

Refinement of painful procedures – are we good enough?

Klas Abelson

Professor of Comparative Medicine



KØBENHAVNS UNIVERSITET



When do we cause pain in the animals?

- Injections, marking and blood sampling
- Surgical procedures
- Induction of models (focus on pain models)

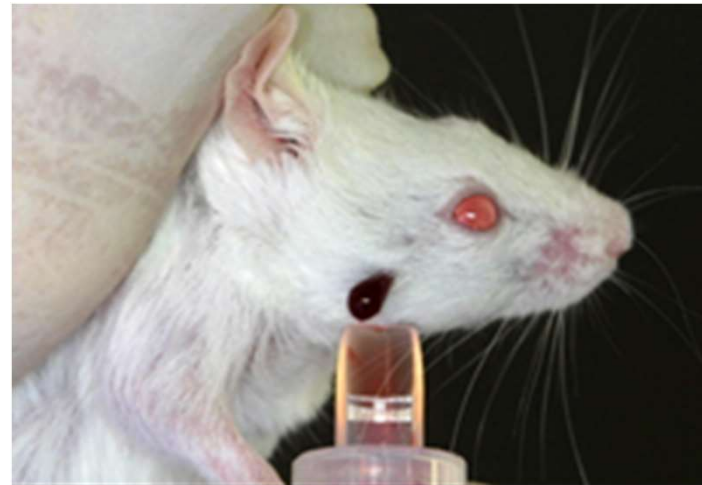
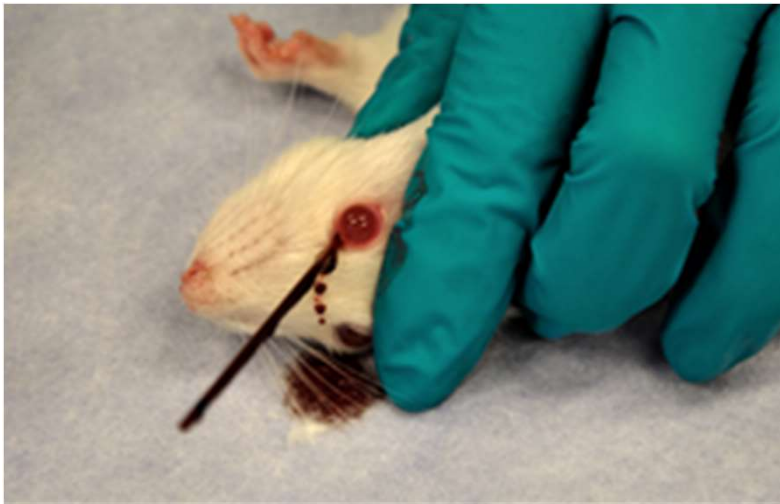
We inflict anything from mild acute pain to substantial chronic pain on the animals

Tremendous need for refinement!



Injectons, marking and blood sampling

- How much does it hurt?



Injections, marking and blood sampling

- Associated with pain and stress of varying degree
- Least invasive method should be used
- Technical skillfulness minimizes trauma and should give less pain and stress
- Where possible – local anaesthetics should be used

Injectons, marking and blood sampling

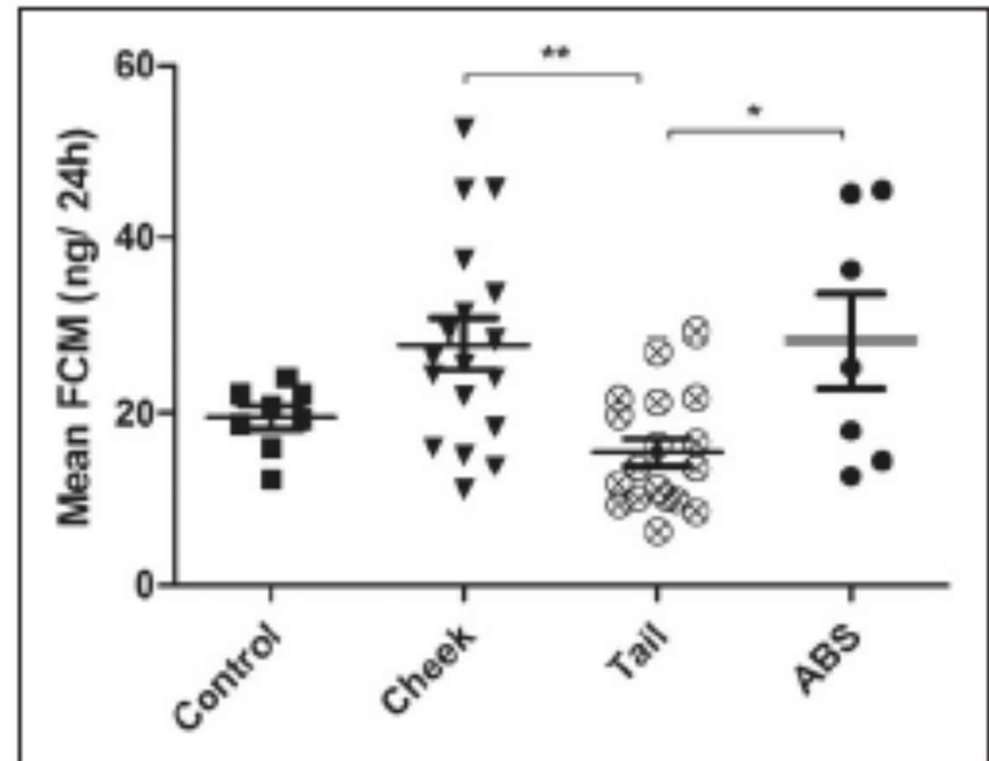
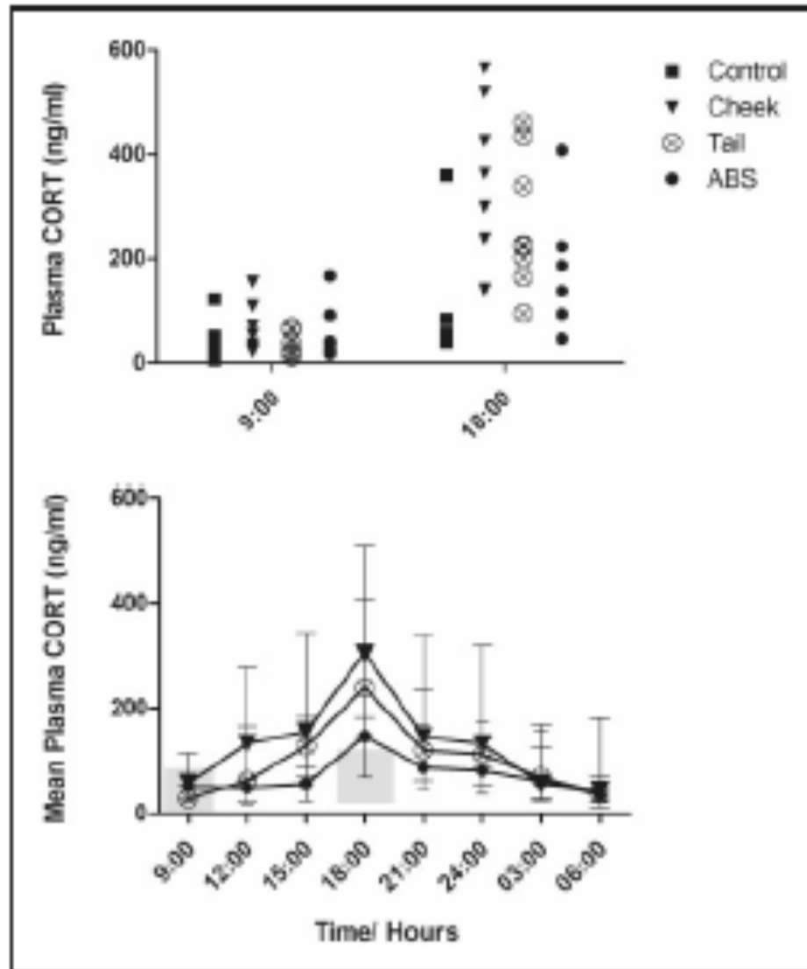
- Examples of various invasiveness in the methods

Manual versus automated blood sampling: impact of repeated blood sampling on stress parameters and behavior in male NMRI mice

A C Teilmann¹, Otto Kalliokoski¹, Dorte B Sørensen², Jann Hau¹
and Klas S P Abelson¹

Laboratory Animals
2014, Vol. 48(4) 278–291
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DOI: 10.1177/0023677214541438
la.sagepub.com







RESEARCH ARTICLE

Physiological and Pathological Impact of Blood Sampling by Retro-Bulbar Sinus Puncture and Facial Vein Phlebotomy in Laboratory Mice

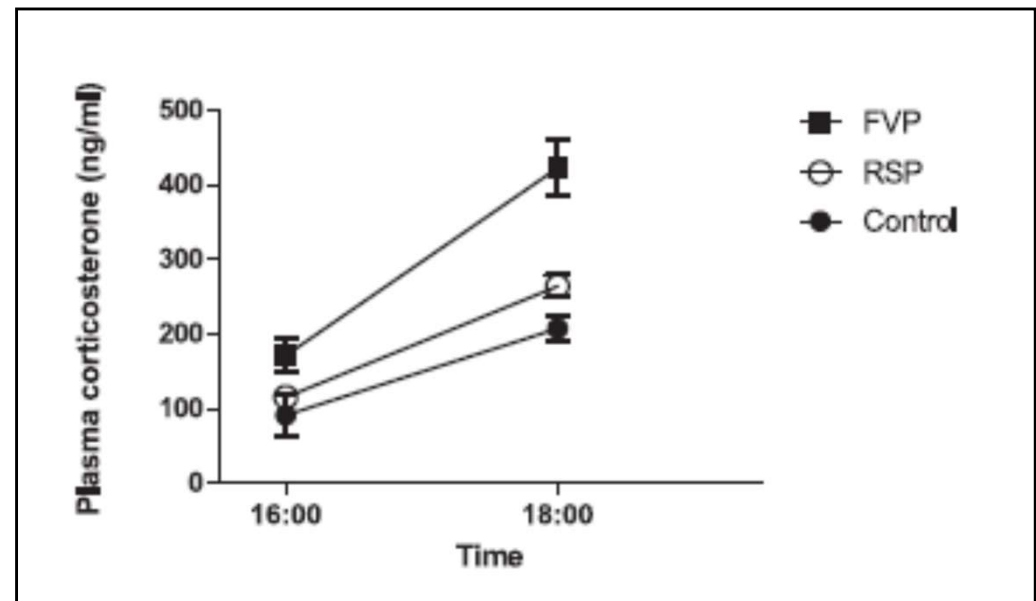
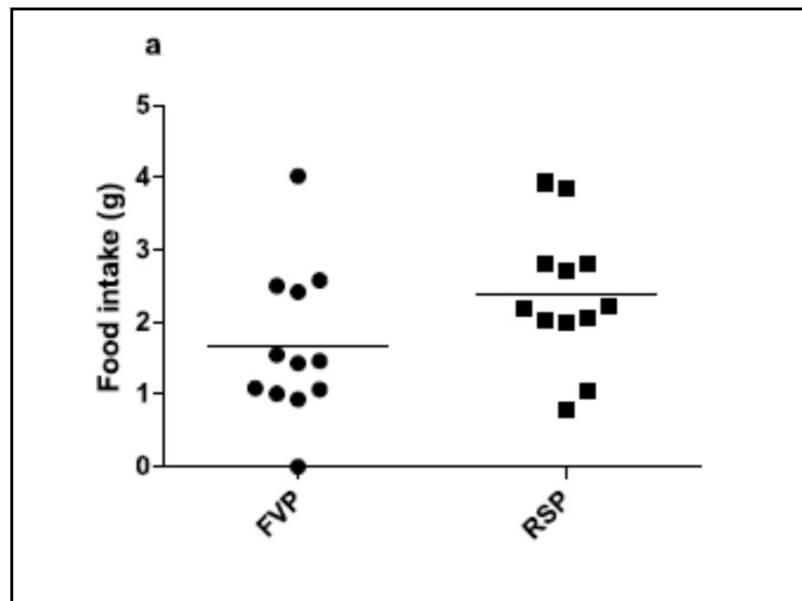
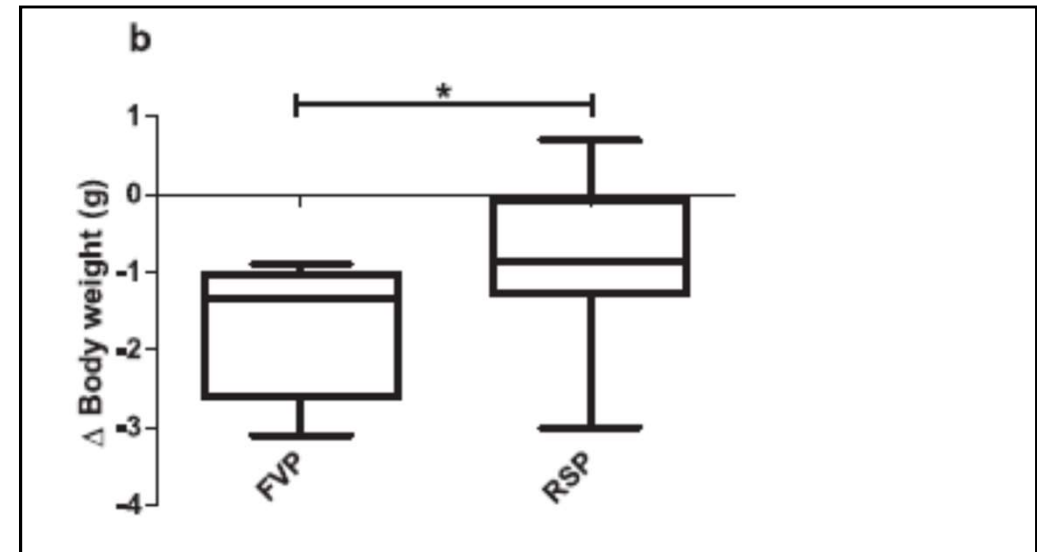
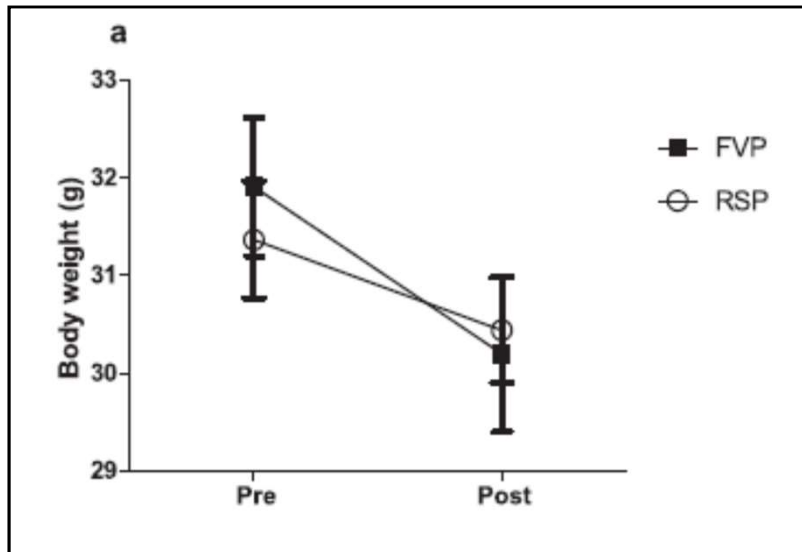
Anne Charlotte Teilmann^{1‡*}, Andreas Nygaard Madsen^{2‡}, Birgitte Holst², Jann Hau¹, Björn Rozell¹, Klas Stig Peter Abelson¹

1. Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 2. The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

*annete@sund.ku.dk

‡ These authors contributed equally to this work.





Injections, marking and blood sampling

Examples of use of local anaesthetics

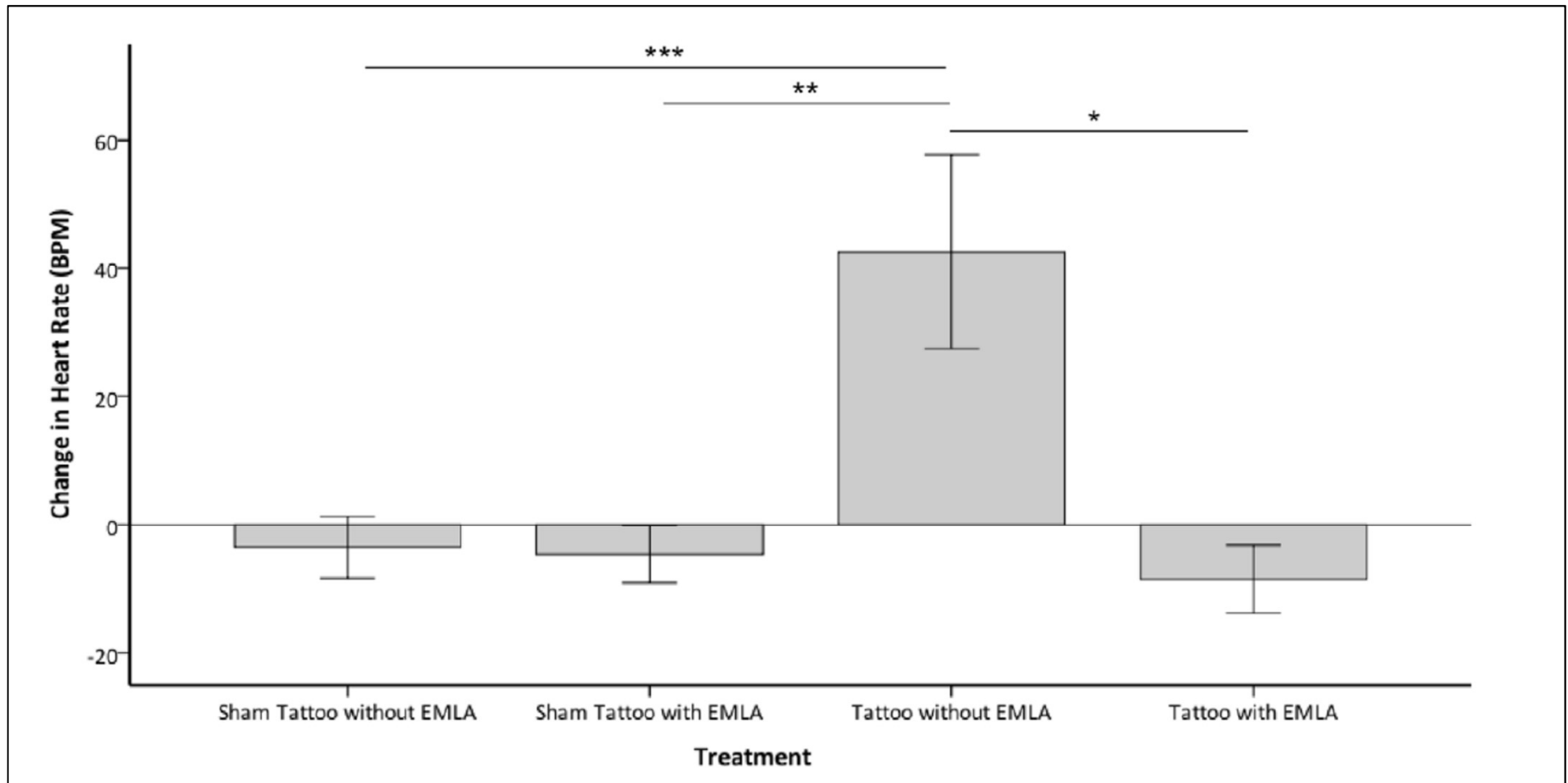
OPEN ACCESS Freely available online

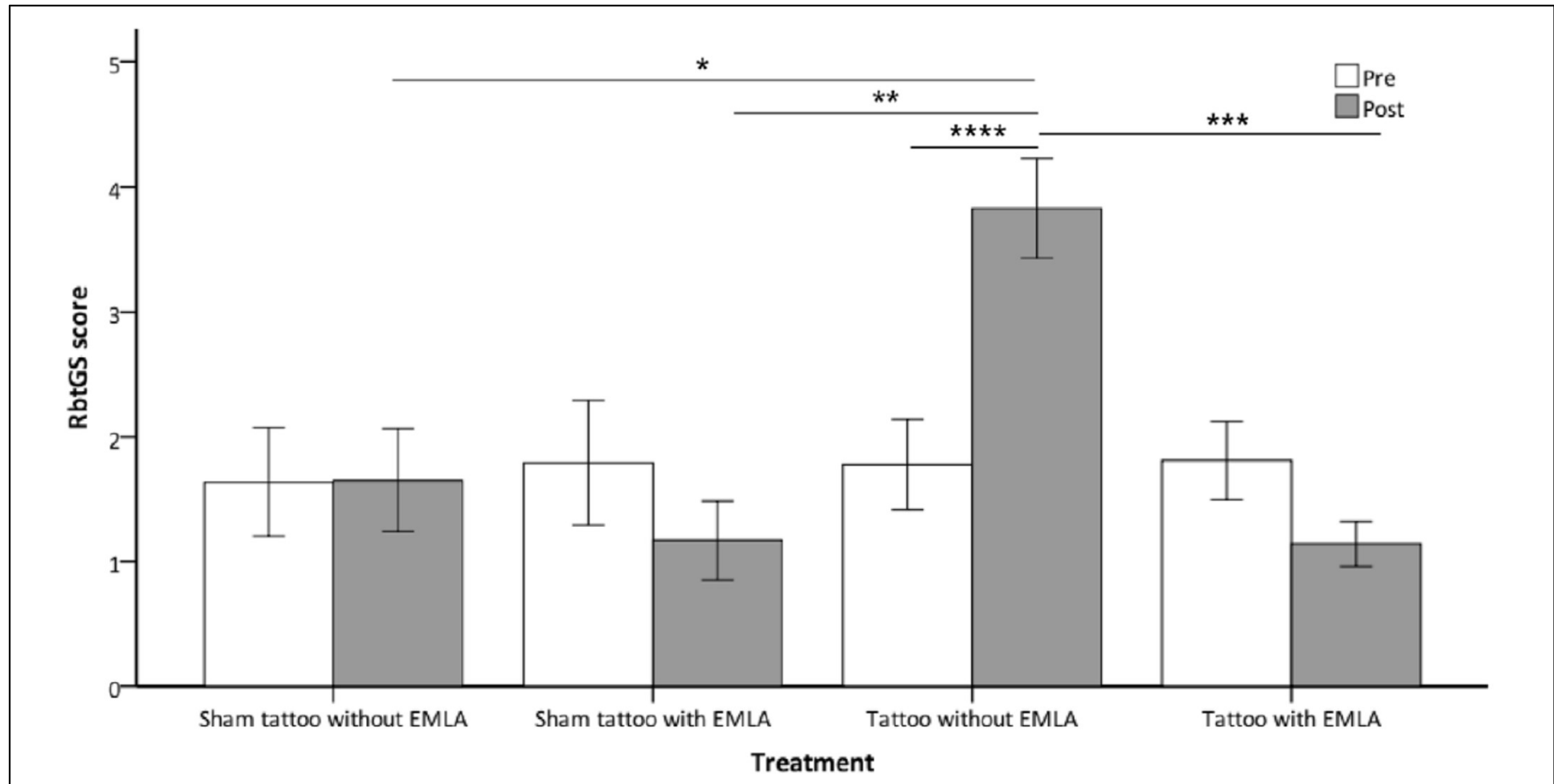


Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses

Stephanie C. J. Keating¹, Aurelie A. Thomas², Paul A. Flecknell², Matthew C. Leach^{2*}

¹ Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, ² Institute of Neuroscience and Comparative Biology Centre, Newcastle University, Newcastle upon Tyne, United Kingdom





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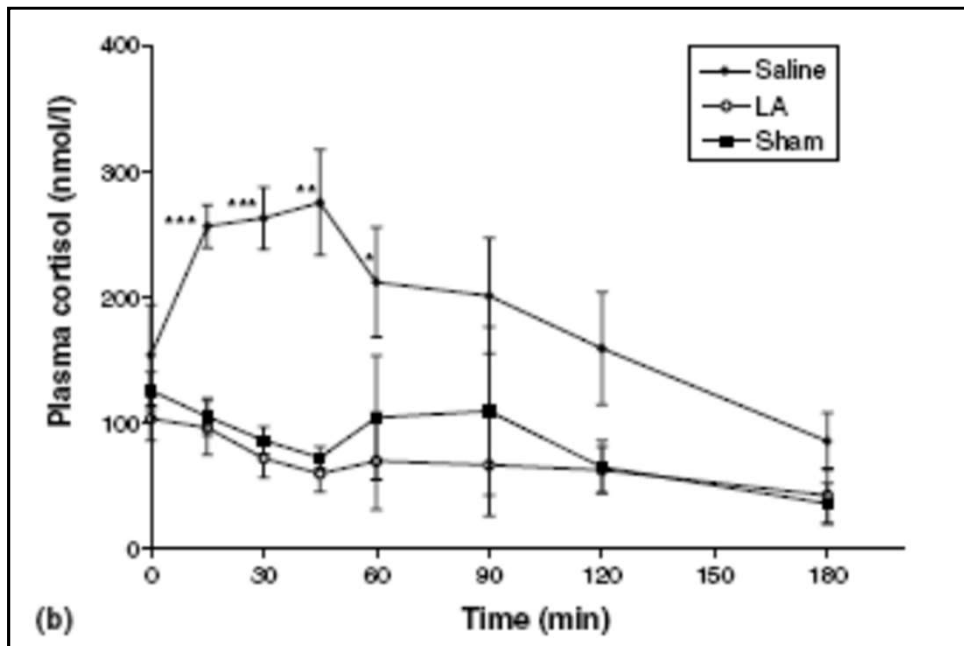
Tremendous need for refinement!



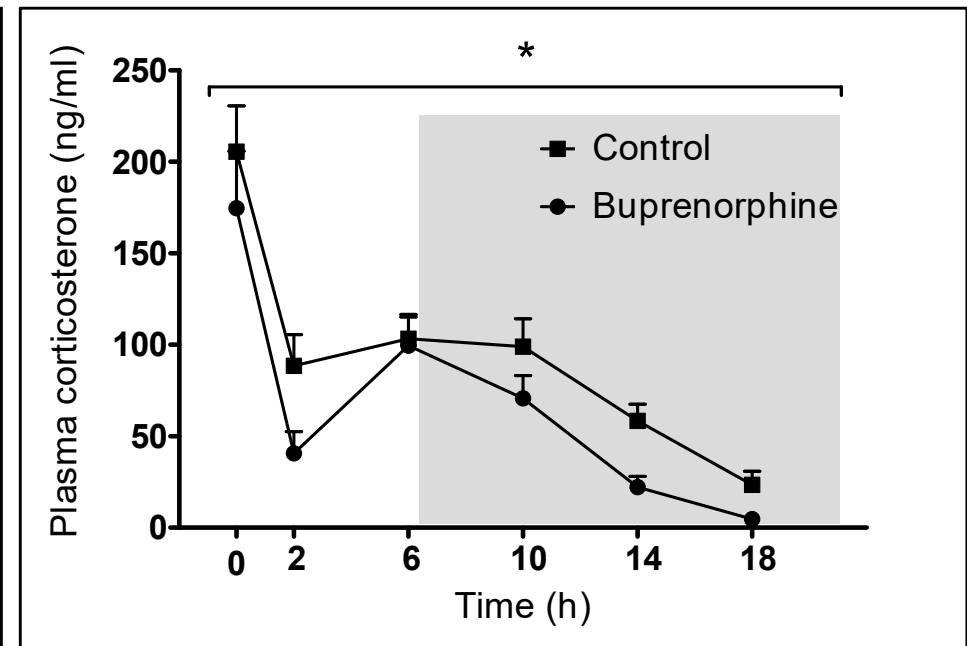
Surgical procedures

- Surgery is associated with pain and stress that can be anything from mild to moderate to severe
- Peri- and postoperative analgesia shall always be given whenever applicable
- Non-invasive delivery of analgesics if possible
- Technical skills and aseptic technique minimizes trauma and risk of infections and thereby less pain and stress

Pain and stress from surgical procedures and effect of analgesia

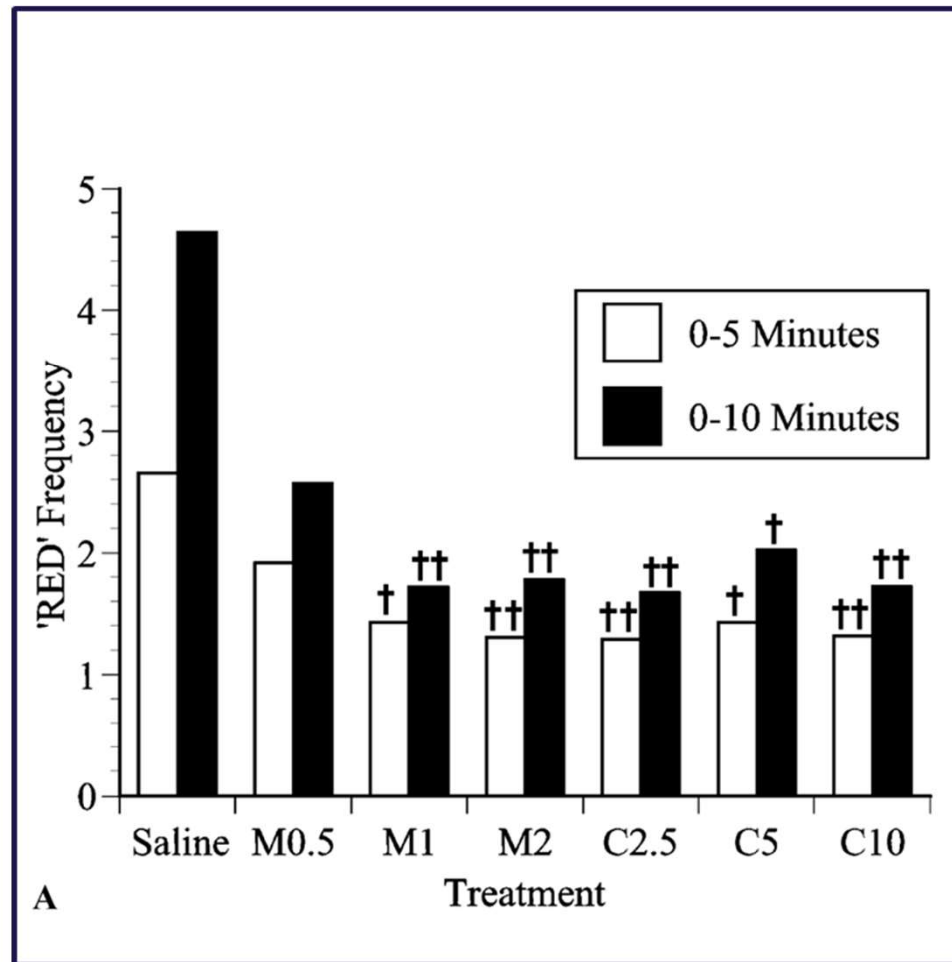


Lykkegaard *et al*, Res. Vet. Sci. 79, 2005



Goldkuhl *et al*, In Vivo, 24, 2010

Effect of laparotomy on behaviour



Roughan and Flecknell, Eur J Pain, vol 7, 2003

Non-invasive delivery of analgesics



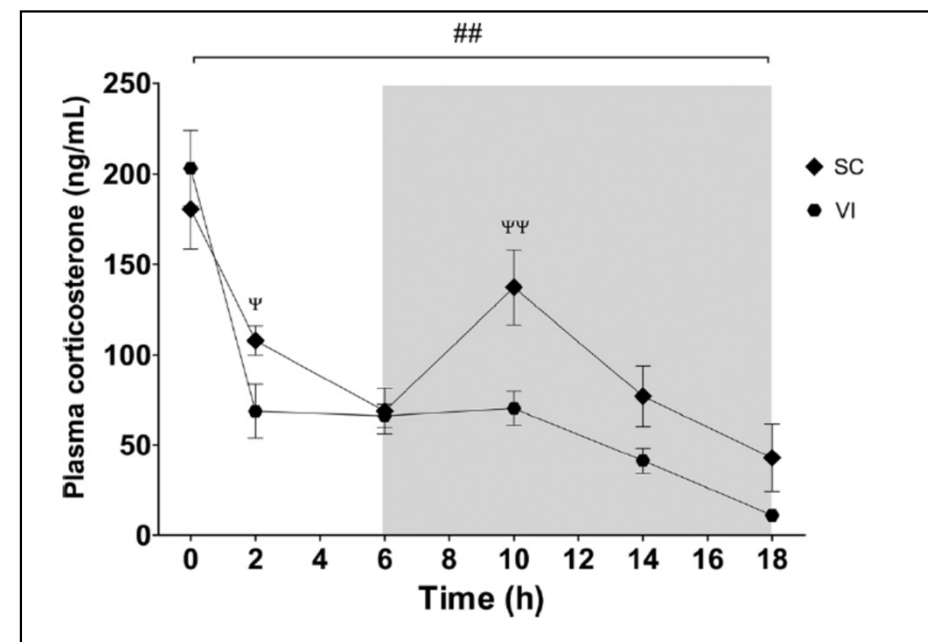
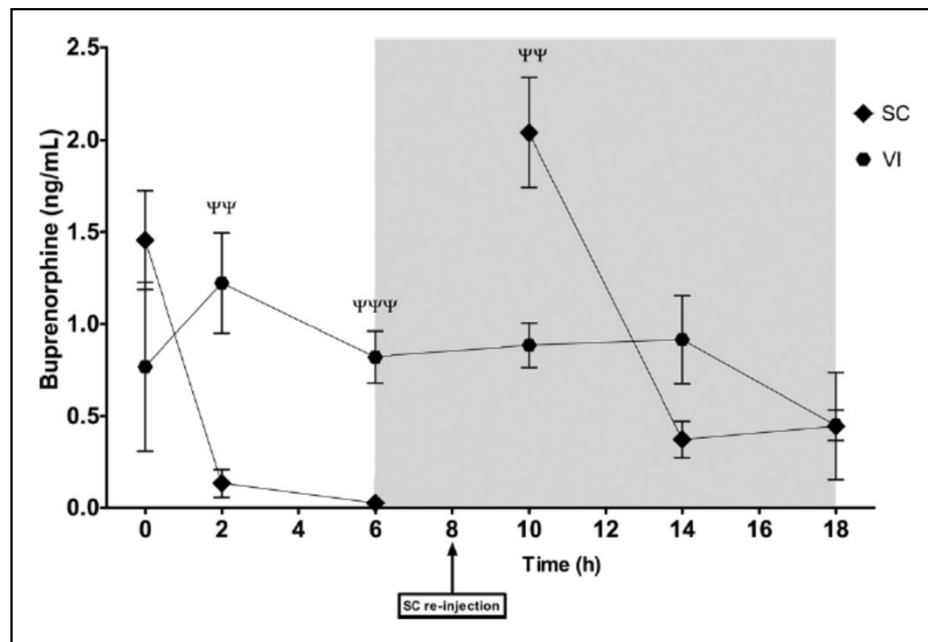
Plasma concentrations of corticosterone and buprenorphine in rats subjected to jugular vein catheterization

Renée Goldkuhl¹, Kirsten R Jacobsen², Otto Kalliokoski², Jann Hau² and Klas S P Abelson^{1,2}

¹Division of Comparative Medicine, Department of Neuroscience, Uppsala University, Uppsala, Sweden; ²Department of Experimental Medicine, University of Copenhagen and National Hospital, Copenhagen, Denmark

Corresponding author: Klas Abelson, Department of Experimental Medicine, University and University Hospital of Copenhagen, Blegdamsvej 3B, DK-2200, Copenhagen N, Denmark. Email: klasab@sund.ku.dk

Laboratory Animals 2010; 44: 337–343. DOI: 10.1258/la.2010.009115



Reduced stress levels and more stable serum concentrations after delivery through voluntary ingestion in hazel nut spread



Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvj



Serum concentrations of buprenorphine after oral and parenteral administration in male mice

Otto Kalliokoski^a, Kirsten R. Jacobsen^a, Jann Hau^a, Klas S.P. Abelson^{a,b,*}

^aDepartment of Experimental Medicine, University and University Hospital of Copenhagen, Copenhagen, Denmark

^bDepartment of Neuroscience, Division of Comparative Medicine, Uppsala University, Uppsala, Sweden

High and stable
serum
concentration of
buprenorphine after
voluntary ingestion
also in mice

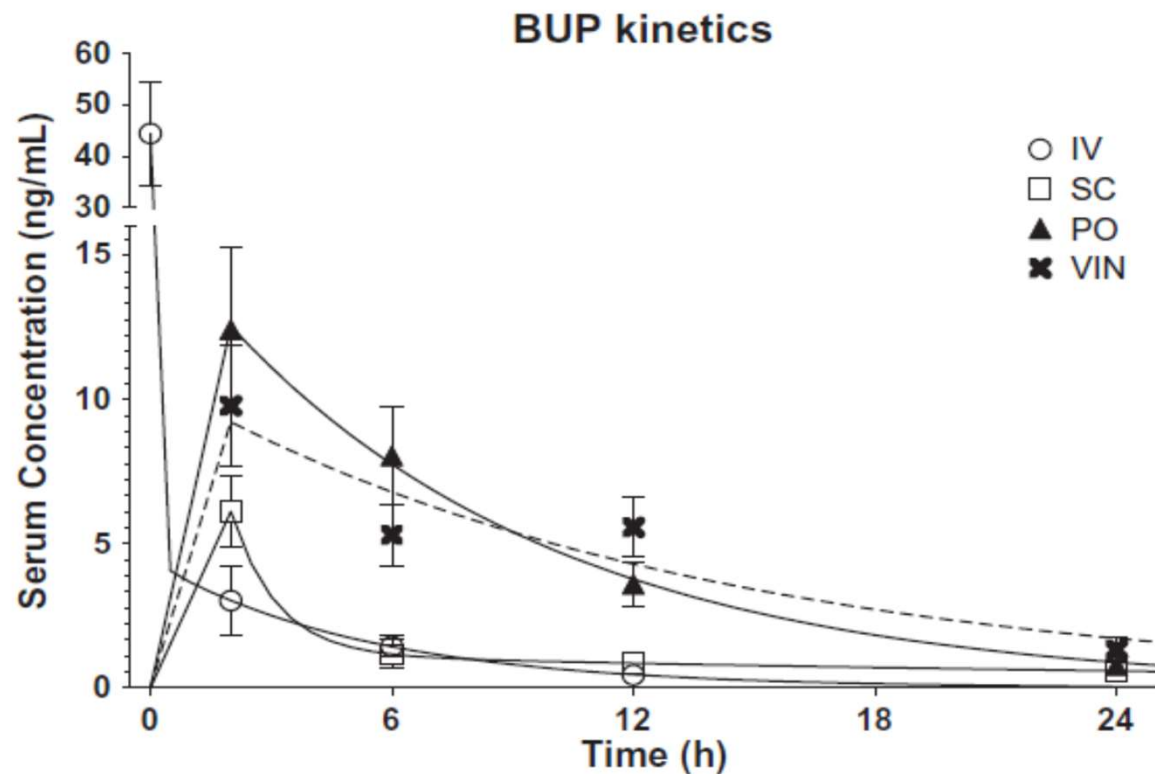


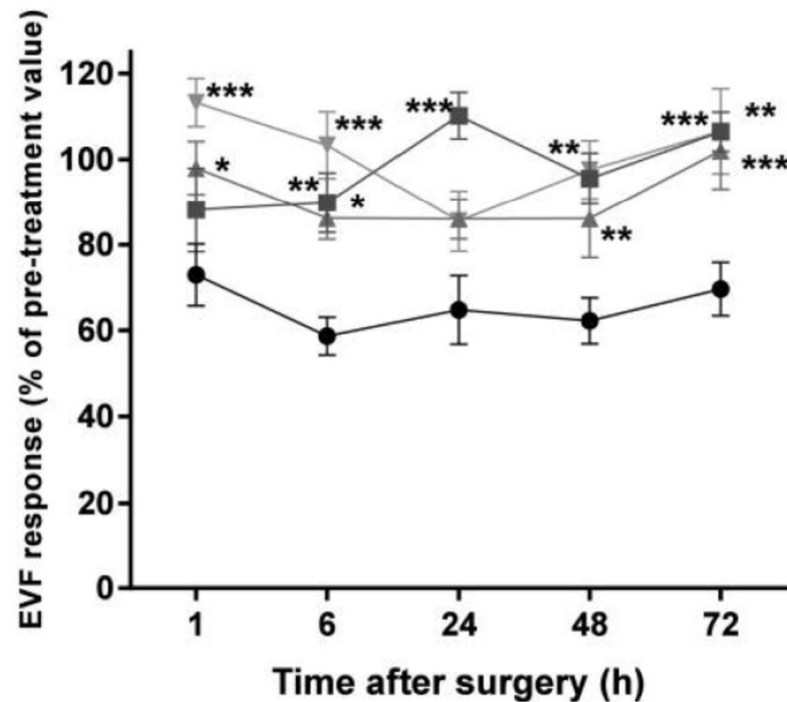
Fig. 1. Serum concentration of buprenorphine over time, means \pm SEM ($n = 6$). Regressions based on a two compartment model are fitted to the parenteral modes of delivery (IV, SC), whereas single compartment models were used for oral routes (PO, VIN). The regression line for VIN is dashed for clarity reasons.

in vivo 32: 713-719 (2018)
doi:10.21873/invivo.11299

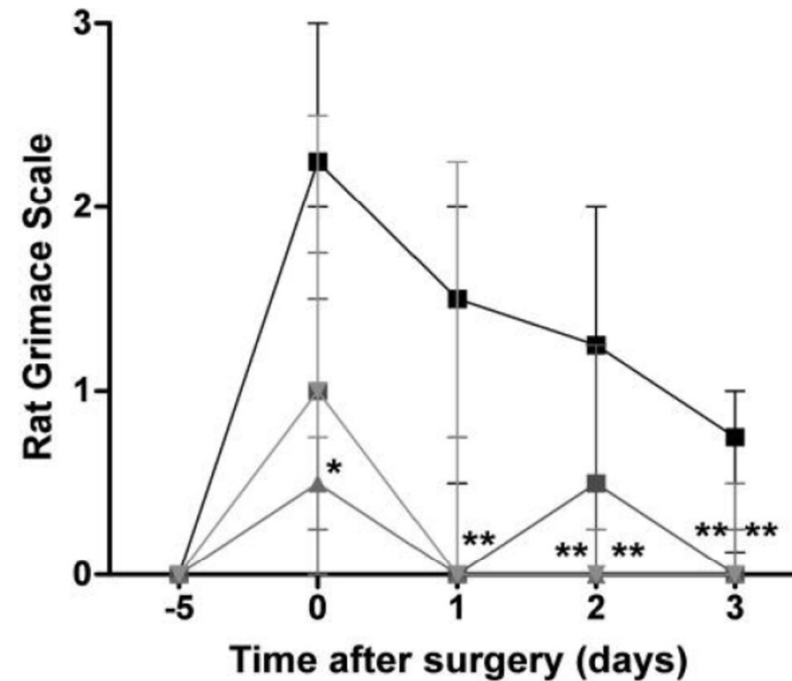
Transdermal Fentanyl Solution Provides Long-term Analgesia in the Hind-paw Incisional Model of Postoperative Pain in Male Rats

JOHANNE CLEMENSEN*, LENA V. RASMUSSEN* and KLAS S.P. ABELSON

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



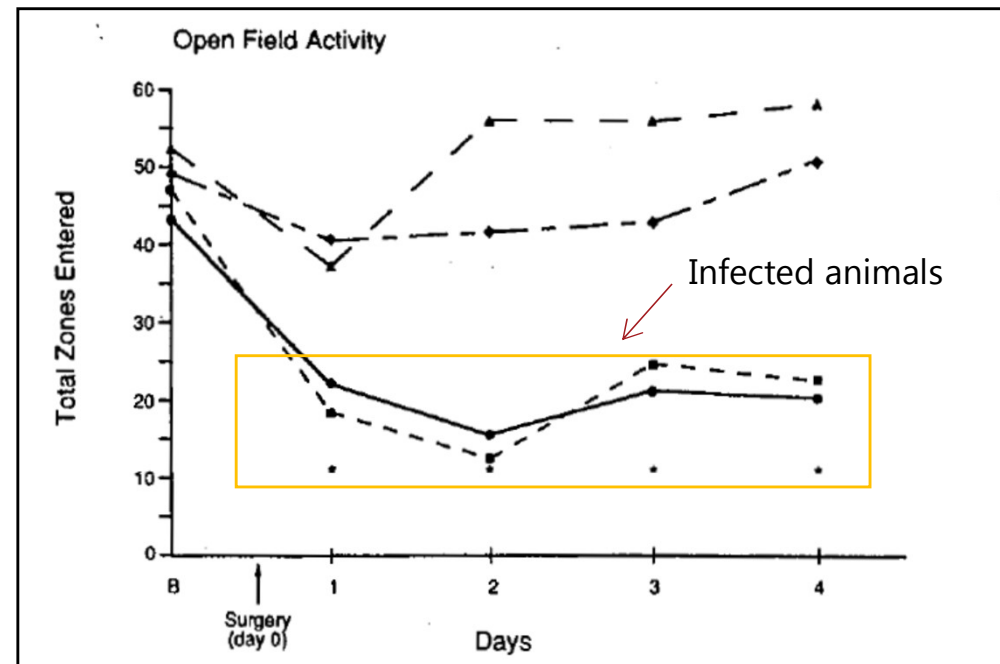
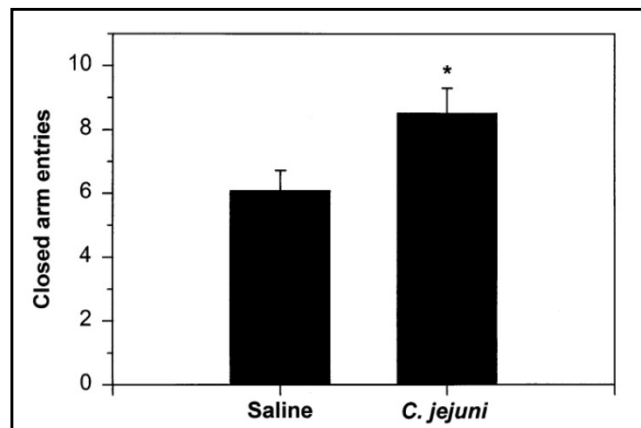
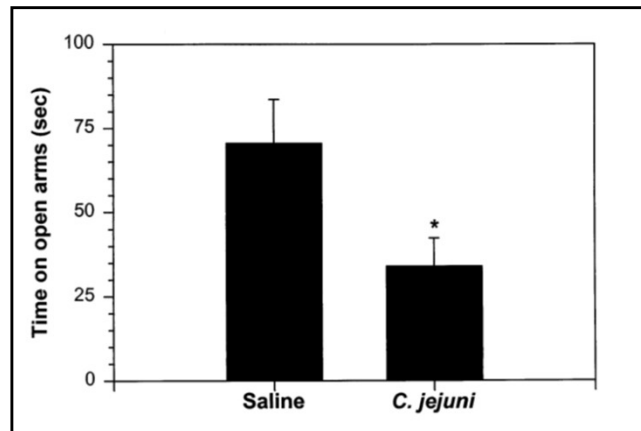
● No analgesia
■ 0.1 mg/kg fentanyl
▲ 0.33 mg/kg fentanyl
▼ 1.0 mg/kg fentanyl



■ No analgesia
■ 0.1 mg/kg fentanyl
▲ 0.33 mg/kg fentanyl
▼ 1.0 mg/kg fentanyl

Aseptic and hygiene

Lack of studies – but here is a couple



Bradfield et al. Lab Anim Sci vol. 42 1992

Lyte et al. Physiology and Behaviour vol. 65 1998

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- Surgical procedures
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We inflict anything from mild acute pain to substantial chronic pain on the animals

Tremendous need for refinement!



Induction of models

- Some animal models are associated with pain and stress that is part of the model and can be anything from mild to moderate to severe
- Analgesic treatment should be provided whenever possible, provided that it does not interfere with experimental data or development of model parameters
- Method for induction should be refined to minimize trauma
- Well-defined humane endpoints and proper welfare monitoring
- Tender love and care!

Analgesia to a neuropathic pain model



RESEARCH ARTICLE

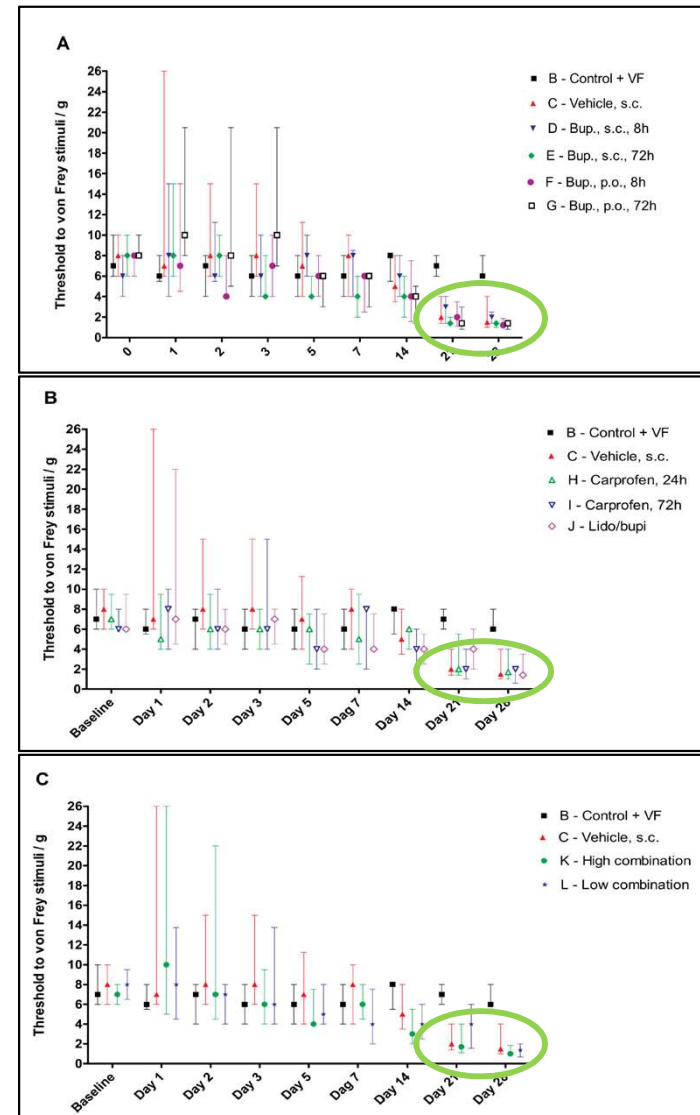
Is there a reasonable excuse for not providing post-operative analgesia when using animal models of peripheral neuropathic pain for research purposes?

Sara Hestehave^{1,2*}, Gordon Munro^{2,3}, Rie Christensen², Tina Brønnum Pedersen⁴, Lars Arvastson⁵, Philip Hougaard⁵, Klas S. P. Abelson¹

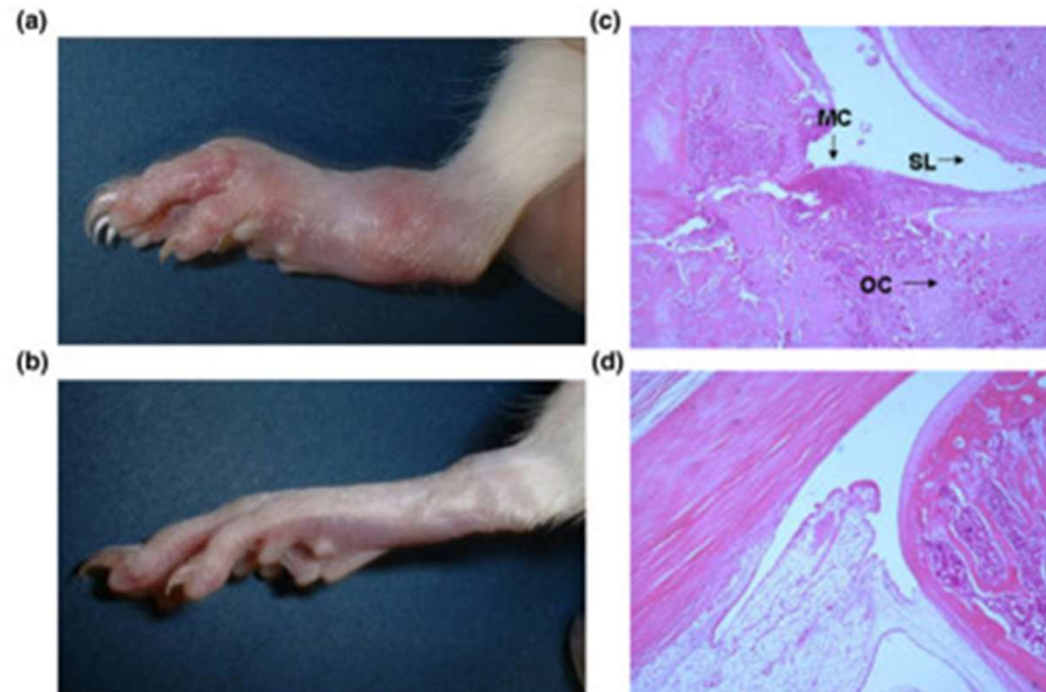
<https://doi.org/10.1371/journal.pone.0188113>

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!



Models for arthritis



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

Analgesic treatment

PLOS ONE

RESEARCH ARTICLE

Effects of buprenorphine on model development in an adjuvant-induced monoarthritis rat model

Mie S. Berke^{1*}, Louise K. D. Fensholdt¹, Sara Hestehave², Otto Kalliokoski¹, Klas S. P. Abelson¹

¹ Dept. of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, ² Dept. of Cell and Developmental Biology, University College London, London, United Kingdom

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Heliyon 8 (2022) e11554



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of buprenorphine on acute pain and inflammation in the adjuvant-induced monoarthritis rat model

M.S. Berke^{a,*}, P. Colding-Jørgensen^a, S. Hestehave^b, O. Kalliokoski^a, H.E. Jensen^c, D. Bratbo Sørensen^c, J. Hau^a, K.S.P. Abelson^a

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

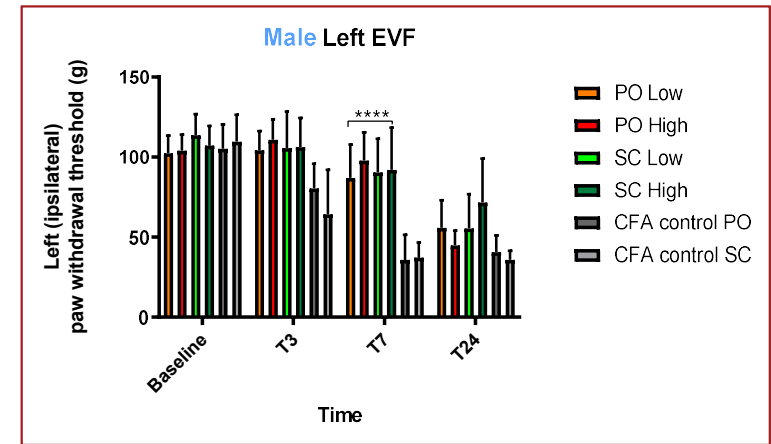
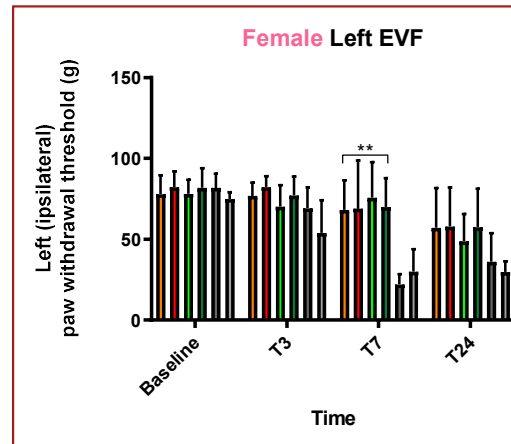
^b Cell & Developmental Biology, University College London, United Kingdom

^c Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

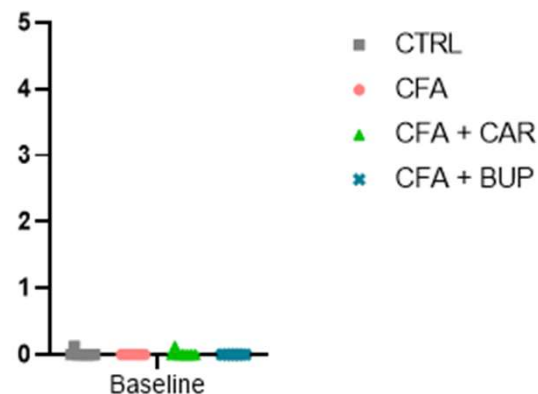
Analgesic treatment

- Buprenorphine (oral and subcutaneous) gives pain relief
- No observable effect on model parameters or disease progression

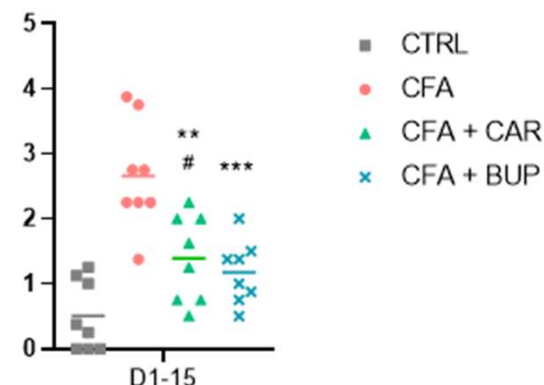
Buprenorphine



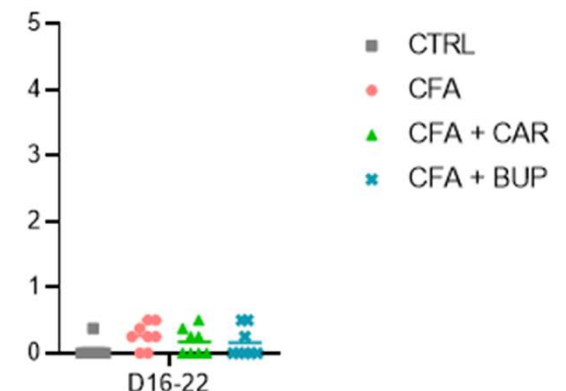
B. RGS AUC (pre-treatment)



C. RGS AUC (treatment period)



D. RGS AUC (post-treatment)



Analgesic treatment

Comparative Medicine
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Vol 72, No 5
October 2022
Pages 320–329

Original Research

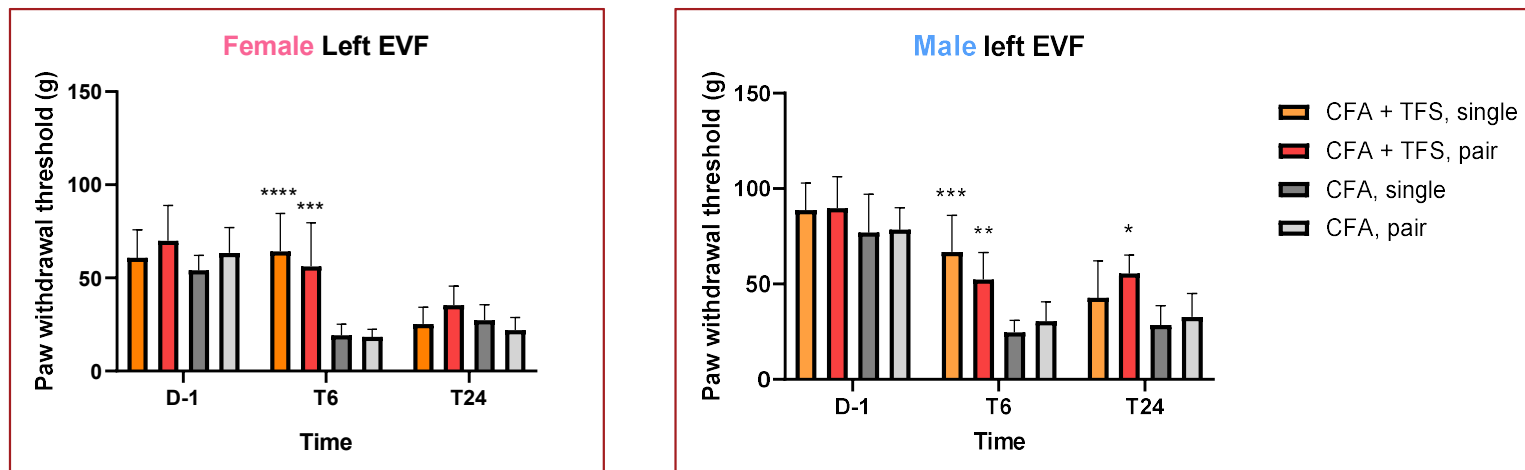
Effects of Transdermal Fentanyl Treatment on Acute Pain and Inflammation in Rats with Adjuvant-induced Monoarthritis

Mie S Berke,^{1,*†} Pernille Colding-Jørgensen,^{1,†} Line G Pedersen,¹ Sara Hestehave,² Otto Kalliokoski,¹ Henrik E Jensen,³
Dorte B Sørensen,³ Jann Hau,¹ and Klas SP Abelson¹

Analgesic treatment

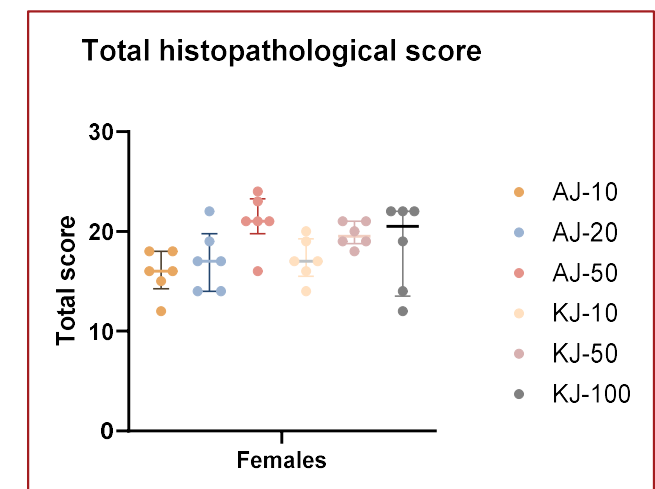
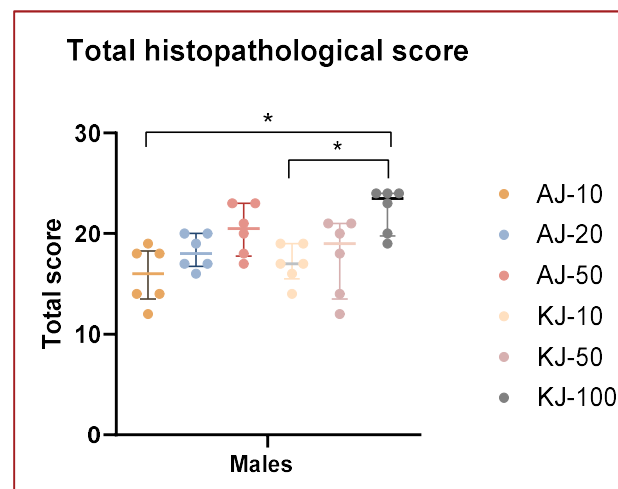
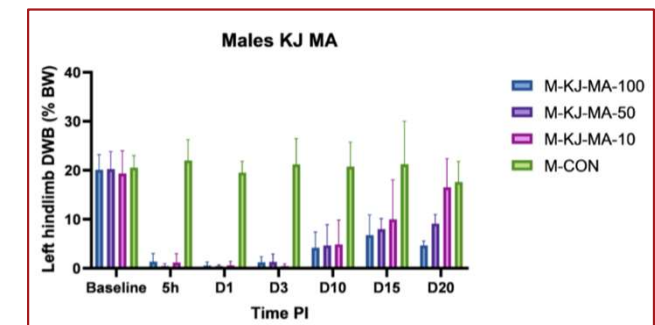
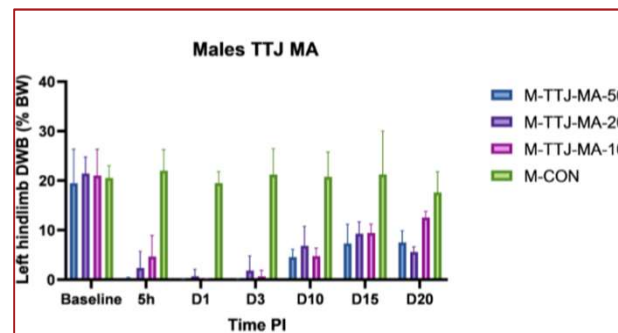
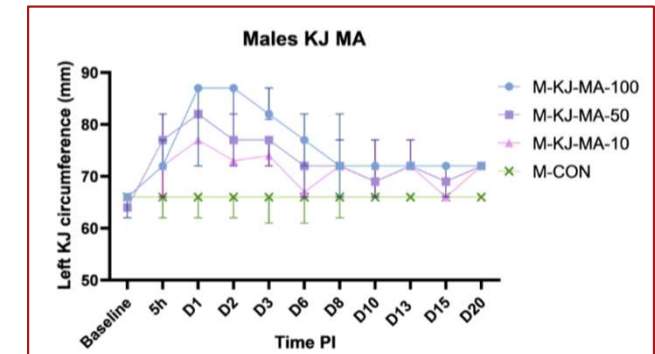
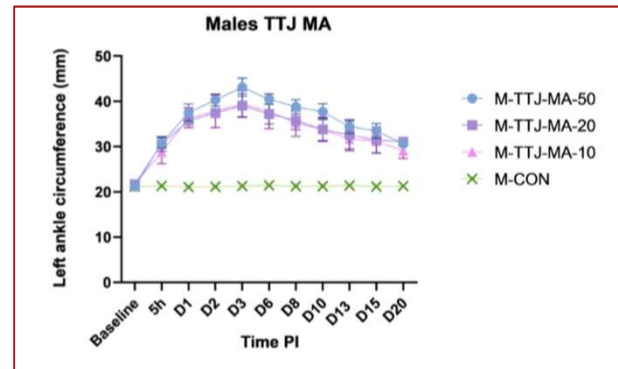
- Transdermal fentanyl gives pain relief to the animals
- No observable effect on model parameters or disease progression
- Associated with adverse effects – use with caution

Transdermal fentanyl



Refinement of induction method

- Injection in the knee joint instead of tibio-tarsal joint: Less leakage and less adverse and irrelevant effects
- No observable effect on model parameters or disease progression
- Injection volume can be considerably reduced, which is a considerable refinement without negative effects on the model
- Manuscript in progress



And we do the same for mice



Master's Thesis

Ditte Grauenhøj Kasahara Stinus (nrq129)

MSc in Human Biology

Refinement of the Mouse Collagen Antibody-Induced Arthritis Model

Refinement of painful procedures – are we good enough?

We may not be here
yet, but we might be on
the right track.





***TO BE
CONTINUED...*** 