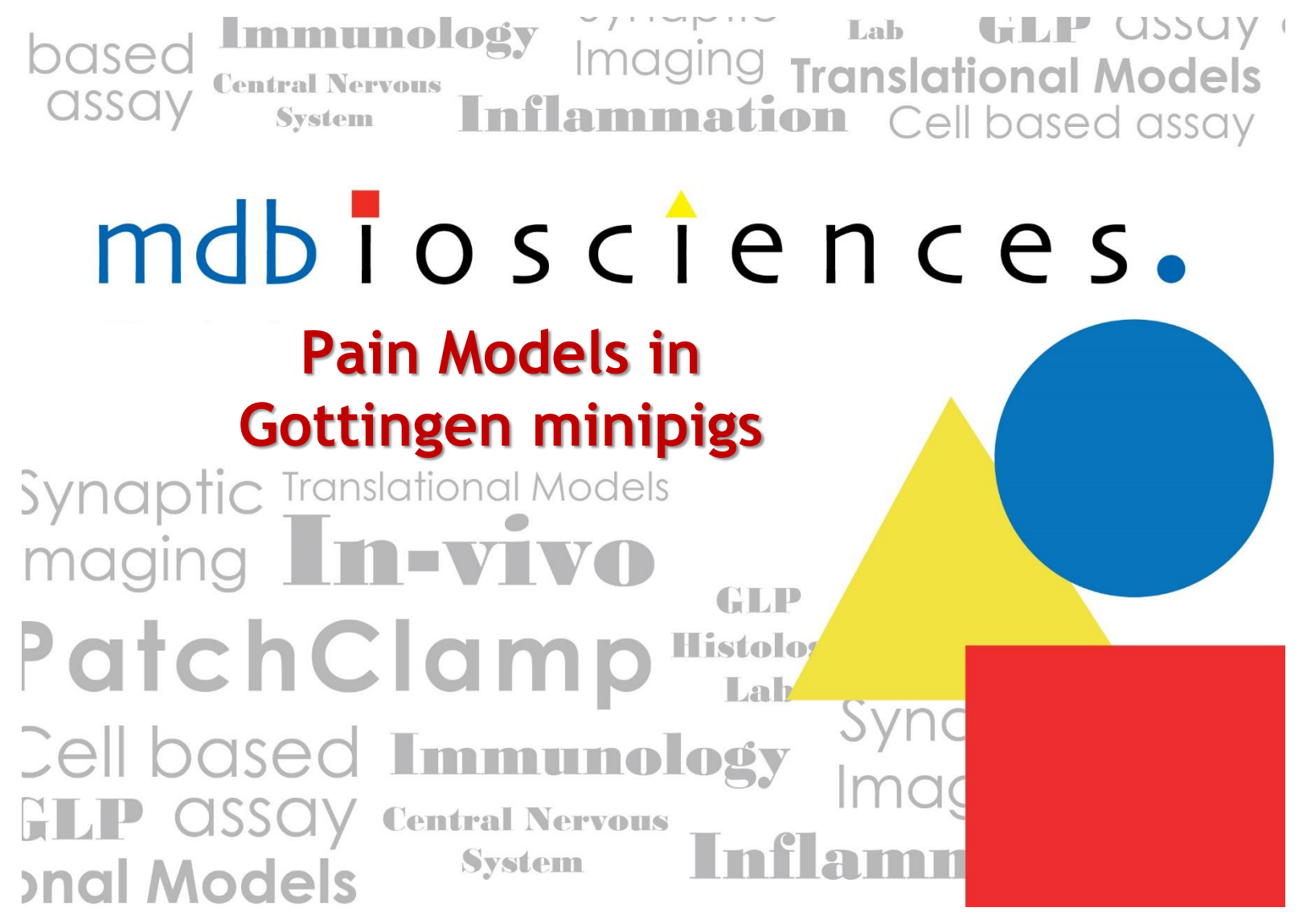
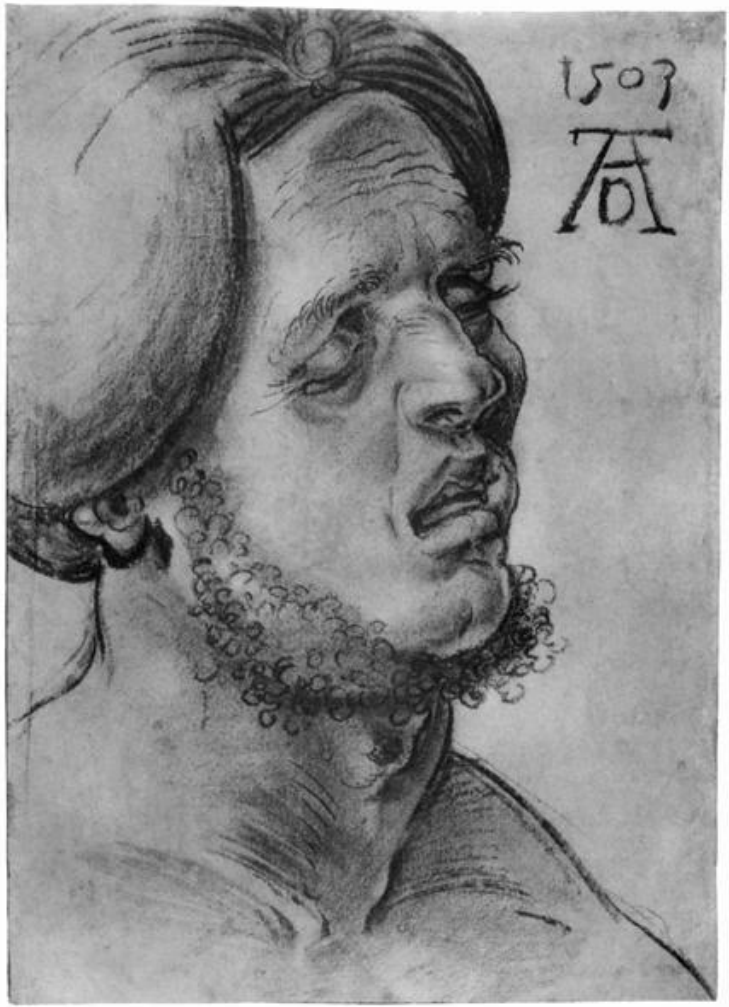

based assay
Immunology
Central Nervous System
Imaging
Inflammation
Lab
Translational Models
Cell based assay
mdbiosciences.
Pain Models in Gottingen minipigs
Synaptic
Translational Models
maging
In-vivo
PatchClamp
GLP
Lab
Cell based
Immunology
GLP assay
Central Nervous System
onal Models



Head of a Suffering Man

Albrecht Durer, 1,503, London, UK

Charcoal, gray washed, on heavily browned paper, 31 × 22.1 cm

Presentation Outline

1. Pain Definition and Preclinical Methods in Pain Research
2. Why Pigs
3. Post-Operative-Pain Models
4. Peripheral nerve injury model for neuropathic pain

Presentation Outline

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Quantitative Sensory Testing
QST

Questionnaires

Pain

An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.

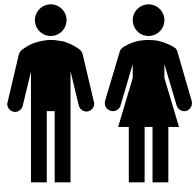
Scales

Verbal description

Part III: Pain Terms, A Current List with Definitions and Notes on Usage. IASP Task Force on Taxonomy, edited by H. Merskey and N. Bogduk, IA… ©1994.

Biomarkers

Pain Assessment in Clinical studies



Pre-Clinical Studies

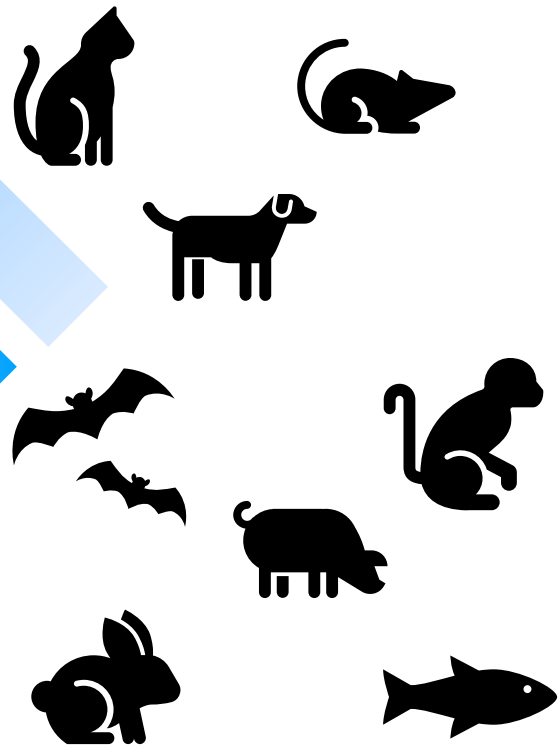
Questionnaires

Verbal description

Scales

Quantitative Sensory Testing
QST

Biomarkers



Preclinical Studies

Induction of Pain

- Acute
- Chronic

Assessment of Pain

Quantitative Sensory Testing
QST

Withdrawal testing

Spontaneous Pain

Scores

Presentation Outline

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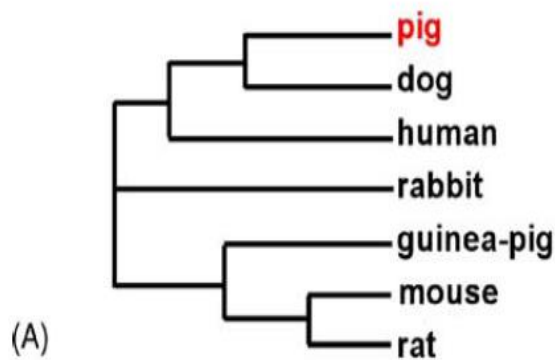
Why Pigs? Sensory Nerve Function Similarities

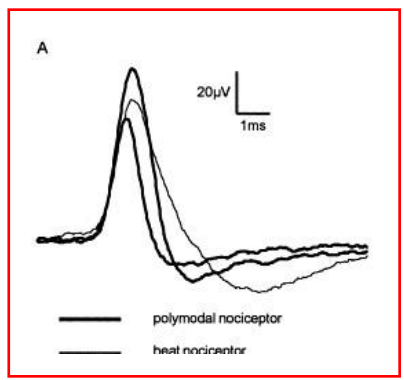
A phylogenic tree of TRPV1:



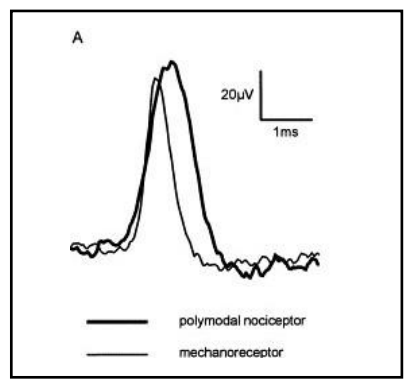
Ohta et al., Biochemical Pharmacology (2005) 173–187

Axonal action potentials recorded from the saphenous nerve:

Pigs/human



Rodents



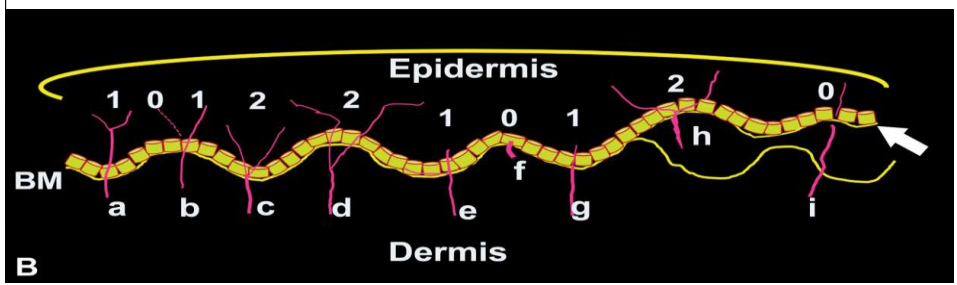
Axon-reflex in skin is mediated by **silent nociceptors like in human**, but unlike in rodents

Lynn et al., Neuroscience Vol. 90, No. 2, pp. 509–518, 1999
Klusch et al., PLOS ONE | https://doi.org/10.1371/journal.pone.0203215 September 27, 2018 1

Why Pigs?

Skin Innervation Similarities

Intraepidermal Nerve Fiber Analysis



Human		Domestic pigs	Minipigs	Rodent
M	F	M	F	M/F
12-14	17-21	12-15	?	30-40

No Broadly Standardized Methods for IENF Analysis in Rodents!

Why Pigs?

Skin Structure & Skin Penetration Properties

Structure: Hair density, healing through re-epithelialization; dermis-epidemis ration. Similarity in >93 skin biomarkers

(Eur J Dermatol. 2013 Jul-Aug;23(4):456-66)

Skin Penetration: Predictability of permeability between pigs and human is 80% (Jung EC and Maibach HI., J. Appl. Toxicol. 2015; 35: 1–10)

Why Pigs? Practicality

1. Large Surface for Topical Treatment
2. Volume of Drug Application is Similar to Human
3. Administration Route (Epidural, IT, Perineural)
4. Device Development (US Ablation)

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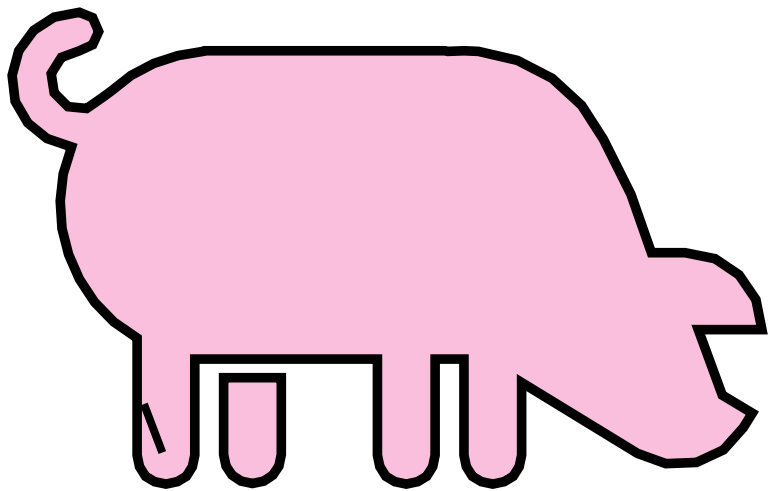
Incisional model in minipigs

A. Procedure

B. Readouts

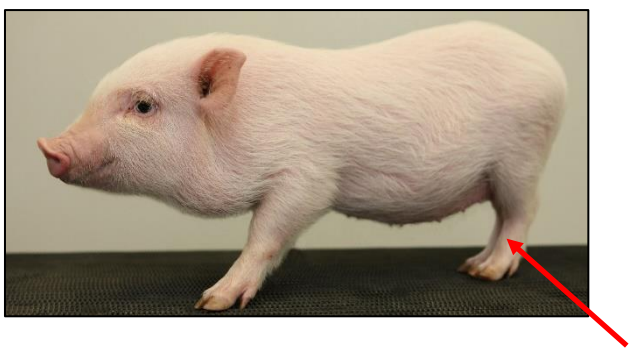
C. Relevant route of administration

D. Representative results

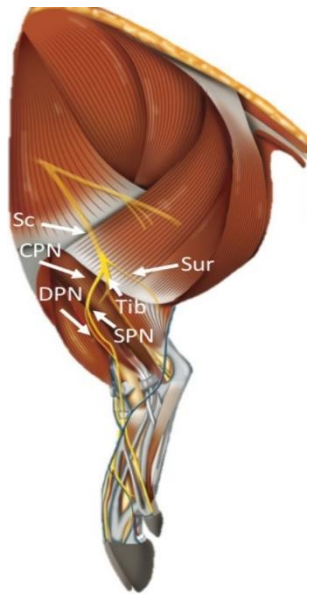


Procedure: Incision (Full Skin and Facia) Location

Gottingen minipig



Sciatic nerve illustration



Location of incision and von Frey testing

Animal Strains

The model was validated in 2 strains:

1. Young Domestic pigs (Danish Landrace × Large White cross-bred)
2. Adult Gottingen minipigs

Readouts

- Response to von Frey
- Behavior score
  (such as protection of injured side)
- Approaching time
- Open field assay
- Electrophysiology

Additional information:

- Pharmacokinetic
- Incision healing
- Safety

Relevant Route of Administration

Perineural US guide injection to the sciatic nerve

Xray guide epidural administration

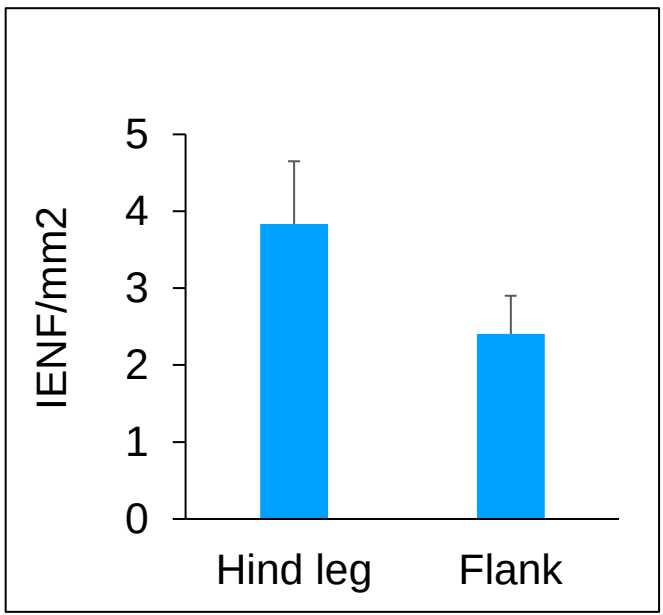
Intrathecal administration (W/WO Xray guide) and CSF collection

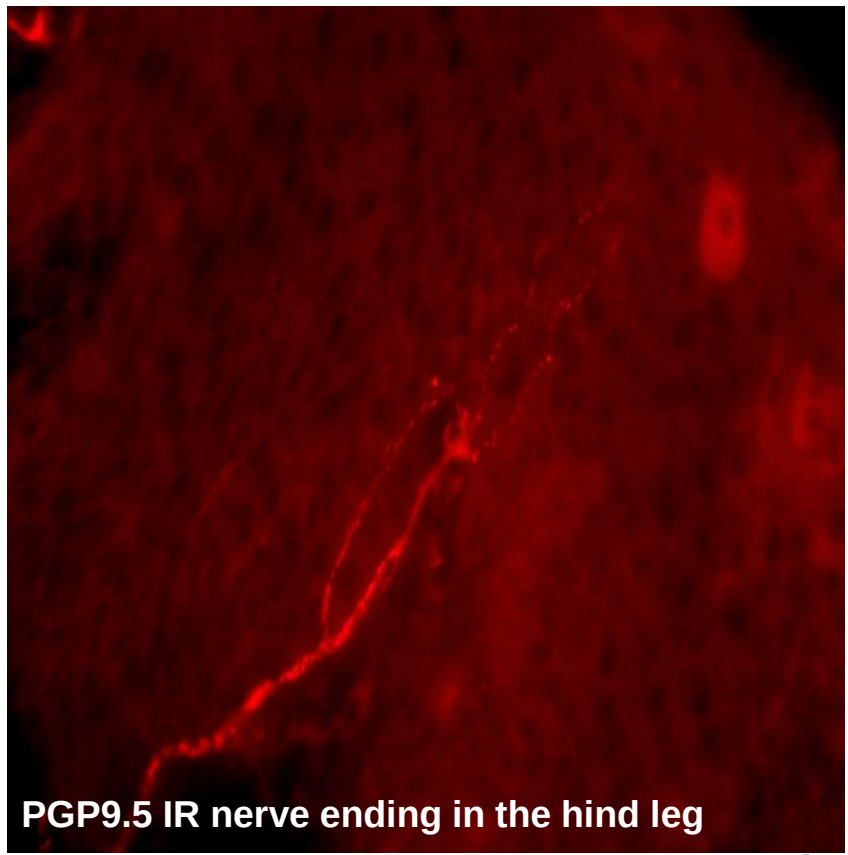
Topicals

Oral administration

Intra Epidermal Nerve Fiber Density (IENF)

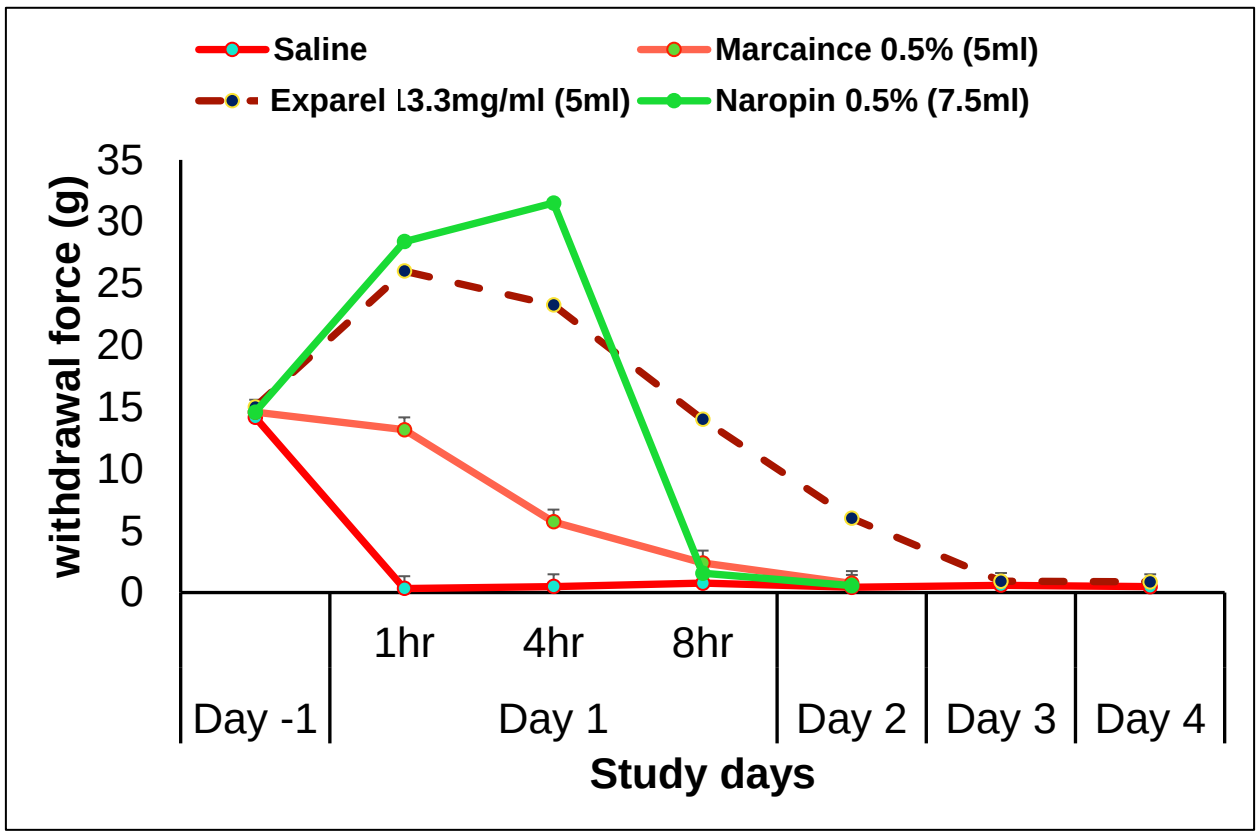
At different body location



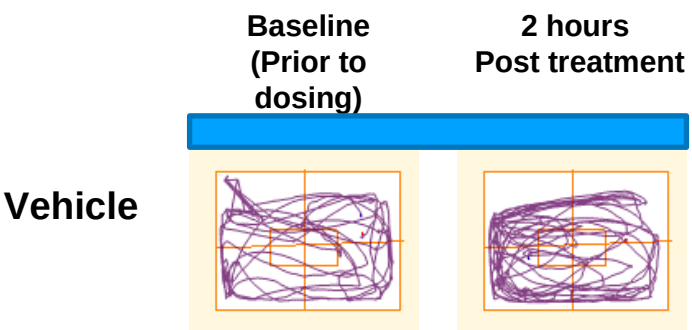

PGP9.5 IR nerve ending in the hind leg

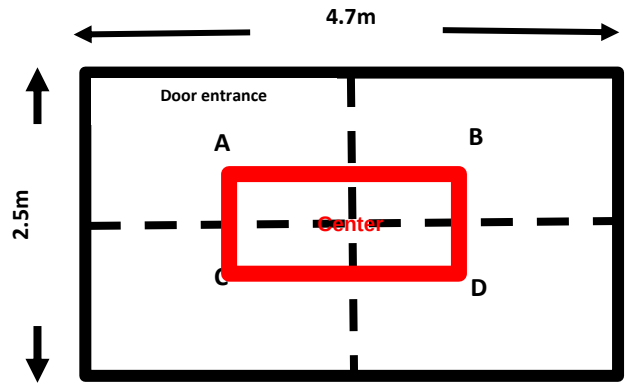
Representative Results

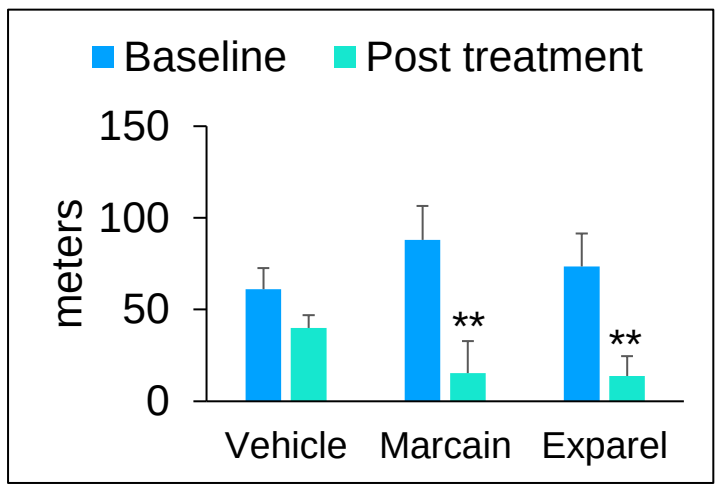
von Frey Assay following perineural injection of local anesthetics (NB)



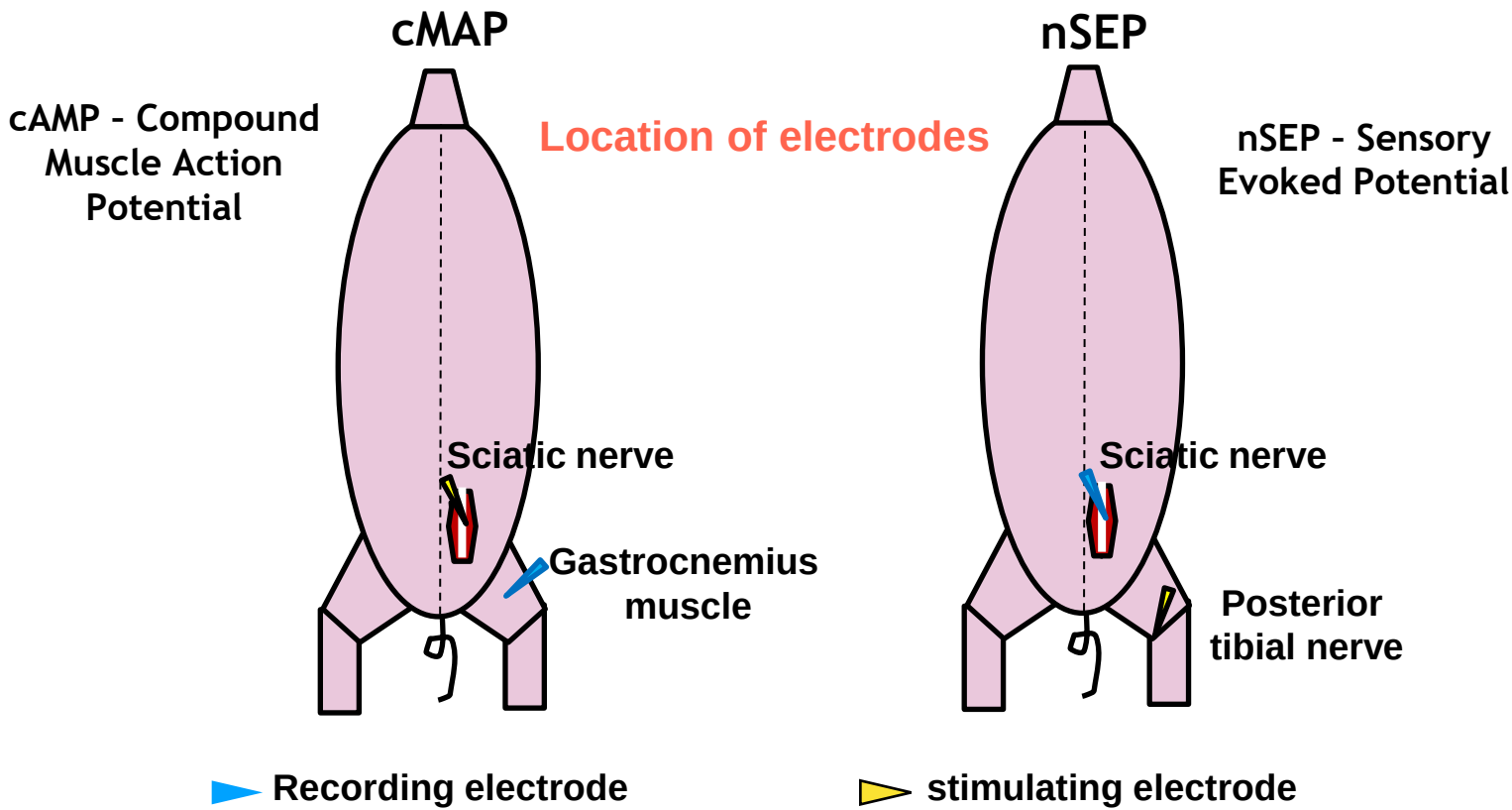
Open Field Assay for Locomotor Activity Post NB Application



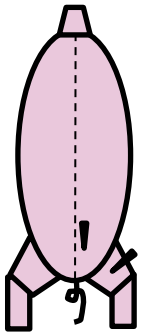




Electrophysiology Approach in Developing New Nerve Blocks

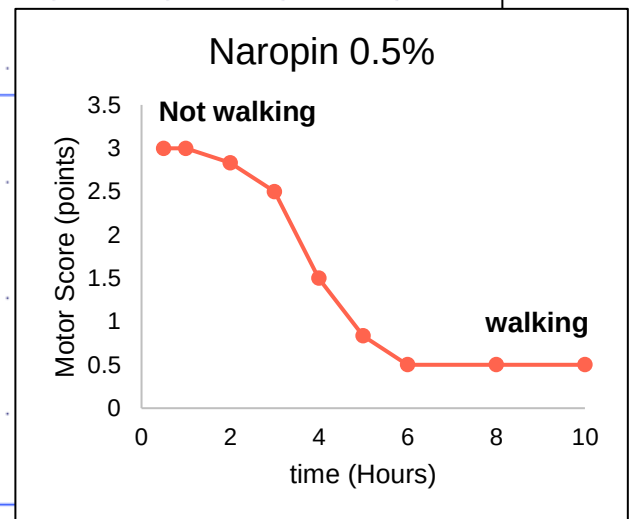


cMAP in Pigs

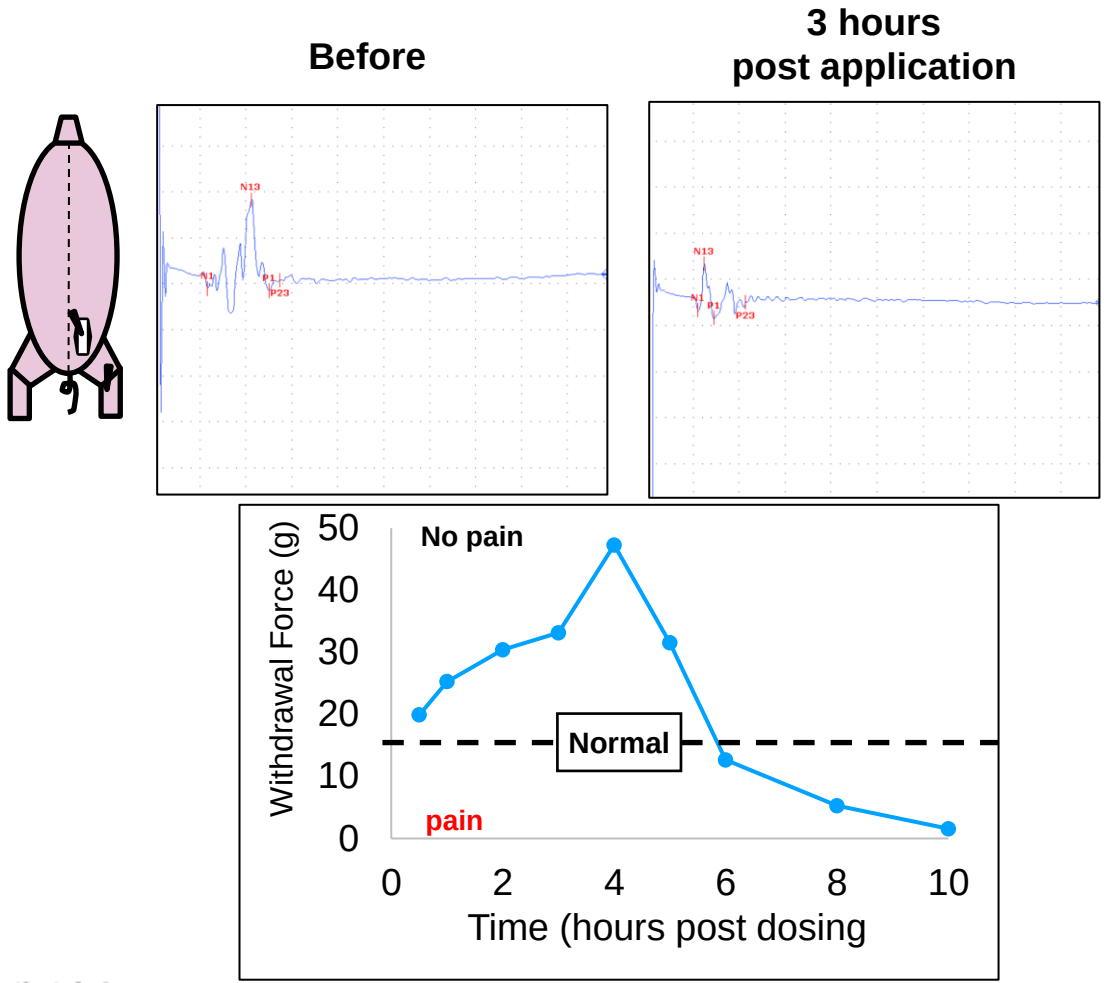


untreated

2 hours post Naropin



Direct SEP from Sciatic Nerve (dnSEP)



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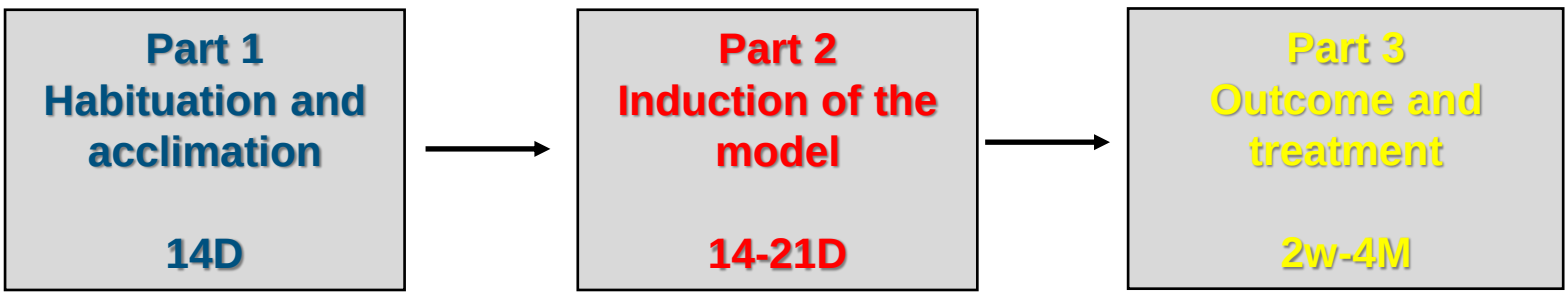
PNT model for neuropathic pain

Model outline

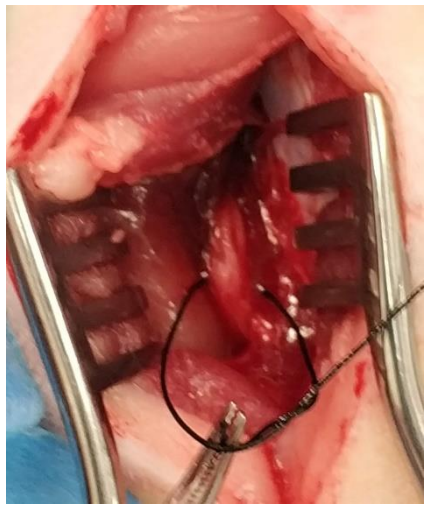


Total study length: 6W ⟶ 6M

Induction of PNT

Sciatic nerve exposure (split 2/3 and a 1/3)

First knot (thread around 2/3 of the sciatic nerve)



Final 3 knot

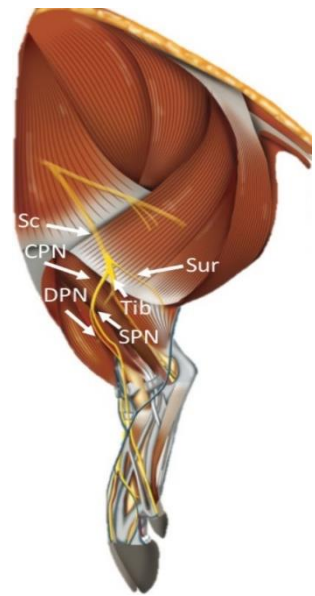
Before returning to home-pen

PNT model for neuropathic pain

Readouts

- Response to von Frey and feather
- Behavior score
- Approaching time ?
- Gait analysis
- Open field assay
- Electrophysiology *(in validation)*
- Biomarker analysis using IHC methods

Sciatic nerve illustration

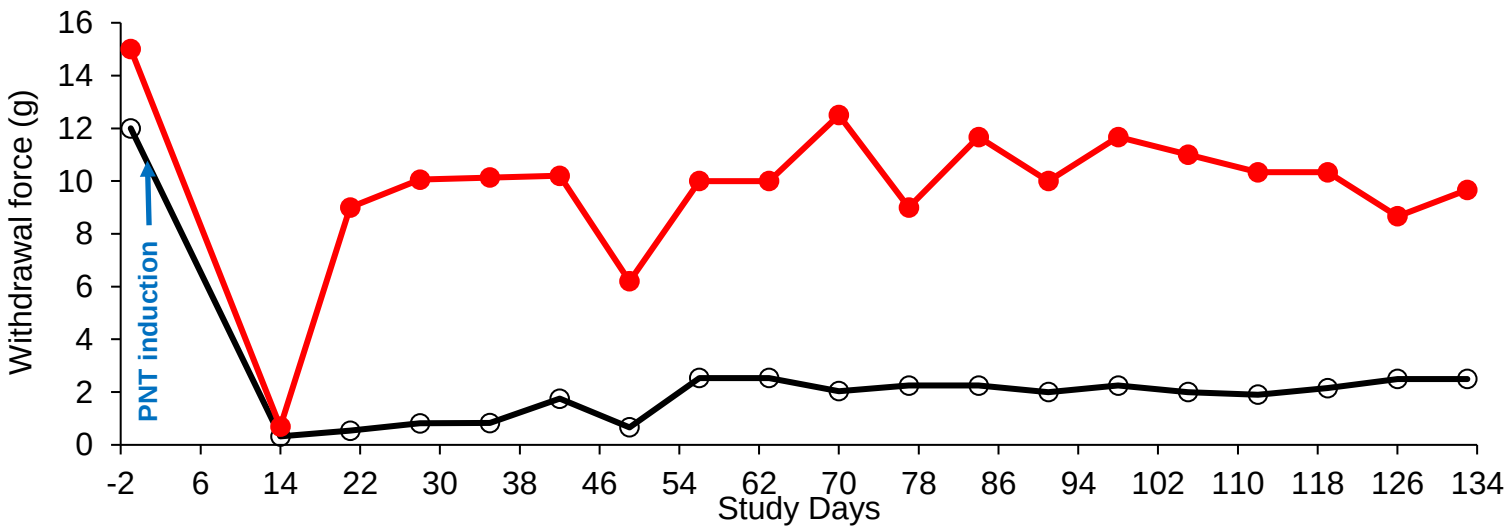


Additional information:

- Pharmacokinetic
- Incision healing
- Safety

PNT model for neuropathic pain

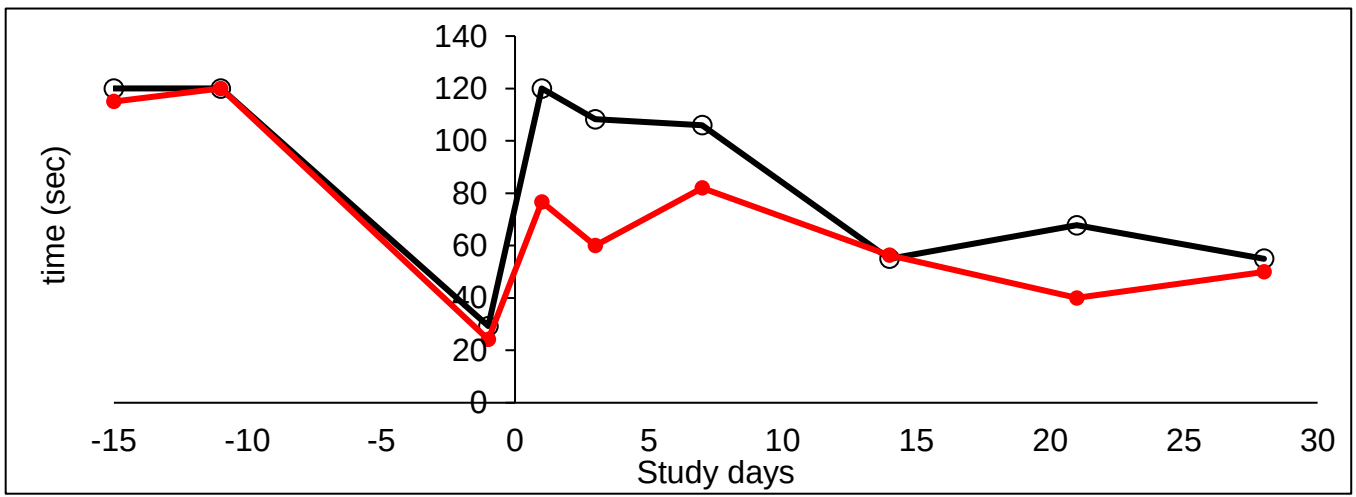
Results: Withdraw response:
von Frey assay



PNT model for neuropathic pain

Results: non withdraw response

Approaching time

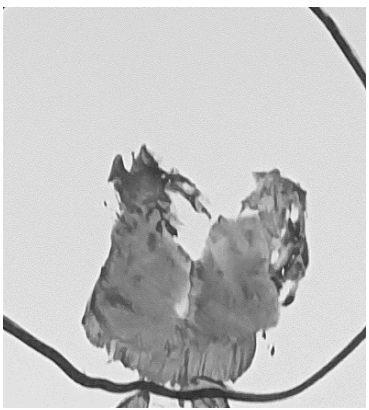


PNT model for neuropathic pain

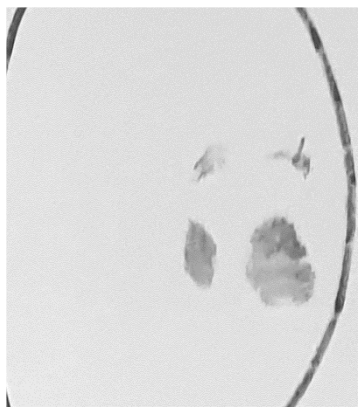
Results: non withdraw response

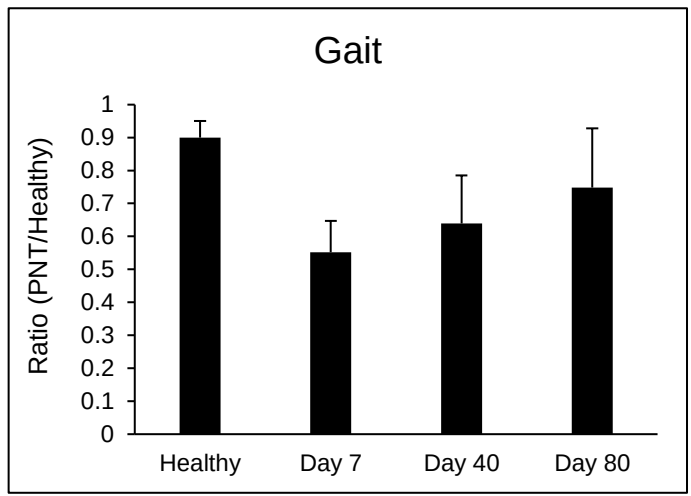
Gate analysis

Healthy



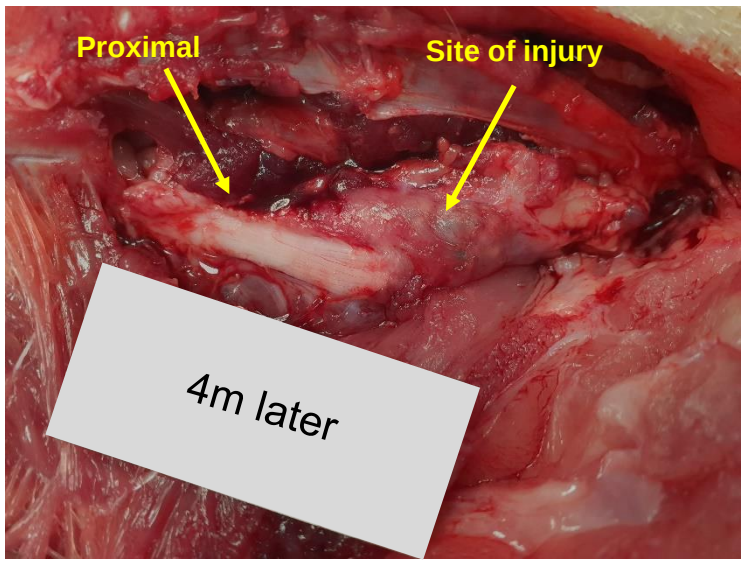
PNT



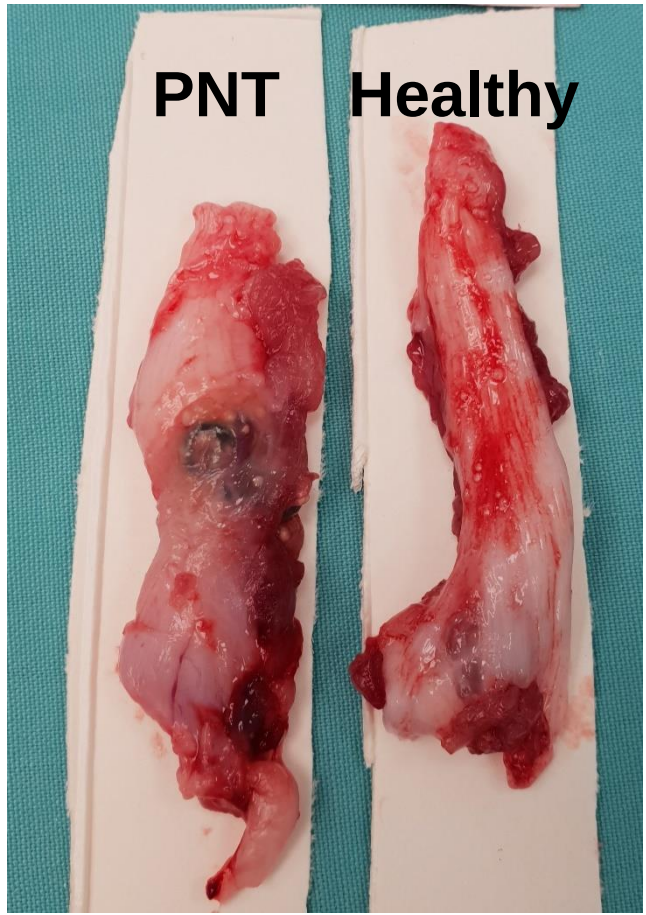


Gait Ratio= OD_{PNT} / $OD_{healthy}$

Gross pathology

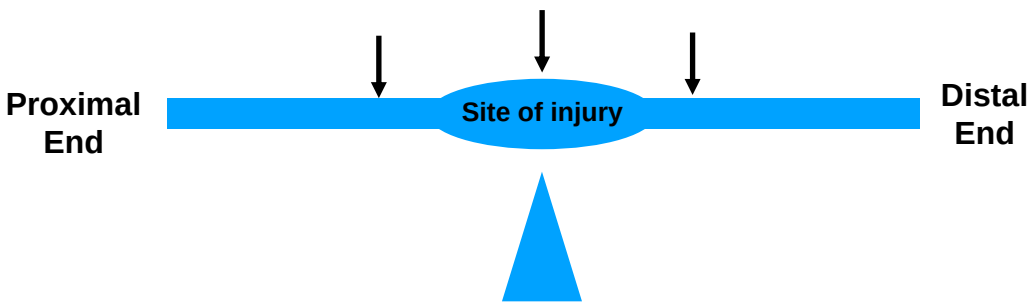


Sciatic nerve
4m post PNT

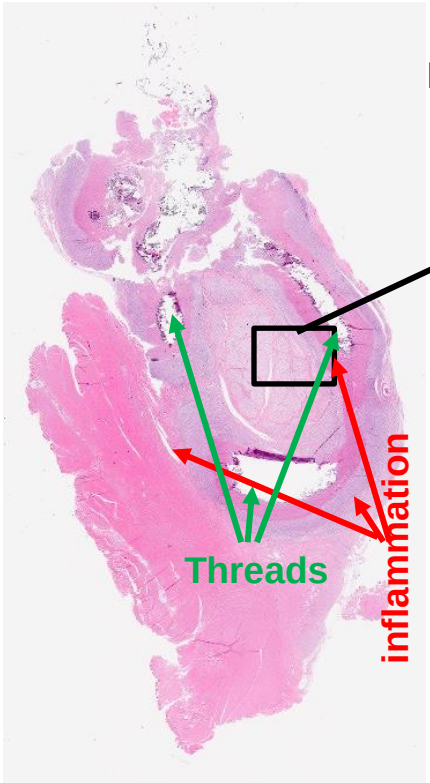


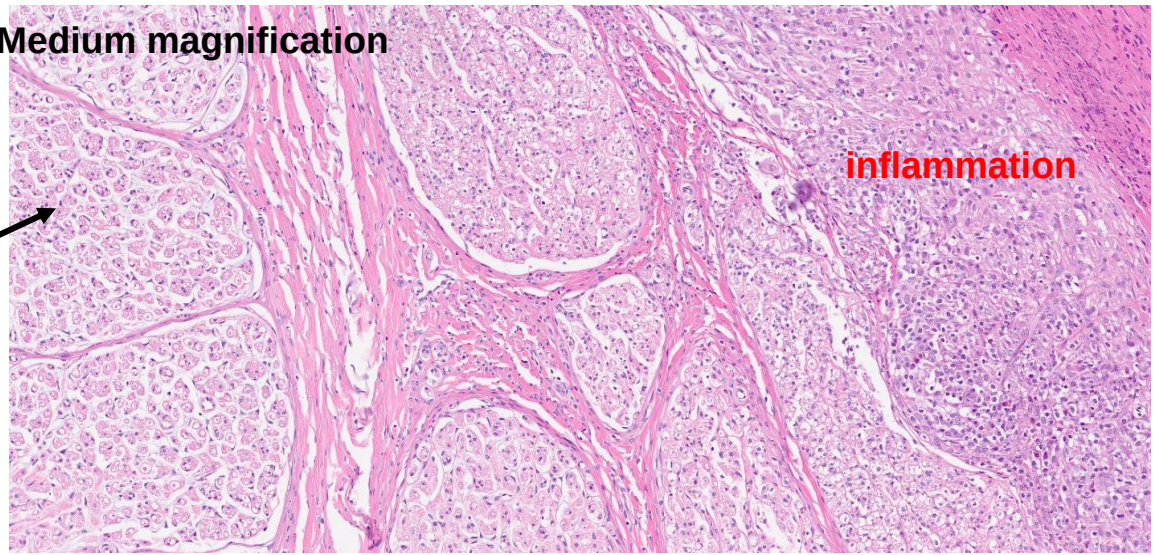
PNT injury, using HE staining:

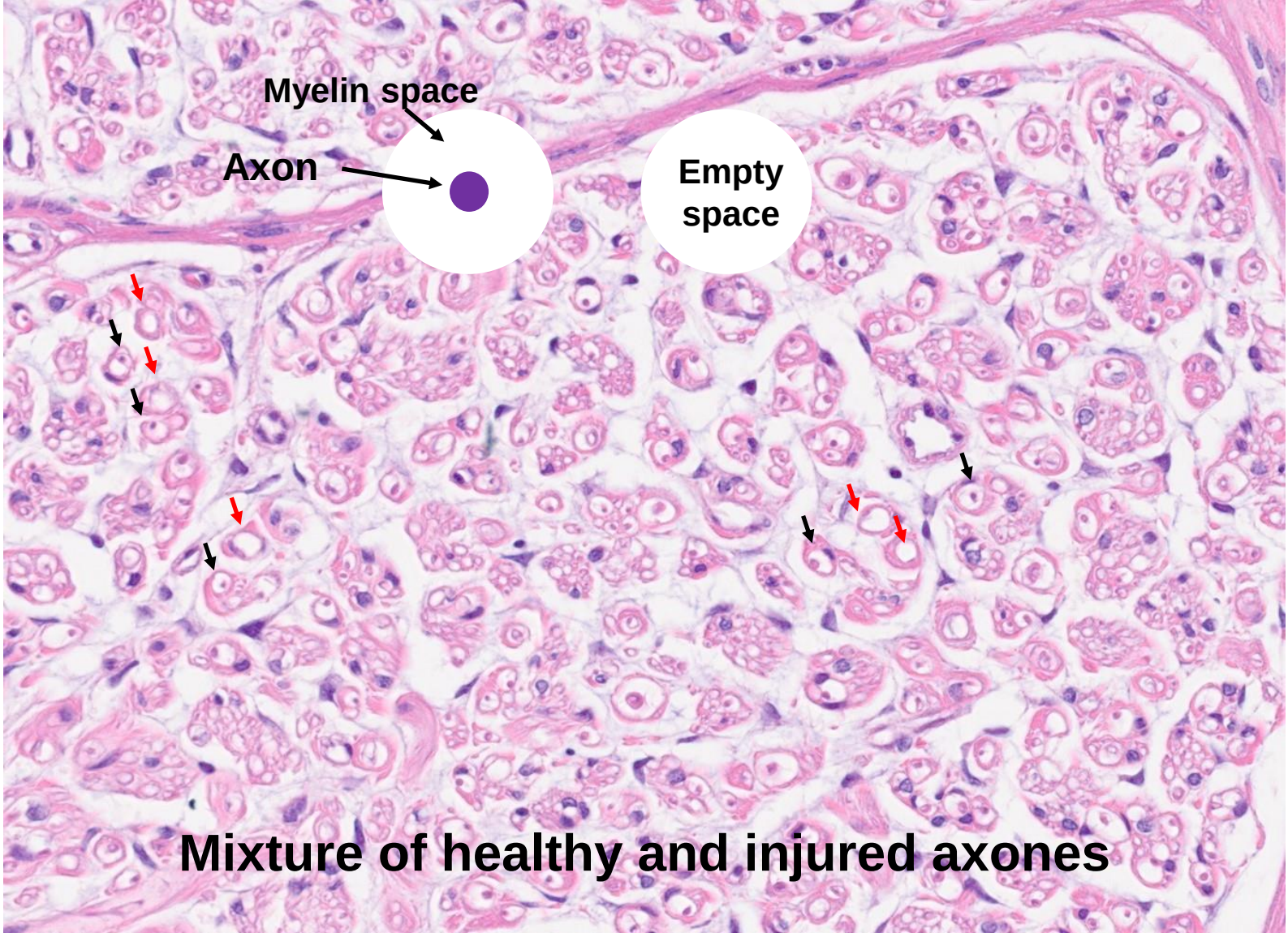
Sectioning diagram



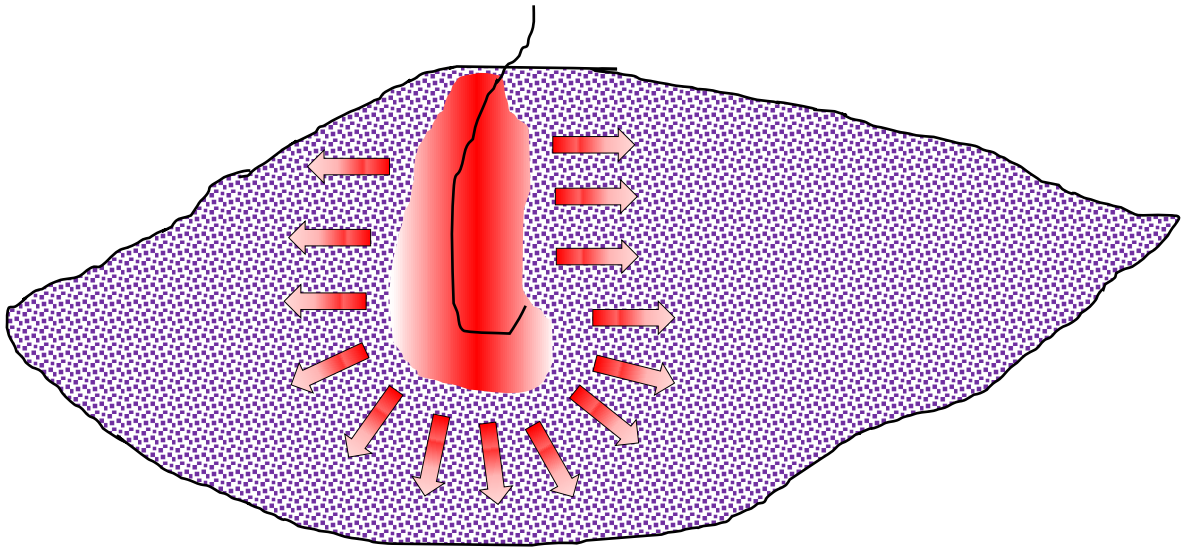
PNT injury, using HE staining:






Myelin space
Axon
Empty space
Mixture of healthy and injured axones

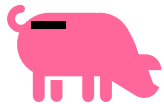
Hypothetic MoA of PNT model: mechanical injury and persist inflammation



Next step:
Further characterization of the inflammation and MoA

Summary of Pain Models in Gottingen minipigs

The PNT Model



- Allows the follow up of more than 3m, the critical length for chronic pain diagnosis in human
- A sensitive model
- Reproducible in adult Gottingen minipigs
- Conpmlex mechanism if action
- Assessment of PK/PD/Tox

Limitations:

- Relatively new- no data is available on biomarkers
- Not yet published

The incision Model

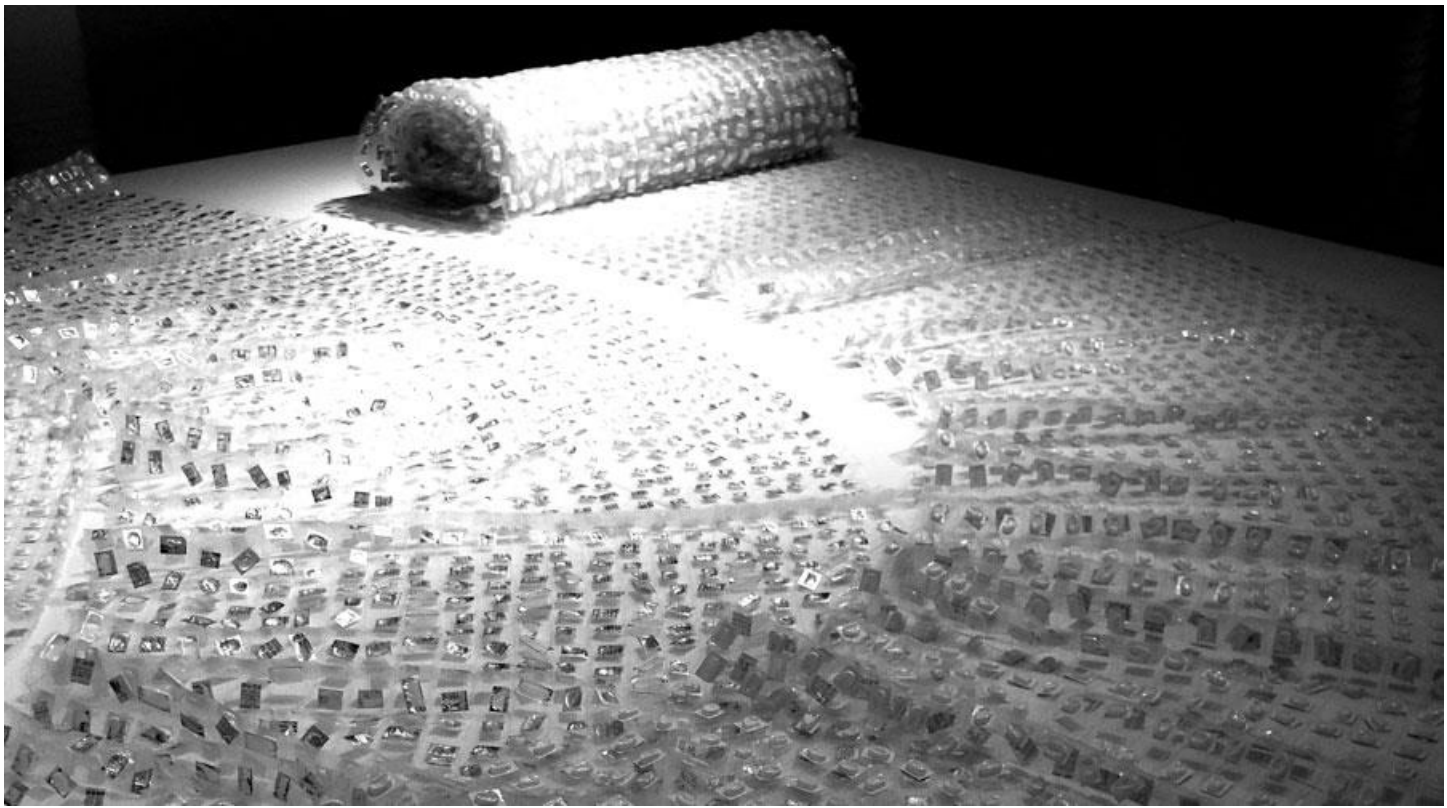
- Increasing use in the last 5 years
- Allows the assessments of new nerve block
- A sensitive model
- Reproducible in adult Gottingen minipigs
- Assessment of PK/PD/Tox

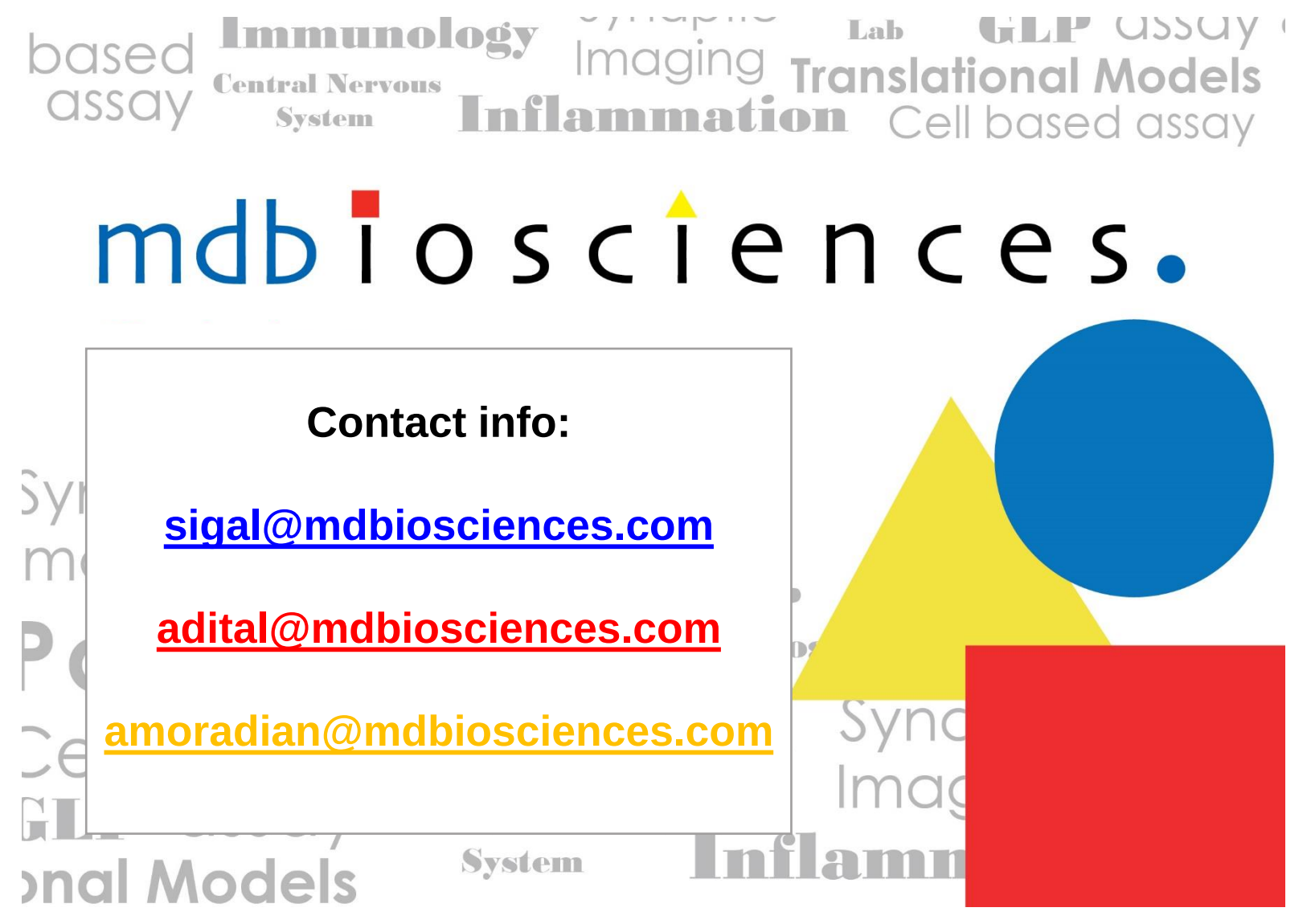
Limitations:

- Not yet published

White Pain by Susie Freeman 2007

Made of pill packaging that remains after one man's lifetime of taking painkillers




based assay
Immunology
Central Nervous System
Imaging
Inflammation
Lab
GLP assay
Translational Models
Cell based assay
mdbiosciences.
Contact info:
sigal@mdbiosciences.com
adital@mdbiosciences.com
amoradian@mdbiosciences.com
System
Inflamm
onal Models