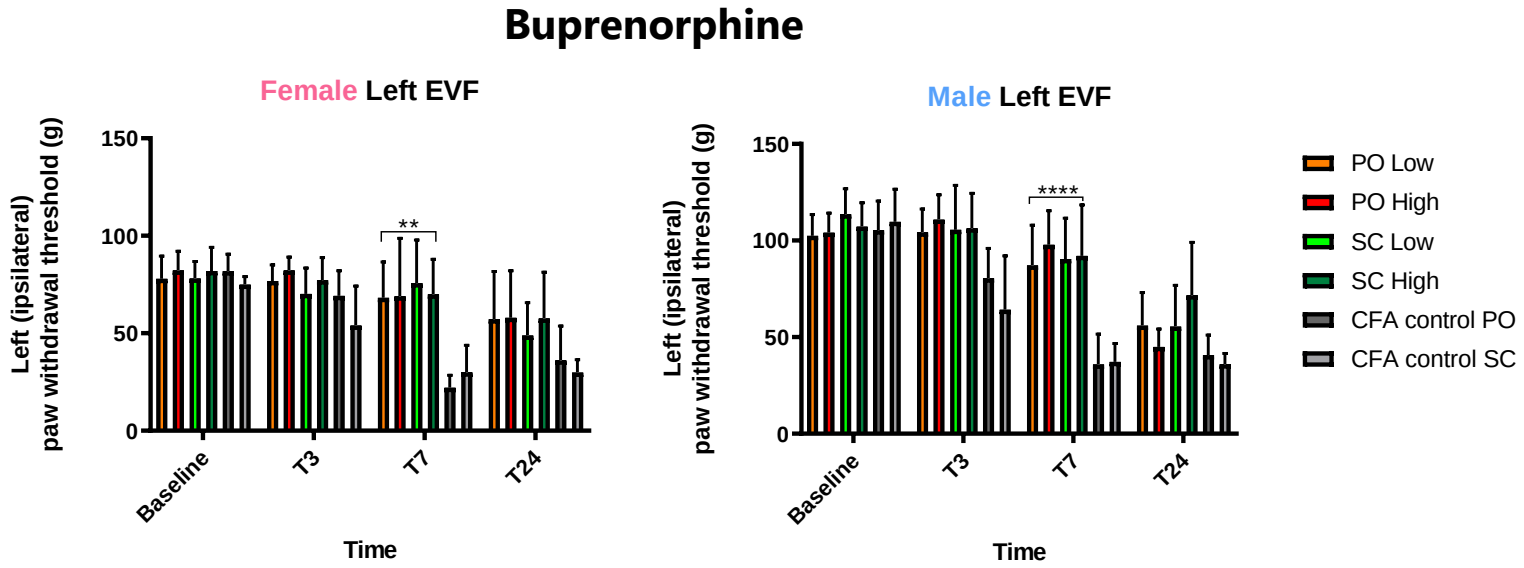
- Refining and optimizing the rat model for monoarthitis, by increasing the success rate with induction and minimizing adverse effects on surrounding tissues, irrelevant to the arthritis

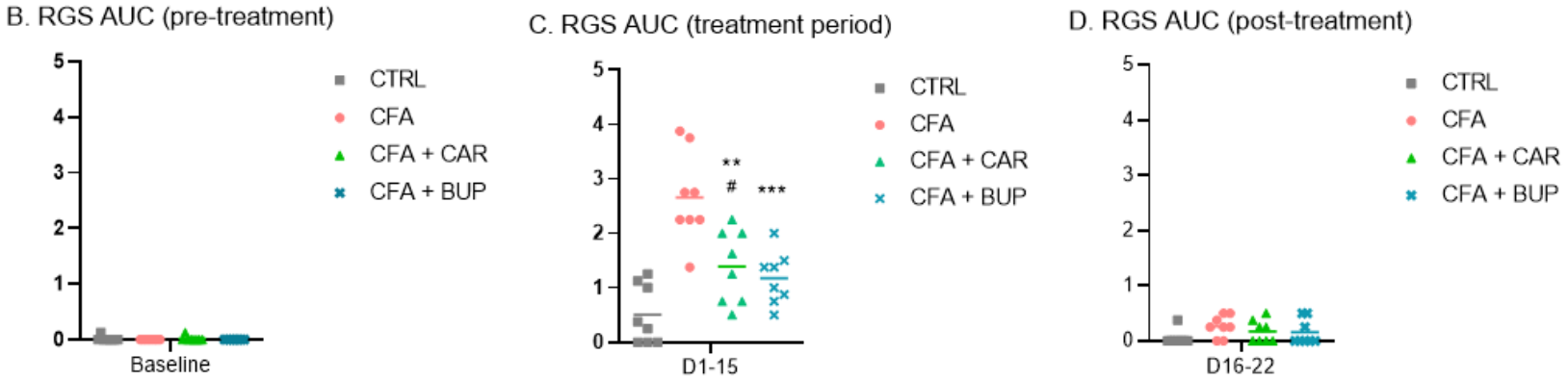
Treatment with buprenorphine

- Oral and parenteral buprenorphine relieves pain in the early phase after induction
- Seems to have no negative impact on the monoarthritic rat model
- One article published

PLOS ONE | https://doi.org/10.1371/journal.pone.0260356

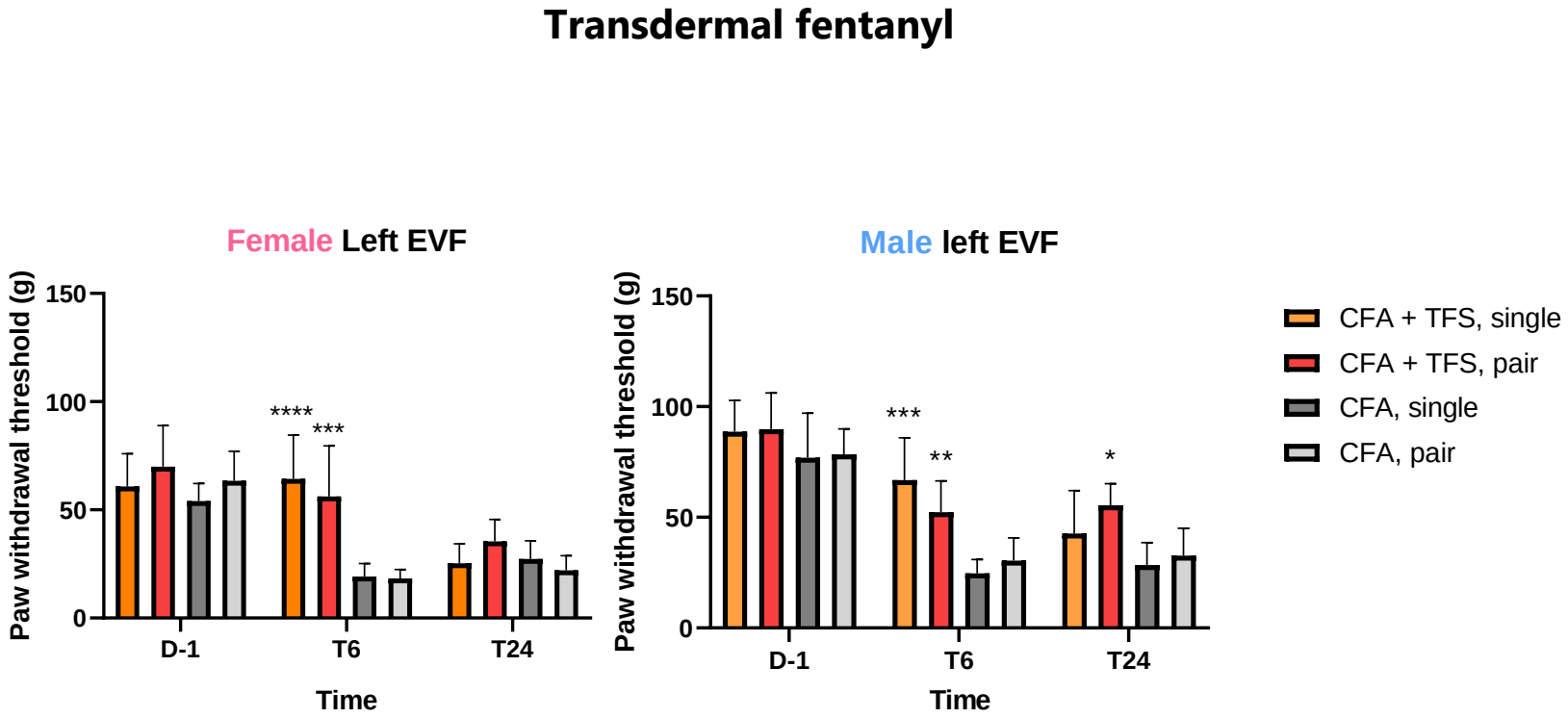
- One manuscript revised for publication





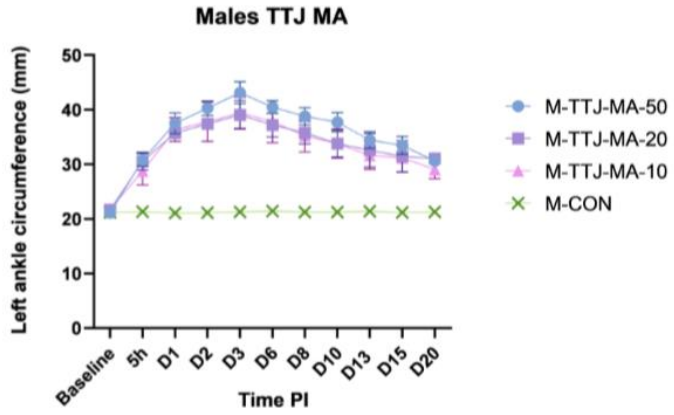
Treatment with fentanyl

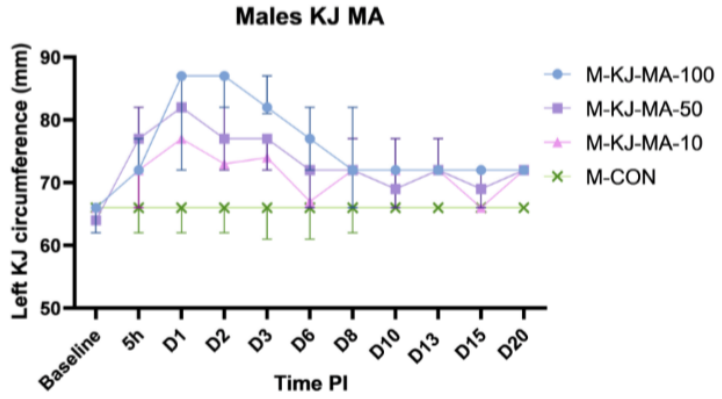
- Transdermal fentanyl relieves pain in the early phase after induction
- Seems to have no negative impact on the monoarthritic rat model
- Manuscript accepted for publication

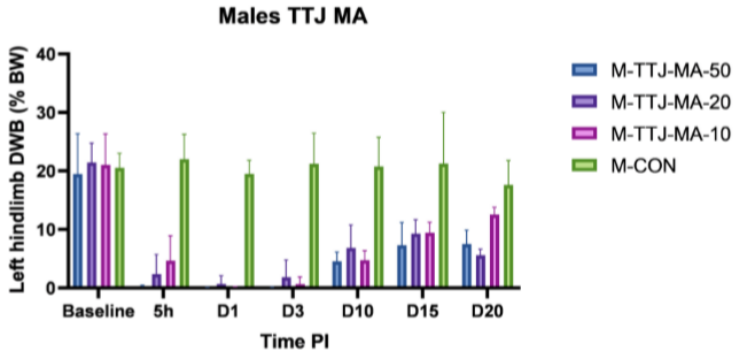


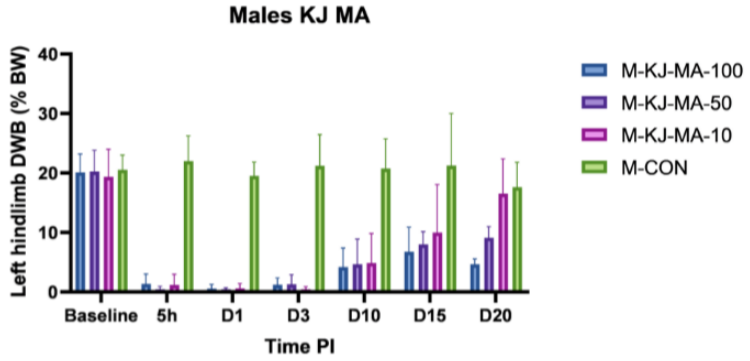
Improving the induction of the model

- Establishing the model by injection into the knee joint instead of the tibio-tarsal joint shows similar disease pattern
  - The knee joint is advantageous since risk of leakage into surrounding tissue is smaller
- The injection volume can be considerably reduced compared to standard volumes with similar disease pattern
  - Less adverse effects and less risk of leakage – refinement and improvement
- Manuscript accepted for publication









So, are we damned?

Do analgesics affect experimental data?

- Yes
- No
- It depends on the model and the analgesic

What should we do to avoid bias by analgesic?

- Choose the most appropriate analgesic regimen

How do we do choose right?

- Study the literature
- Consult experts and discuss with colleagues
- Investigate if and how the model actually is affected

Whenever pain relieve is not possible use other means of refinement!

Whenever pain relieve is not possible

- Introduce **early experimental endpoints**
  - The animals do not have to be very sick in order to obtain relevant data

- **Strictly defined humane endpoints** with actions specified

- If animals are clearly affected within approved severity, **facilitate the situation** by easy access to food and water, comfortable bedding, etc.
  - ➢ **Talk to the technicians!**


Thank
You
Obrigado
Asante
Merci
Multumesc
Spasibo
Maake
Grazie
Kiitos
Mochchakkeram
Matondo
Dank Je
Nirringrazzjak
Mamana
Ua Tsaug Rau Koj
Chokrane
Welalin
Juspaxar
Kia Ora
Vinaka
Cam on ban
Terma Kasih
Raibh Maith Agat
Matur Nuwun
Salamat
Dankon