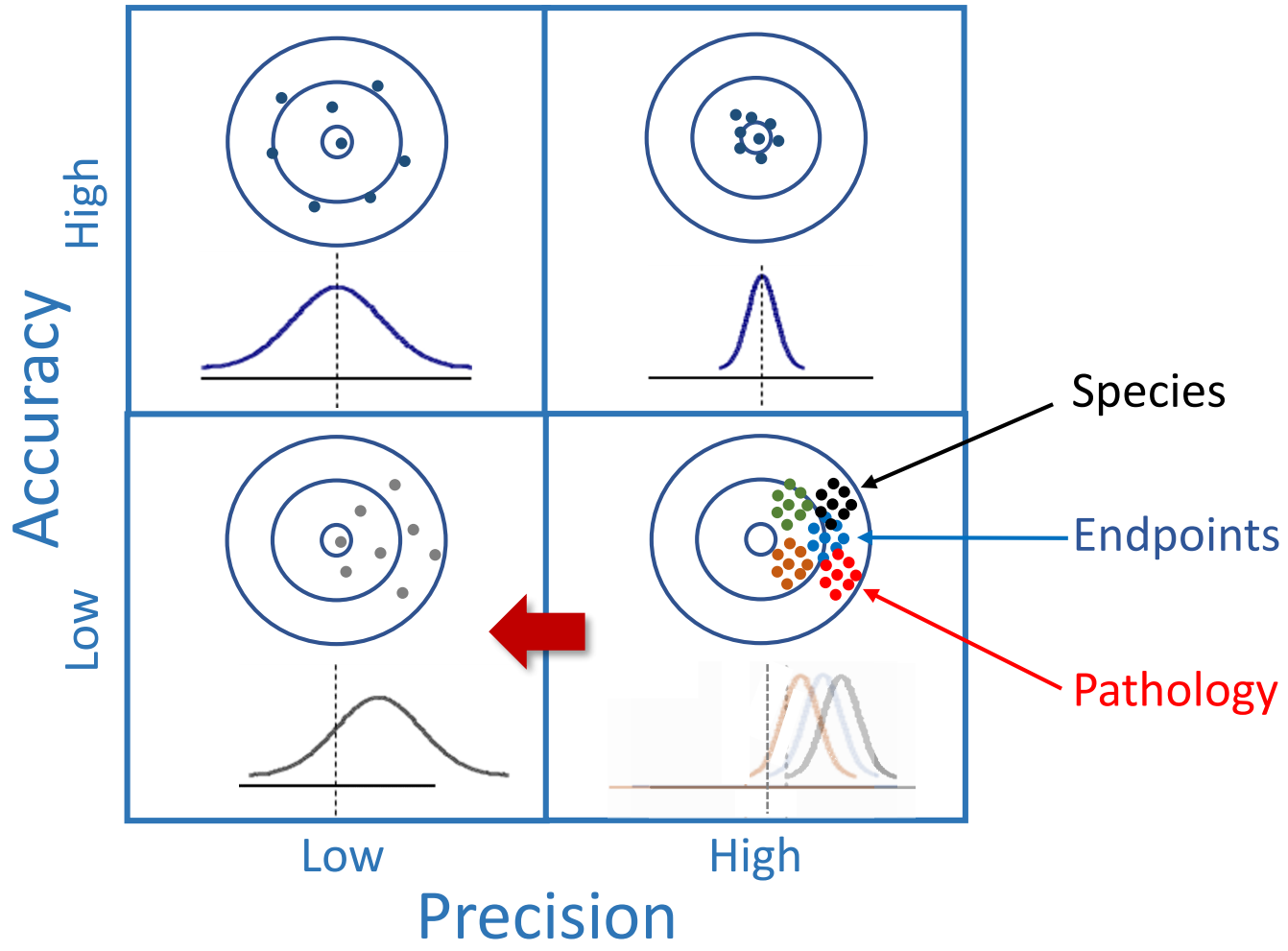
NOVEL STRATEGIES FOR TRANSLATIONAL RESEARCH
in Pain Drug Discovery

Andre Ghetti, Ph.D.
AnaBios Corporation

Addressing the Weaknesses of the Current Preclinical Strategy to Generate More Predictive Data



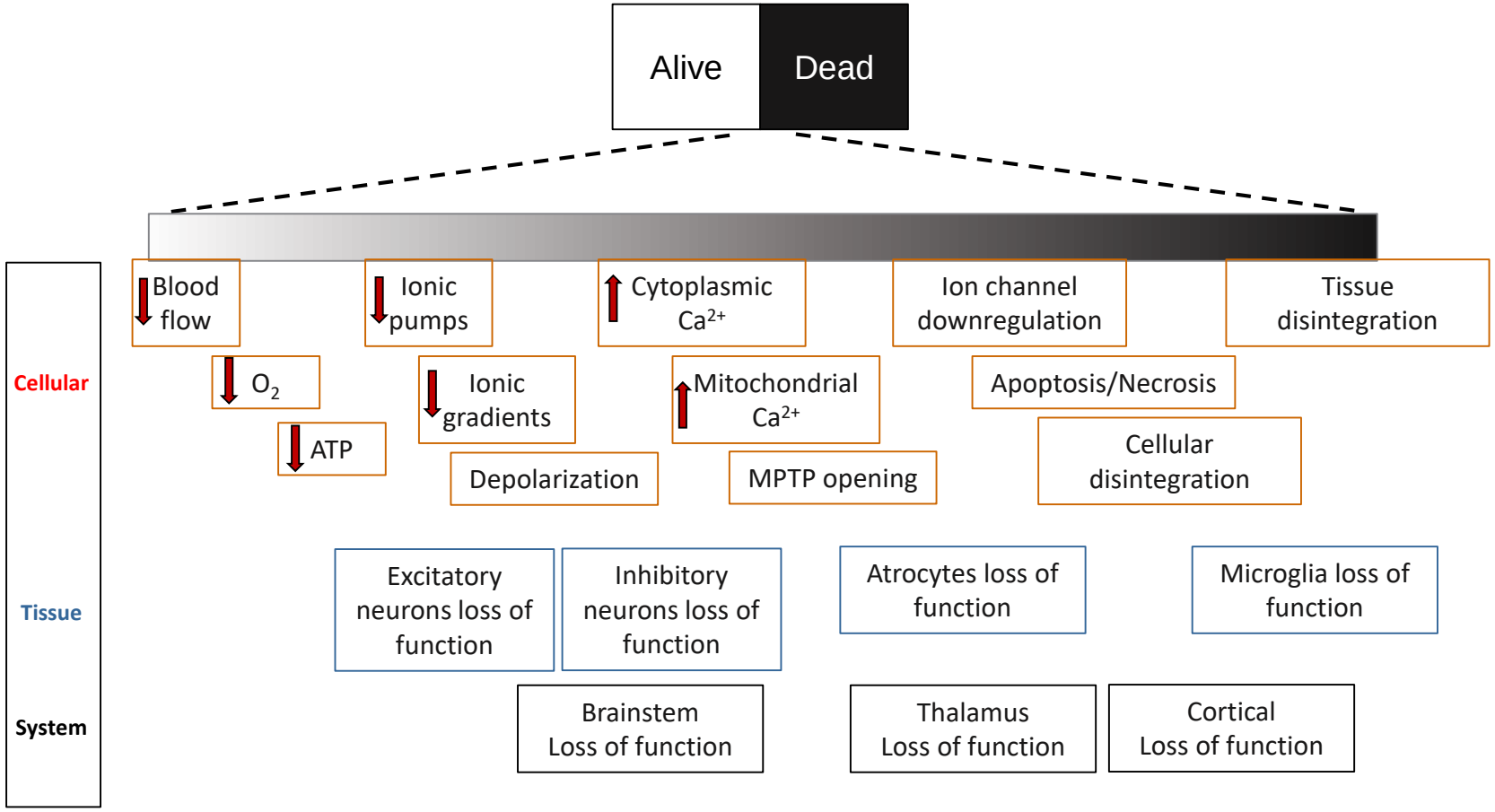
AnaBios studies drug effects directly on

isolated human organs and tissues

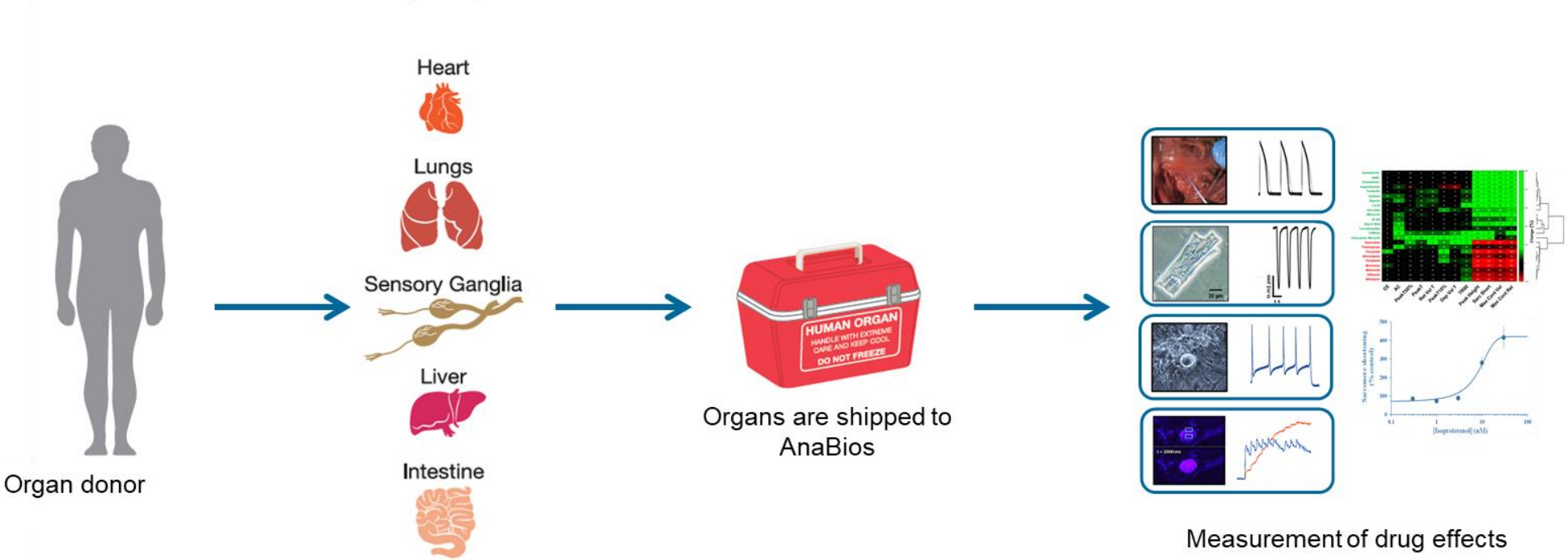
Challenges to the Use of Human Tissue in Research

- *Viability*
- *Access*
- *Reproducibility*

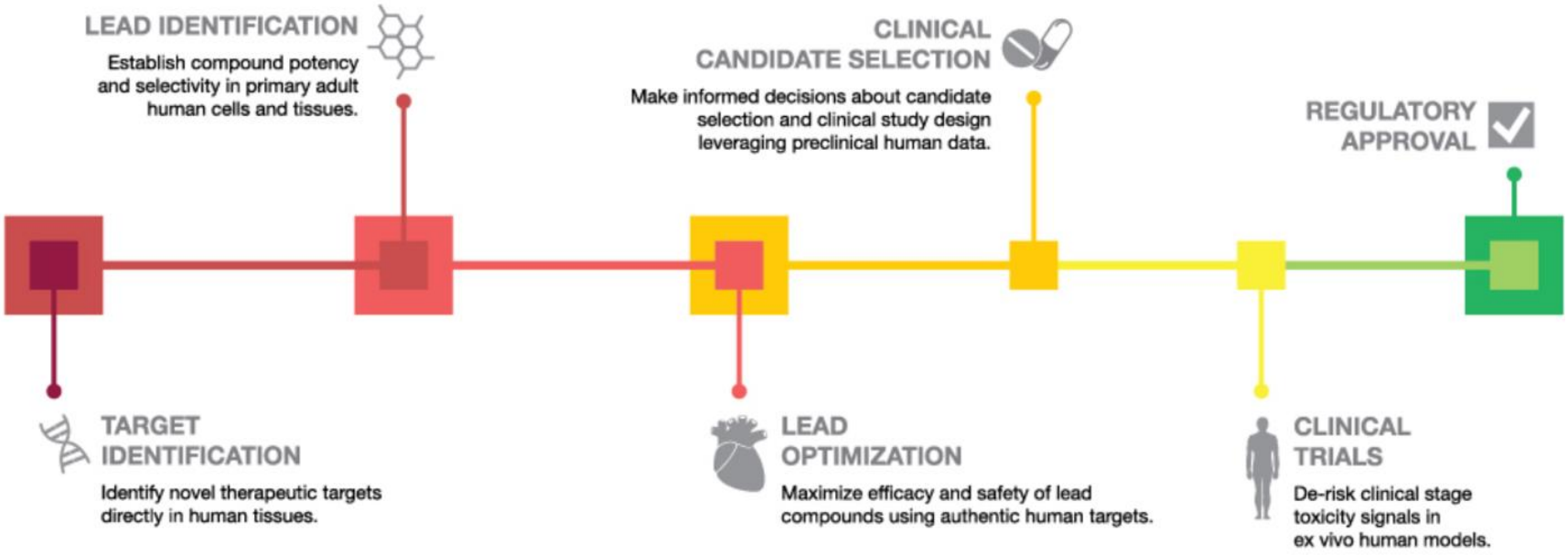
Cellular and Tissue Loss of Function is a Process



Enabling Drug Discovery in Human Tissues



Human Tissue Can Provide Critical Data
at Multiple Steps in the Development Process



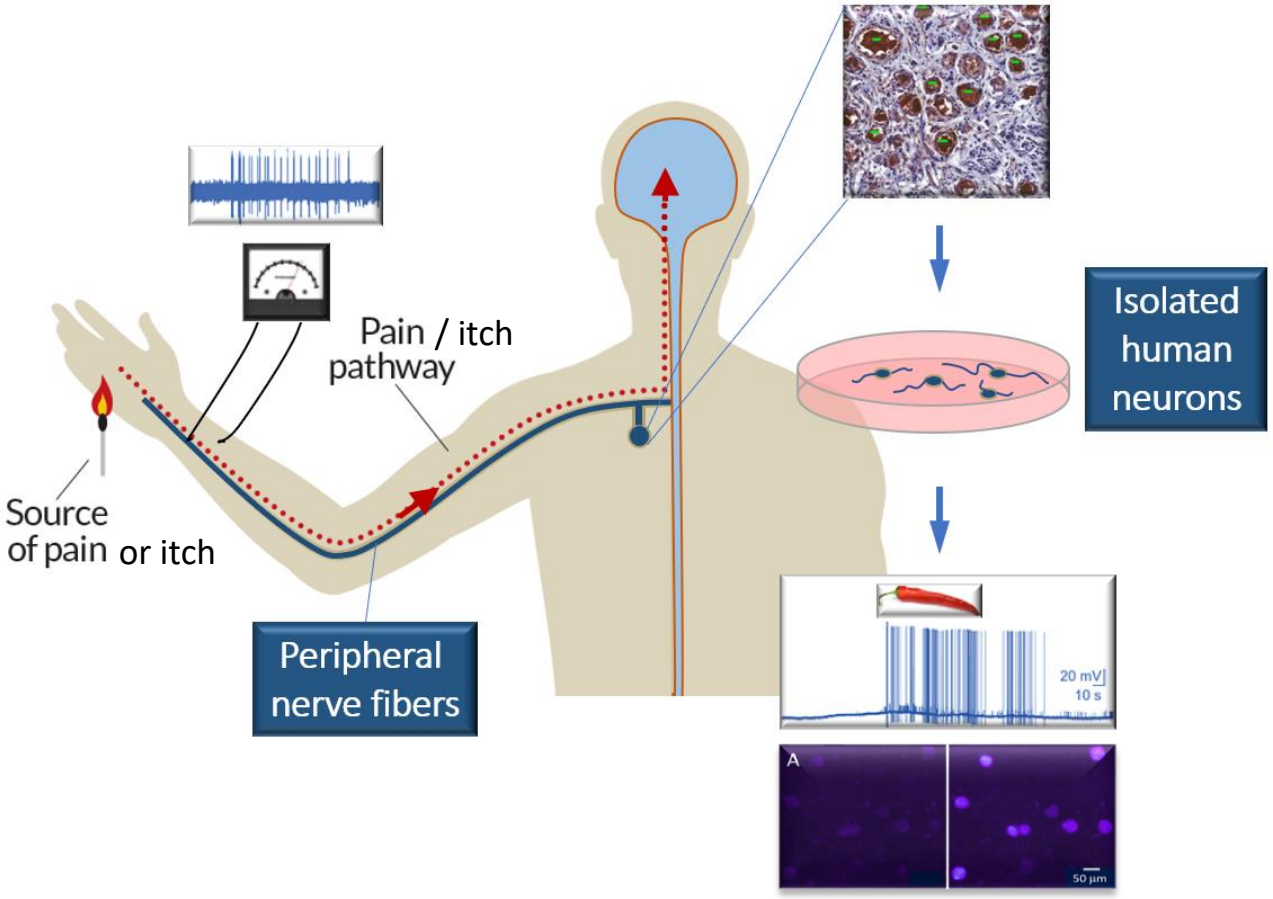
Predictive of clinical outcomes

Lower development risks related to interspecies differences

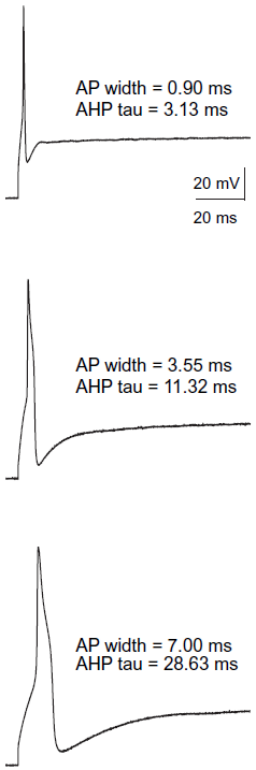
Study of drug action in healthy or pathological states

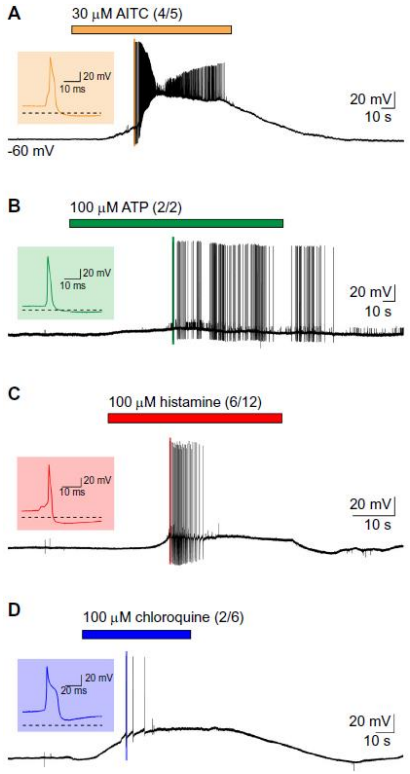
Reliable assessment of potency to guide first in human dosing

Studying the Activation of Peripheral Sensory Neurons in Vitro



hDRG Neurons in Culture Exhibit Stable Phenotype and the Expected Nociceptive Properties





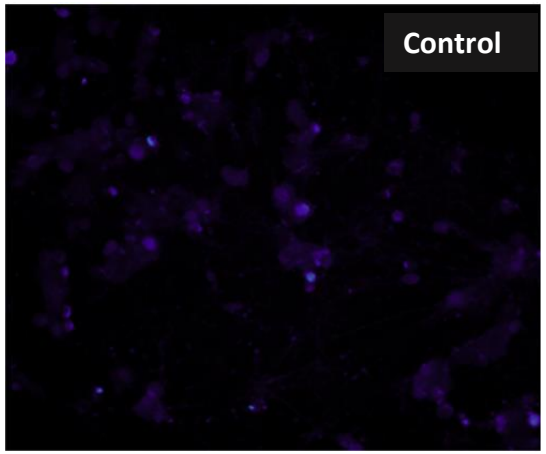
Davidson et al., PAIN (2014)

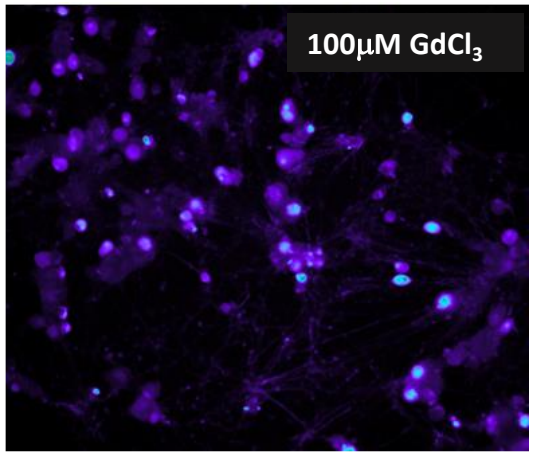
- Exhibit expected biophysical and pharmacological properties
- Respond to nociceptive agents
- Amenable to electrophysiology, calcium imaging, electrical field stimulation, gene delivery
- Useful for studying a variety of targets:
- Voltage gated Na^+, Ca^{2+}, K^+, Cl^- channels
- TRP channels
- GluR channels, mGluR receptors
- GABA receptors
- Opioid receptors

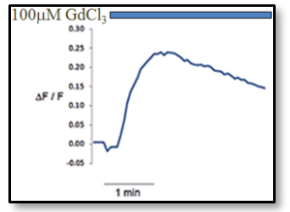
Agonistic and Antagonistic

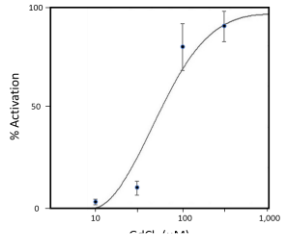
Species-specific Activity: Effects of Gadolinium

Human DRG

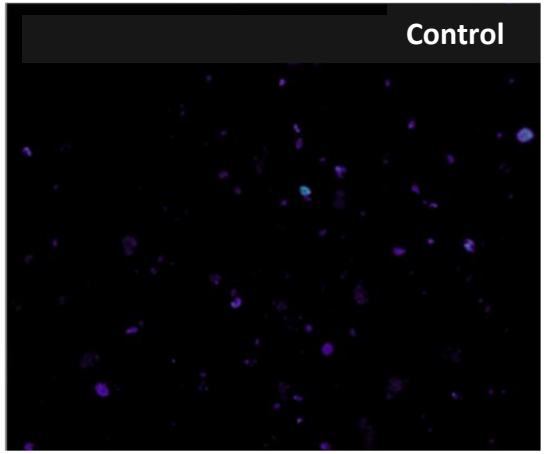


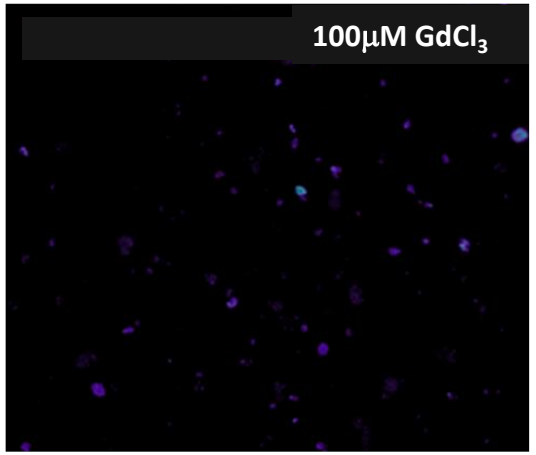


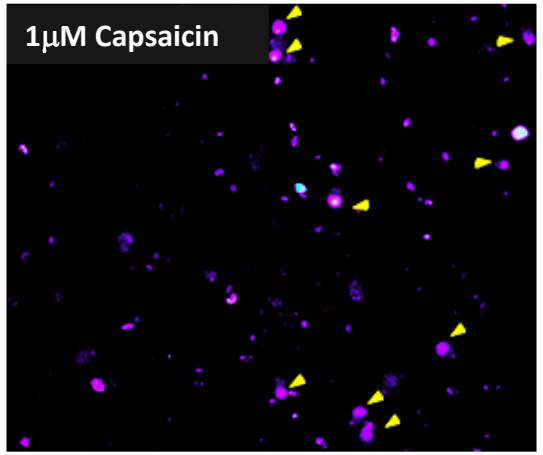




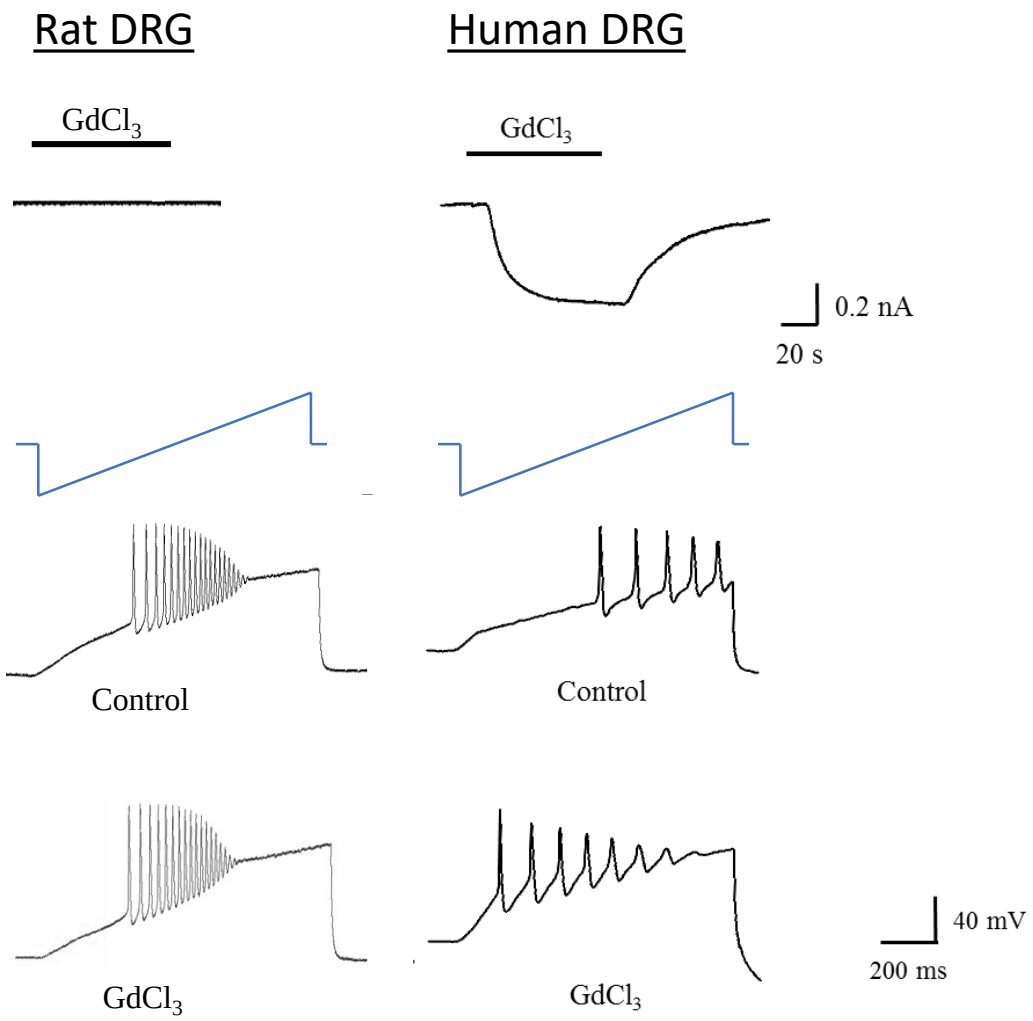
Rat DRG

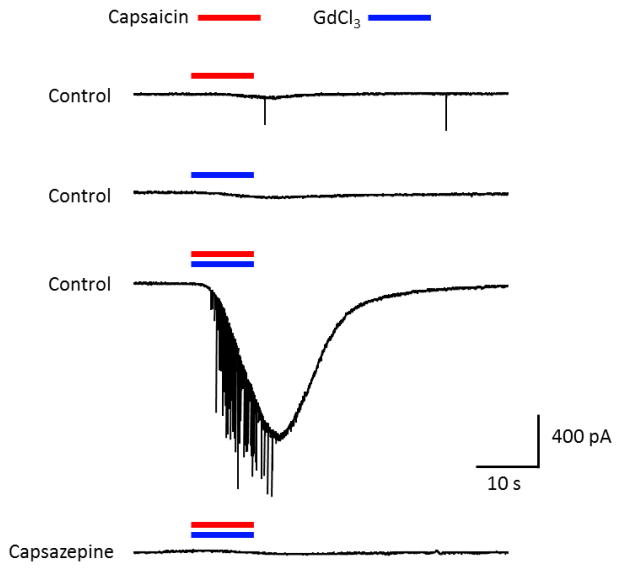


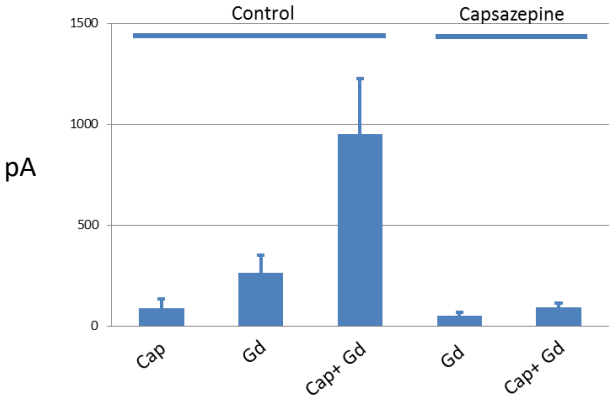




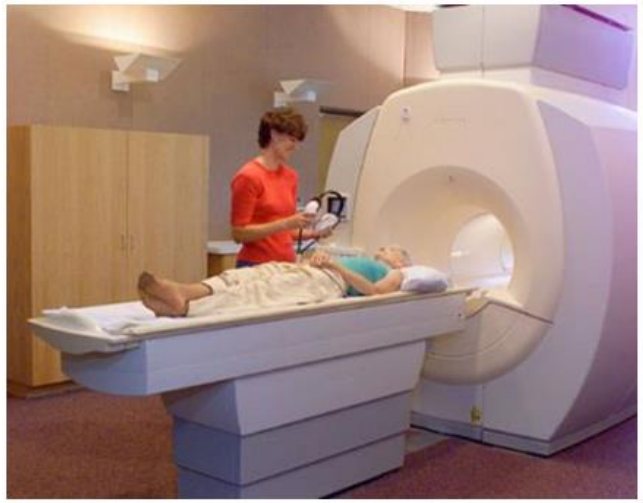
Gadolinium Sensitizes Human Sensory Neurons

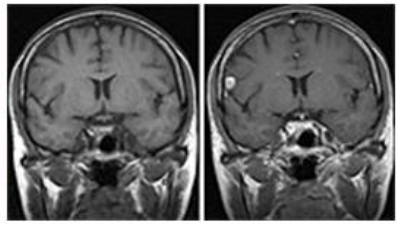


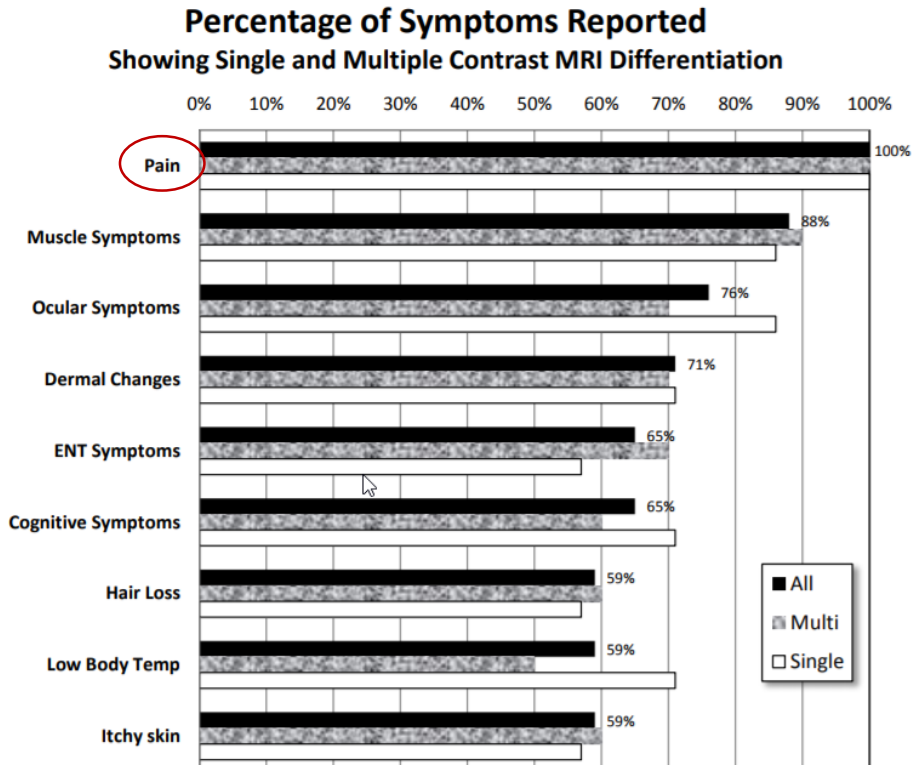




The Sensitization of Human Nociceptors Explains Clinical Side Effects of Gadolinium-based Contrast Agents





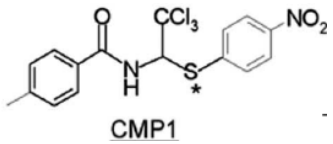


In patients with renal failure / poorly functioning kidneys that are unable to properly eliminate gadolinium:

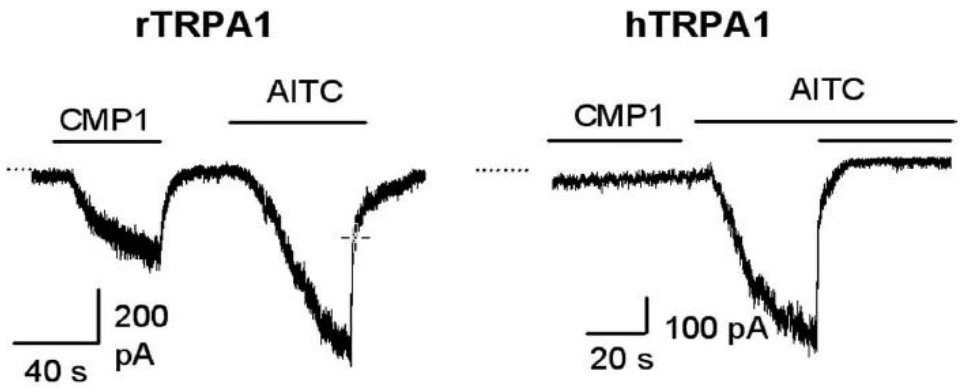
- Nephrogenic Systemic Fibrosis
- Strong generalized pain (especially in the joints)

Agonistic and Antagonistic

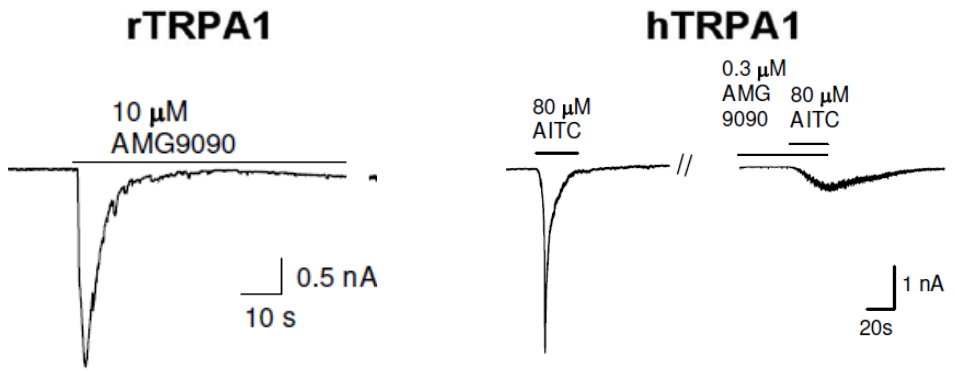
Species-specific Activity: Effects of Small Molecule Ligands



CMP1



Chen et al. *J.Neurosc* (2008)



Klionsky et al. *Mol.Pain* (2007)

S6

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rTRPA1 (934)LAYPVLTFGQLIAFTMFVPIVLMNLLIGLAVGDI
hTRPA1 (931)LAHPVLSFAQLVSFTIFVPIVLMNLLIGLAVGDI
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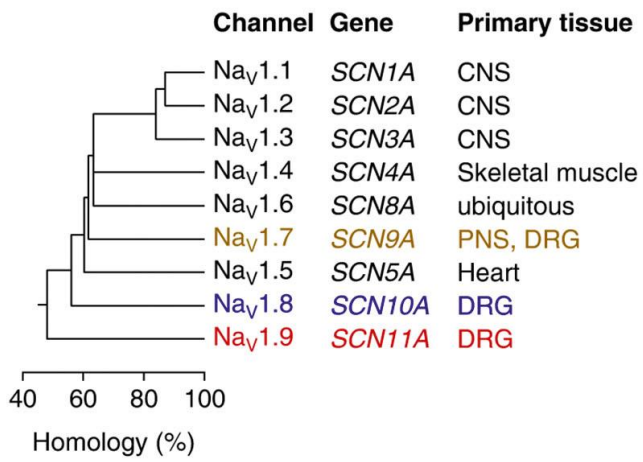
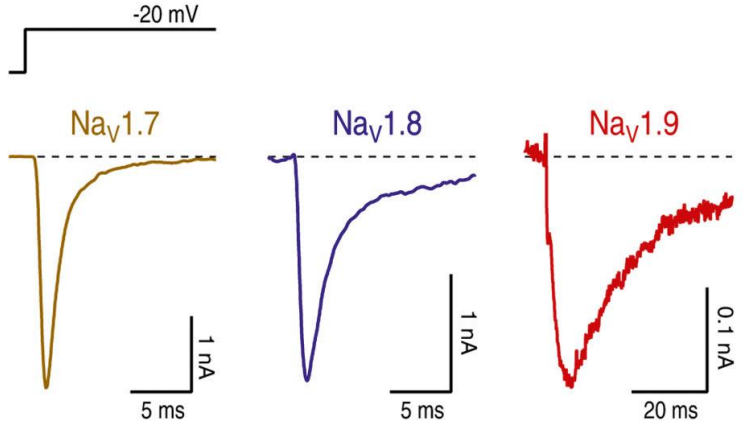
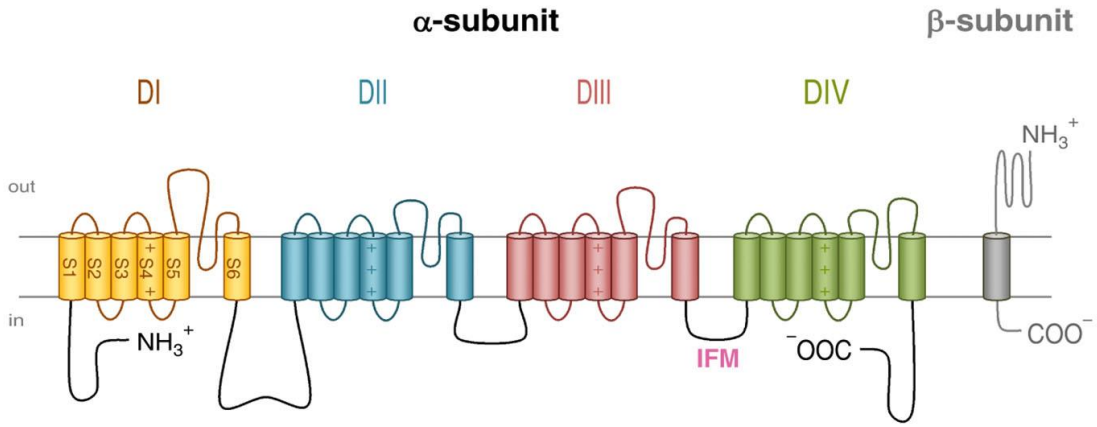
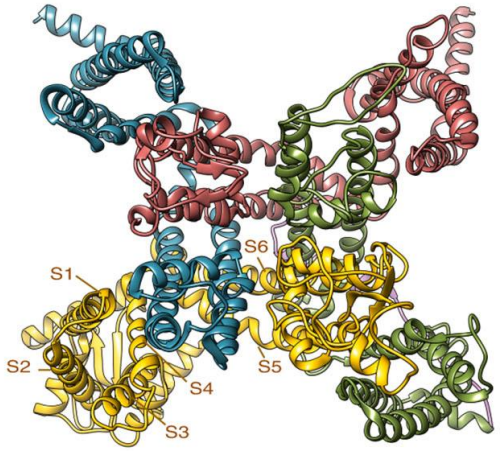
Voltage Gated Sodium Channels Subtypes:

Drug Selectivity is Essential for Favorable Efficacy and Safety









Nau & Leipold, *Neuroforum* (2017)

Human-focused Drug Discovery

Targeting Na_v Channels for the Treatment of Pain

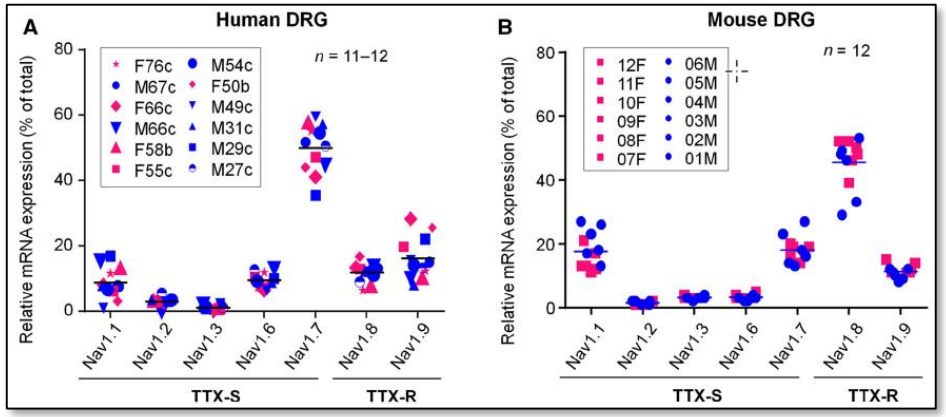
1) Selection of model species

2) Measurement of drug selectivity

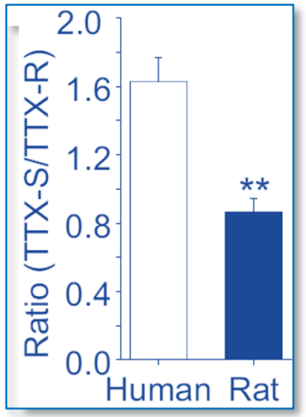
3) Target selection (Na_v subtype)

4) Assessment of the therapeutic potential

Expression of Nav Subtypes
Varies Across Species

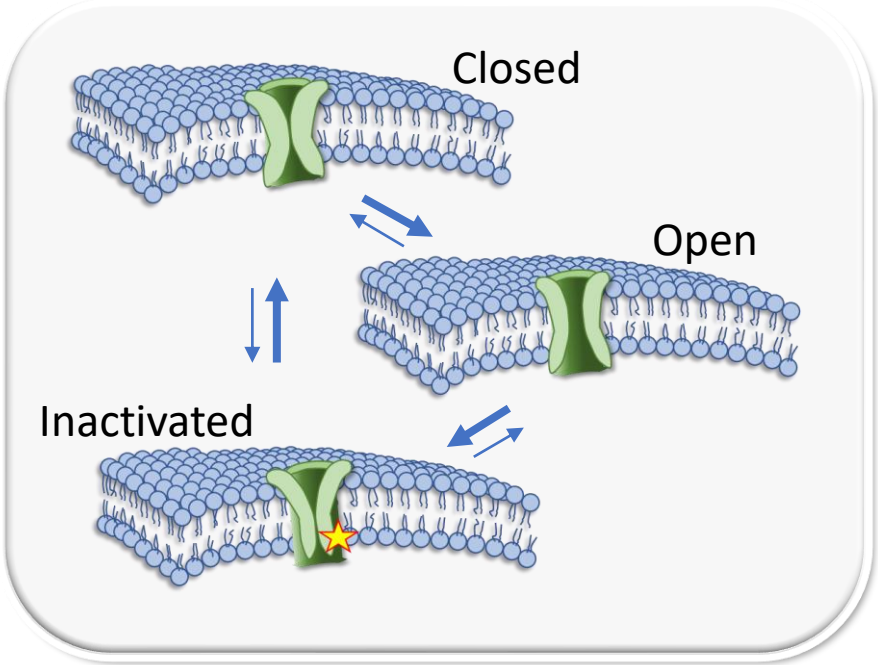


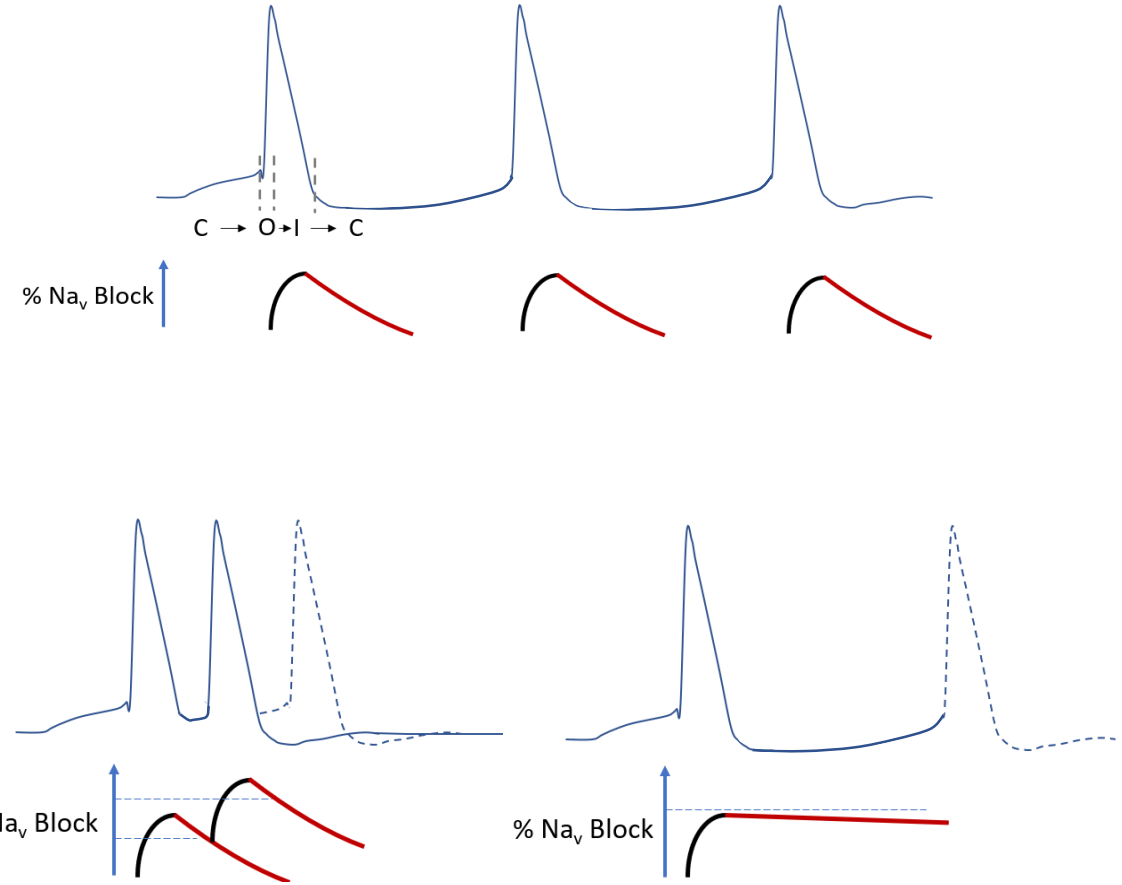
Chang et al., *Neurosci. Bull*., 2018



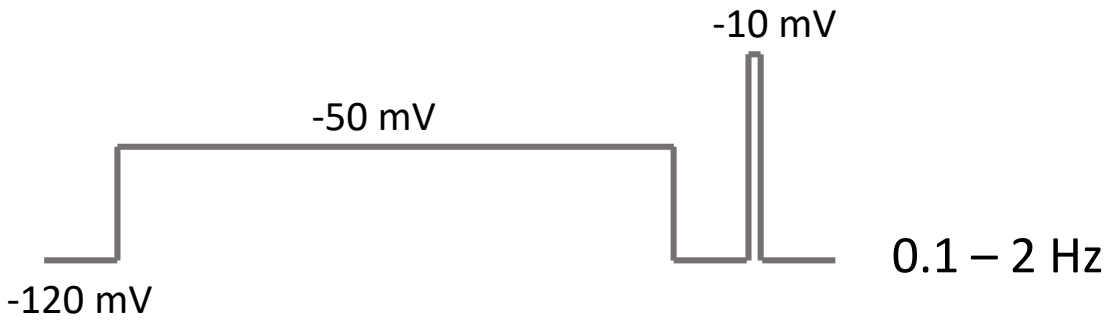
Zhang et al., *eLife*., 2017

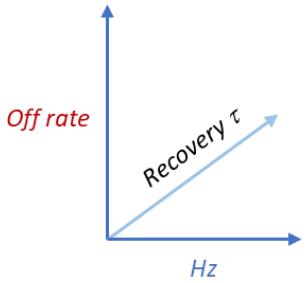
Sodium Channel Inhibition by Most Small Molecules is
State-dependent



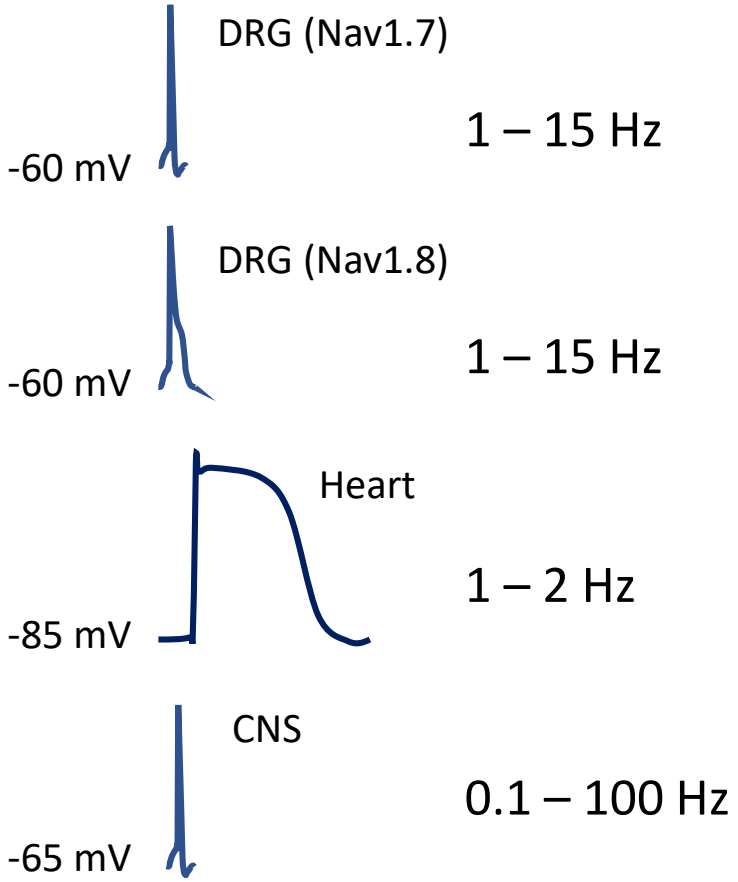


Measuring the Relevant Endpoints:
Assessment of Ion Channel Selectivity





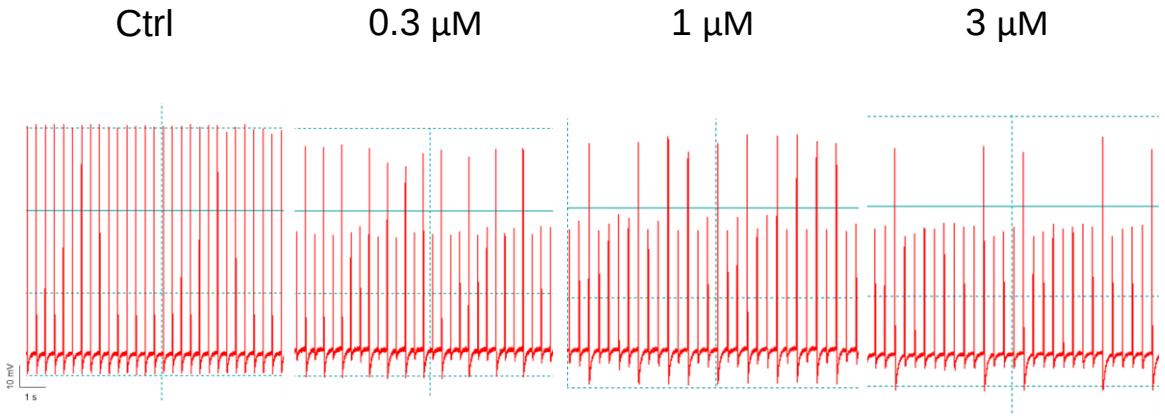
500ms

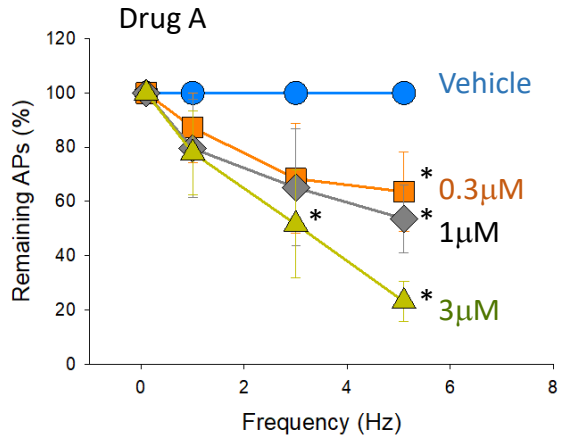


Measuring Drug Effects on Human Nociceptor Action Potentials

Drug A	Nav1.7	Nav1.8	Nav1.5
IC_{50} (nM)	700	800	4,000

From channels expressed in HEK cells



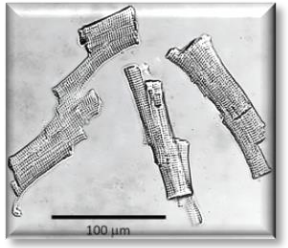


Assay features:

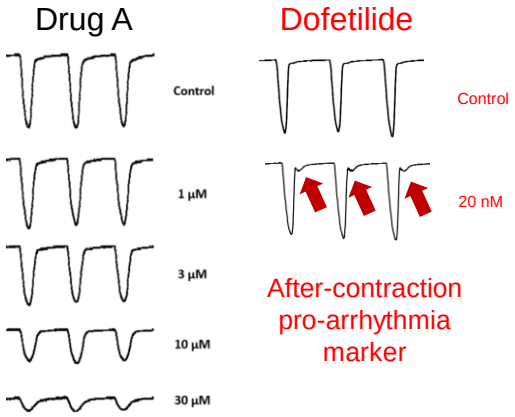
- *Physiological response*
- *Quantitative assessment of potency*
- *Assessment of use dependence*

Cardiac Safety Assessment
in Human Heart Ex-vivo

Assessment of pro-arrhythmia risk in human isolated primary cardiomyocytes

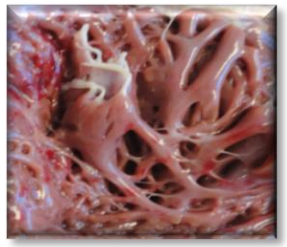


Human adult cardiomyocytes

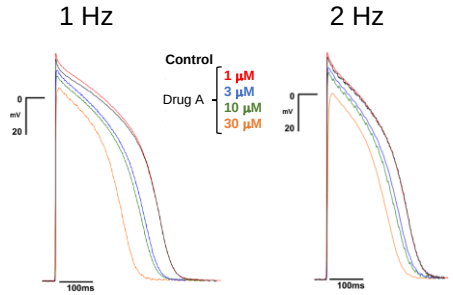


After-contraction pro-arrhythmia marker

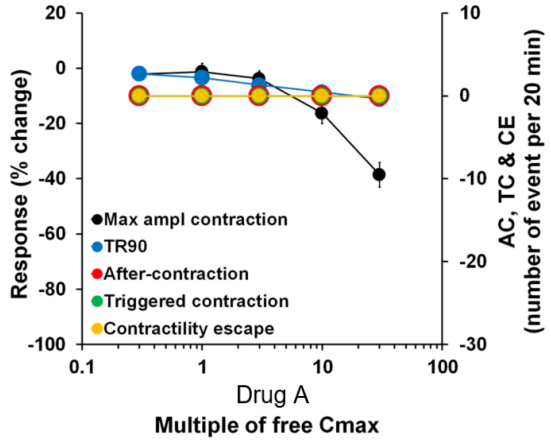
Assessment of pro-arrhythmia risk in human ventricular tissue



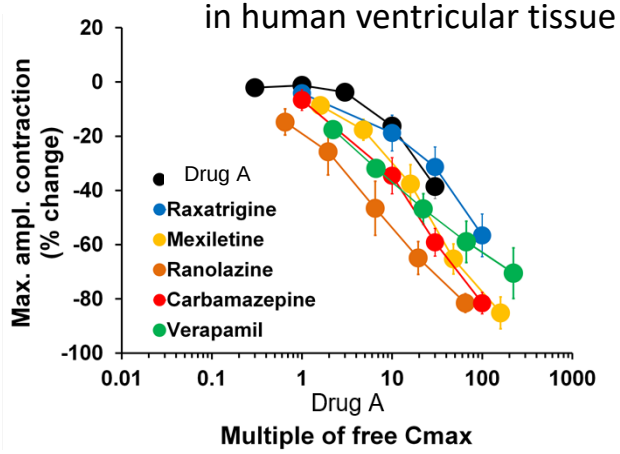
Human adult ventricular trabeculae



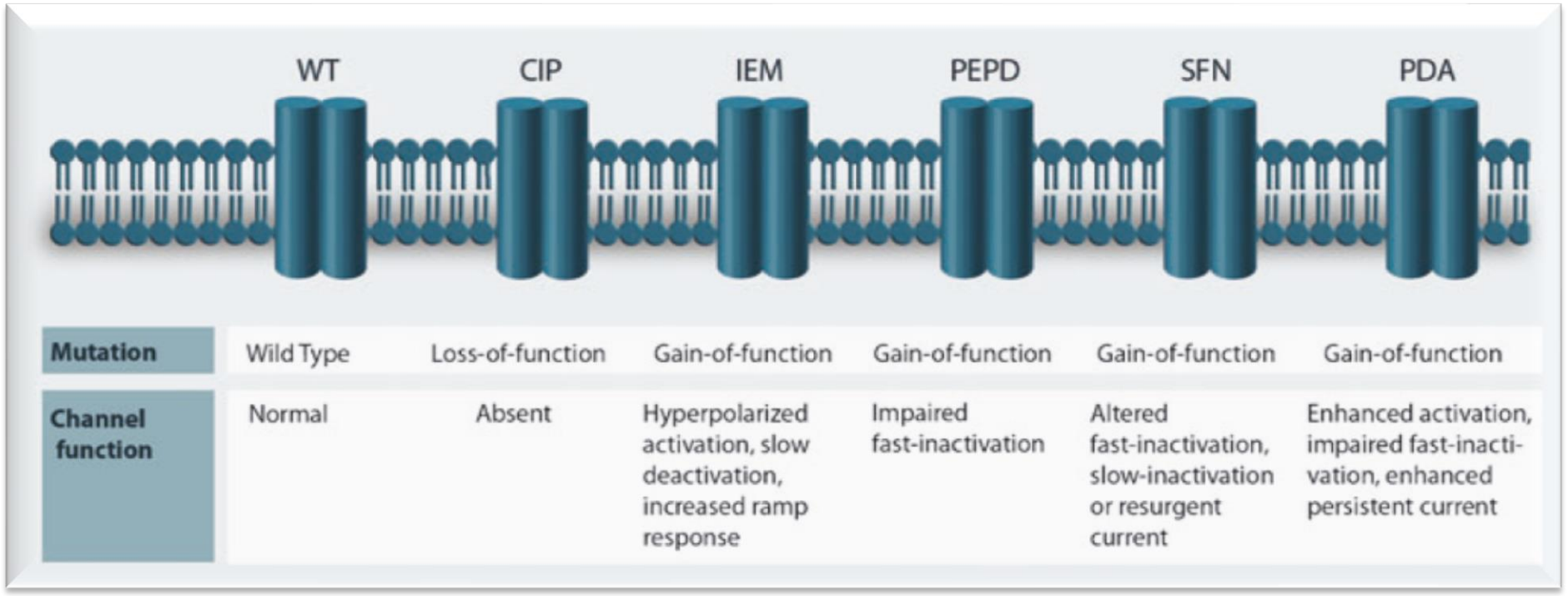
Assessment of contractility risk in human isolated primary cardiomyocytes



Assessment of contractility risk in human ventricular tissue



Human Genetics Validate $Na_v1.7$ as a Pain Target

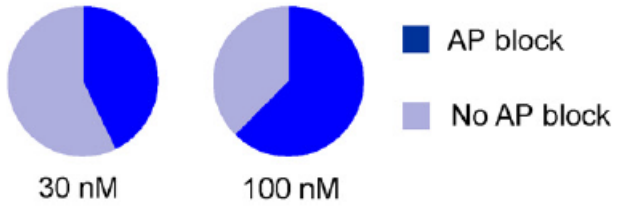


Mutation	Wild Type	Loss-of-function	Gain-of-function	Gain-of-function	Gain-of-function	Gain-of-function
Channel function	Normal	Absent	Hyperpolarized activation, slow deactivation, increased ramp response	Impaired fast-inactivation	Altered fast-inactivation, slow-inactivation or resurgent current	Enhanced activation, impaired fast-inacti-vation, enhanced persistent current

- Human congenital pain disorders validate $Na_v1.7$ as a premier target for the treatment of pain
- $Na_v1.7$ is upregulated in some forms of neuropathic pain

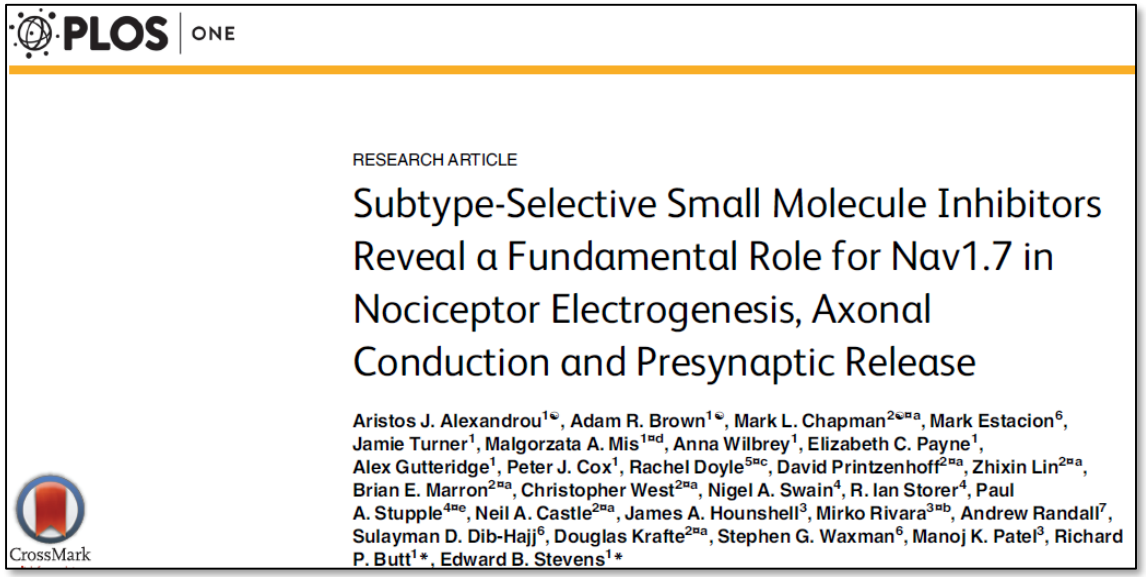
Human Genetics Validate $Na_v1.7$ as a Pain Target
But Additional Na_v Subtypes Are Also Critical

- Selective $Na_v1.7$ blocker PF-05089771 *does not* suppress action potential firing in a large proportion of hDRG neurons



(IC_{50})
At this concentration Nav1.7 selective

(IC_{90})
At this concentration also blocking Nav1.2, Nav1.6



PLOS ONE

RESEARCH ARTICLE

Subtype-Selective Small Molecule Inhibitors Reveal a Fundamental Role for Nav1.7 in Nociceptor Electrogenesis, Axonal Conduction and Presynaptic Release

Aristos J. Alexandrou[1☯], Adam R. Brown[1☯], Mark L. Chapman[2☯¤a], Mark Estacion[6], Jamie Turner[1], Malgorzata A. Mis[1¤d], Anna Wilbrey[1], Elizabeth C. Payne[1], Alex Gutteridge[1], Peter J. Cox[1], Rachel Doyle[5¤c], David Printzenhoff[2¤a], Zhixin Lin[2¤a], Brian E. Marron[2¤a], Christopher West[2¤a], Nigel A. Swain[4], R. Ian Storer[4], Paul A. Stupple[4¤e], Neil A. Castle[2¤a], James A. Hounshell[3], Mirko Rivara[3¤b], Andrew Randall[7], Sulayman D. Dib-Hajj[6], Douglas Krafte[2¤a], Stephen G. Waxman[6], Manoj K. Patel[3], Richard P. Butt[1]*, Edward B. Stevens[1]*

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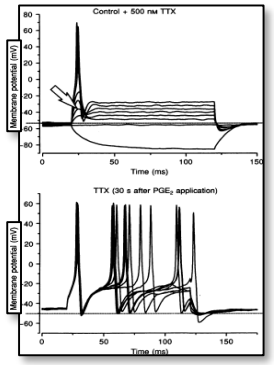
$Na_V1.8$ as a Critical Target for Inflammation-related Pain

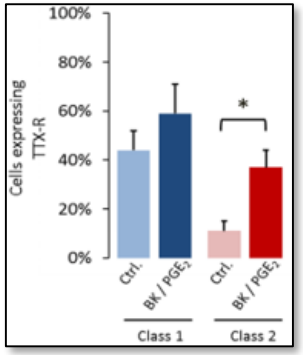
- Inflammatory agents sensitize sensory DRG neurons and drive the functional upregulation of $Na_V1.8$



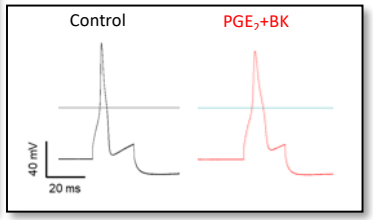
Gold et al., *Proc.Natl.Acad.Sci.USA*, 1996

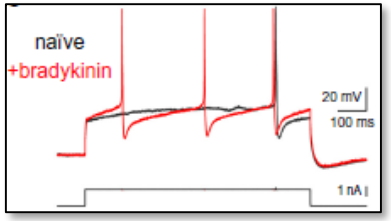
England et al., *J.Physiol.*, 1996

- AnaBios' data from hDRG neurons supports the role for $Na_V1.8$ in inflammatory pain
  - Subpopulation of hDRG neurons expressing TRPV1, treatment with PGE_2 and Bradykinin leads to upregulation of TTX-R responses
  - Inflammatory agents increase hDRG neuron excitability and slow the kinetics of the action potentials, consistent with $Na_V1.8$ upregulation



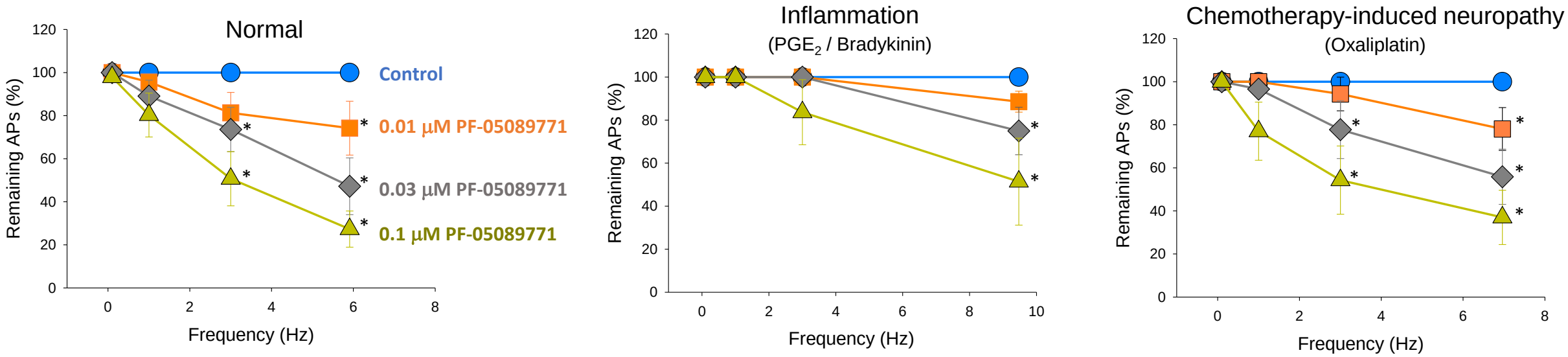
Davidson et al., *PAIN*, 2014





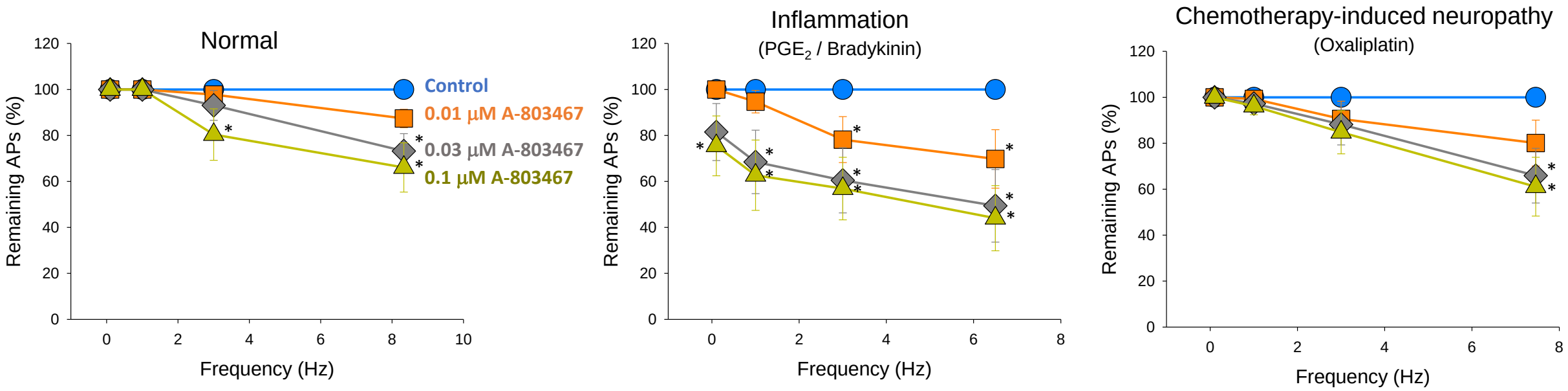
- Gain of function mutations of Nav 1.8 result in painful neuropathy

$Na_v1.7$ Selective Blocker: PF-05089771



- Inhibition is more effective at the higher frequency rates
- Less effective in inflammatory conditions

$Na_v1.8$ Blocker: A-803467



- Inhibition is more effective at the higher frequency rates
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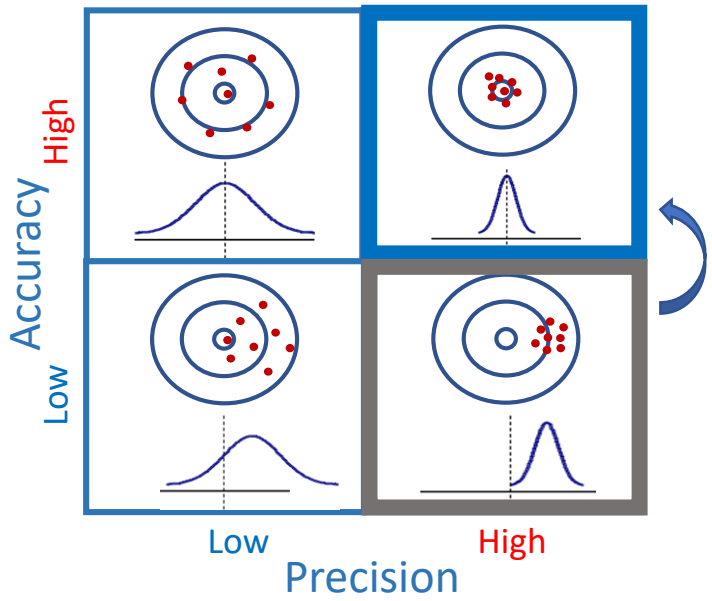
Summary



Assessment of drug effects in ex vivo *human* models

Study of drug action in the context of pathological states

Bypass cross-species differences

Measure drug effects and potency across authentic human targets