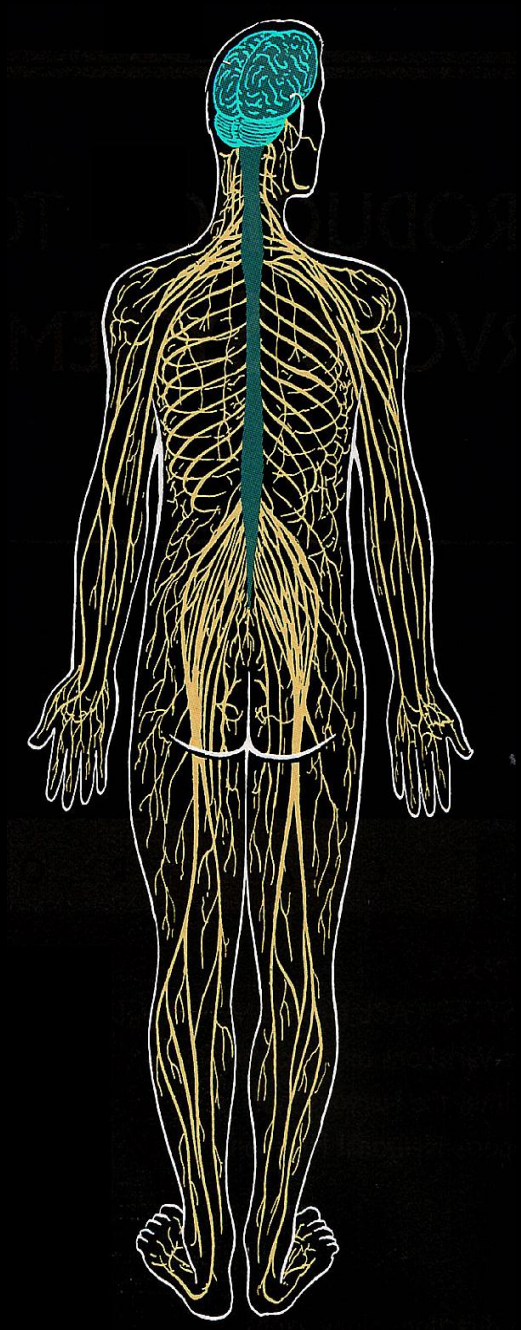


Identifying pain genes in humans

Stephen McMahon
London Pain Consortium
King's College London

The London Pain Consortium

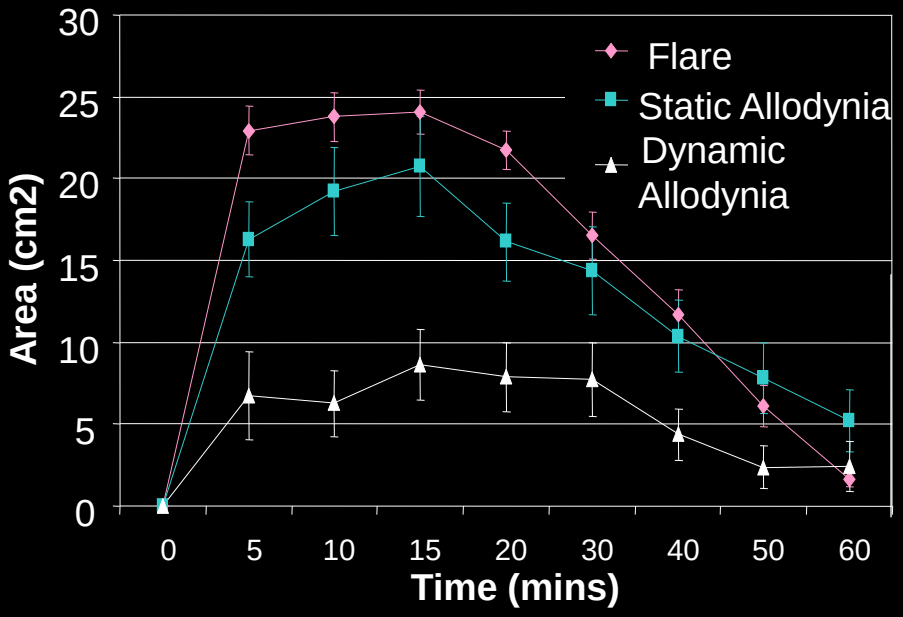
Identifying pain genes in humans

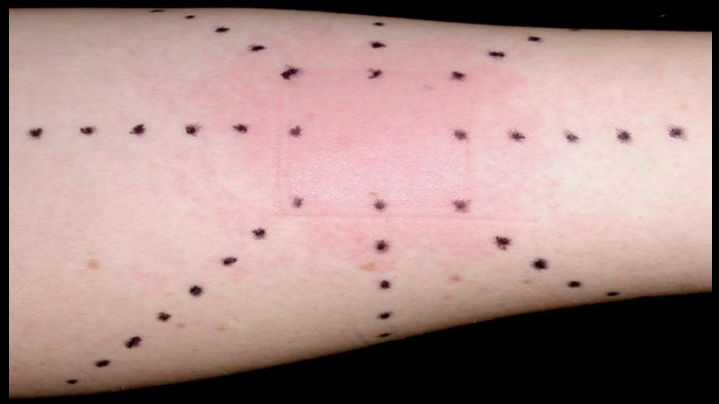
1. Twin studies & exome sequencing in experimental pain states.
2. Identifying novel pain mediators in tissue bopsies.

Genetic influences on Pain – Twin Studies

MONOZYGOTIC DIZYGOTIC

- Heat Pain Threshold (HPT)
- Pain during creation of 45°C thermal burn
- Primary & secondary hyperalgesia after burn
- Pain during iontophoresis of Acid + ATP
- Itch after iontophoresis of Histamine

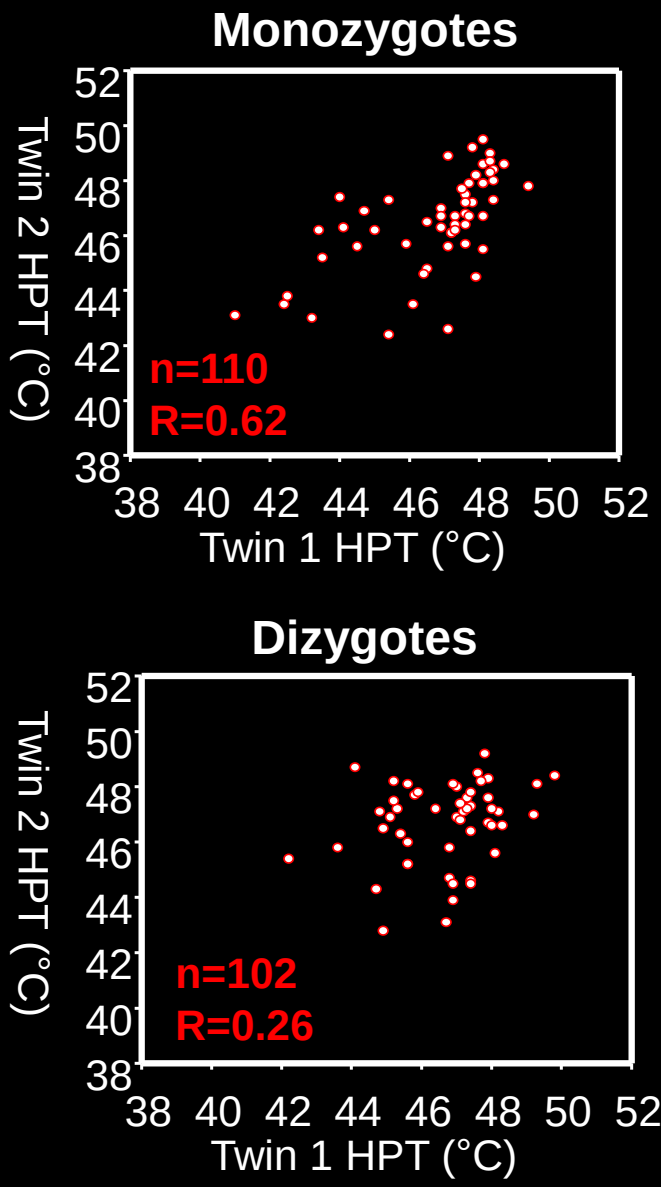




45 ºC for 330sec

Tim Norbury

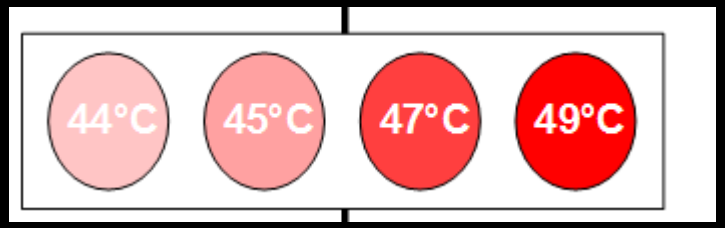
Heritability of pain traits – twin studies



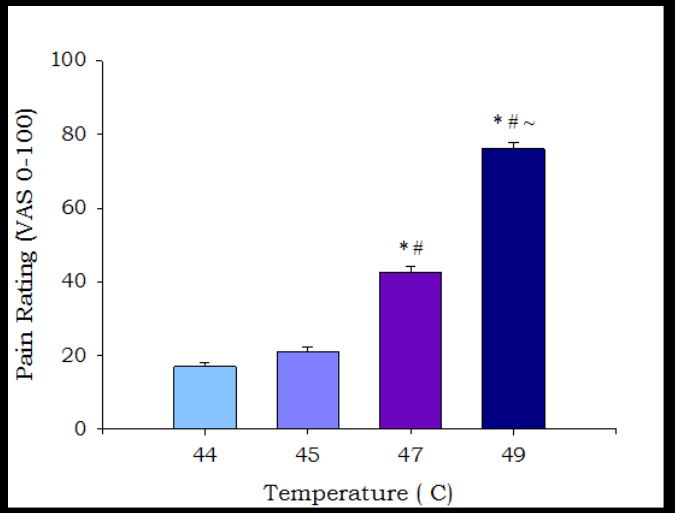
Modality	Rmz	Rdz	Heritability	Shared Environment
Pre-Burn HPT	0.62 (0.46-0.79)	0.26 (0.01-0.52)	**0.57** (0.40-0.70)	0
Post-Burn HPT	0.49 (0.29-0.69)	0.28 (0.03-0.54)	**0.45** (0.26-0.60)	0
Pain Rating during burn creation	0.30 (0.06-0.54)	0.08 (0.00-0.35)	**0.30** (0.05-0.51)	0
Pain during ATP iontophoresis	0.27 (0.02-0.52)	0.00 (0.00-0.29)	**0.24** (0.00-0.45)	0
Pain during acid iontophoresis	0.28 (0.03-0.52)	0.13 (0.00-0.41)	**0.27** (0.04-0.47)	0
Itch after histamine iontophoresis	0.34 (0.12-0.62)	0.2 (0.00-0.35)	**0.19** (0.09-0.52)	0
2°pinprick hyperalgesia area	0.60 (0.43-0.77)	0.29 (0.04-0.54)	**0.59** (0.41-0.73)	0
2°brush evoked allodynia area	0.55 (0.36-0.74)	0.38 (0.19-0.57)	**0.35** (0.00-0.74)	0
2°skin flare area	0.76 (0.66-0.87)	0.56 (0.38-.075)	0	**0.69** (0.57-0.78)
HPT Replication Cohort (n=150)			**0.72**	0

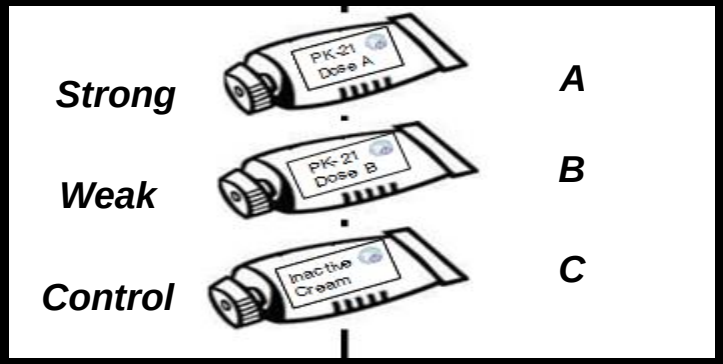
Norbury et al., 2007

Are placebo responses heritable?

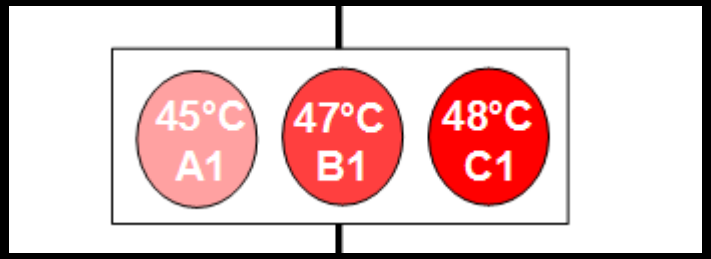


Sensitivity

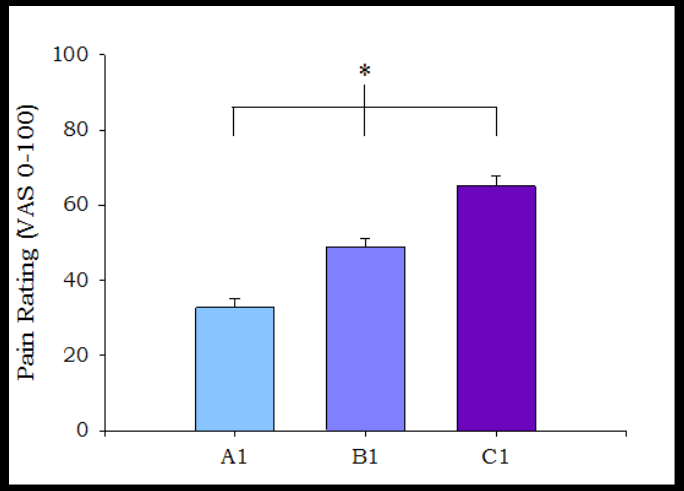


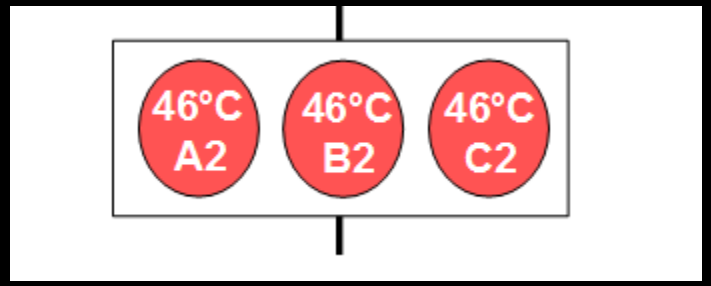


Expectation



Reinforcement



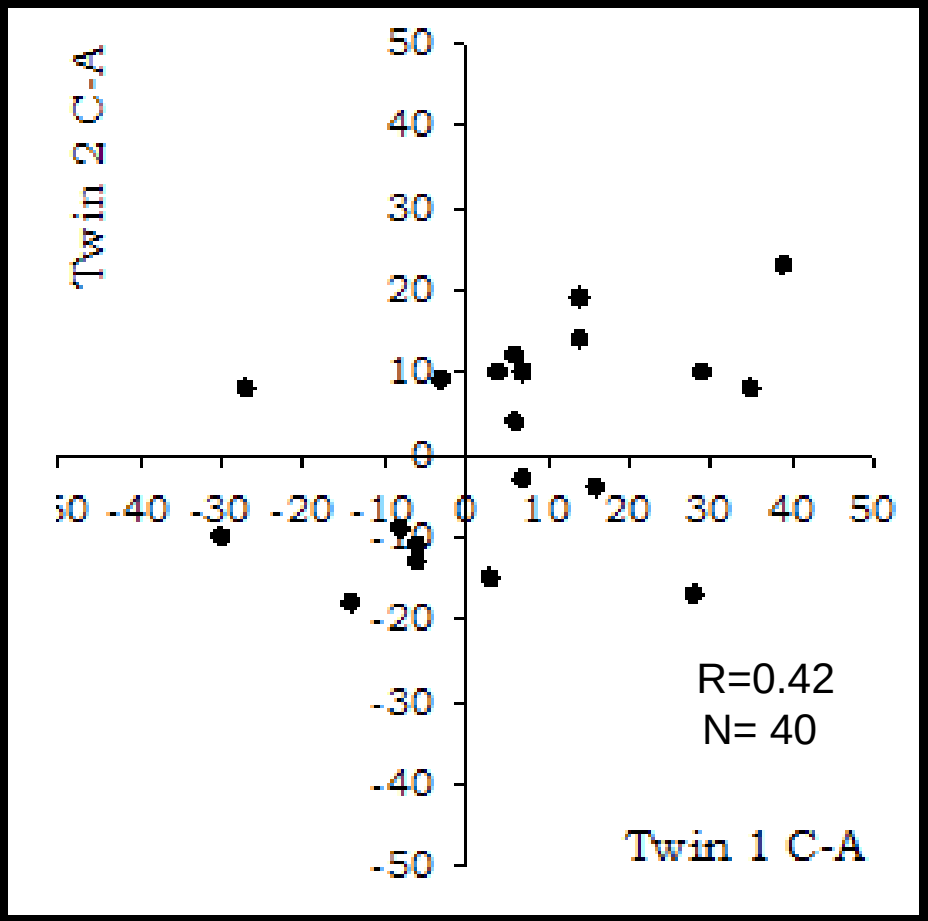


Placebo trial

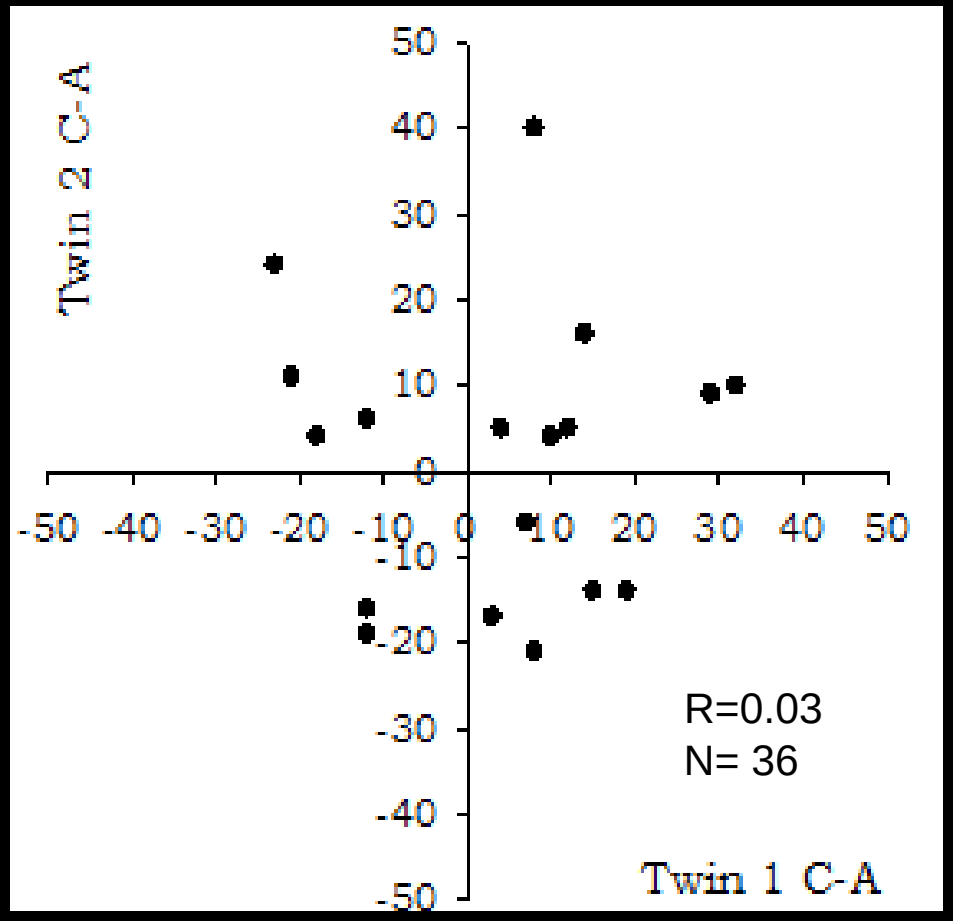
Placebo response

Are placebo responses heritable?

MZ twins



DZ twins

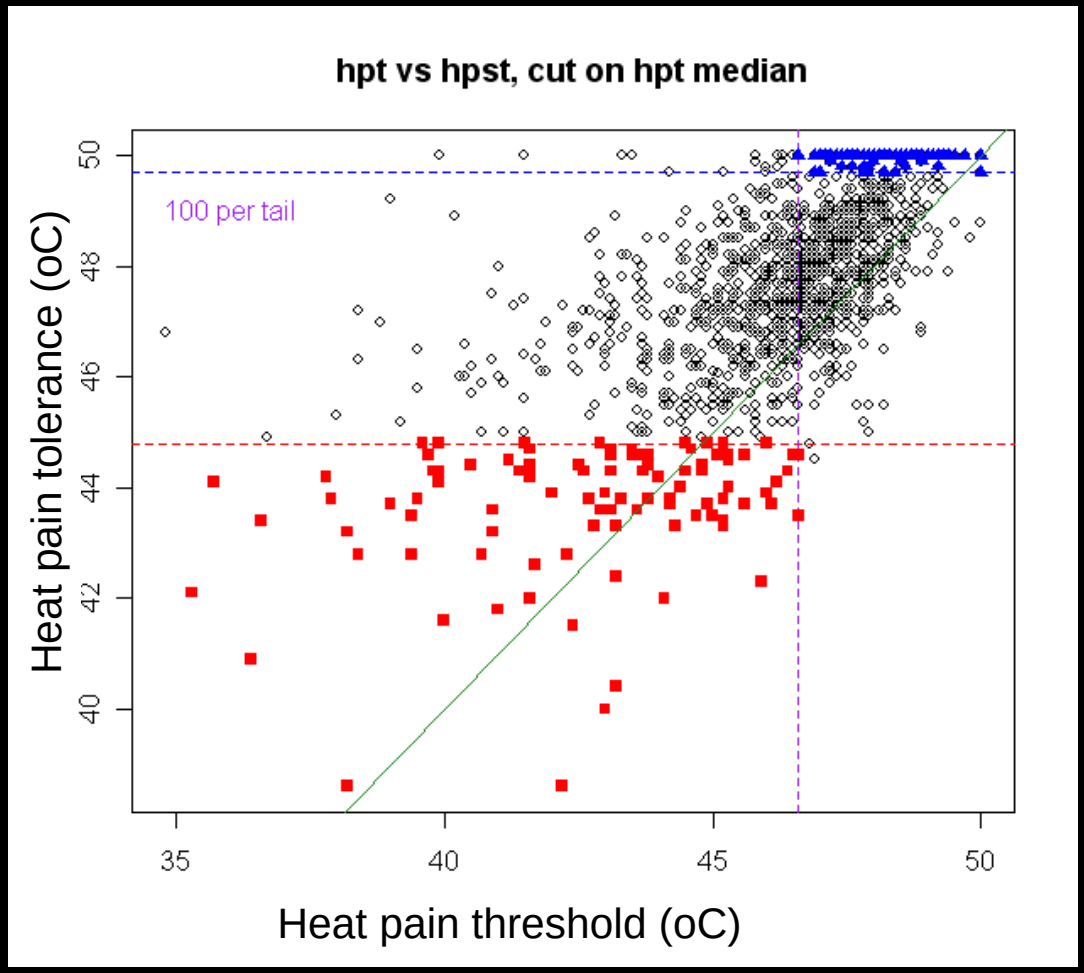


MZ correlation	**DZ correlation**	**AE**	**CE**	**ACE**	ADE	**Heritability**
0.42 (-.03-0.7)	-0.03 (-.49-0.44)	-4.1	-3.0	-2.1	-2.7	**0.33 (0.0-0.65)**

Identification of low frequency and common genetic determinants of pain in the normal population

SUBJECTS

- Top & bottom 10% of selected
- Upper tail: HPST≥49.2
  Lower tail: HPST≤45.5
- HPT scores are required to reside on the same side of HPT median
- Roughly half selected in either end
- For MZs, one twin from each pair was used as long as their twin was in the same tail
- Discovery 203; replication 210



KING'S College LONDON

Pfizer

wellcome trust sanger institute

The London Pain Consortium

SNVs identified in discovery and replication sets

Functional Effects	Discovery Set	Replication Set
NO. Mb SEQUENCED	32	44
NUMBER OF EXONS	180k	300k
NON_SYNONYMOUS_CODING	60,353	82,293
PARTIAL_CODON	4	3
SPLICE_SITE	8,155	11,060
STOP_GAINED	1,100	1,728
STOP_LOST	76	124
SYNONYMOUS_CODING	44,878	56,993

The significance of SNVs was tested in 6 ways

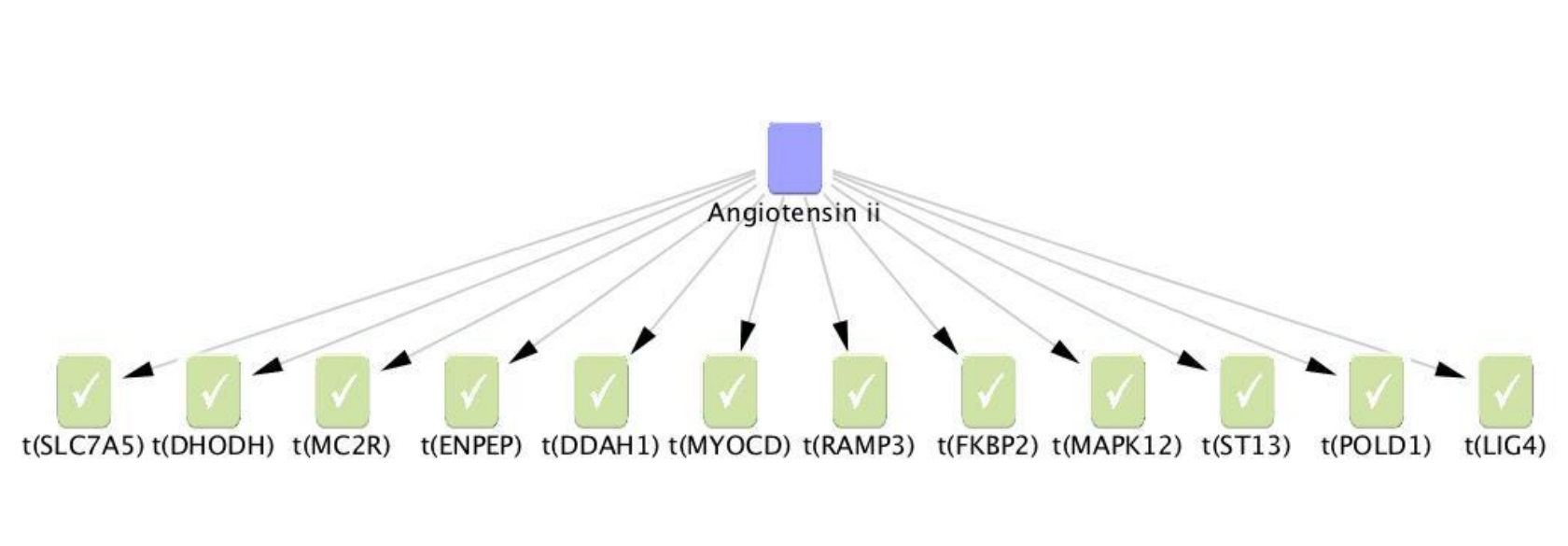
- AMELIA: Allele Matching Empirical Locus-specific Integrated Association test. Multivariate test considering both common and rare variants, and is based on genotypic similarity rather than rare allele accumulation
- aSum: Data adaptive sum test. A regression based collapsing approach, which takes account of the direction of effect of the alleles. This type of method is expected to tolerate misclassification eg. if alleles with different functions are collapsed together
- SSU (Sum of Squares Test): a test analogous to traditional multivariate analysis on a binary trait
- simple threshold test: a case/control by subject on carriers with one or more variants having MAF<0.05. It is similar to the CAST method
- CCRaVAT (using Pearson test): collapsing method examining the accumulation of rare alleles using analysis of contingency tables. Like ARIEL, it is sensitive to linkage disequilibrium, however it evaluates the presence or absence of individual rare alleles in cases or controls (rather than the proportion rare variants)
- Madsen and Browning using polyphen weights (MB pphen): method combines variants by weighting based on allele frequency and, optionally, polyphen predictions (selected here)

SNVs which may mediate pain sensitivity

Gene	List Source	Evidence	Ch	Gene annotation	Lowest Pval	MERGED Pval
GZMM	Primary+Repl	Very high	19	granzyme M (lymphocyte met-ase 1)	0.00010	6.86E-05
CCNJL	Primary+Repl	High	5	cyclin J-like	0.00010	0.00025
ZNF767	Primary+Repl	High	7	zinc finger family member 767	0.00036	0.00070
LAMA4	Primary+Repl	High	6	laminin, alpha 4 [Homo sapiens]	0.00041	0.00117
OR5F1	Primary+Repl	High	11	olfactory receptor, family 5/F/1	0.00074	0.00033
TBK1	Primary+Repl	High	12	TANK-binding kinase 1	0.00083	0.00030
DDAH1	Primary+Repl	High	1	dimethylarginine dimethylaminohydrolase	0.00165	0.00028
PDHA2	Merged	Medium	4	pyruvate dehydrogenase (lipoamide) alpha 2	-	0.00060
FBXW7	Merged	Medium	4	F-box and WD repeat domain containing 7	-	0.00063
DLD	Merged	Medium	7	dihydrolipoamide dehydrogenase	-	0.00078
RHEB	Merged	Medium	7	Ras homolog enriched in brain	-	0.00097
CCDC111	Primary+Repl	Medium	4	coiled-coil domain containing 111	0.00075	0.00056

Bonferroni cut off for significance of multiple testing 14,109 genes: p<3.0e-06

Causal reasoning identifies Angiotensin II as highly significant upstream regulator of pain genes associated high heat pain sensitivity



Name	Correctness p (Bonferroni corrected p)	Enrichment p (Bonferroni corrected p)	No. connections (no. possible connections)
Angiotensin II -	1.2 x 10-8 (1.4 x 10-5)	3.4 x 10-7(3.8 x 10-4)	12 (204)

Identifying novel pain mediators

Are there novel peripheral pain mediators?

Many persistent pain states are maintained by peripheral drive

Lidocaine patch

Capsaicin patch

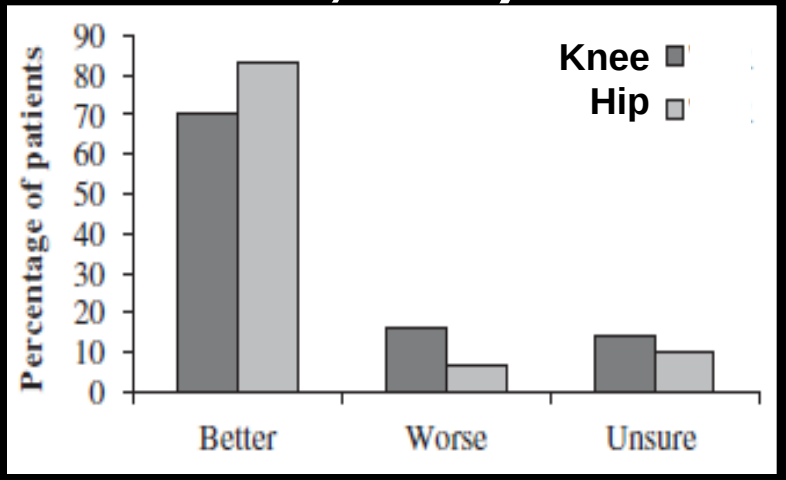
Joint replacement

TopicalNSAIDs

Local glucocorticoids

Biologics (Anti-TNFa, IL1b, NGF)

	Total Knee replacement (n=632)	Total Hip replacement (n=662)
No pain	56%	73%
Mild pain	12%	9%
Mod. pain	17%	11%

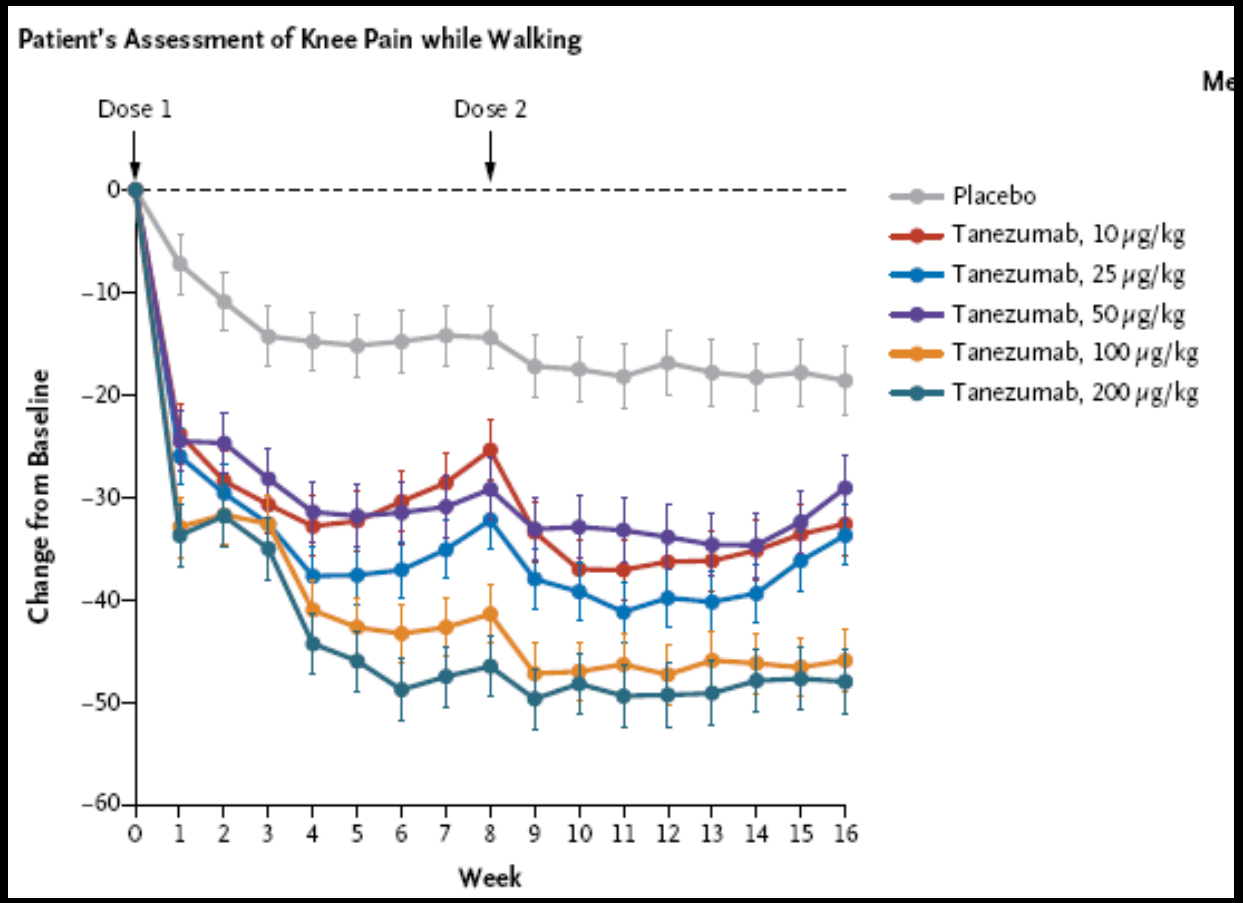


Wylde et al 2011

The NEW ENGLAND JOURNAL of MEDICINE

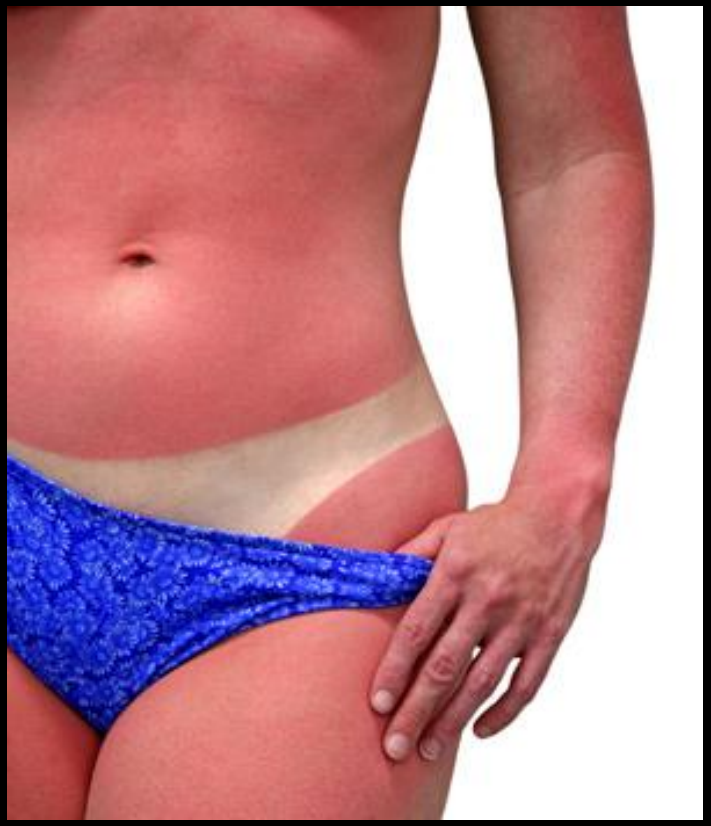
Tanezumab for the Treatment of Pain from Osteoarthritis of the Knee

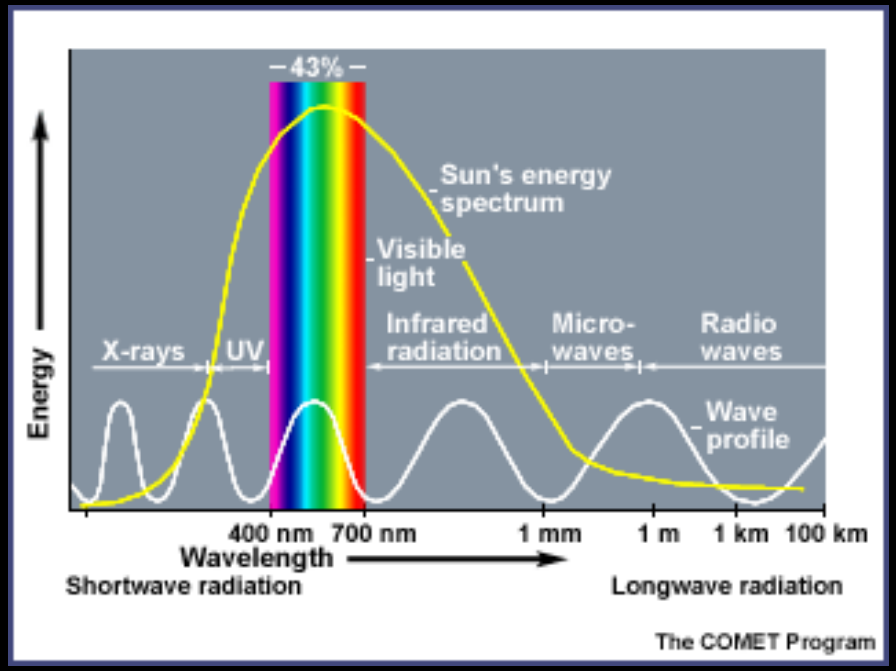
Nancy E. Lane, M.D., Thomas J. Schnitzer, M.D., Ph.D., Charles A. Birbara, M.D., Masoud Mokhtarani, M.D., David L. Shelton, Ph.D., Mike D. Smith, Ph.D., and Mark T. Brown, M.D.



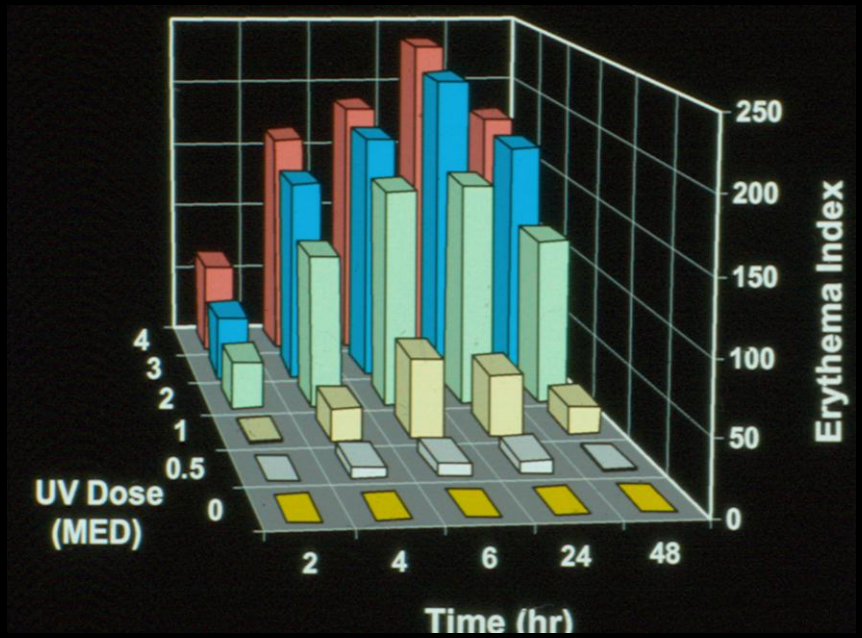
Lane et al., New England J Medicine, 2010 Oct 14;363(16):1521-31.

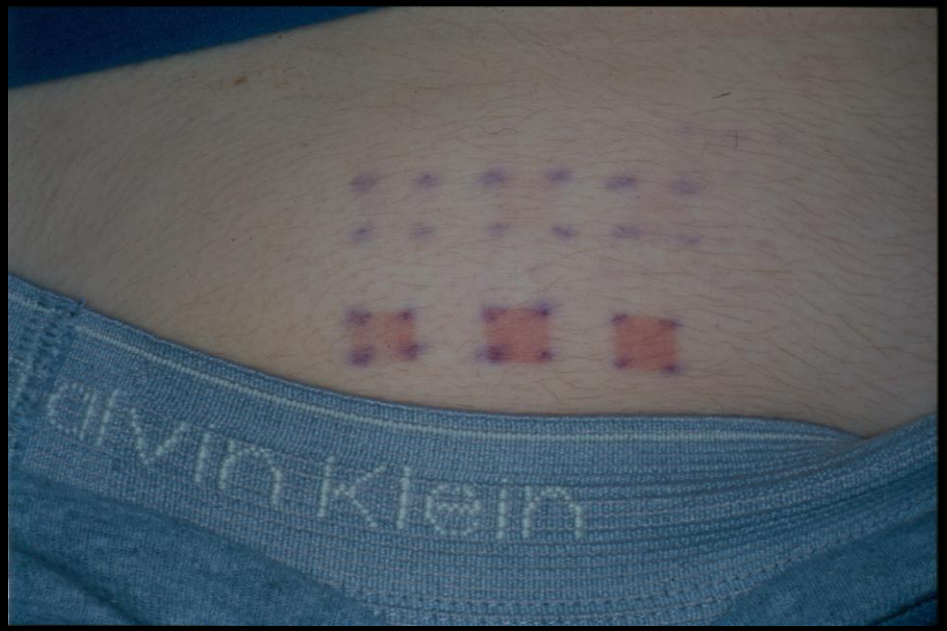
Sunburn is a well recognised cause of hyperalgesia





UVB inflammation is a useful translational model of pain

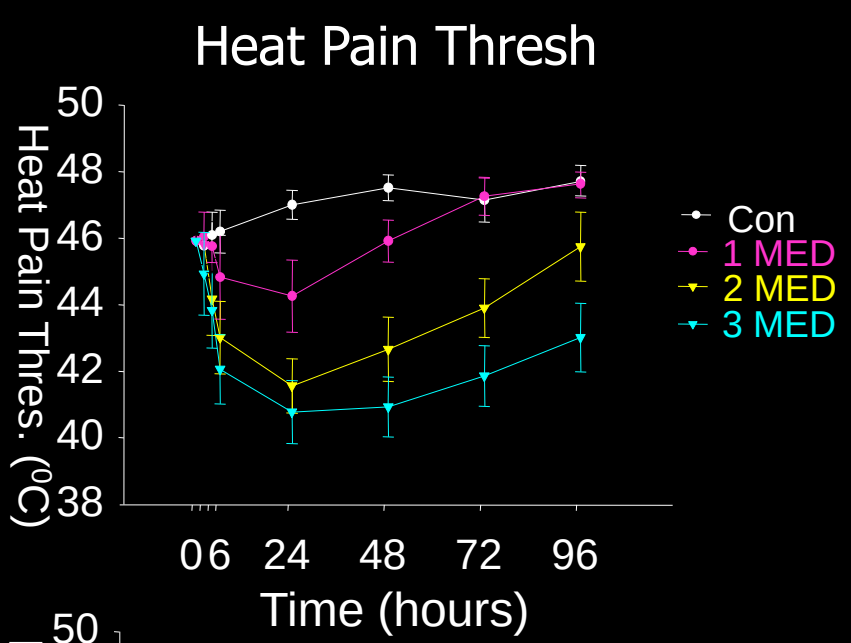


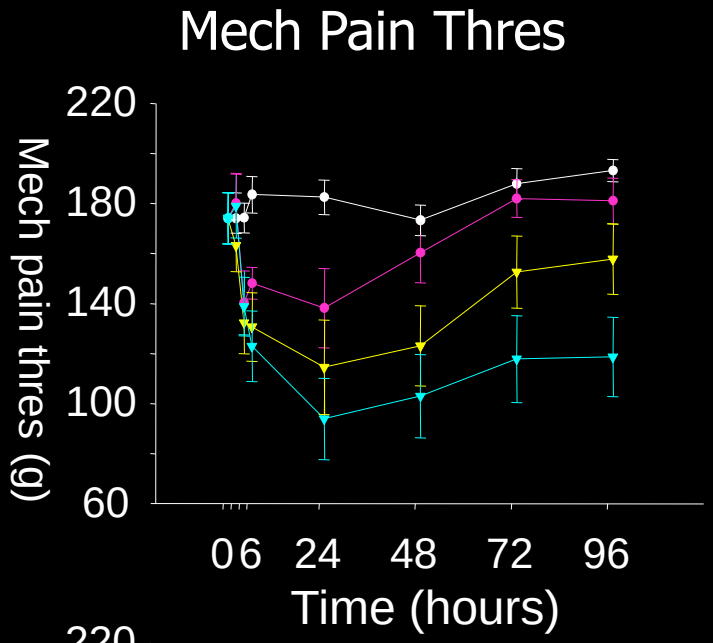


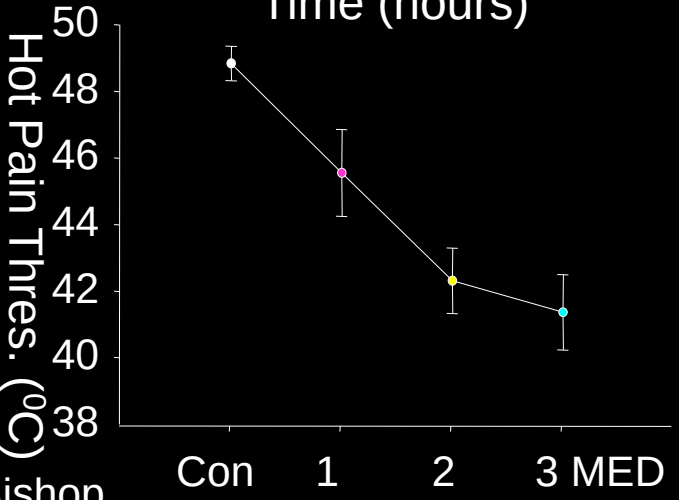
- No spontaneous pain.
- Only primary sensory changes

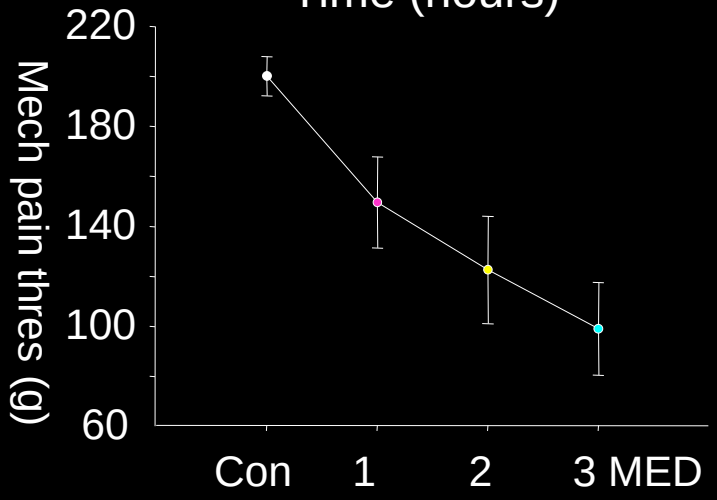
Graham Harrison

UVB promotes long lasting erythema and sensory changes in human skin



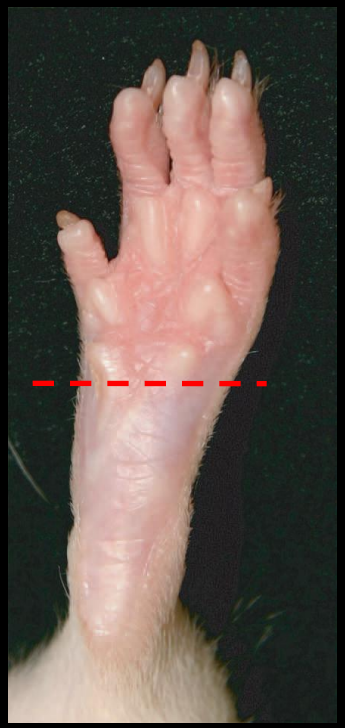




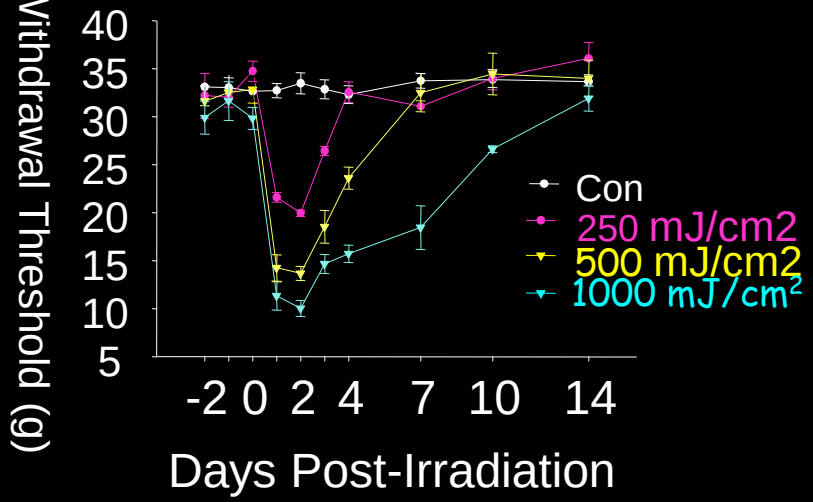


Tom Bishop

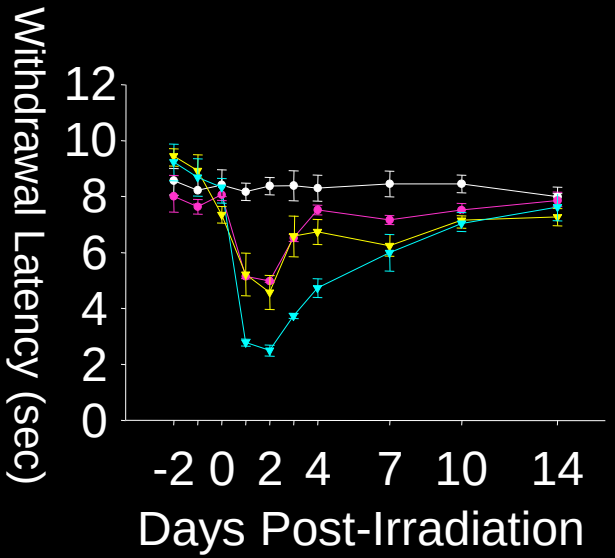
UVB promotes long lasting erythema and sensory changes in rat skin

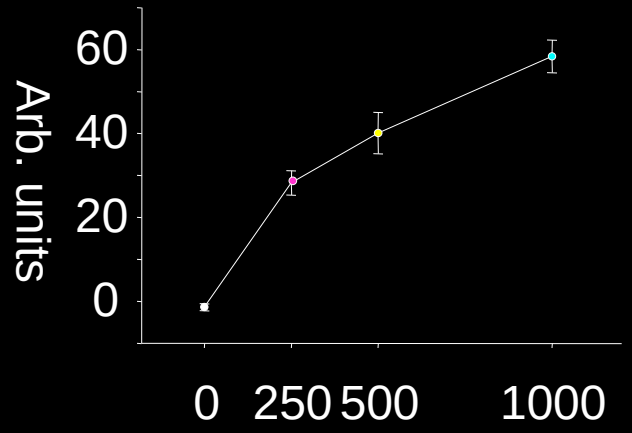


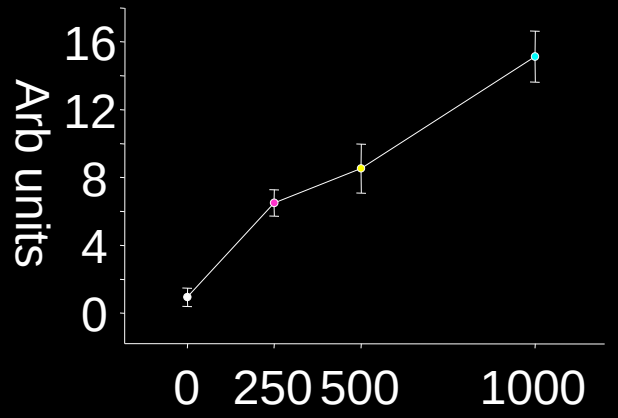
Mech. Hyperalgesia



Therm. Hyperalgesia



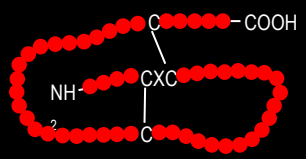




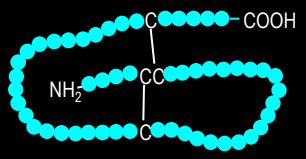
Tom Bishop

Chemotactic Cyto**kines** (Chemokines)

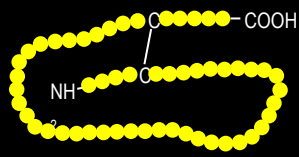
A large family of secreted proteins characterised by a 4-cysteine motif, important in the chemotaxis of leukocytes and the activation of immune cells.



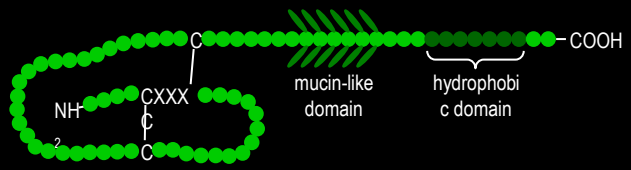
CXC (α) chemokines



CC (β) chemokines



C (γ) chemokines

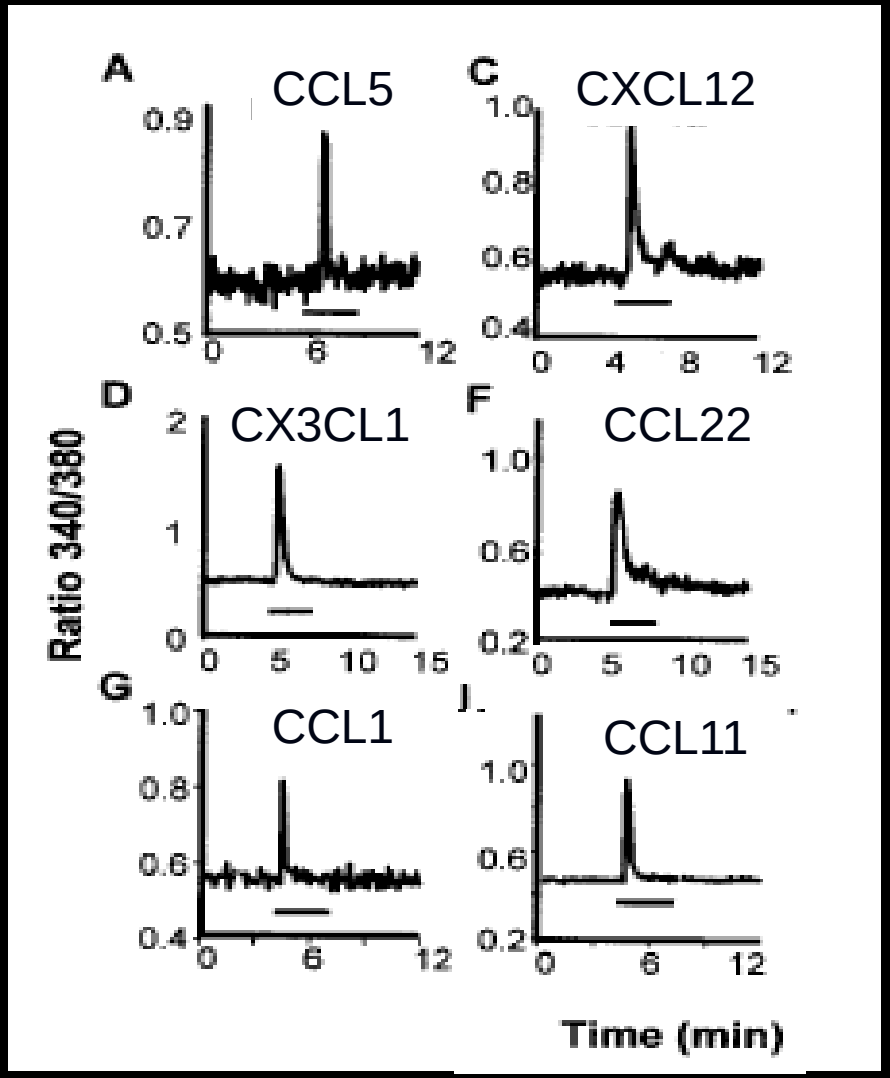


CX3C (δ) chemokines

CCR1	CCL3, 5, 7, 8, 9†, 13, 14, 15, 16, 23
CCR2	CCL2, 7, 8, 12†, 13, 14, 16
CCR3	CCL5, 7, 8, 11, 13, 15, 24, 26, 28
CCR4	CCL17, 22
CCR5	CCL3, 4, 5, 8, 11, 14, 16
CCR6	CCL20
CCR7	CCL19, 21
CCR8	CCL1, 4, 17
CCR9	CCL25
CCR10	CCL26, 27, 28
?	CCL6†, 18
CXCR1	CXCL1, 5, 6, 8
CXCR2	CXCL1, 2, 3, 5, 6, 7, 8
CXCR3a	CXCL9, 10, 11
CXCR3b	CXCL4, 9, 10, 11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, 12
?	CXCL14, 15, 17
CX3CR1	CX3CL1
XCR1	XCL1, 2

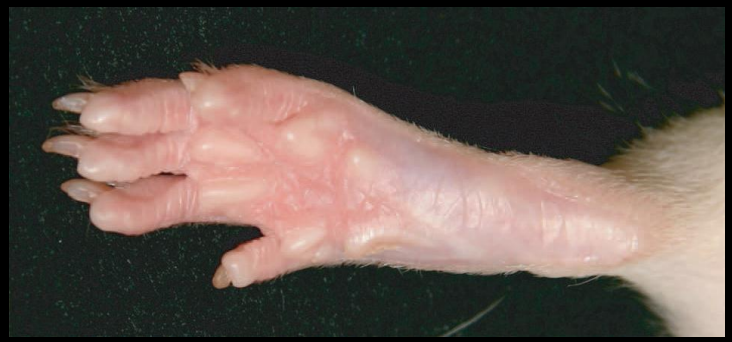
Sensory neurones respond to many chemokines

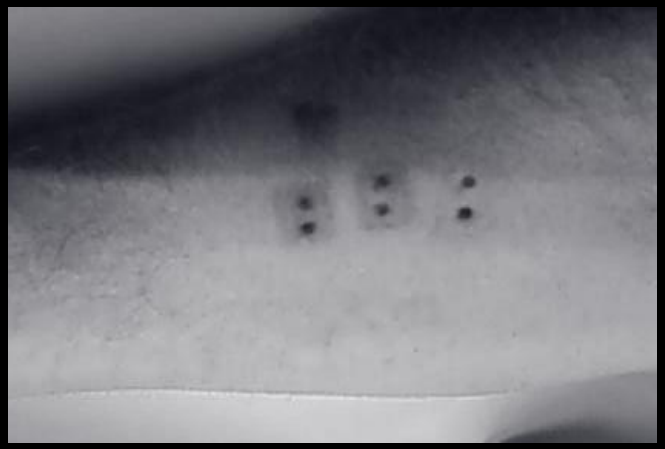
Receptors tested	% responding
CCR1, CCR3, CCR5, CCR9	6%
CCR2, CCR9	22%
CCR3	15%
CCR4	30%
CCR4	11%
CCR5, CCR9	12%
CCR6	12%
CCR7	36%
CCR8	8%
CXCR1, CXCR2	5%
CXCR3	12%
CXCR4	30%
CXCR5	31%
CX3CR1	9%
CXCR4	6%
CCR5	11%
CCR8	9%
CCR3, CCR8	12%
CCR4	4%
(Capsaicin)	55%



Oh et al., J. Neruosci. 2001

Low density arrays can measure multiple chemokines





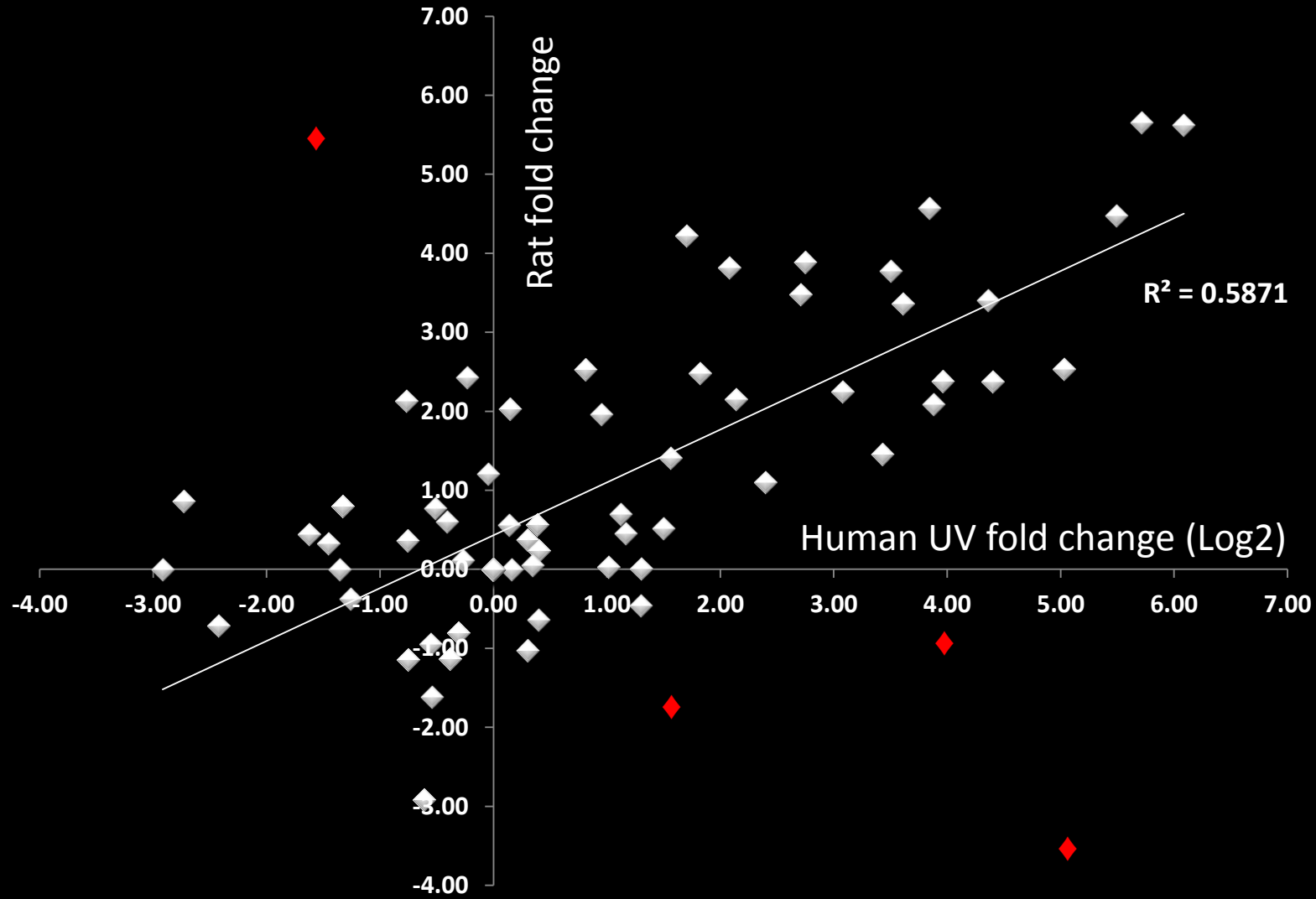
BDNF	NGF	ARTN	CCL1	CCL2	CCL3	CCL4	CCL5	CCL7	CCL8	CCL11	CCL13
CCL14	CCL16	CCL17	CCL18	CCL19	CCL20	CCL21	CCL22	CCL23	CCL24	CCL25	CCL26
CCL27	CCL28	CXCL1	CXCL2	CXCL3	CXCL4	CXCL5	CXCL6	CXCL7	CXCL8	CXCL9	CXCL10
CXCL11	CXCL12	CXCL13	CXCL14	CXCL16	CXCL17	XCL1	CX3CL1	CSF1	CSF2	CSF3	IL1a
IL1b	IL2	IL3	IL4	IL5	IL6	IL7	IL9	IL10	IL11	IL12a	IL12b
IL13	IL14	IL15	IL16	IL17	IL18	IL19	IL20	IL21	IL22	IL23a	IL24
IL25	IL26	IL27a	IL27b	IL28	IL29	IL31	IL32	IL33	IL34	TNF-a	COX-2
PTGES	END1	KGF	iNos	MIF	TRPV3	TRPV4	TRPA1	B2m	18s	GAPDH	βActin

= Human and Rat PCR array

= Human PCR array

John Dawes, Kathryn Paterson

UVB induces similar transcriptional changes in human and rat skin



John Dawes

Normalised to GAPDH

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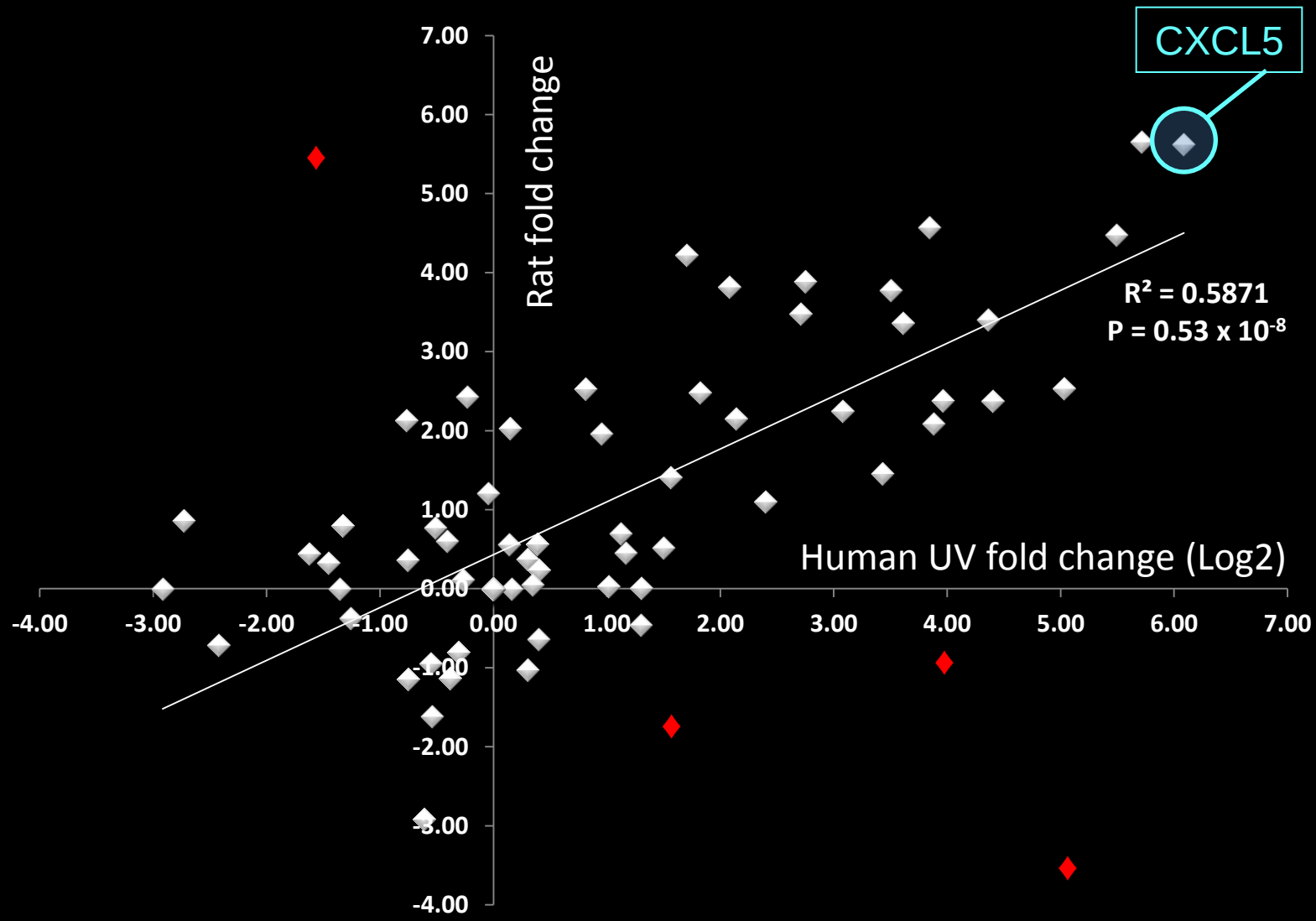
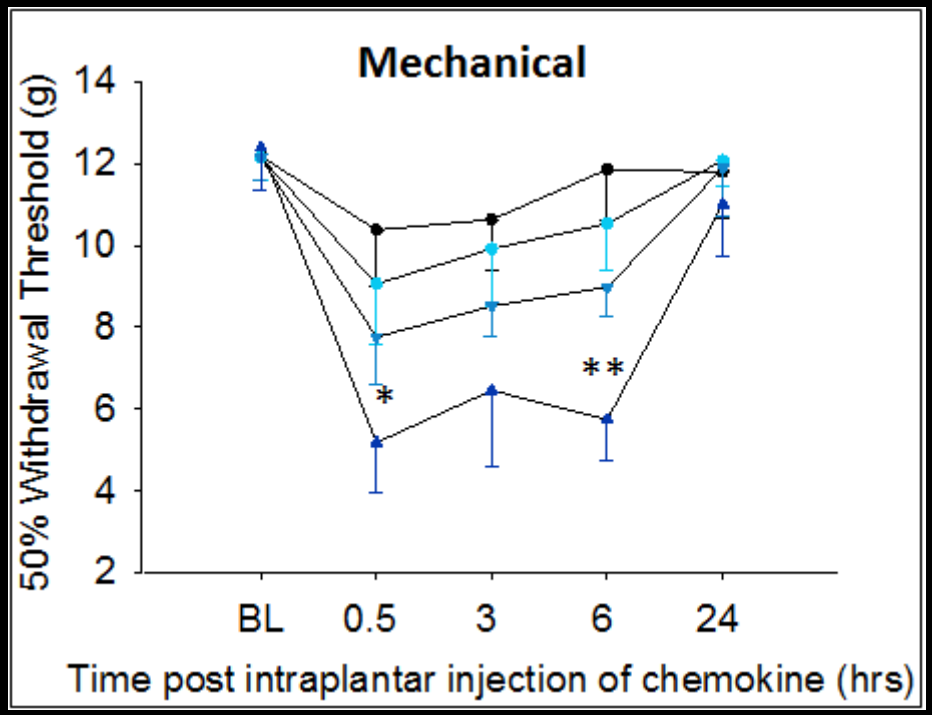
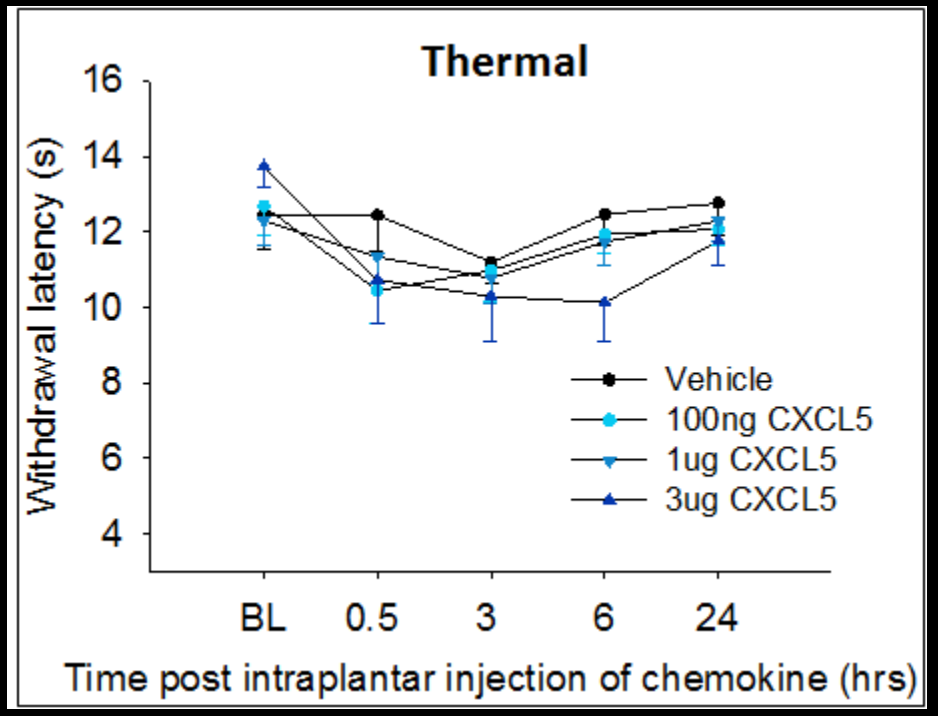


John Dawes

Normalised to GAPDH

CXCL5 induces dose dependent mechanical pain related hypersensitvity in the rat





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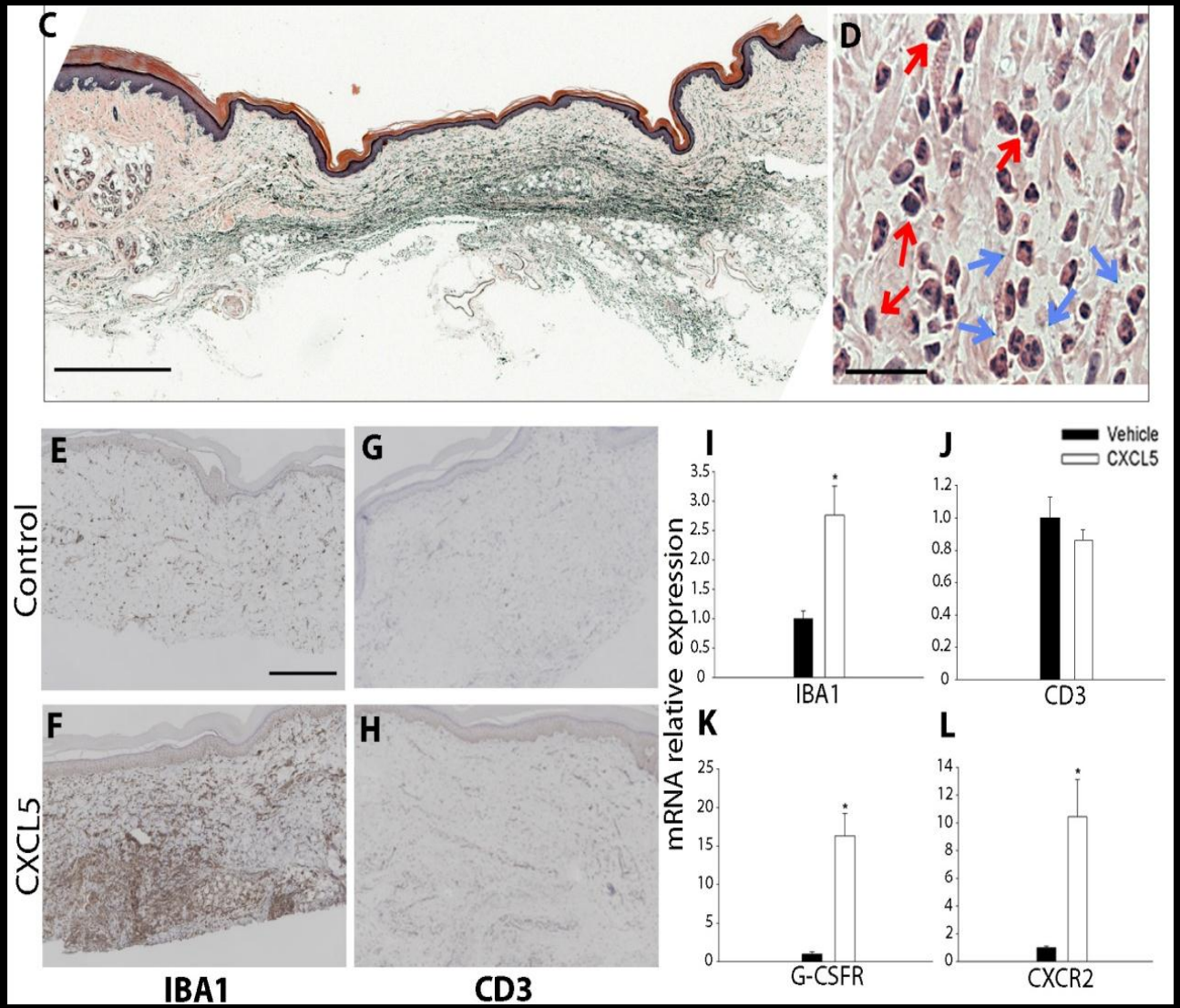
CXCL5 induces immune cell recruitment



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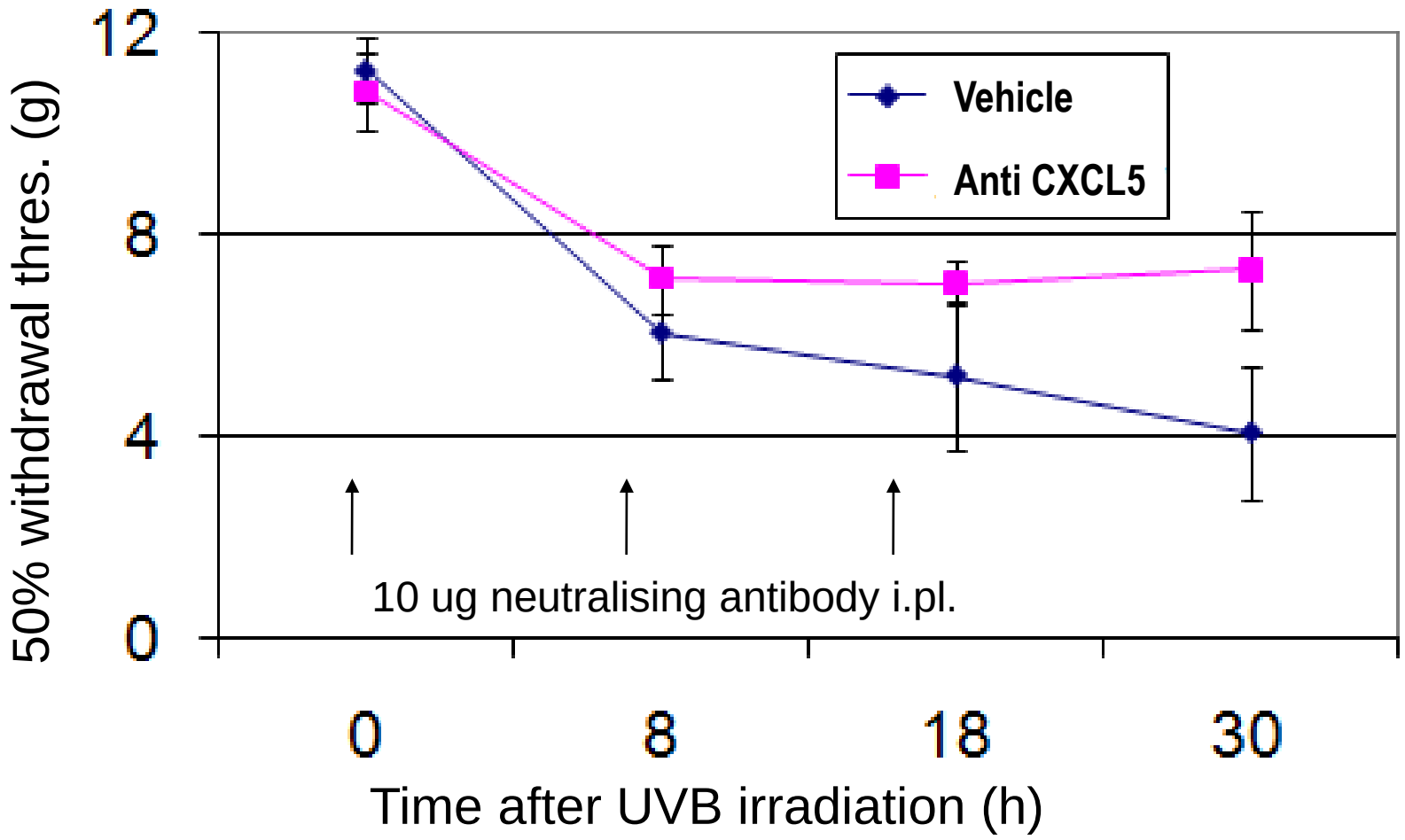
Local chemokine neutralisation can attenuate mechanosensitization after UVB



John Dawes

There are multiple potential applications of the approach of reverse translation

Painful bladder syndrome (Interstitial cystitis)
Ulcerative colitis
Chronic cough
Osteoarthritis
Vulvadynia

immune competency predict post-surgical pain?

Questions

1. How useful are GWAS/sequencing approaches to identify novel pain targets?

2. Is translational failure a result of an obsession of screening over biology?

3. Are there more opportunities for developing peripherally acting drugs?