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

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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 Projections
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
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spont behavior</th>
<th>Evoked response</th>
<th>Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPLT Formalin</td>
<td>Flinching</td>
<td>None</td>
<td>0-0.2</td>
</tr>
<tr>
<td>Ph1:</td>
<td>Flinching</td>
<td>None</td>
<td>0.2-1</td>
</tr>
<tr>
<td>Ph2:</td>
<td>None</td>
<td>TA</td>
<td>124</td>
</tr>
<tr>
<td>Ph3:</td>
<td></td>
<td>Wt bearing Ambulation</td>
<td></td>
</tr>
<tr>
<td>Paw / Knee Carrageenan</td>
<td>Guard</td>
<td>TH/TA Press Wt bearing Ambulation</td>
<td>1-48</td>
</tr>
<tr>
<td>Paw / Knee Freunds Adj</td>
<td>Guard</td>
<td>TH/TA Pressure Wt bearing Ambulation</td>
<td>4-96</td>
</tr>
<tr>
<td>Paw Burn</td>
<td>Guard</td>
<td>TH/TA</td>
<td>1-2</td>
</tr>
<tr>
<td>Paw Incision</td>
<td>Guard</td>
<td>TA</td>
<td>1-48</td>
</tr>
<tr>
<td>Visceral irritant Distention</td>
<td>Guarding vocalization</td>
<td>TA</td>
<td>1-&gt;24h</td>
</tr>
<tr>
<td>KBxN</td>
<td>Guard</td>
<td>TA Ambulation</td>
<td>Early: inflamed 0-15d Late: neuropath 15-30d</td>
</tr>
<tr>
<td>CAIA</td>
<td>Guard</td>
<td>TA Ambulation</td>
<td>Acute: inflamed 0-15d Late: neuropath 15-30d</td>
</tr>
</tbody>
</table>
BEHAVIORAL MODELS
NERVE INJURIES

Mononeuropathies: Somatotopically localized 1° and 2° hyperpathia.
Polyneuropathies: Whole body hyperpathia / paws

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spont behavior</th>
<th>Evoked response</th>
<th>Time (wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bennett:</strong> (CCI) 4 loose ligatures</td>
<td>Guard</td>
<td>TH &gt; TA</td>
<td>1 - 4</td>
</tr>
<tr>
<td><strong>Chung:</strong> L5 ligation</td>
<td>Guard</td>
<td>TA, CA</td>
<td>1 &gt; 8</td>
</tr>
<tr>
<td><strong>Shir / Seltser</strong> Hemiligation of sciatic n</td>
<td>Guard</td>
<td>TH/TA/CA</td>
<td>1 - &gt;12</td>
</tr>
<tr>
<td><strong>Spared nerve (SNI)</strong></td>
<td>Guard</td>
<td>TH/TA/CA</td>
<td>1 - &gt;12</td>
</tr>
<tr>
<td><strong>IFN ligation</strong></td>
<td>Guard</td>
<td>TA</td>
<td>1 - 6(?)</td>
</tr>
<tr>
<td><strong>Cisplatin</strong></td>
<td>Guarding</td>
<td>TA/CA</td>
<td>1 - &gt;8</td>
</tr>
</tbody>
</table>

[Diagram of nerve injuries]
## PRECLINICAL PHARMACOLOGICAL PROFILES FOR VARIOUS PAIN MODELS

**SURVEY OF PRECLINICAL STUDIES: ACTIONS OF DRUG CLASSES IN PAIN MODELS THROUGH 2017.**

<table>
<thead>
<tr>
<th>DRUG CLASS</th>
<th>ACUTE* (Thermal)</th>
<th>TISSUE INJURY INFLAMMATION⁵</th>
<th>MONO NEUROPATHY⁶</th>
<th>POLY NEUROPATHY⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ OR-ag</td>
<td>+ (50)</td>
<td>+ (50)</td>
<td>+/- (5)</td>
<td>+ (3)</td>
</tr>
<tr>
<td>δ OR ag</td>
<td>+ (11)</td>
<td>+ (7)</td>
<td>+/- (4)</td>
<td>?</td>
</tr>
<tr>
<td>α2 adren ag</td>
<td>+ (14)</td>
<td>+ (14)</td>
<td>+ (4)</td>
<td>+ (3)</td>
</tr>
<tr>
<td>COX2 inhib</td>
<td>0 (12)</td>
<td>+ (16)</td>
<td>+/- (3)</td>
<td>+ (3)</td>
</tr>
<tr>
<td>Aden A1 ag</td>
<td>+ (5)</td>
<td>+ (4)</td>
<td>+ (3)</td>
<td>?</td>
</tr>
<tr>
<td>Acetominophen</td>
<td>+/- (4)</td>
<td>+ (16)</td>
<td>+ (2)</td>
<td>+ (2)</td>
</tr>
<tr>
<td>Gabapentinoids</td>
<td>0 (6)</td>
<td>+ (21)</td>
<td>+ (22)</td>
<td>+ (2)</td>
</tr>
<tr>
<td>NK1 antag</td>
<td>0 (10)</td>
<td>+ (13)</td>
<td>+/- (4)</td>
<td>?</td>
</tr>
<tr>
<td>AMPA</td>
<td>+ (6)</td>
<td>+ (7)</td>
<td>+ (4)</td>
<td>?</td>
</tr>
<tr>
<td>AMPA-CP</td>
<td>+ (2)</td>
<td>+ (3)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>NMDA</td>
<td>0 (6)</td>
<td>+ (14)</td>
<td>+ (21)</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Cav 2.2blk</td>
<td>0 (3)</td>
<td>+ (11)</td>
<td>+ (14)</td>
<td>+ (2)</td>
</tr>
<tr>
<td>NaV blkr</td>
<td>+ (4)</td>
<td>+ (6)</td>
<td>+ (13)</td>
<td>+ (3)</td>
</tr>
</tbody>
</table>

(Number of published studies in PubMed though 2017)  

*Acute: Hot Plate, Hargreaves, Paw Pressure;  
Tissue injury: Formalin (Phase2 ,Carrageenan / Burn/ Incision;  
Mononeuropathy: Bennett / Chung /Shir /SNI;  
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.
<table>
<thead>
<tr>
<th>Target</th>
<th>Drug/Molecules</th>
<th>(+) Clinical Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic uptake inh</td>
<td></td>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>Alpha2 agonist</td>
<td>Dexmedetomidine</td>
<td>Sedative/analgesic</td>
</tr>
<tr>
<td>CGRP CGRP-r Ab</td>
<td>ALD-403, Erenamab Galcanezumab, TEV-48125,</td>
<td>Migraine</td>
</tr>
<tr>
<td>AT2-R Blker</td>
<td>EMA 401</td>
<td>PHN</td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>clodronate, pamidronate, zoledronic acid</td>
<td>Bone Mets</td>
</tr>
<tr>
<td>CaV2.2 blker</td>
<td>Prialt</td>
<td>NP</td>
</tr>
<tr>
<td>CGRP-r anti</td>
<td>Olcegepant, Telcagepant, Rimagepan, BI44370TA, Atogepant, Ubrogepant</td>
<td>Migraine</td>
</tr>
<tr>
<td>COX-2 inhibitors</td>
<td>Celebrex</td>
<td>OA/RA/JRA</td>
</tr>
<tr>
<td>Gabapentinoids</td>
<td>Gabapentin/ Pregablin</td>
<td>DPN, PHN Fibromyalgia</td>
</tr>
<tr>
<td>KOR ag (Periph)</td>
<td>CR845</td>
<td>OA,</td>
</tr>
<tr>
<td>Kv7</td>
<td>Flupirtine</td>
<td>Post op</td>
</tr>
<tr>
<td>MOR</td>
<td>TRV130 (biased)</td>
<td>Post op</td>
</tr>
<tr>
<td>Mu-opioid Combo</td>
<td>Q8003</td>
<td>Bunionectomy</td>
</tr>
<tr>
<td>Anti-NGF (ab)</td>
<td>tanezumab, fulranumab</td>
<td>OA</td>
</tr>
<tr>
<td>TRPV1 antagonists</td>
<td>Resiniferatoxin (IT), Capsaicin (topical)</td>
<td>Cancer PHN</td>
</tr>
<tr>
<td>TTX (sc)</td>
<td>Halneuron</td>
<td>Cancer pain</td>
</tr>
</tbody>
</table>
ANALGESIC TARGETS:
CLINICAL EFFICACY TRANSLATIONAL FAILURES

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

**Likelihood of Approval (LOA) for Drug to Progress from Phase 1 to Phase 2 and Phase 2 to Phase 3 (2003-2011)**

<table>
<thead>
<tr>
<th></th>
<th>Pain</th>
<th>Psychiatry</th>
<th>Infectious Disease</th>
<th>Rheumatoid</th>
<th>Solid Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph 1 to Ph 2</td>
<td>10.8%</td>
<td>7.2%</td>
<td>16.7%</td>
<td>10.3%</td>
<td>5.7%</td>
</tr>
<tr>
<td>Ph 2 to Ph 3</td>
<td>15.9%</td>
<td>12.0%</td>
<td>25.4%</td>
<td>13.9%</td>
<td>8.6%</td>
</tr>
</tbody>
</table>

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 landmark 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.

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
R Ramachandran, PhD
Ashley Wiese, DVM
X-Y Xu, PhD

Mayo Clinic
Andreas Beutler, MD, PhD

University of Wisconsin
Sabine Pellett, Ph.D
Eric Johnson, PhD

Karolinska Institute
Camilla Svensson, Ph.D

Medtronics
Keith Hildebrand, DVM

OHSU
Marjorie Grafe, MD, PhD

Univ College London
SuEllen Walker, MD, PhD

R01 NIDA 15353
R01 DE022757
R01 NS102432
R01 NS099338
PRECLINICAL MODELS

Surrogate ............... Target

Surrogate Validation: Face-Construct ... Predictive

Mechanism
System Pharmacology
Functional phenotype

HUMAN PHENOTYPE
POST TISSUE INJURY PAIN STATES

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

IPLT carrageenan: 0.5 - 48 hrs

Graphs show changes in:
- Paw thickness (PAW THICKNESS)
- Thermal escape (THERMAL ESCAPE)
- Tactile threshold (TACTILE THRESHOLD)
- Weight bearing (WEIGHT BEARING)
K/BxN arthritis...Acute to Chronic

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

Christiansen, et al
ACUTE POST TISSUE INJURY PAIN STATES

- Incision of plantar surface $\rightarrow$ 2° Tactile allodynia

Plantar paw incision

1° and 2° tactile allodynia and mechan hyperalgesia

Onset in mins, persist for 48-96 hrs.

(Brennan, et al, 1996)
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

- **Weight Bearing**
  Asymmetry 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?

IT Clonidine vs Veh in CFA rat (Okun, et al 2011)
SUMMARY:
Published KOs / transgenic mice in pain studies

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

Broad range of reported KO/TGs

Caveats:
• All pain end points are not the same
• Background stains for appropriate controls.

Courtesy: Z David Luo, MD
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.

**Formalin evoked licking**

- **Phase 1**
- **Phase 2**

Mouse Strains:

- 129P3
- AB
- BALBc
- C3HHe
- C57Bl6
- CBA
- CD-1
- SW

Facilitated Component covaries with cFOS and Strain

Adapted from Mogil
FACTORS IN CHARACTERIZING ANALGESICS.

Pain /analgesic mechanisms may vary by strain
- Basis for genetic linkage studies
- Definition of underlying mechanisms

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

Are we 129s or BALBc or ??
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