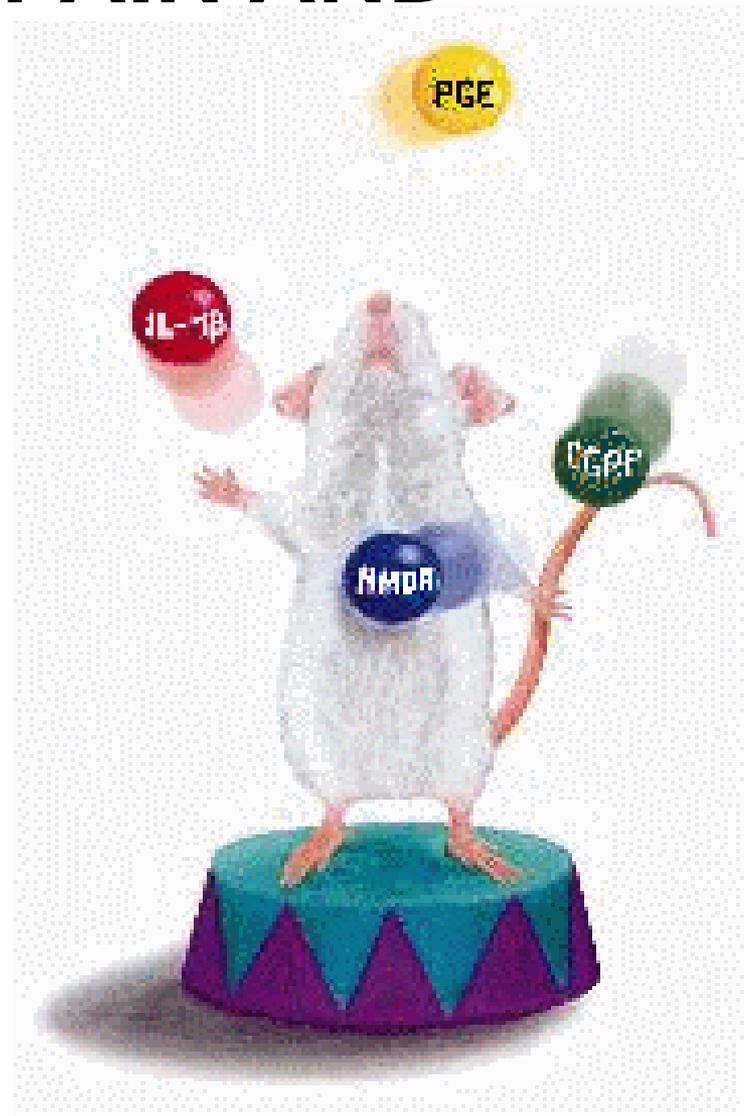


IN VIVO - PRECLINICAL STUDIES USE IN DEFINING PAIN AND ANALGESICS...

Tony Yaksh, Ph.D.

University of California

San Diego



1. Massive expansion in understanding of system physiology and cell biology of central and peripheral systems mediating pain sensation / behavior.

Peripheral terminal

Local milieu
Local release
Sensitization

DRG

Ectopic activity
Sprouting
Satellite cells
In migration

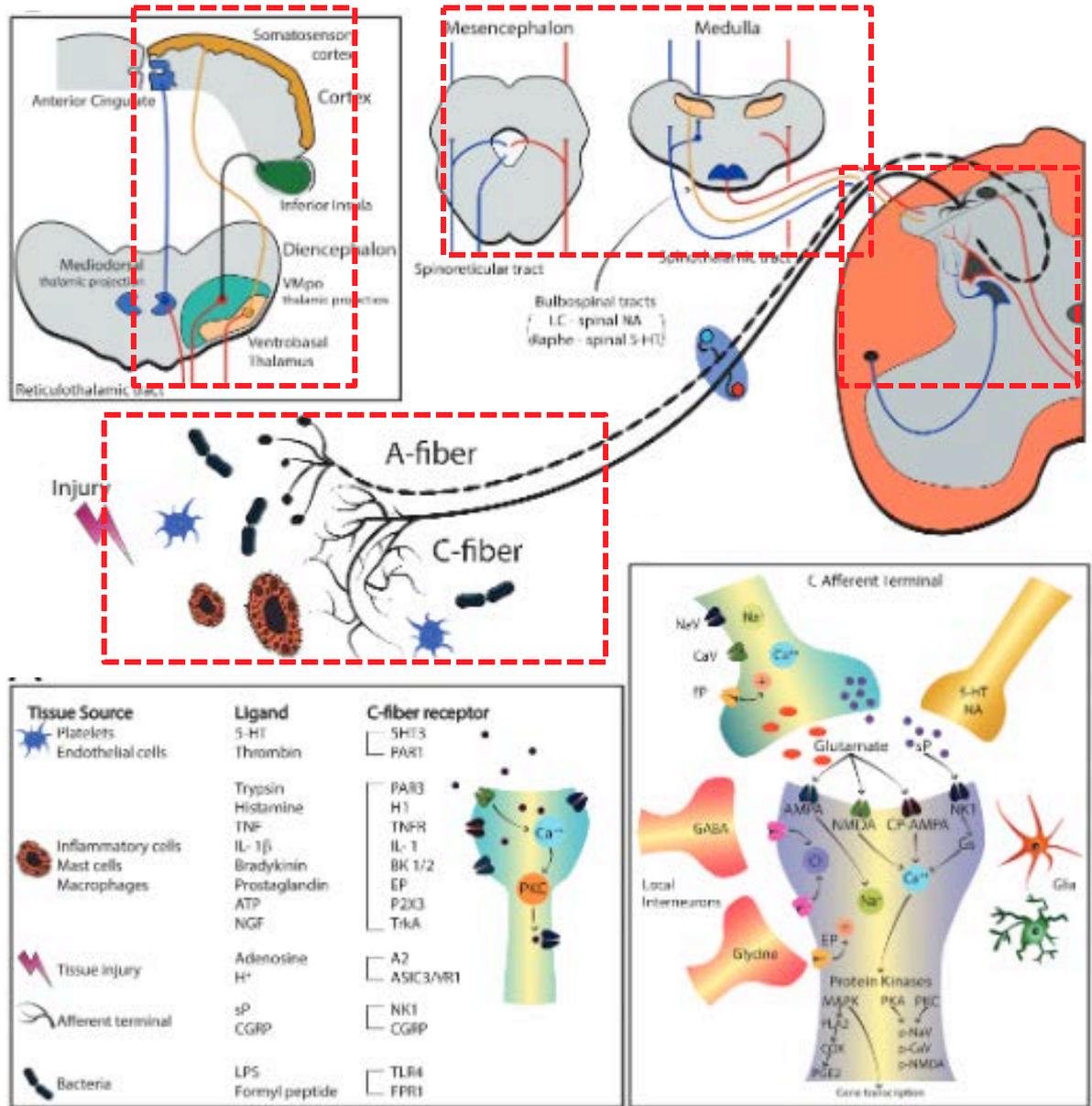
Dorsal horn

Tripartite synapse
Astrocytes/microglia
Local interneurons
Bulbospinal projections
Dynamic processing

Ascending Projection

Sensory - discriminative
 Classic SS pathways
Affective - motivation
 Limbic forebrain

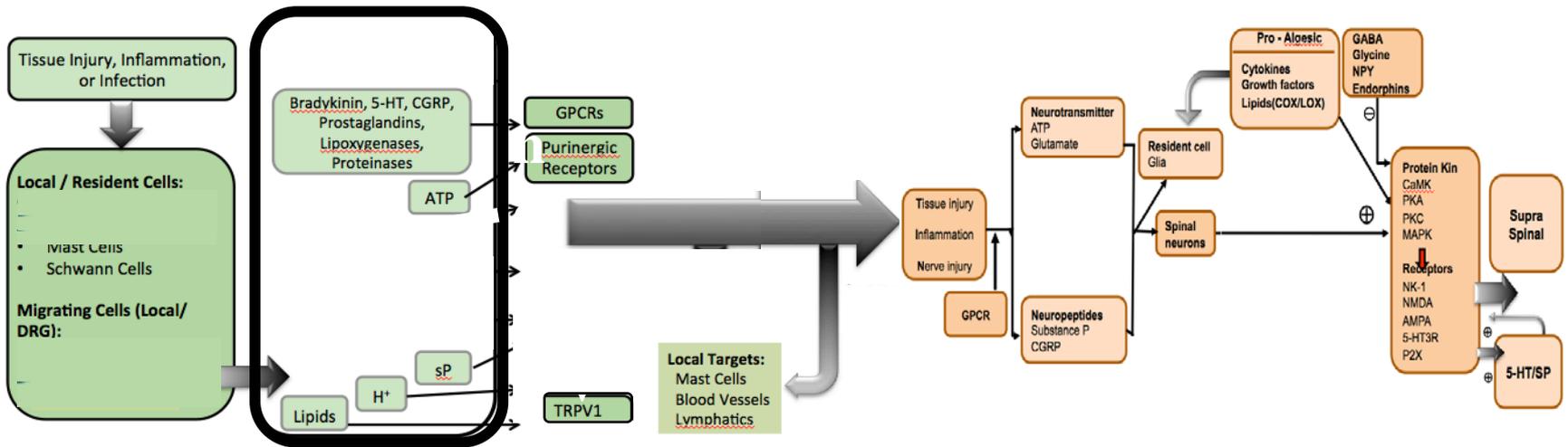
Descending Projections...



Neuraxial systems mediating nociceptive processing is a target rich environment...things have grown over 30 years



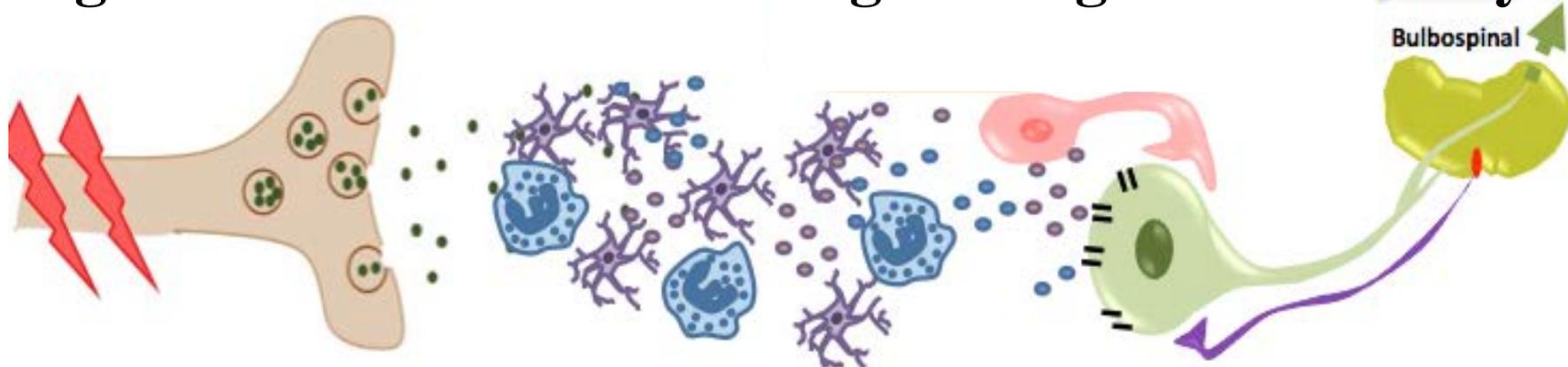
Mid 80's



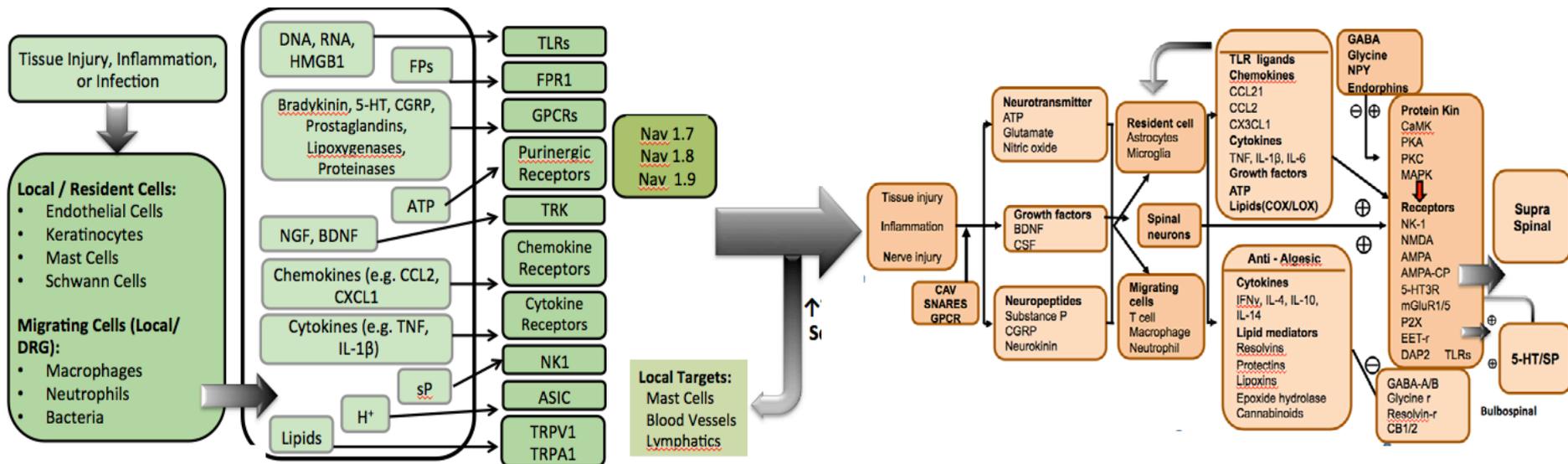
Peripheral Terminal /DRG

Dorsal horn

Neuraxial systems mediating nociceptive processing is a target rich environment...things have grown over 30 years



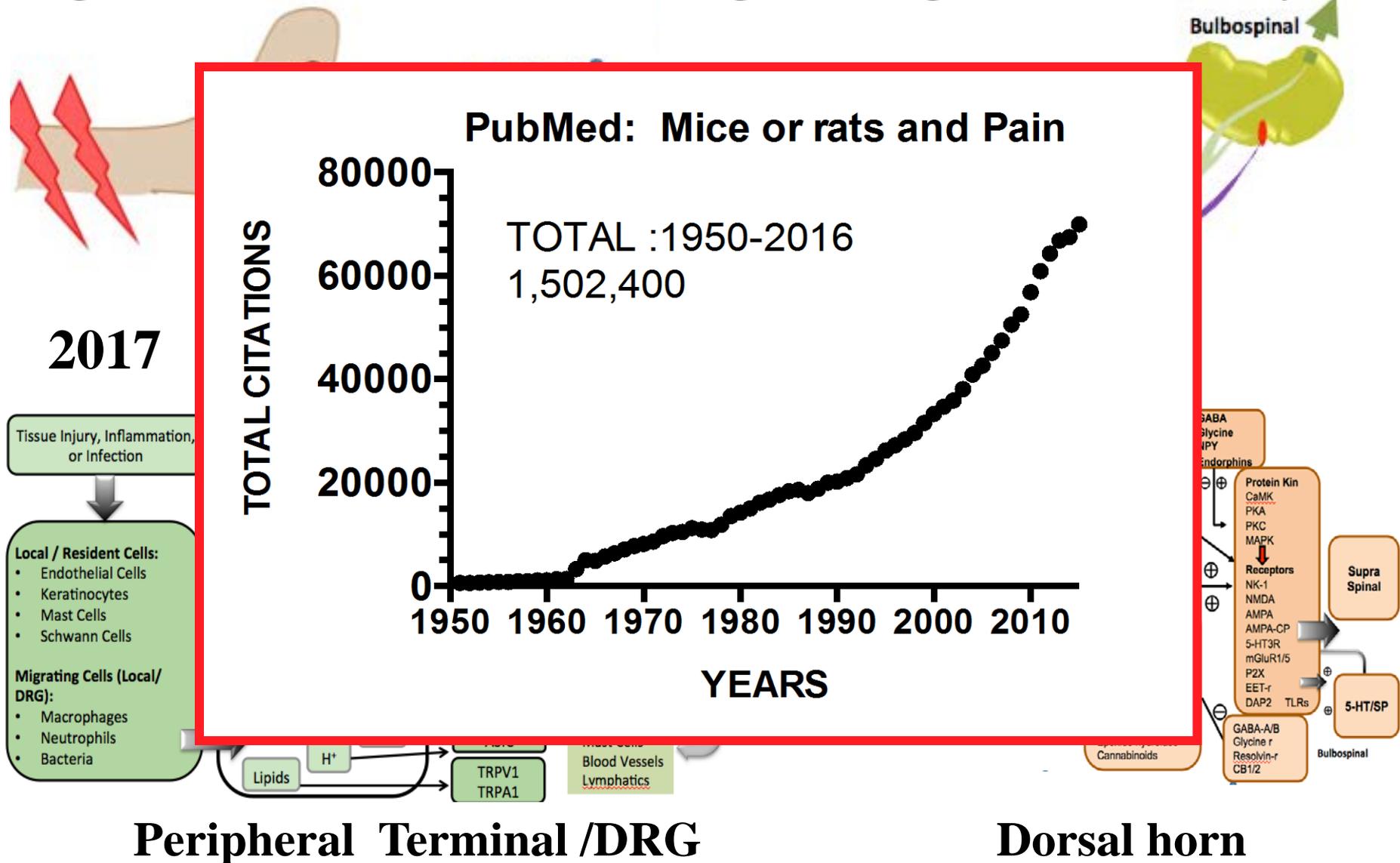
2017



Peripheral Terminal /DRG

Dorsal horn

Neuraxial systems mediating nociceptive processing is a target rich environment...things have grown over 30 years



2. Role of cellular components in “pain processing” is based on demonstrating their role in defining the behavior of the intact / unanesthetized organism in a painful state.

MODELING PAIN STATES

Acute Stimulation

Thermal...> 42 °C / < 4 °C

Mechanical Distortion

Tissue injury

Trauma/Post operative

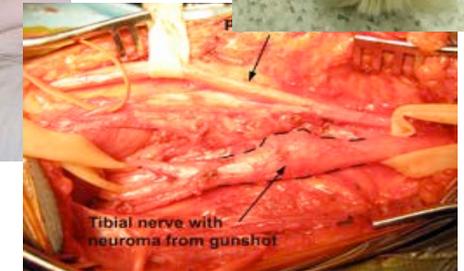
Arthritis

Nerve Injury

Physical

Chemical

Immune



BEHAVIORAL MODELS

TISSUE INJURY/INFLAMMATION

- Acute vs persistent

• “Spontaneous”
 Flinching/guarding/
 altered ambulation

- Evoked:
 1° Hyperalgesia
 2° Allodynia

Treatment	Spont behavior	Evoked response	Time (hr)
IPLT Formalin Ph1: Ph2: Ph3:	Flinching Flinching None	None None TA	0-0.2 0.2-1 124
Paw / Knee Carrageenan	Guard	TH/TA Press Wt bearing Ambulation	1-48
Paw / Knee Freund's Adj	Guard	TH/TA Pressure Wt bearing Ambulation	4-96
Paw Burn	Guard	TH/TA	1-2
Paw Incision	Guard	TA	1-48
Visceral irritant Distention	Guarding vocalization	TA	1->24h
KBxN	Guard	TA Ambulation	Early: inflamed 0 -15d Late: neuropath 15 - >30d
CAIA	Guard	TA Ambulation	Acute: inflamed 0 -15d Late: neuropath 15 - >30d

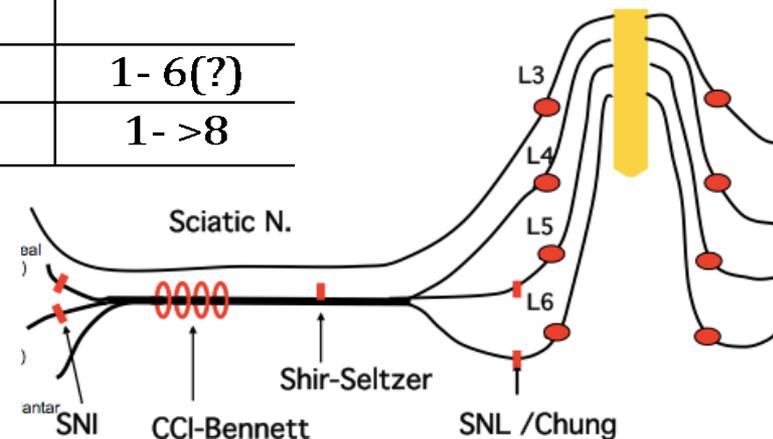
BEHAVIORAL MODELS

NERVE INJURIES

Mononeuropathies: Somatotopically localized 1° and 2° hyperpathia.

Polyneuropathies: Whole body hyperpathia / paws

Treatment	Spont behavior	Evoked response	Time (wks)
Bennett: (CCI) 4 loose ligatures	Guard	TH > TA	1 - 4
Chung : L5 ligation	Guard	TA, CA	1 > 8
Shir / Seltser Hemiligature of sciatic n	Guard	TH/TA/CA	1- >12
Spared nerve (SNI)	Guard	TH/TA/CA	1- >12
IFN ligation	Guard	TA	1- 6(?)
Cisplatin	Guarding	TA/CA	1- >8



PRECLINICAL PHARMACOLOGICAL PROFILES FOR VARIOUS PAIN MODELS

SURVEY OF PRECLINICAL STUDIES: ACTIONS OF DRUG CLASSES IN PAIN MODELS THOUGH 2017.				
DRUG CLASS	ACUTE ^a (Thermal)	TISSUE INJURY INFLAMMATION ^b	MONO NEUROPATHY ^c	POLY NEUROPATHY ^d
μ OR-ag	+ (>50)	+ (>50)	+/- (5)	+ (3)
δ OR ag	+ (11)	+ (7)	+/- (4)	?
α 2 adren ag	+ (14)	+ (14)	+ (4)	+ (3)
COX2 inhib	0 (12)	+ (16)	+/- (3)	+ (3)
Aden A1 ag	+ (5)	+ (4)	+ (3)	?
Acetaminophen	+/- (4)	+ (16)	+ (2)	+ (2)
Gabapentinoids	0 (6)	+ (21)	+ (22)	+ (2)
NK1 antag	0 (10)	+ (13)	+/- (4)	?
AMPA	+ (6)	+ (7)	+ (4)	?
AMPA-CP	+ (2)	+ (3)	?	?
NMDA	0 (6)	+ (14)	+ (21)	+ (4)
Cav 2.2blkr	0 (3)	+ (11)	+ (14)	+ (2)
NaV blkr	+ (4)	+ (6)	+ (13)	+ (3)

(Number of published studies in PubMed though 2017) ^a Acute: Hot Plate, Hargreaves, Paw Pressure; ^bTissue injury: Formalin (Phase2 ,Carrageenan / Burn/ Incision; ^c Mononeuropathy: Bennett / Chung /Shir /SNI; ^d Poly neuropathy: Cisplatin/Taxol, DPN etc.

3. There have been a number of “successful” drug translations. e.g. What worked in the preclinical models resulted in analgesia in the human exposure.

ANALGESIC TARGETS:

CLINICAL EFFICACY TRANSLATIONAL **SUCCESSSES**

Target	Drug/Molecules	(+) Clinical Efficacy
Adrenergic uptake inh		Fibromyalgia
Alpha2 agonist	Dexmedetomidine	Sedative/analgesic
CGRP CGRP-r Ab	ALD-403, Erenamab Galcanezumab, TEV-48125,	Migraine
AT2-R Blker	EMA 401	PHN
Bisphosphonates	clodronate, pamidronate,, zoledronic acid	Bone Mets
CaV2.2 blker	Prialt	NP
CGRP-r anti	Olcegepant, Telcagepant Rimagepan, BI44370TA, Atogepant, Ubrogepant	Migraine
COX-2 inhibitors	Celebrex	OA/RA/JRA
Gabapentinoids	Gabapentin/ Pregabalin	DPN, PHN Fibromyalgia
KOR ag (Periph)	CR845	OA,
Kv7	Flupirtine	Post op
MOR	TRV130 (biased)	Post op
Mu-opioid Combo	Q8003	Bunionectomy
Anti-NGF (ab)	tanezumab, fulranumab	OA
TRPV1 antagonists	Resiniferatoxin (IT) Capsaicin (topical)	Cancer PHN
TTX (sc)	Halneuron	Cancer pain

ANALGESIC TARGETS: CLINICAL EFFICACY TRANSLATIONAL **FAILURES**

Target

		pain. [4][5][6] diabetic neuropathy,
--	--	---

3. The preclinical pain models have many failures...How do they compare to other modeling targets?

LIKLIHOOD OF APPROVAL (LOA) FOR DRUG TO PROGRESS FROM PHASE 1 TO PHASE 2 AND PHASE 2 TO PHASE 3 (2003-2011)

	PAIN	PSYCHIATRY	INFECTIOUS DISEASE	RHEUMATOID	SOLID TUMOR
Ph 1 to Ph 2	10.8%	7.2%	16.7%	10.3%	5.7%
Ph 2 to Ph 3	15.9%	12.0%	25.4%	13.9%	8.6%

Extracted from Hayes, et al, 2014; Table 5-8. Courtesy [John H. Kehne, Ph.D](#)

FUTURE DIRECTIONS TO ADDRESS CURRENT SHORT COMINGS IN PRECLINICAL PAIN MODELS

Move to longer term studies.

Inflammation: KBxN/CAIA weeks to month

Joint remodeling/sprouting

Closer attention to recapitulating human phenotype

Irritable bowel syndrome: inflammation vs early stressors

Joint remodeling

Address spontaneous vs evoked (threshold) behaviors

Diurnal activity, incidence of rearing, gait, wt bearing
traversing of aversive environment

Assessment of re-enforcing effects of “pain relief”

Variations on conditioned place preference.

Naturally occurring pathology

IATROGENIC PAIN STATES

Rodent / Non Rodent

- Chemotherapy-induced neuropathy: vincristine, taxol, cisplatin
- Viral neuropathy: HSV-1, HIV injection
- Inflammatory neuritis : perisciatic Zymosan injection -
- Neuroma : nerve transection, cryoneurolysis of sciatic nerve
- Spinal cord injury: contusion, ischemia
- Spinal stenosis : silicone rubber, steel rod
- Gout: urate crystals in joint spaces
- Cystitis: cyclophosphamide ip
- Charcot-Marie-Tooth: chr17p11.2, PMP22 transgenic
- Guillian-Barre experimental. autoimmune neuritis (EAN);
- Demyelinating polyneuropathy (CIDP): peripheral myelin+Freunds adj
- Irritable Bowel syndrome. (Neonatal stressors).
- Osteosarcoma (syngenic cells in marrow)
- CRPS (femoral fracture)

WHAT ARE WE (trying) TO MODELING?

Fibromyalgia
10 million in
US....estimated
3-6% of world
population.

Unique syndrome diagnosed based on symptoms and is of uncertain etiology... female > male

Symptomatology: musculoskeletal pain, fatigue, anxiety, affective disorders, TMJ, visceral hyperalgesia, dysautonomia, and sleep disorders..

Covariance with the presence of abnormal sensitization and temporal summation of second pain

Preclinical models

**Intramuscular hypertonic saline
Reserpine induced myalgia
Cold stress**

4. A broader issue....

preclinical research: Reproducibility

- Amgen chose 53 land mark papers in cancer research....Replicated .. 6 of them. Begley and Ellis, 2012**
- Bayer reported that about 25 % of published preclinical findings could be replicated. Rubin and Gilliland, 2012**
- Pharma success in internally replicating external reports in the pain field?**
- Yaksh laboratory. (29-30 LSAs /yr
Failure to recapitulate in house data from four sponsors**

NEED FOR THE OBVIOUS

Appropriate and detailed reporting of methods and results

- **Randomization**
- **Blinding**
- **A priori sample size estimation**
- **Data handling**
- **Stronger emphasis upon effect size**
- **Routine inclusion of active controls**

- **Emphasis upon replication**
 internal and independent
- **Sourcing of negative data.**

Prinz, et al, 2011, Begley and Ellis, 2012; Landis, et al, 2013; Martic-Kehl and Shubiger, 2016; (and many others)

A modest proposal.....

Recapitulate in part the Epilepsy program

ANALGESICS SCREENING CONSORTIUM

Aim: Develop a mechanism for developing high quality, well controlled data for analgesic candidate molecules.

Organization: Establish a directorate (intra and extramurally funded (FNIH?))...Directorate is administrative unit: establish precise protocols, provide training for consortium participants (and students), perform data management and quality control...

Undertake organized research by consortium. Each molecule would be examined by multiple groups.

Anesthesiology Research Laboratory, UCSD



UCSD

Marypat Corr, MD
Yury Miller, MD
Jeff Allen, PhD
Alvaro Cisternas, DVM
Kelly Eddinger, BS
Tyler Hochman, DVM
A. Myanohara, PhD
Shelle Malkmus, BS
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Ashley Wiese, DVM
X-Y Xu, PhD

Mayo Clinic

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Camilla Svensson, Ph.D.

Medtronics

Keith Hildebrand, DVM
Linda Page, Pharm D.

OHSU

Marjorie Grafe, MD, PhD

Univ College London

SuEllen Walker, MD, PhD

R01 NIDA 15353

R01 DE022757

R01 NS102432

R01 NS099338

PRECLINICAL MODELS

SurrogateTarget



Surrogate Validation: Face-Construct ...Predictive

Mechanism

System Pharmacology

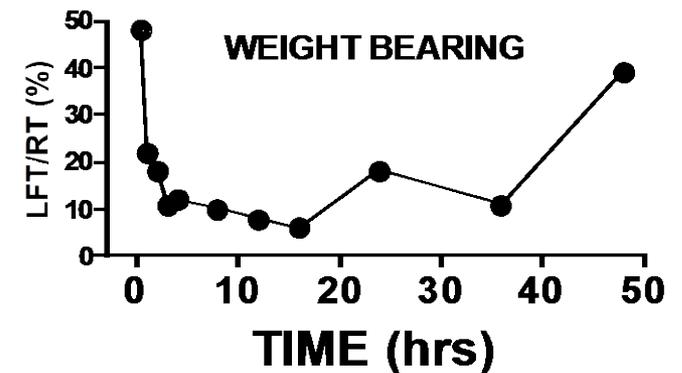
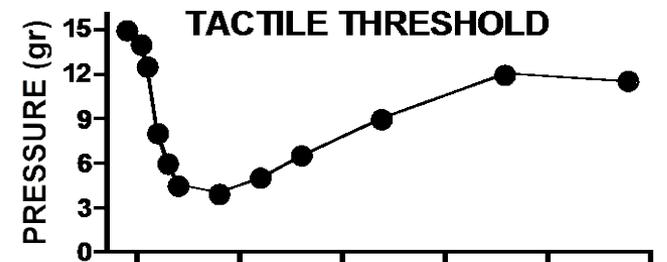
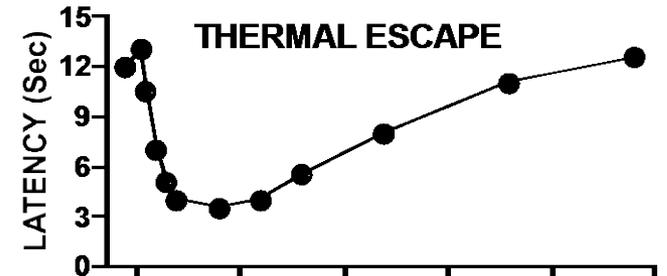
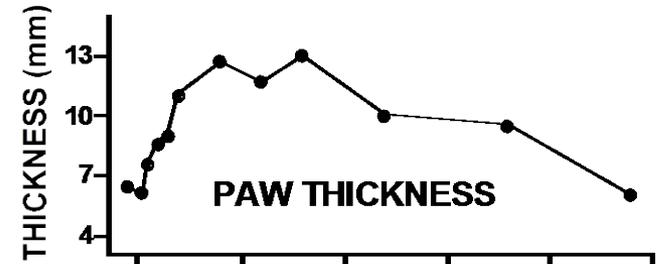
Functional phenotype

**HUMAN
PHENOTYPE**

POST TISSUE INJURY PAIN STATES

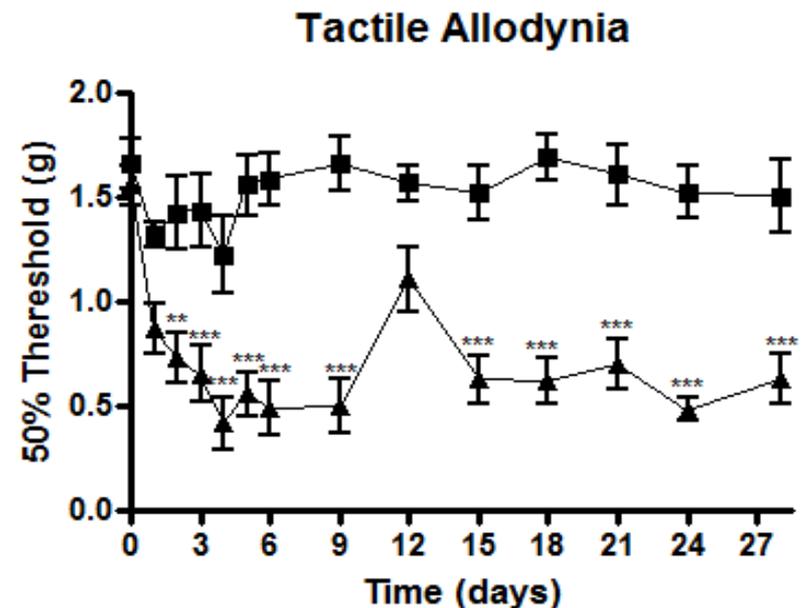
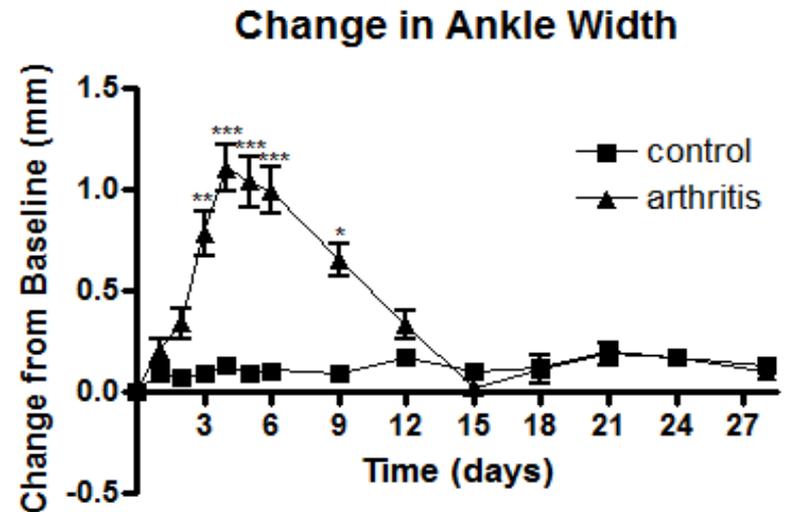
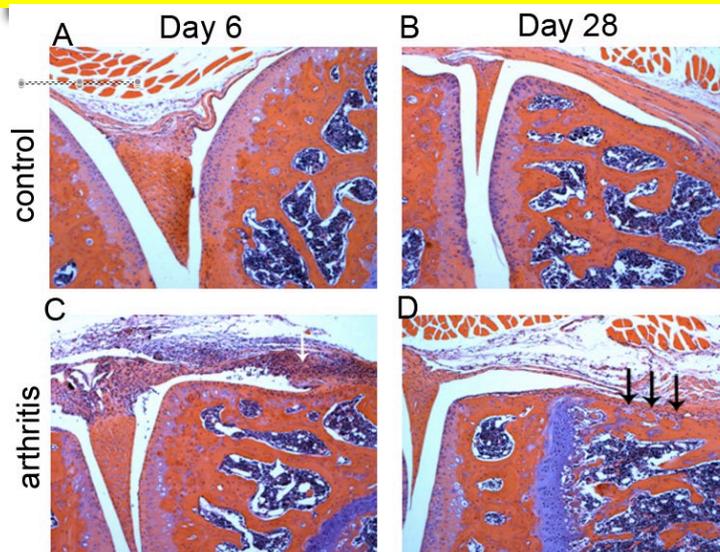
IPLT carrageenan: 0.5 - 48 hrs

- ↑ Paw Swelling
- ↑ Thermal Hyperalgesia
- ↑ Tactile allodynia
- ↓ weight bearing
- ↓ Spontaneous activity



K/BxN arthritis...Acute to Chronic

- Extremity inflammation (1-15 days)
- Neutrophils (early)
- Joint remodeling (late)
- Mechanical allodynia persists after decrease in inflammation.



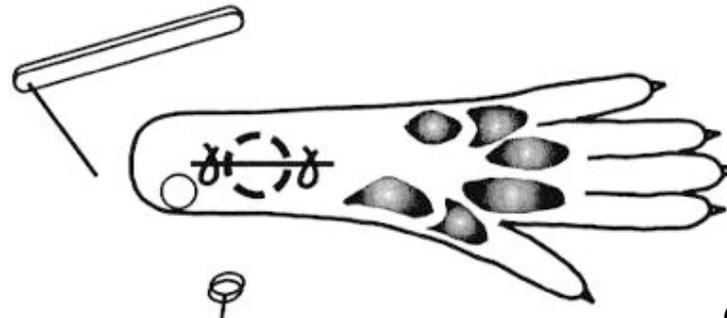
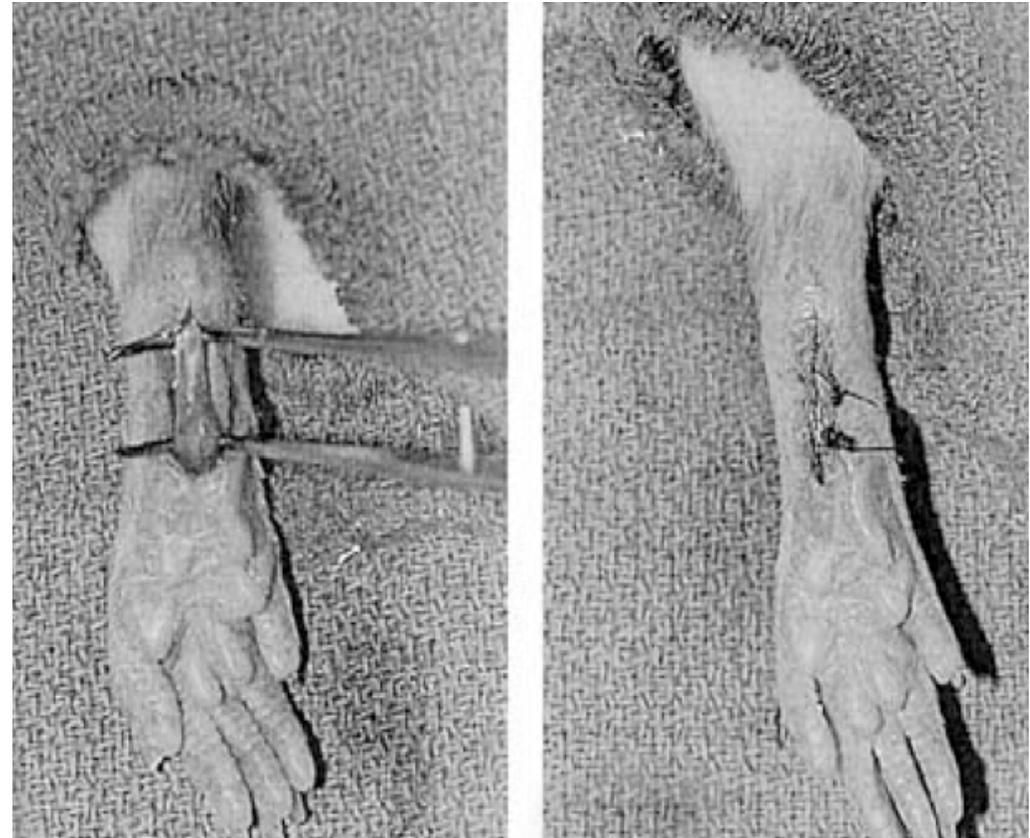
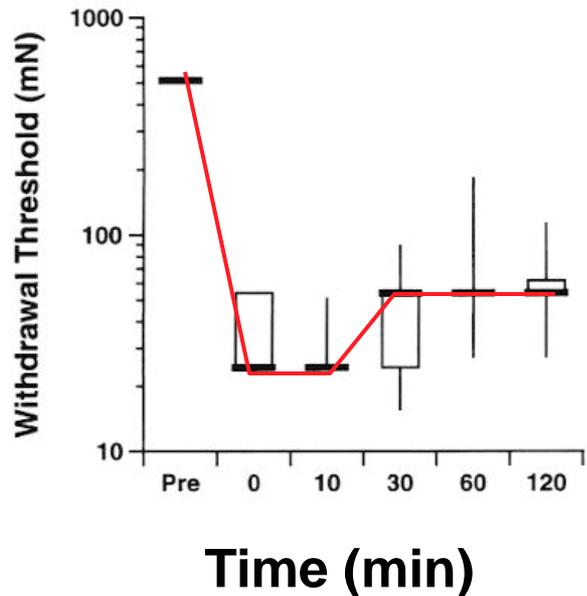
ACUTE POST TISSUE INJURY PAIN STATES

- Incision of plantar surface → 2° Tactile allodynia

Plantar paw incision

1° and 2° tactile allodynia
and mechan hyperalgesia

Onset in mins, persist for
48-96 hrs.



POST TISSUE INJURY PAIN STATES

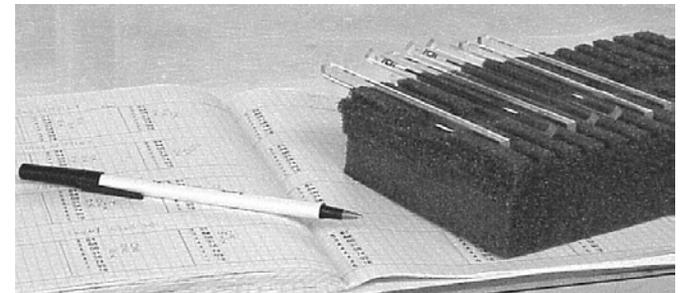
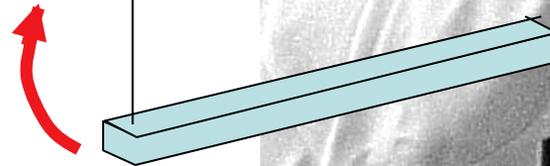
- Incision of plantar surface → 2° Tactile allodynia



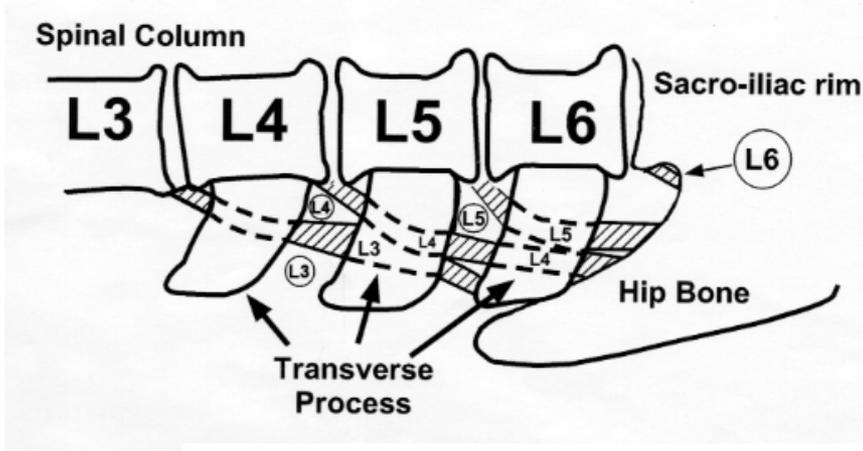
FIGURE 27.—Longitudinal heel-splitting incision used to debride infected calcaneus and plantar heel wounds.

VON FREY HAIR TESTING

- Assessment of local Tactile thresholds of skin
- Response is withdrawal to touch by probe
- Thresholds assessed by:
 - % response to repeated application of a fixed stimulus
 - “up-down” method
- Normally innocuous.....
Thresholds > 15 gms
- With local “sensitization” thresholds fall.

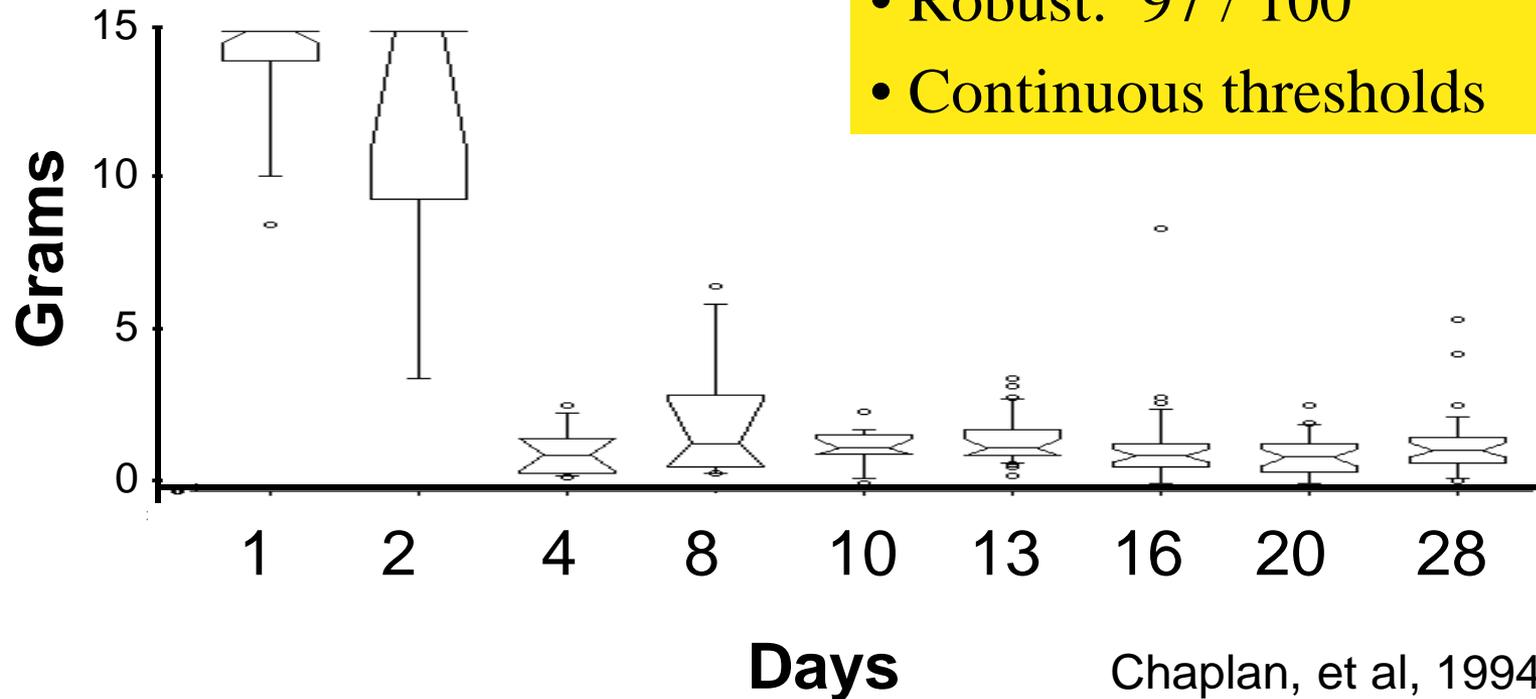


NERVE INJURY MODEL: Chung (L5/6 Ligation)



CHUNG MODEL- RAT

- Minimal motor impairment
- Modest change in wt gain
- Stable allodynia > 24 days
- Robust: 97 / 100
- Continuous thresholds



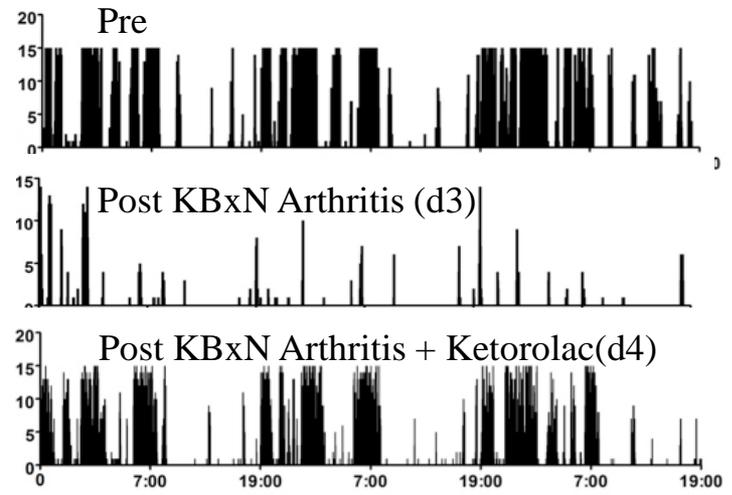
Chaplan, et al, 1994

ACTIVITY: Ambulation / Weight bearing

Pain...suppresses/ inhibits normal motor function

- **Spontaneous activity**

Depression of 24 activity cycle in chronic arthritis



Mohammed Suhail

- **Weight Bearing**

Asymetry in Inflamed vs Un-inflamed limb

- **Ambulation**

Cat walk type devices....walking
/ wt bearing

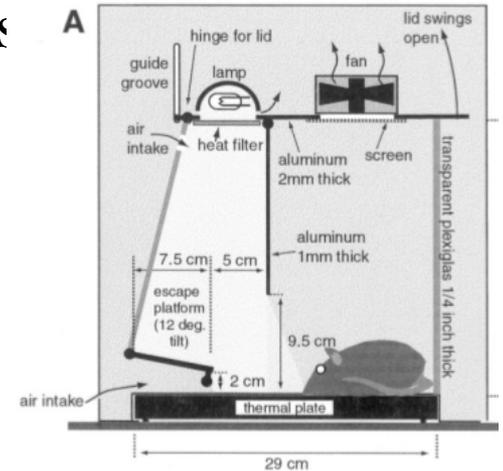
Recovery of function with analgesic



Operant escape models

Pain..negatively reinforcing experience.. Animals behave to avoid painful / stressful experiences

Operant.. Device provide a thermal stimulus which forces the animal to escape to a stressful environment (bright light).



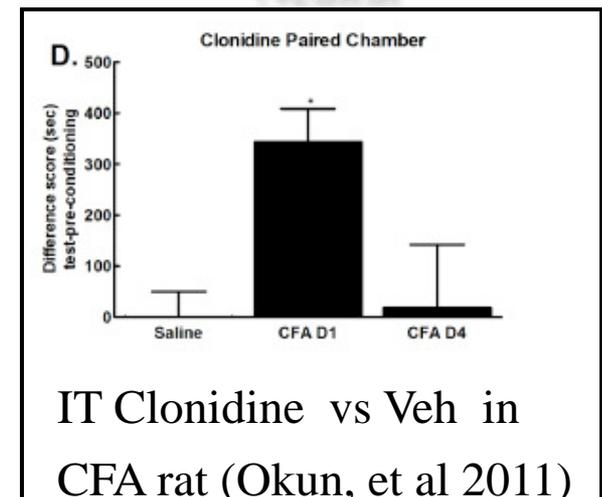
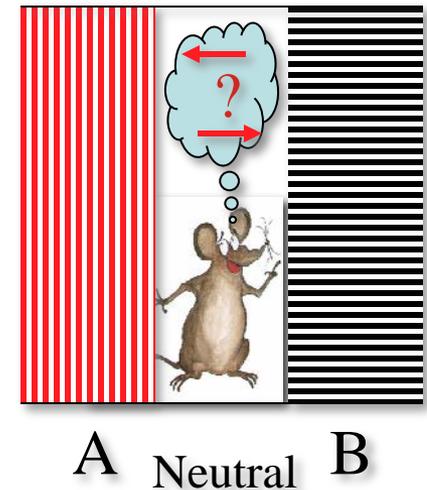
Mauderli, et al, 2000

Conditioned Place Preference Paradigm

Pain..negatively reinforcing experience.. Animals behave to avoid painful / stressful experiences....Conversely, animals will seek environment that reduces negative reinforcing properties of pain (e.g. as with an analgesic).

To test an analgesic..

1. Initiate a pain state (e.g. nerve injury / CFA)
2. Assess preference of the rat to either A or B after placement in Neutral chamber
3. Adapt animal over days.
4. Initiate daily Tx, placing animal in chamber A to receive drug or B to receive vehicle
5. 5. On test day, place animal in neutral chamber and give no drug.. Where does rat go?



SUMMARY: Published KOs / transgenic mice in pain studies

Broad range of
reported KO/TGs

Caveats:

- All pain end points are not the same
- Background stains for appropriate controls.

Genotype	Phenotype
β -endorphin KO	Abolished opioid analgesia induced by stress.
Galanin OE	Elevation of nociceptive threshold to thermal stimulation
G prot Go- α o KO	Thermal hyperalgesic and motor control impairment
IFN- γ KO	Abolished pain behavior after sciatic section
IL-6 KO	Reduced analgesic effects of morphine
κ -opioid R KO	Enhanced sensitivity to visceral pain. No effect of κ -opioid
NET KO	Enhanced opiate analgesia
NGF KO NGF OE	Mediate inflame-induced peripheral/central sensitization. Hyperalgesic to mechanical and thermal stimulation
NK-1 R	.Attenuation in response to heat hyperalgesia
NMDA-NR2B, OE	Enhanced responsiveness to formalin /CFA
NPY KO	Increased autotomy behavior after sciatic transection
PKA RI β KO	Reduction in tissue injury-induced persistent pain,
PKC- γ KO	Blocked neuropathic syndrome after sciatic injury
Prodynorphin KO	Accelerated recovery after nerve ligation
ppENK KO	Reduced supraspinal, but not spinal, pain threshold
μ -opioid R KO	Shortened latencies on thermal escape
δ -opioid R KO	Abolished spinal δ -opioid analgesia
Substance P OE	Facilitation o opiate-mediated antinociceptive mechanism
TNF OE	Enhanced allodynia after spinal nerve transection

EFFECT OF MOUSE STRAIN SELECTION ON FORMALIN EVOKED RESPONSES

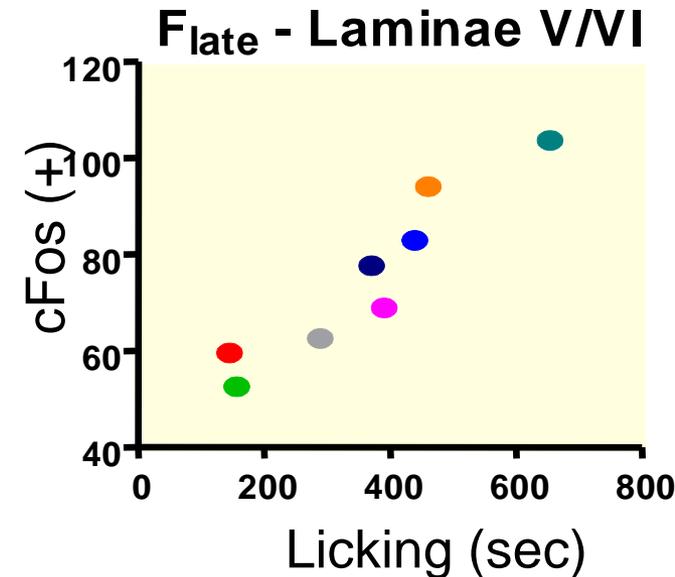
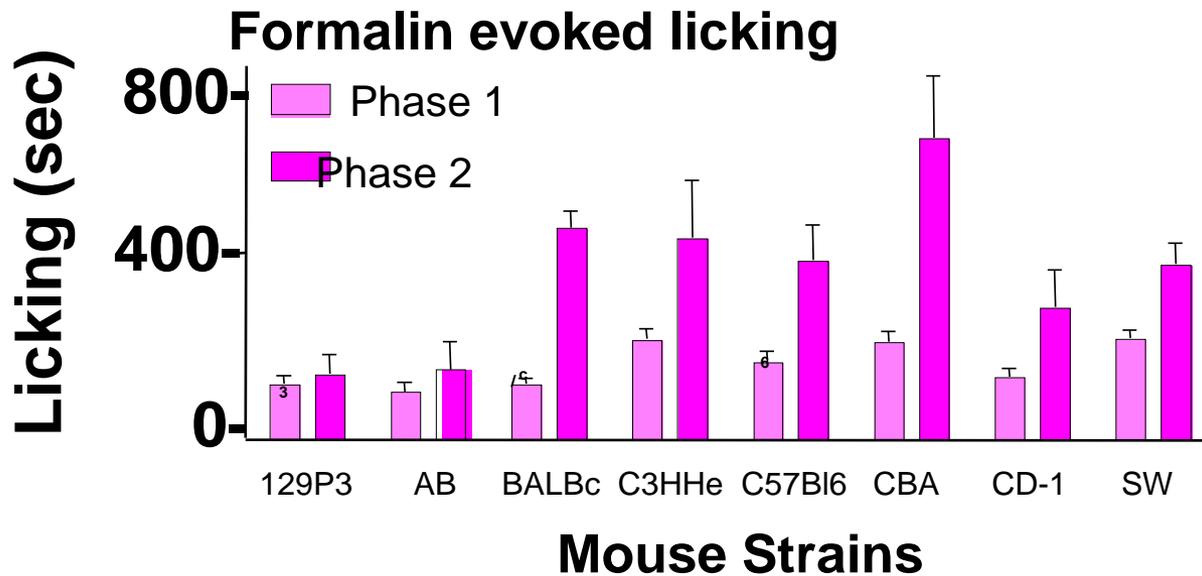
Screening of murine strains: Intraplantar formalin

Phase 1/2-Licking

CBA > BALBc > C3HHe > C57Bl6 > SW > CD1 > AB > 129P3A

Dorsal horn cFos activation

Covariance with Phase 2 response .



Facilitated Component covaries with CFOS and Strain

FACTORS IN CHARACTERIZING ANALGESICS.

Pain /analgesic mechanisms may vary by strain

- Basis for genetic linkage studies
- Definition of underlying mechanisms

Are we 129s or BALBc or ?



Mechanisms may vary by sex

Analgesic effects are impacted by model characteristics.

- Drug effects dependent upon stimulus intensity
(Increased stimulus intensity shifts DR curve to right)
- Same end point different pharmacology?
(Nerve vs Inflammation evoked allodynia)

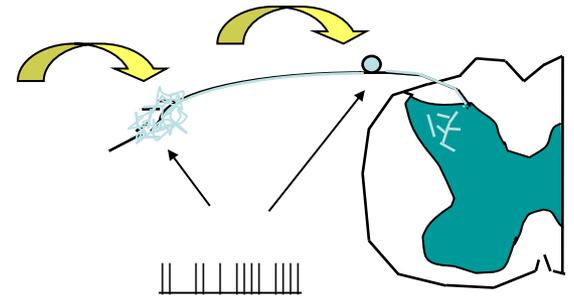
Analgesic effects are mechanism dependent

- All pain is not the same.
- Choice of test models depends upon hypothesized mechanisms

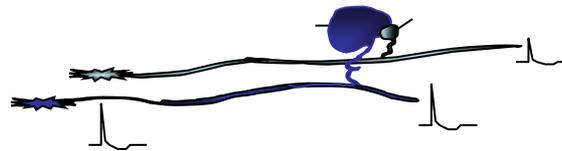
**NERVE
INJURY** →

**Spontaneous afferent activity
Mechanical sensitivity**

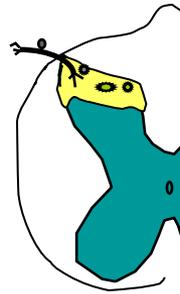
Ectopic activity: Neuroma / DRG



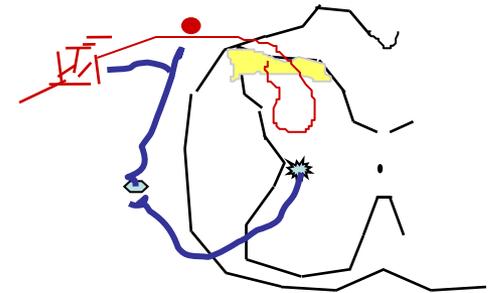
Afferent cross talk:



**Loss of inhibition
GABA/Glucine**



Sprouting: Neuroma
Post gang sympathetics



The Future of Pain research: *Dasypus novemcinctus*

