Pain Research: Translational Scientists New Best Friends, and What They Tell Us.

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Comparative Pain Research and Education Center,

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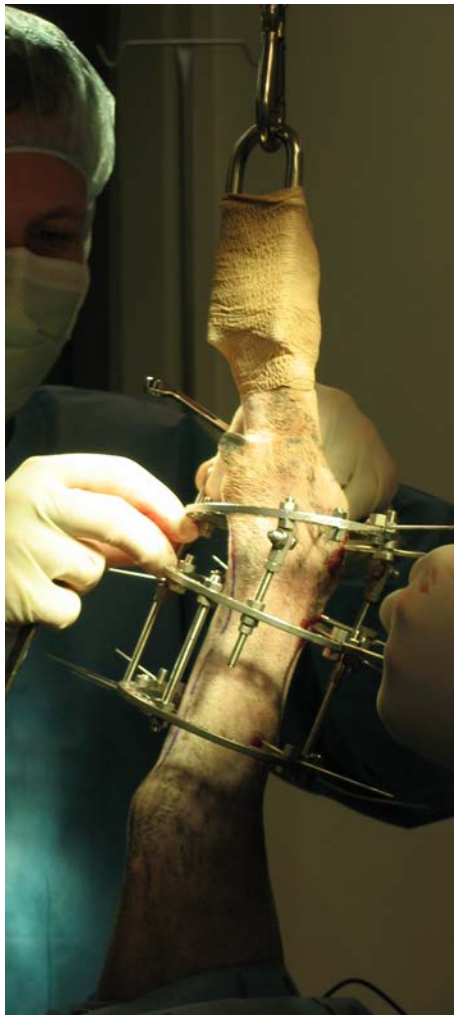

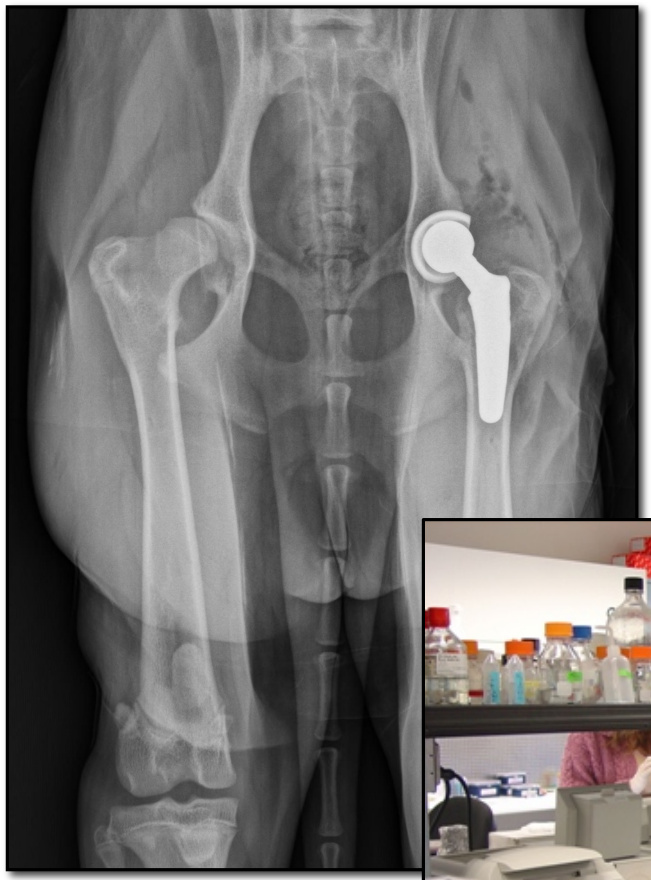

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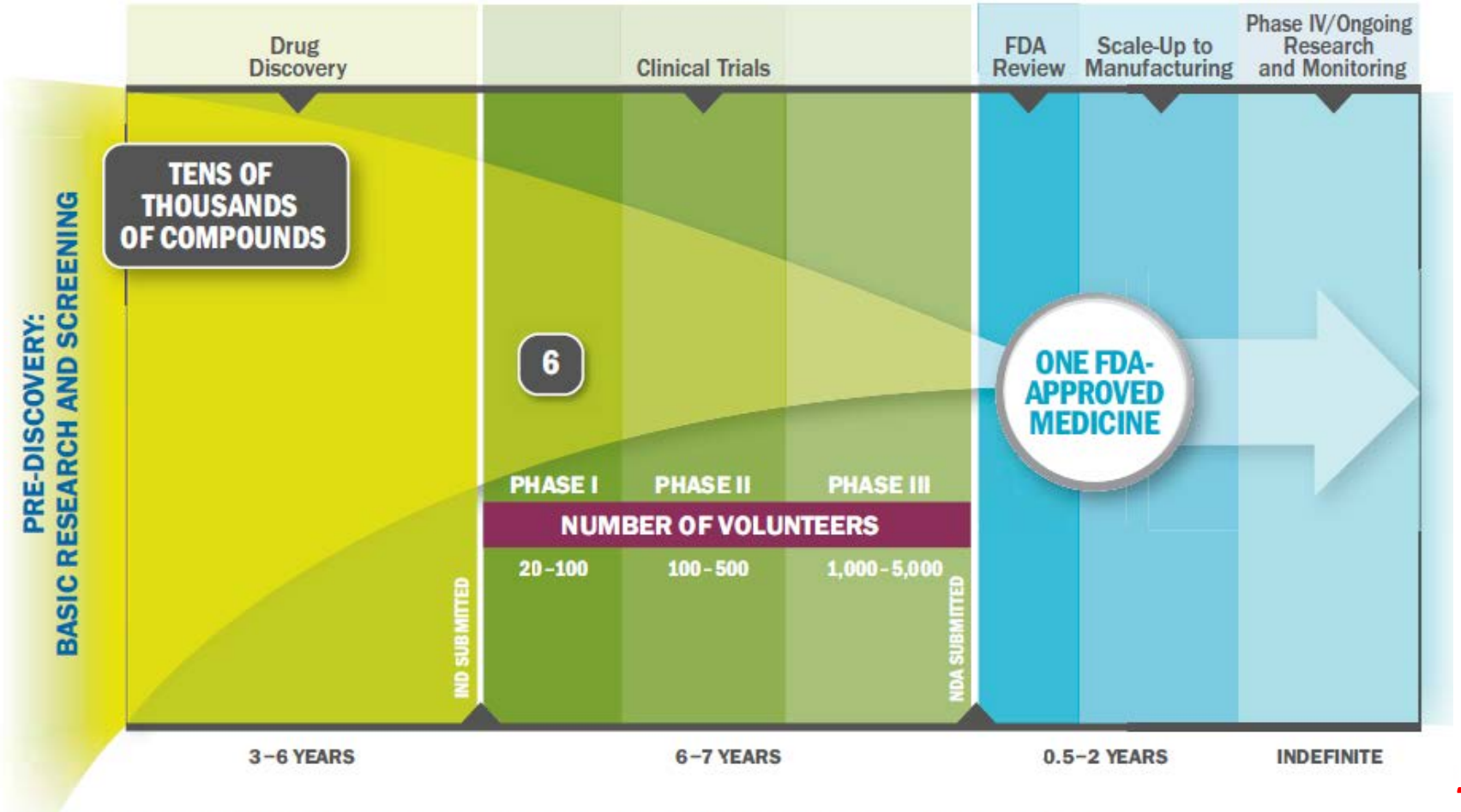
Translational pain research is not producing new analgesics

Where does translational pain research 'go wrong'?



SOURCE: Pharmaceutical Research and Manufacturers of America. "Drug Discovery and Development: Understanding the R&D Process." Washington, DC: PhRMA, 2014.

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Published in final edited form as:
Sci Transl Med. 2014 August 13; 6(249): 249sr3. doi:10.1126/scitranslmed.3008320.

Lost but making progress—Where will new analgesic drugs come from?

David Borsook[1], **Richard Hargreaves**[1], **Chas Bountra**[2], and **Frank Porreca**[3,*]

SPECIAL ARTICLE

Preclinical Pain Research

Can We Do Better?

J. David Clark, M.D., Ph.D.

Animal models of pain: progress and challenges

Jeffrey S. Mogil

Pain 135 (2008) 7–10

PAIN

www.elsevier.com/locate/pain

Topical review

Clinical and pre-clinical pain assessment: Are we measuring the same thing?

C.J. Vierck [a,*], P.T. Hansson [c], R.P. Yezierski [b]

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Multiple mechanisms have been tested in pain — how can we improve the chances of success?

Ann G Hayes[1], Lars Arendt-Nielsen[2] and Simon Tate[3]

Topical review

PAIN

Sensory profiling in animal models of neuropathic pain: a call for back-translation

Andrew S.C. Rice[a,*], Nanna B. Finnerup[b,c], Harriet I. Kemp[a], Gillian L. Currie[d], Ralf Baron[e]

BJP British Journal of Pharmacology

DOI:10.1111/bph.12645
www.brjpharmacol.org

REVIEW

Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain

N Percie du Sert[1] and A S C Rice[2,3]

Correspondence
Nathalie Percie du Sert, National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK. E-mail: nathalie.percie.dusert@nc3rs.org.uk

Commissioning Editor
Tom Blackburn

Keywords
animal model; translation; neuropathic pain; analgesic; preclinical model

Received

Where does preclinical translational pain research ‘go wrong’?

Where could preclinical translational pain research benefit?

Enhancements to:

- Models (the induced pain state)
- Outcome Measures used in the models
- Relevance of the Target

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Models (the induced pain state)

"One can ask whether peripheral nerve injury (e.g. nerve ligation), the acetic acid writhing test, the orofacial injection of formalin, or the intra-articular injection of Freud's complete adjuvant (FCA) actually mirror any clinical conditions?"

Lascelles & Flecknell 2010. IASP Pain Clinical Updates

Models

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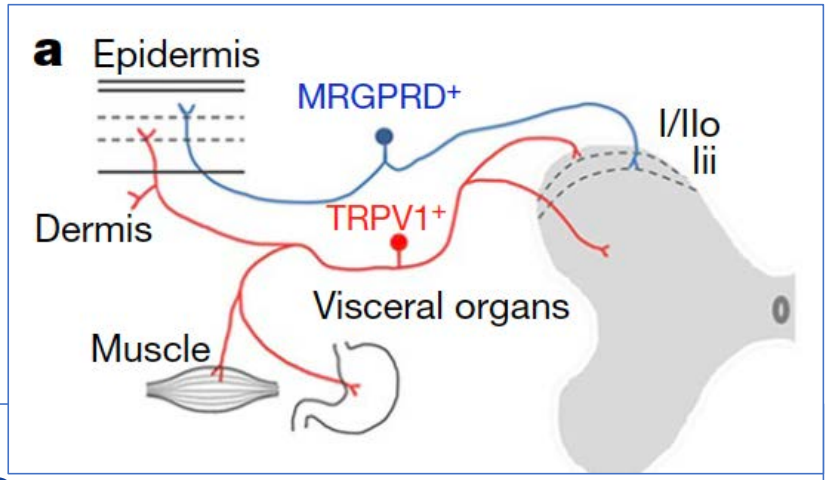
Outcome measures

https://www.youtube.com/watch?v=jWqC-5_zXHc



LETTER

https://doi.org/10.1038/s41586-018-0793-8

Identifying the pathways required for coping behaviours associated with sustained pain

Tianwen Huang[1,2,8], Shing-Hong Lin[1,2,8], Nathalie M. Malewicz[3], Yan Zhang[1,4,5], Ying Zhang[1,6], Martyn Goulding[7], Robert H. LaMotte[3] & Qiufu Ma[1,2]*

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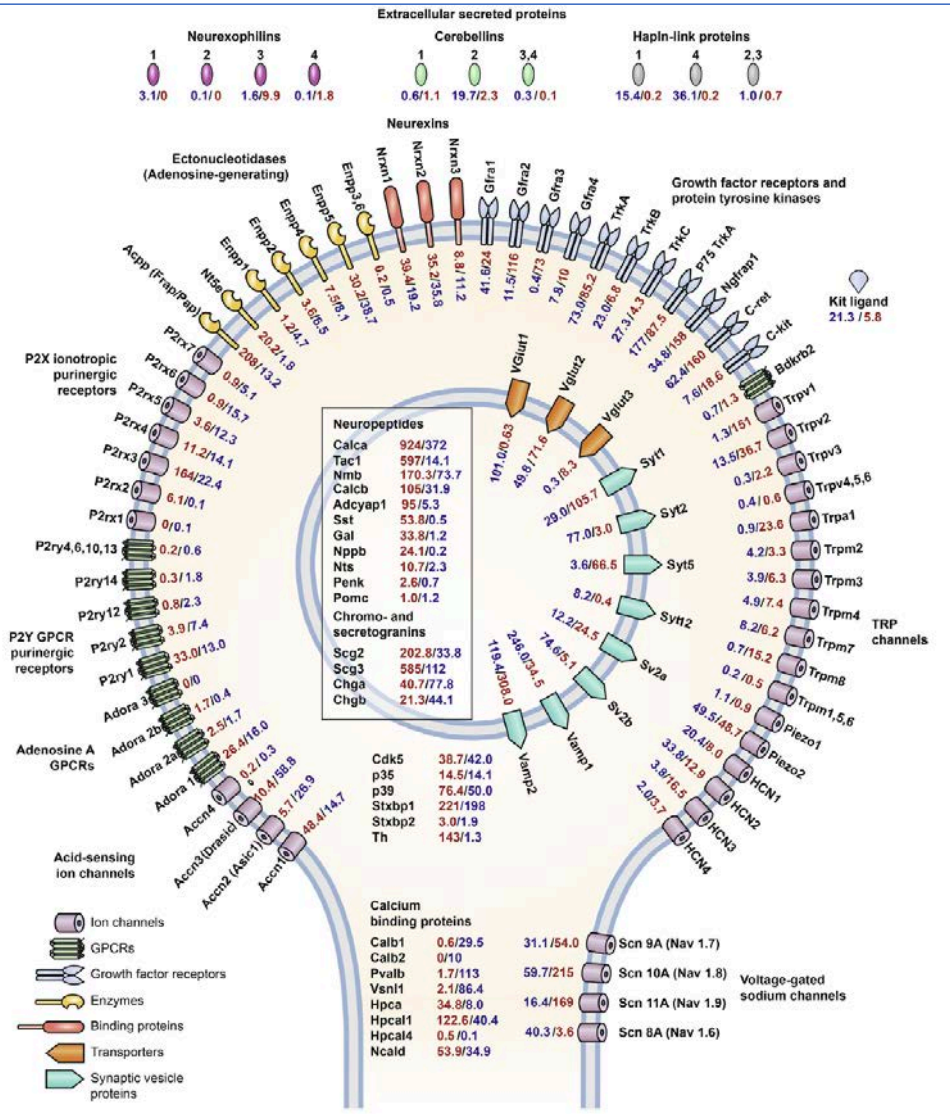
Relevance of target:

Does it play a role in the target disease?
How important is that role?

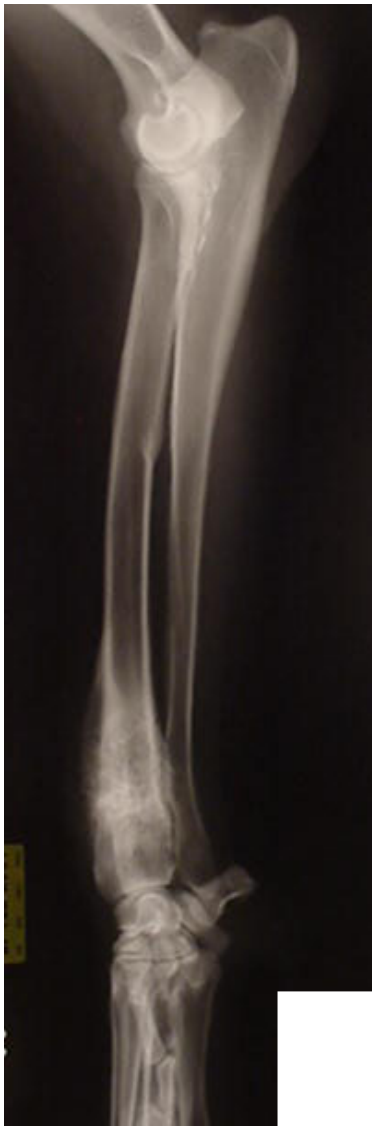


Goswami et al. 2014

American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 15, No 12 (December), 2014: pp 1338-1359
Available online at www.jpain.org and www.sciencedirect.com

Molecular Signatures of Mouse TRPV1-Lineage Neurons Revealed by RNA-Seq Transcriptome Analysis



A proposition:

Companion animals with naturally-occurring painful disease may enhance the translational pain research paradigm

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These naturally occurring painful disease ‘models’ may better reflect the complex genetic, environmental, temporal and physiological influences present in humans.

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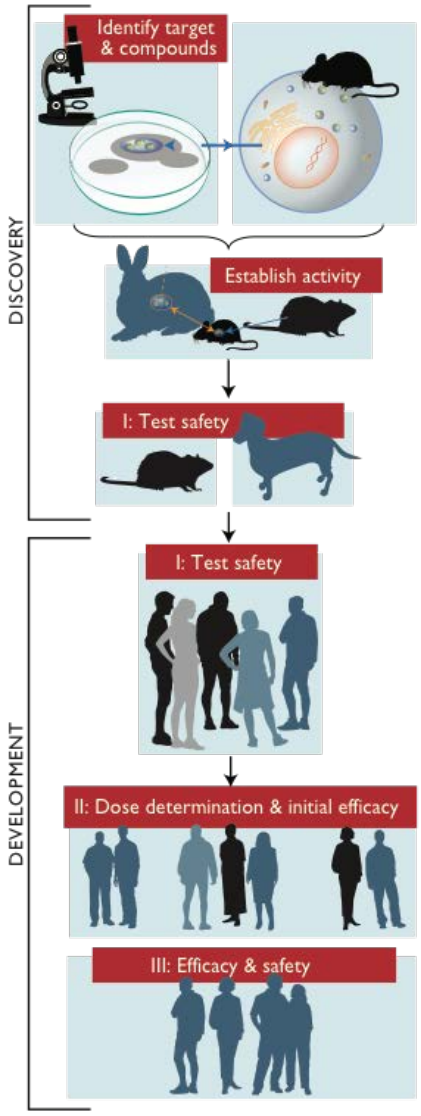

Verification Bridge: Inform the critical 'Go / No-Go' decision making point

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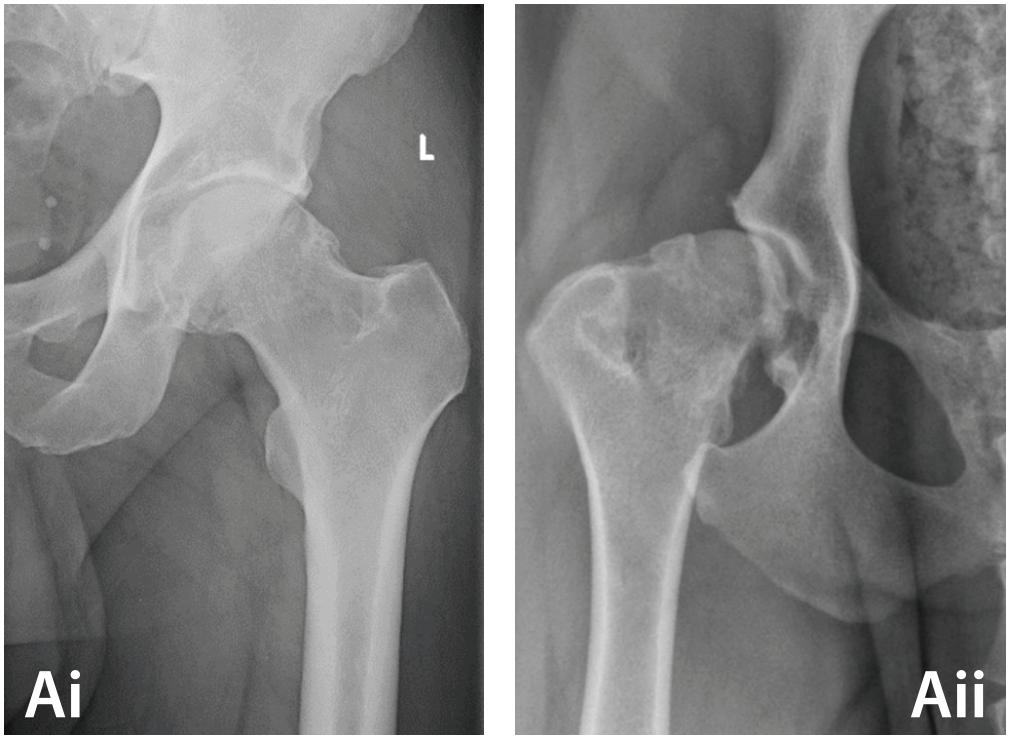
Model fidelity: Osteoarthritis

- Biomechanically, structurally, histologically, genomically, and molecularly human, canine, feline and equine OA are similar.



Clements et al. Arthritis Res Ther. 2006; **8:** R158.
Little & Hunter. Nat Rev Rheumatol. 2013; **9:**485-497
McCoy. Vet Pathol. 2015; **52:**803-818.

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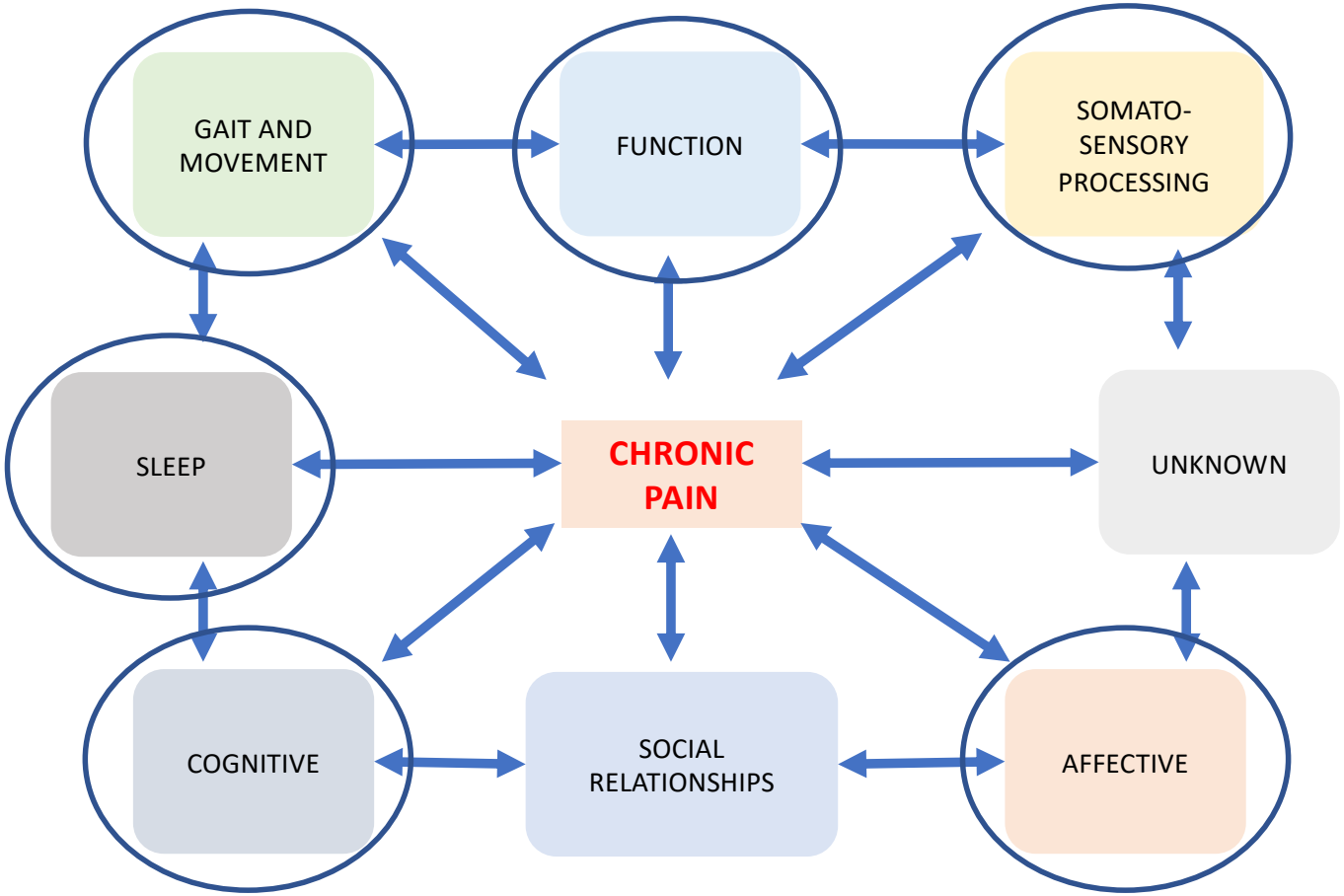
Outcome Measures:
Measurable dimensions impacted by pain in humans



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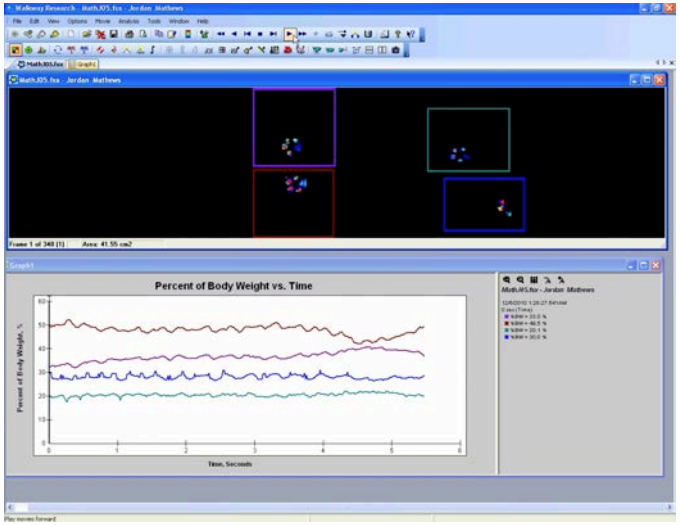
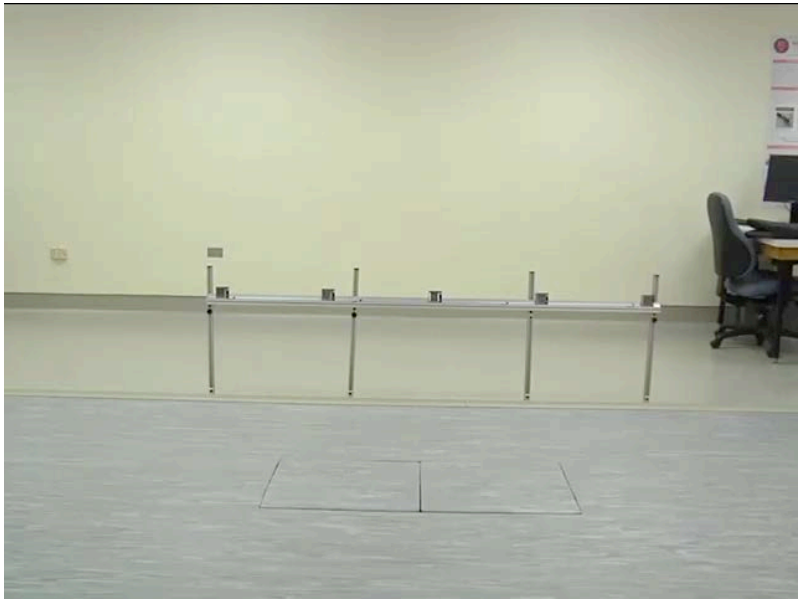
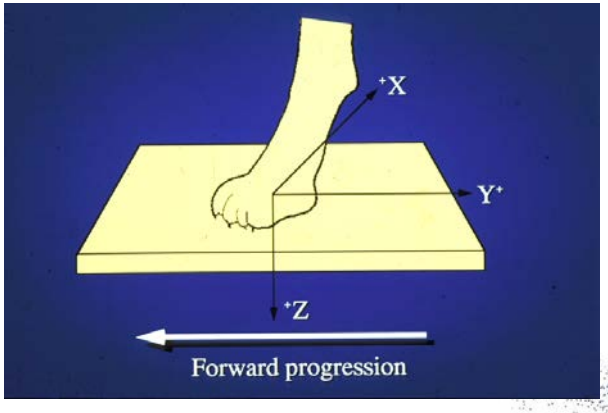
Gait (joint pain, via limb use)







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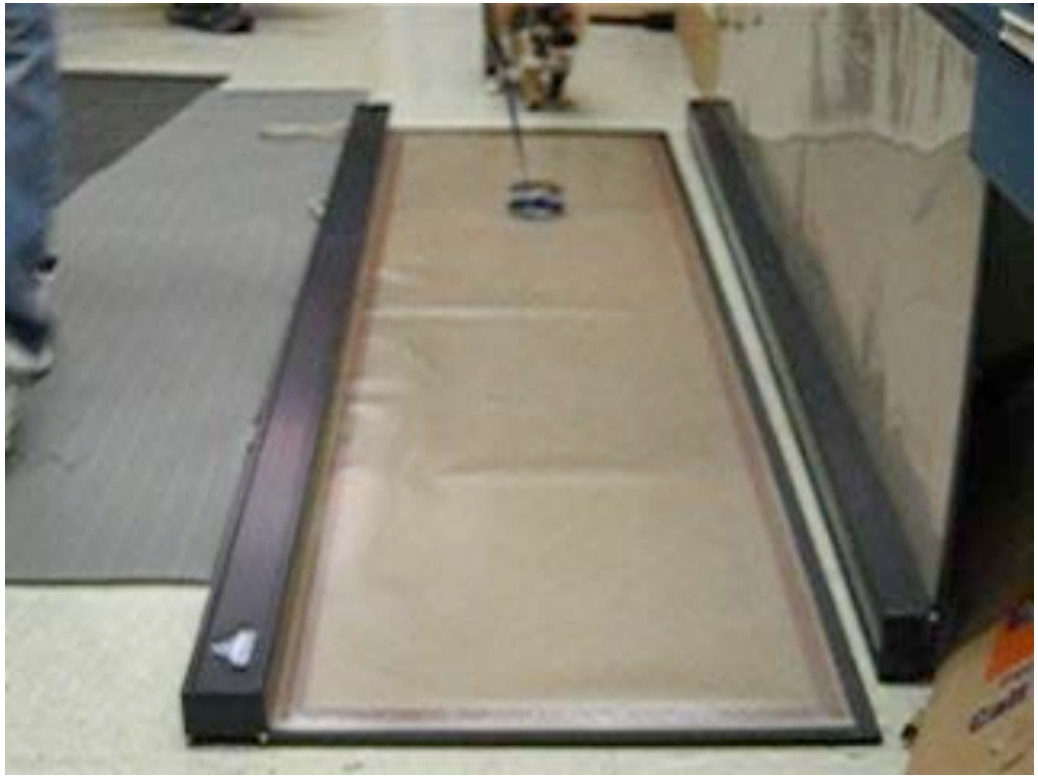
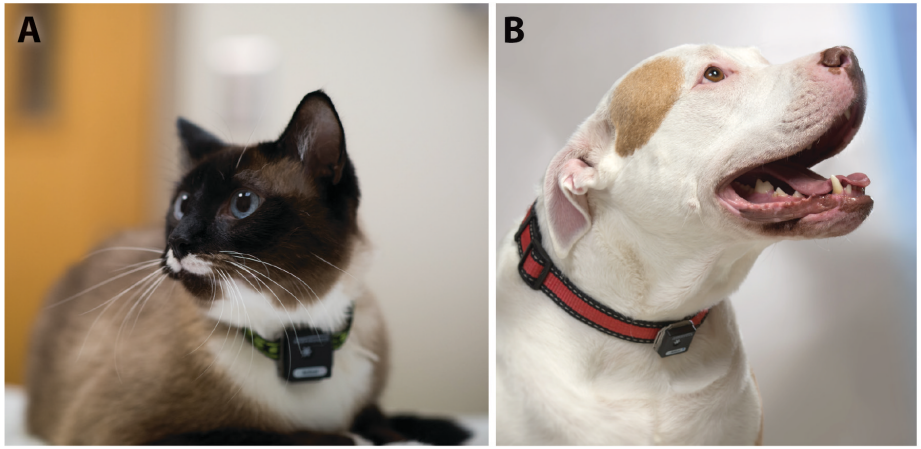
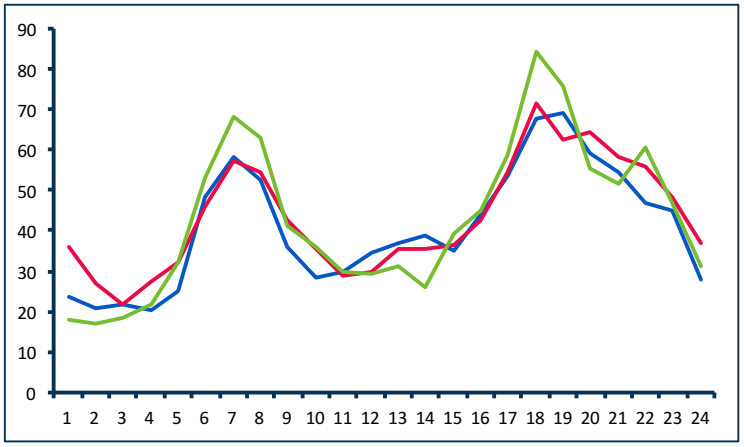


Activity



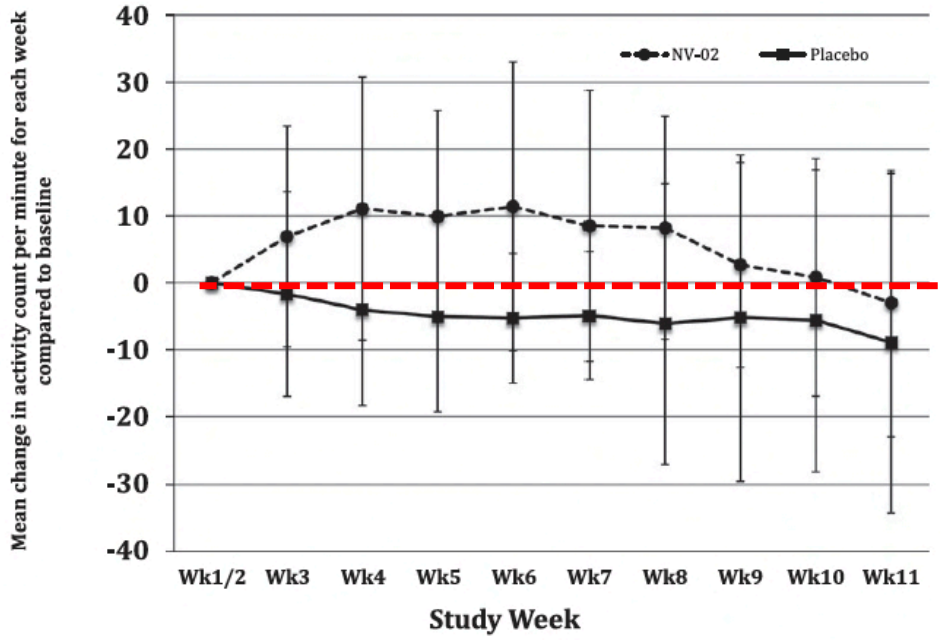


Anti-NGF mAb for OA Pain

Journal of Veterinary Internal Medicine
ACVIM
Open Access
J Vet Intern Med 2016

A Feline-Specific Anti-Nerve Growth Factor Antibody Improves Mobility in Cats with Degenerative Joint Disease–Associated Pain: A Pilot Proof of Concept Study

M.E. Gruen, A.E. Thomson, E.H. Griffith, H. Paradise, D.P. Gearing, and B.D.X. Lascelles



Activity

Improved sleep with NSAID in OA-pain states

www.nature.com/scientificreports

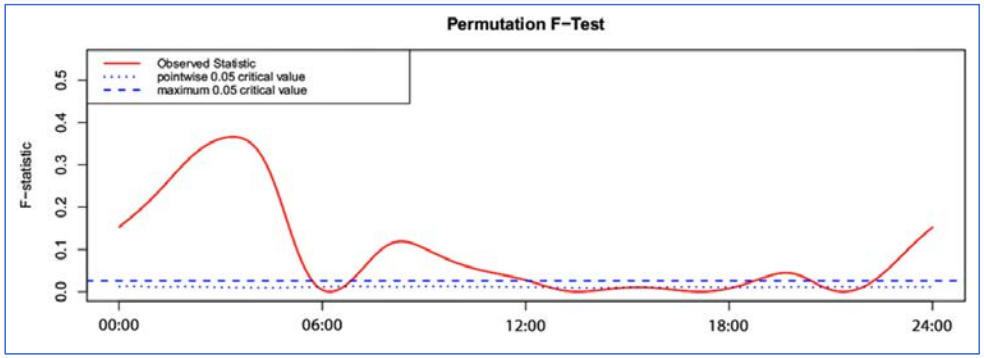
SCIENTIFIC REPORTS
nature research

OPEN

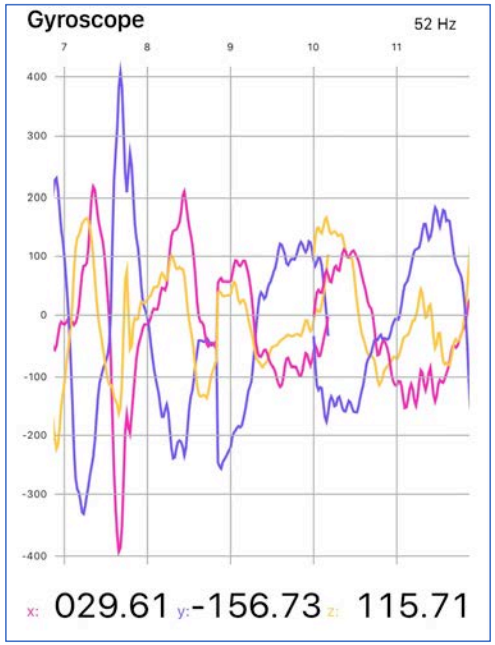
Functional linear modeling of activity data shows analgesic-mediated improved sleep in dogs with spontaneous osteoarthritis pain

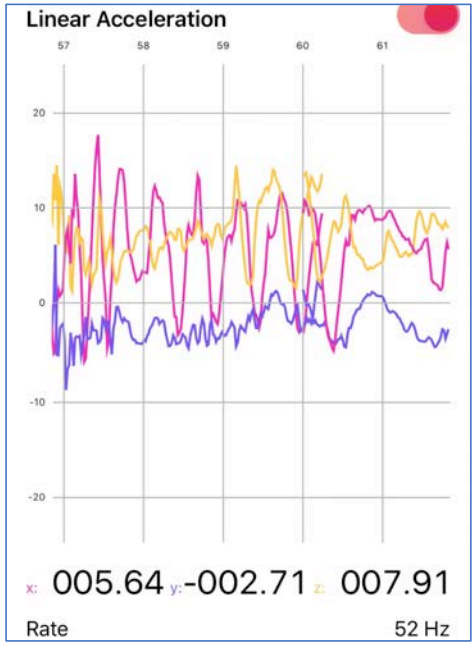
Received: 24 June 2019
Accepted: 12 September 2019
Published online: 02 October 2019

M. E. Gruen[1,2], D. R. Samson[3] & B. D. X. Lascelles[1,2,4,5,6]



Measuring smoothness of motion





Function

Fill in the oval next to the one number that describes how during the past 7 days **pain has interfered** with your dog's:

7. Ability to Rise to Standing From Lying Down

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Does not Interfere — Completely Interferes

Caine Brief Pain Inventory (CBPI)

8. Ability to Walk

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Does not Interfere — Completely Interferes

9. Ability to Run

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Does not Interfere — Completely Interferes

12. How disabled is your dog by his/her lameness?

Not at all disabled	☐	Severely disabled	☐
Slightly disabled	☐	Extremely disabled	☐
Moderately disabled	☐		

Liverpool Osteoarthritis in Dogs (LOAD) index

13. How active is your dog?

Extremely active	☐	Slightly active	☐
Very active	☐	Not at all active	☐
Moderately active	☐		

14. What is the effect of cold, damp weather on your dog's lameness?

No effect	☐	Severe effect	☐
Mild effect	☐	Extreme effect	☐
Moderate effect	☐		

Sensory Function: QST Integrity of CPM

NSR 2020 10; 1654

SCIENTIFIC REPORTS
nature research

OPEN

The effect of spontaneous osteoarthritis on conditioned pain modulation in the canine model

King Wa Chiu[1], Jon Hash[1], Rachel Meyers[1] & B. Duncan X. Lascelles[1,2,3,4*]

Research Paper

PAIN®

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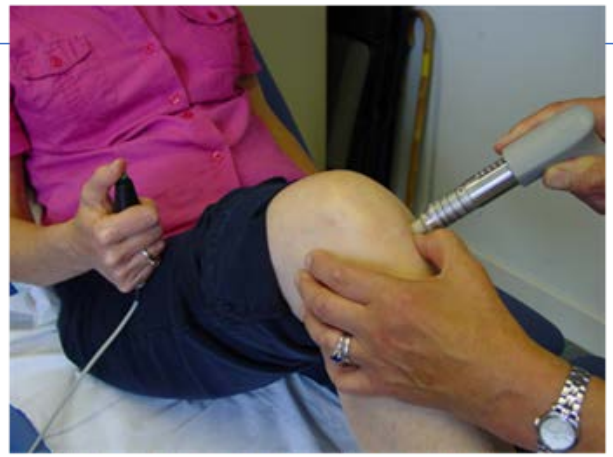
Widespread somatosensory sensitivity in naturally occurring canine model of osteoarthritis

David Knazovicky[a], Erika S. Helgeson[b], Beth Case[a], Margaret E. Gruen[a,c], William Maixner[d], B. Duncan X. Lascelles[a,c,d,*]



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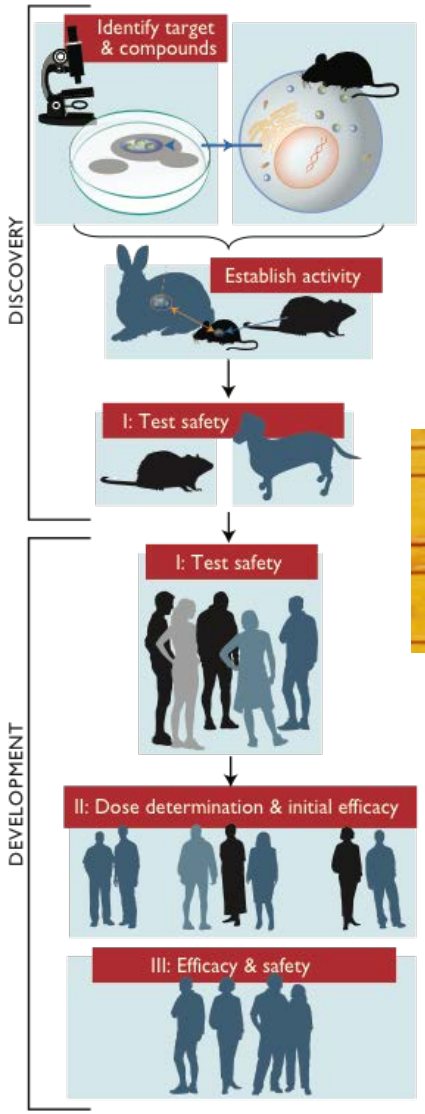
Proof of Concept (POC) Studies



Verification Bridge: Informing the critical 'Go / No-Go' decision making point

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SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

OSTEOARTHRITIS

A randomized clinical efficacy study targeting mPGES1 or EP4 in dogs with spontaneous osteoarthritis

Carol Robertson-Plouch[1]*, John R. Stille[1†], Peng Liu[1‡], Claire Smith[2§], Dorothy Brown[3,4], Margaret Warner[1], Leijun Hu[1], Matthew J. Fisher[1]

Predictability of POC studies in companion animals

Drug	Efficacy in Rodents	Efficacy in Dogs	Efficacy in Humans	References
NSAIDs	Yes	Yes	Yes	Brown, JVIM, 2013 Bannuru, Ann Intern Med 2015
Anti-NGF mAbs	Yes	Yes	Yes	Lascelles, BMC Vet Res, 2015 Lane, NEJM, 2010 Gruen, JVIM, 2016
TRPV1 antagonist	Yes	No	No	Malek, BMC Vet Res, 2012 Miller, Comtemp Clin Trials, 2014
Resiniferatoxin	Yes	Yes	Yes	Brown, Pain, 2015 Brown, Pharmaceuticals, 2016
NK1 antagonist	Yes	No	No	Ma, Curr. Opin. Invest. Drugs, 1999 Dionne, Curr. Opin. Invest. Drugs 1999
Substance P-saporin	Yes	(Yes)	(ongoing)	Brown, Anesthesiology, 2013
EP4 receptor antagonist	Yes	Yes	(ongoing)	Rausch-Derra, JVIM, 2016;
Capsaicin (IA)		Yes	Yes	Stevens, Arthritis Rheumatol, 2019; Lascelles, IASP, 2020

The practicalities of running POC studies in companion animals

- Sites and expertise available, allowing veterinary oversight
- Veterinary CRO assistance available
  - GCP; Protocol; Agreements;
- Recruitment (owners; appropriate phenotype)
- Do need sufficient toxicity data in order to test in pet animals
- Ethical Regulations; IACUC; owner consent
- Expense

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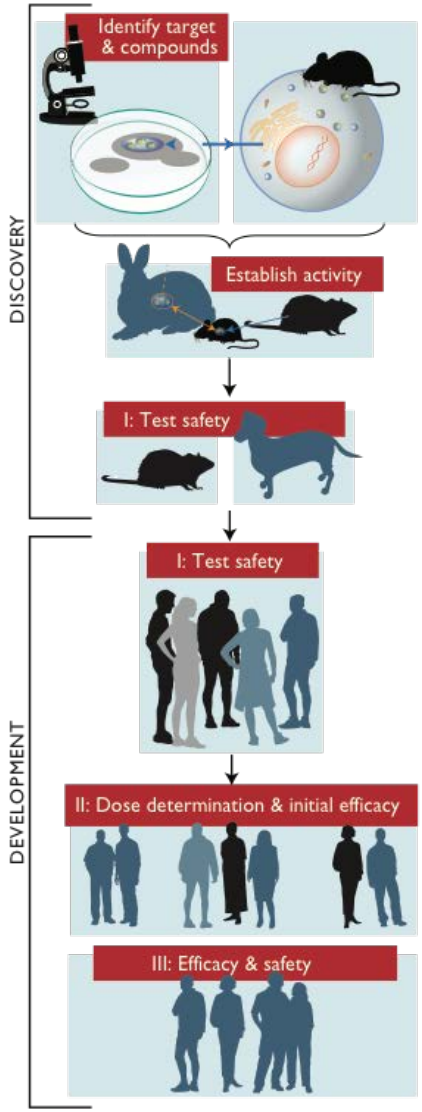
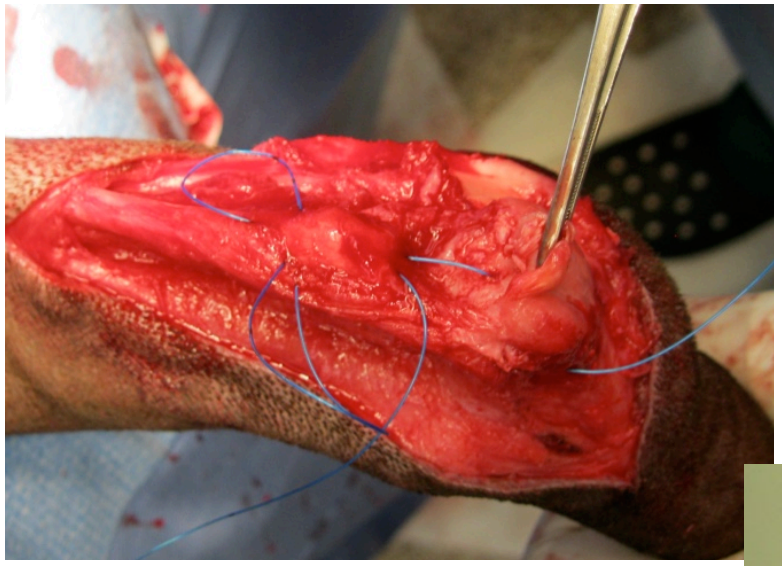
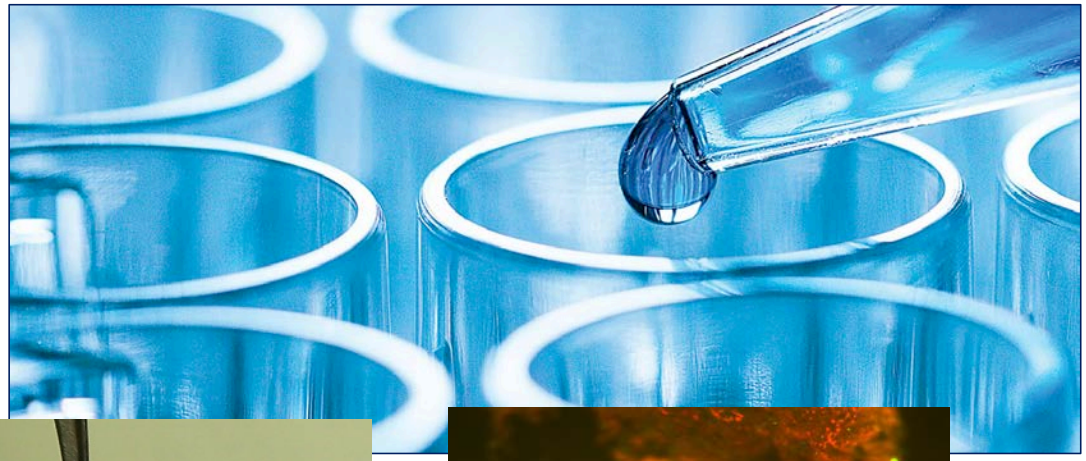


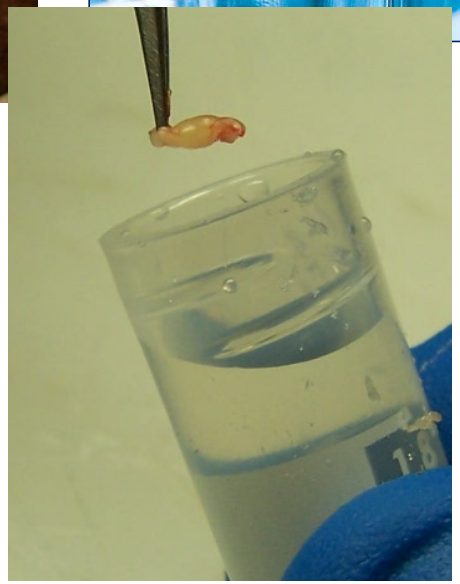
Discovery:
face validity &
relevance of the target

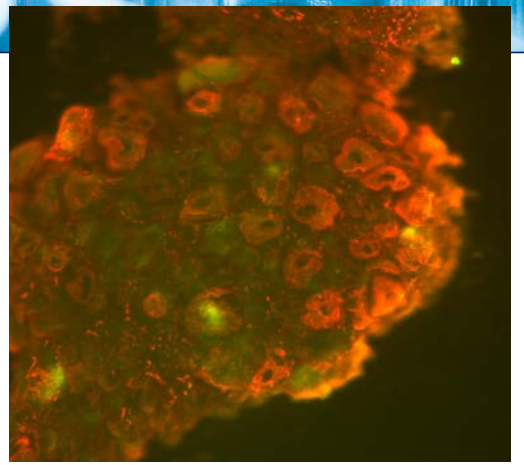
Discovery

- Companion animals can be pain phenotyped
- Veterinarians have unprecedented access to biological samples
- Interrogate target tissues for target of interest, or *in an unbiased way*









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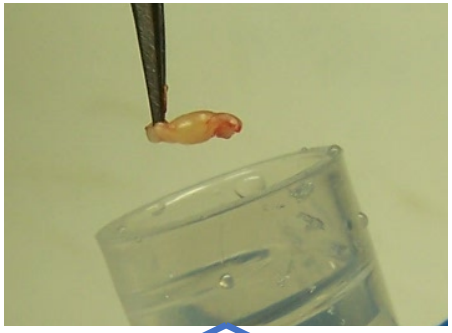
Uncovering a potential role of artemin/GFRα3 signaling in OA-pain



Ipsilateral and contralateral L4-7 DRG from dogs with unilateral hip OA

No Pain

OA-Pain

American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY

PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 15, No 12 (December), 2014: pp 1338-1359
Available online at www.jpain.org and www.sciencedirect.com

Molecular Signatures of Mouse TRPV1-Lineage Neurons Revealed by RNA-Seq Transcriptome Analysis

RT-PCR for a variety of receptors expressed on TRPV1

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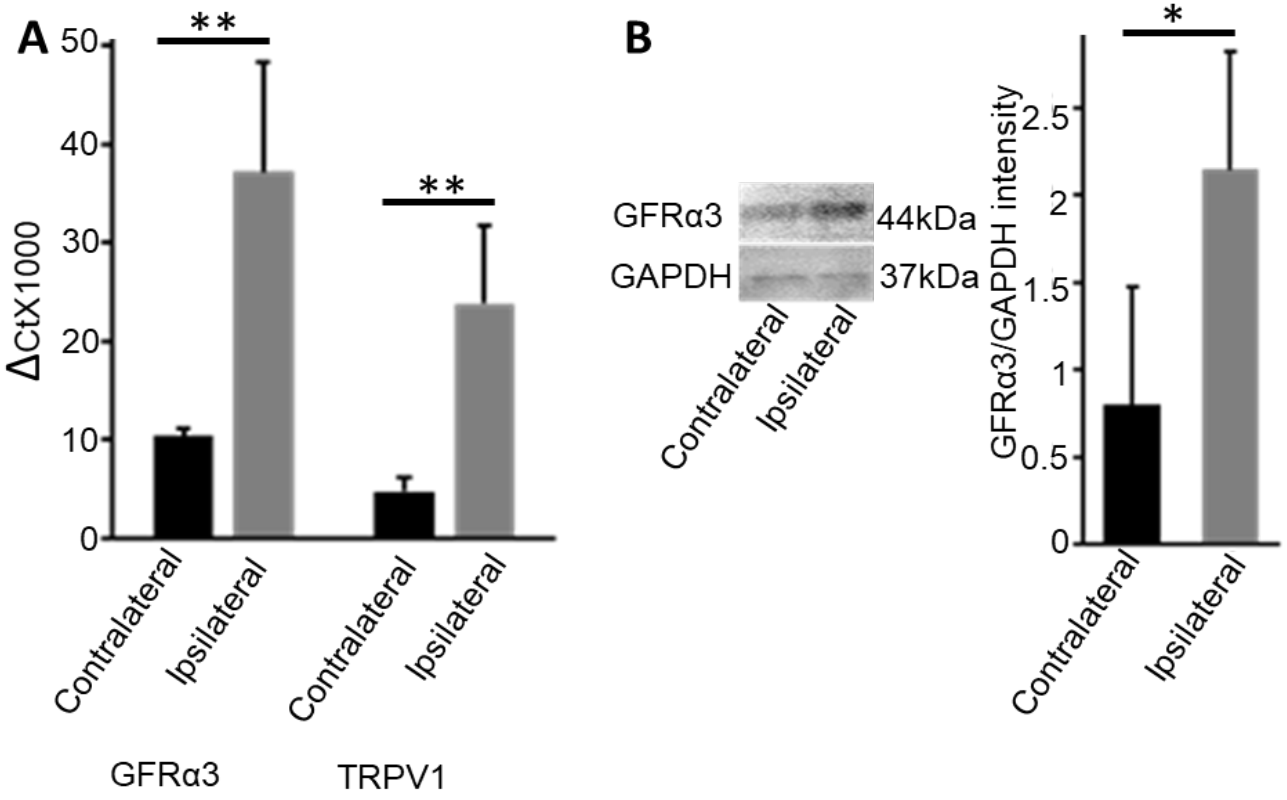
GFRα3 is upregulated in dogs with naturally occurring OA pain; DRG serving OA-pain joints versus DRG serving normal joints



European Journal of Neuroscience, Vol. 13, pp. 2177–2182, 2001

SHORT COMMUNICATION
GFRalpha3 is expressed predominantly in nociceptive sensory neurons

Olivia E. Orozco, Lee Walus, Dinah W. Y. Sah, R. Blake Pepinsky and Michele Sanicola
Biogen, 14 Cambridge Center, Cambridge MA 02142, 617 679-3307, USA

- Most DRG neurons that are GFRα3 positive are:
  - TRPV1 +ve
  - TrkA +ve
  - RET +ve
  - Peripherin +ve

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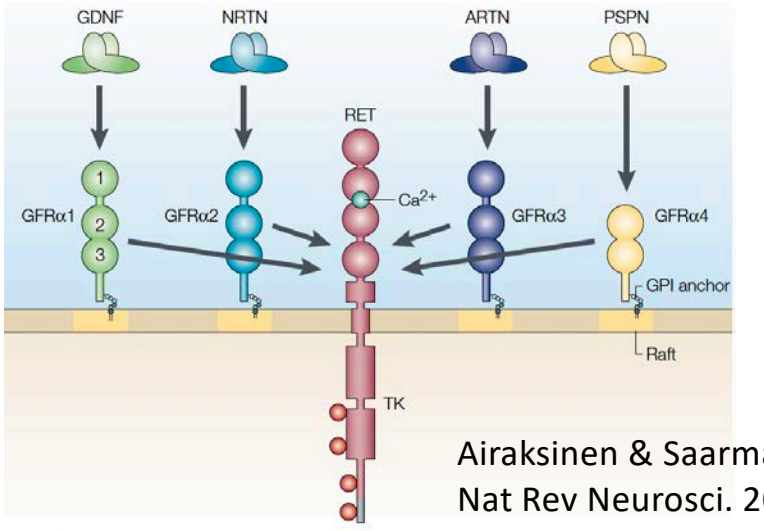
GFRα3 is a GFL growth factor family receptor that complexes with the RET tyrosine kinase to activate intracellular signaling.

GDNF Family of Ligands (GFL) receptors:

1.GDNF ⟷ GFRα1
2.NRTN ⟷ GFRα2
3.ARTN ⟷ **GFRα3**
4.PSPN ⟷ GFRα4

RET ^{P} ⟹ Intracellular signaling



Airaksinen & Saarma
Nat Rev Neurosci. 2002

GDNF family ligands (GFLs):

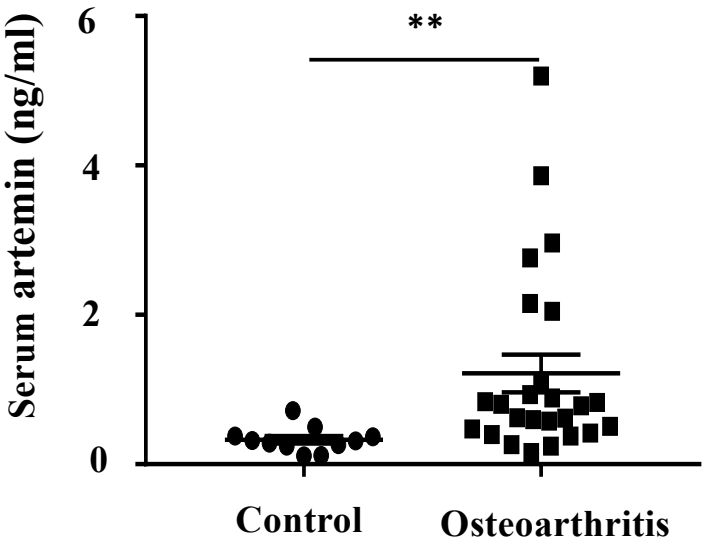
1.Glial cell line-derived neurotrophic factor (GDNF)
2.Neurturin (NRTN)
3.Artemin (ARTN)
4.Persephin (PSPN)

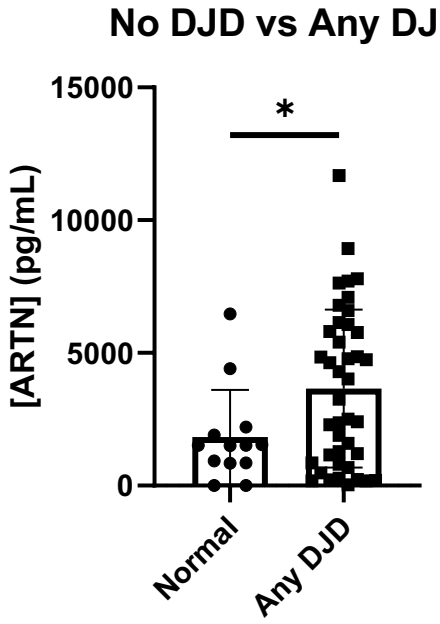
Intra-cellular signaling

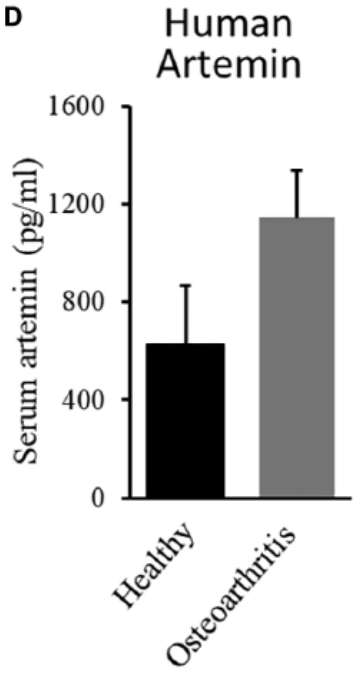
TRPV1
TRPA1
TRPM8
ASIC 1
ASIC 3
TRPV3

TRiP

Serum ARTN is elevated in dogs and cats (& humans) with OA-pain compared to healthy controls





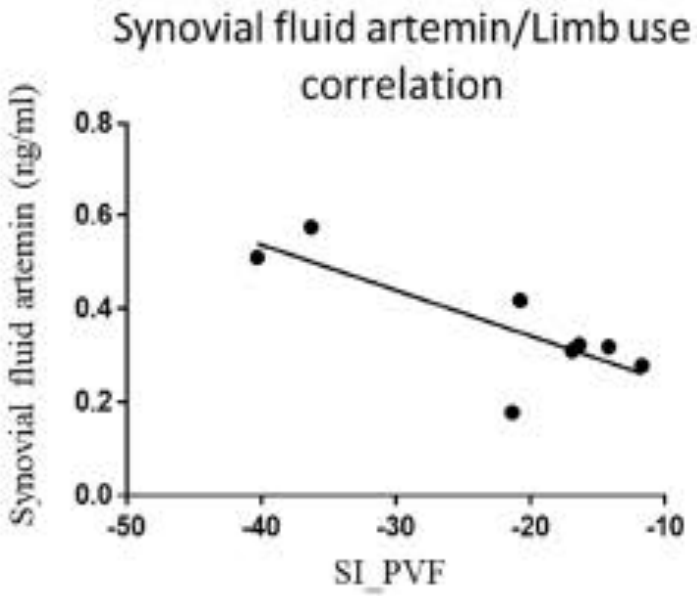


- OA dogs and normal dogs (n=26 OA ; n=11 normal; p=0.007)

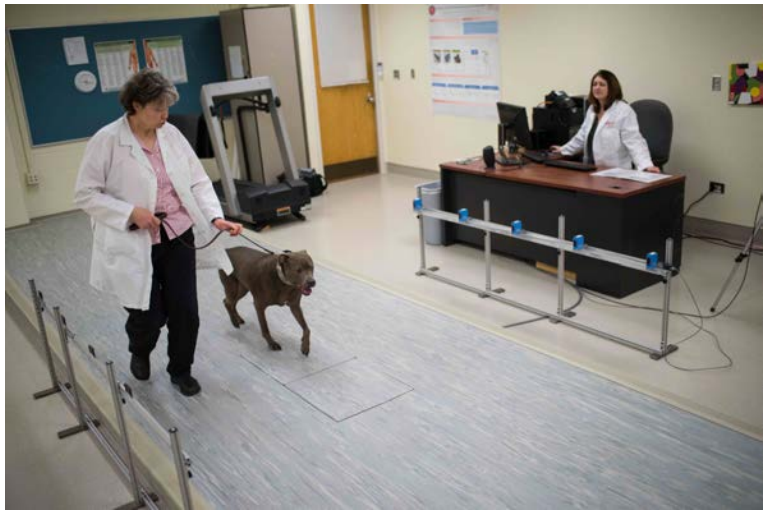
DJD-pain cats and control cats (n=41 DJD-pain ; n=13 control; p=0.011)

TRiP

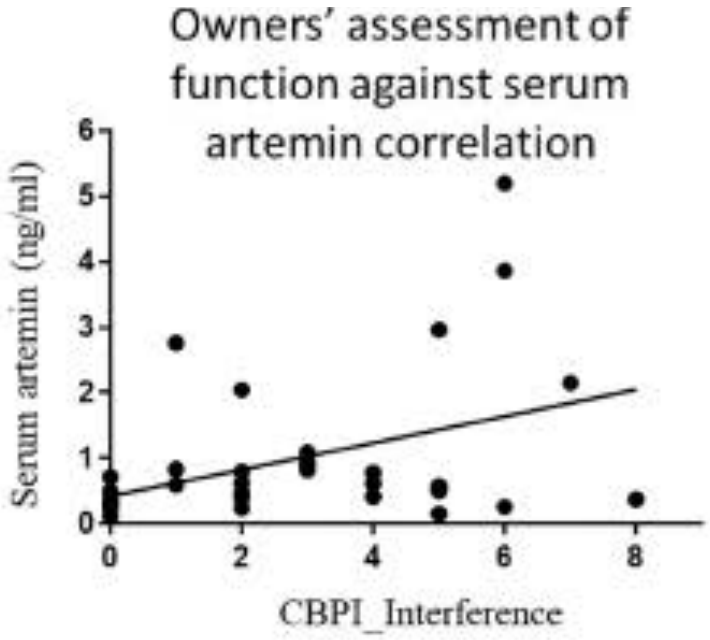
Artemin Concentrations in Synovial Fluid Correlate to Limb-use



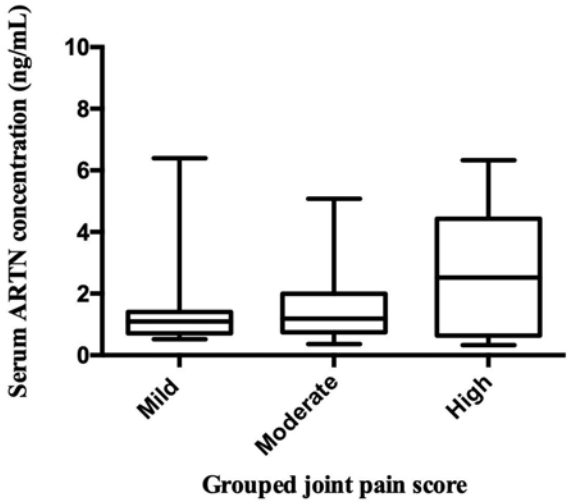
- Negative values of Symmetry Index (SI) correspond to decreased limb use.
- Increased synovial fluid concentrations of artemin correspond to less limb use (n=8; R^2=0.62; p=0.02).



Owner-assessed disability shows some relationship to serum ARTN concs (Minnema et al 2020; confirmed with larger 'n' in Gupta, submitted)



Total joint pain scores do positively correlate with serum ARTN (Gupta, submitted)



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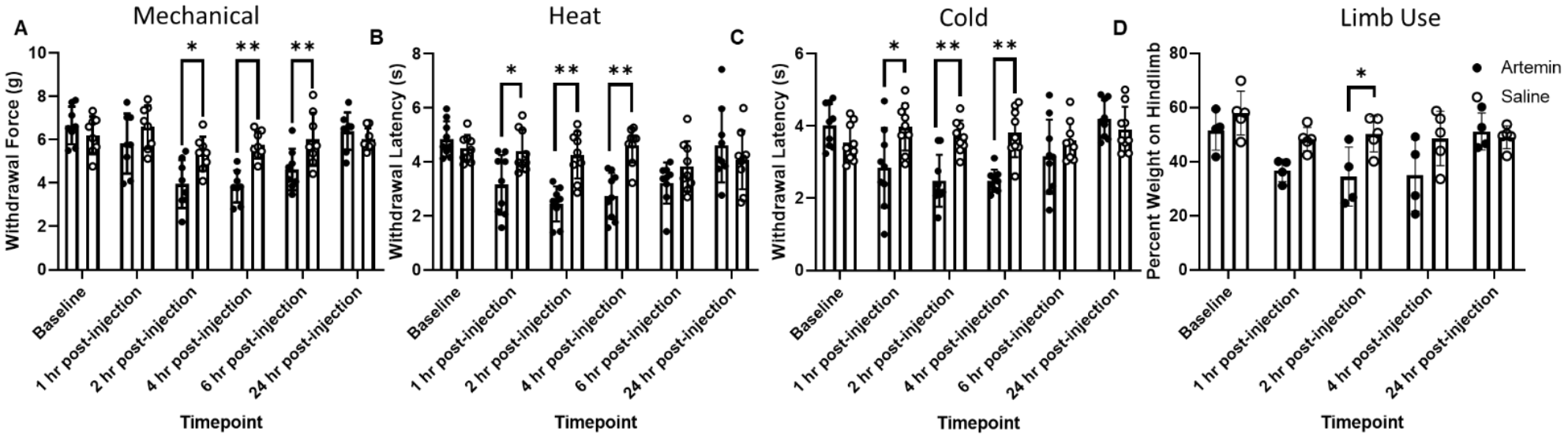
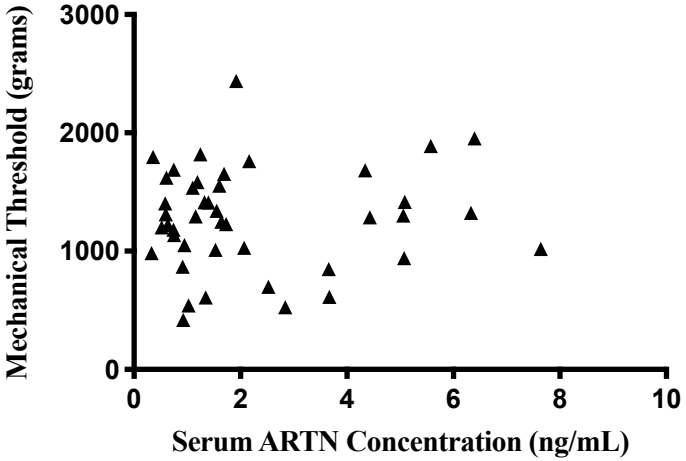
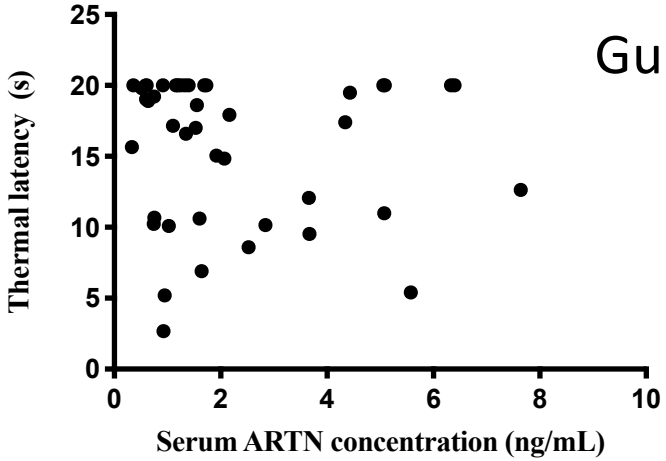
Mouse: 200ng artemin (into paw) produces mechanical, heat and cold sensitivity, in association with decreased limb use



Within a cohort of dogs with OA-pain, serum ARTN does is not associated with remote (from OA joint) sensitivity as measured by QST (n=43)





Gupta, submitted

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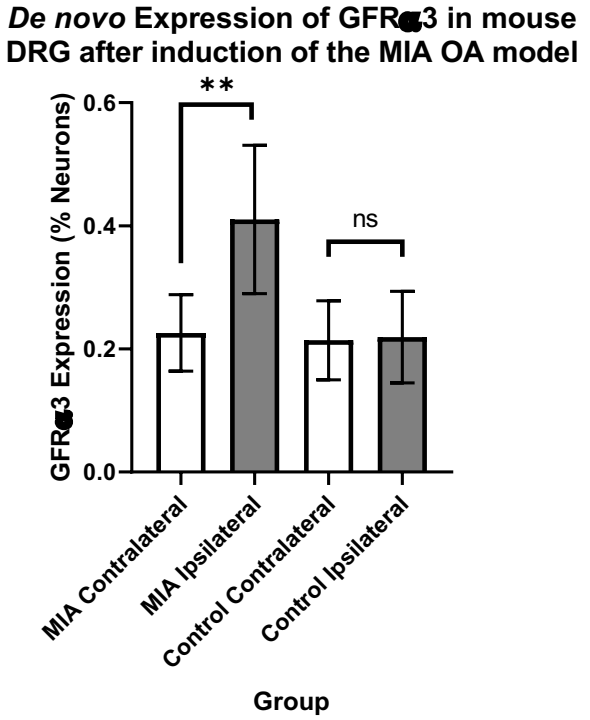
Early data in mouse model of OA

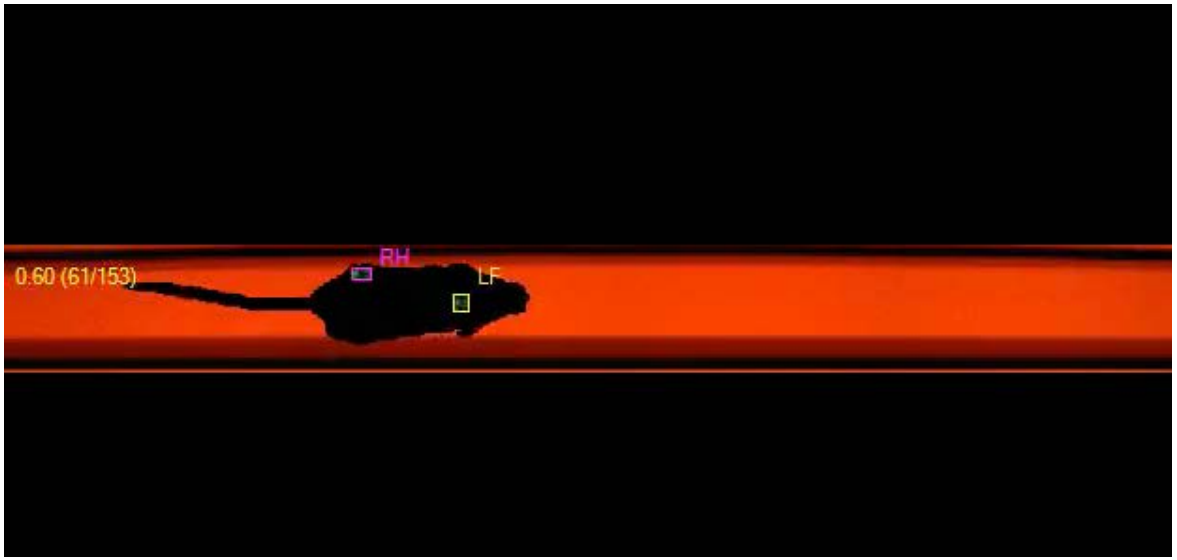
- ARTN (SC, paw) induces mechanical, heat and cold hypersensitivity
- Increase in GFRα3 in DRG (IHC) with MIA
- No increase in serum ARTN in 'single joint' MIA, versus controls; no increase in serum ARTN in bilateral DMM (samples courtesy of A-M Malfiat)
- IA injection of anti-GFRα3 Ab does not improve limb use (MIA model)
- IP injection of anti-ARTN mAb significantly decreases mechanical, heat and cold hypersensitivity in MIA model

N = 8-12 in all groups; replication ongoing



10 ug in 10 ul of a polyclonal anti-GFRα3 antibody (abcam; Cat. ab2028) or IgG isotype control (R&D; rabbit IgG) dissolved in PBS

PBS or 25 ug of anti-artemin monoclonal antibody (R&D, cat: MAB10851-500) dissolved in PBS



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Next steps:

- Detail the expression and co-expression of GFRα3 with TRP channels
- Understand the role of ARTN and GFRα3 in initiating (early) and maintaining (late) OA in different models
  - DMM; MIA
  - Anti-bodies; GFRα3 KO; GFRα3 mutants (non-functional)
- Understand parallel and alternative signaling (GFRα3 vs. GFRα1; RET vs. NCAM)
- Elucidate the downstream targets (TRP receptors)
- Verify findings using *ex vivo* and *in vivo* work in pet dogs

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Companion animal ‘models’ can contribute to translational pain research in two basic ways

1) Discovery / Face Validity of Target: Tissue from naturally occurring disease states may provide vital information about the neurobiology of pain in the natural disease state.

1) Verification bridge between rodent preclinical and human clinical studies, testing drugs for efficacy prior to human clinical studies

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