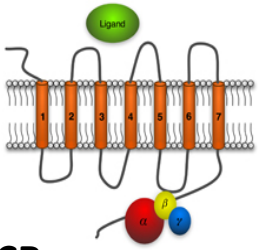
Phenotypic drug discovery at

ESTEVE

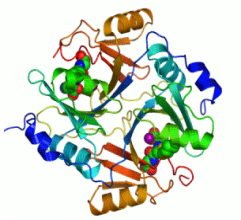
Focus on PAIN

Drug Discovery at ESTEVE → Focus on Pain

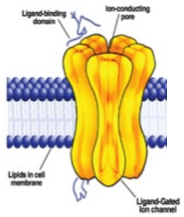
- Target-based screening



GPCRs

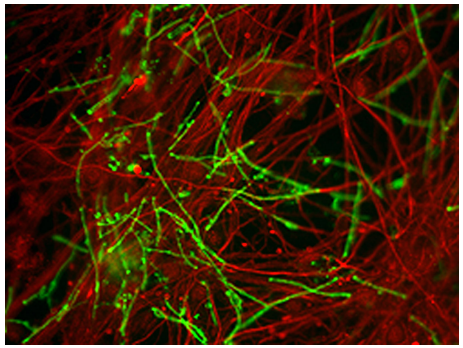


Enzymes



Ion Channels & Transporters

- Cell-based screening



- In Vivo screening

ESTEVE

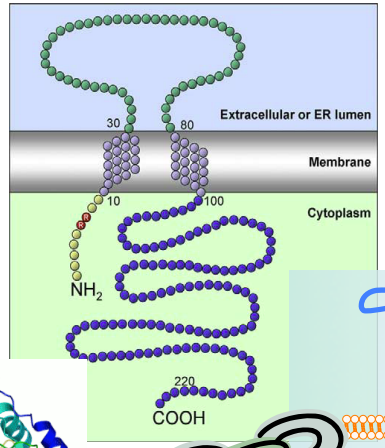
Target-based

- Single target approach

  Sigma 1 receptor

- Multimodal approach

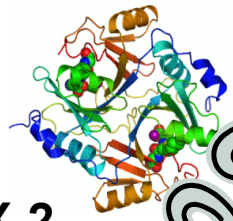
  SACO



Currently at Phase II

μ-opioid receptors

Phase II OK



COX-2

Other

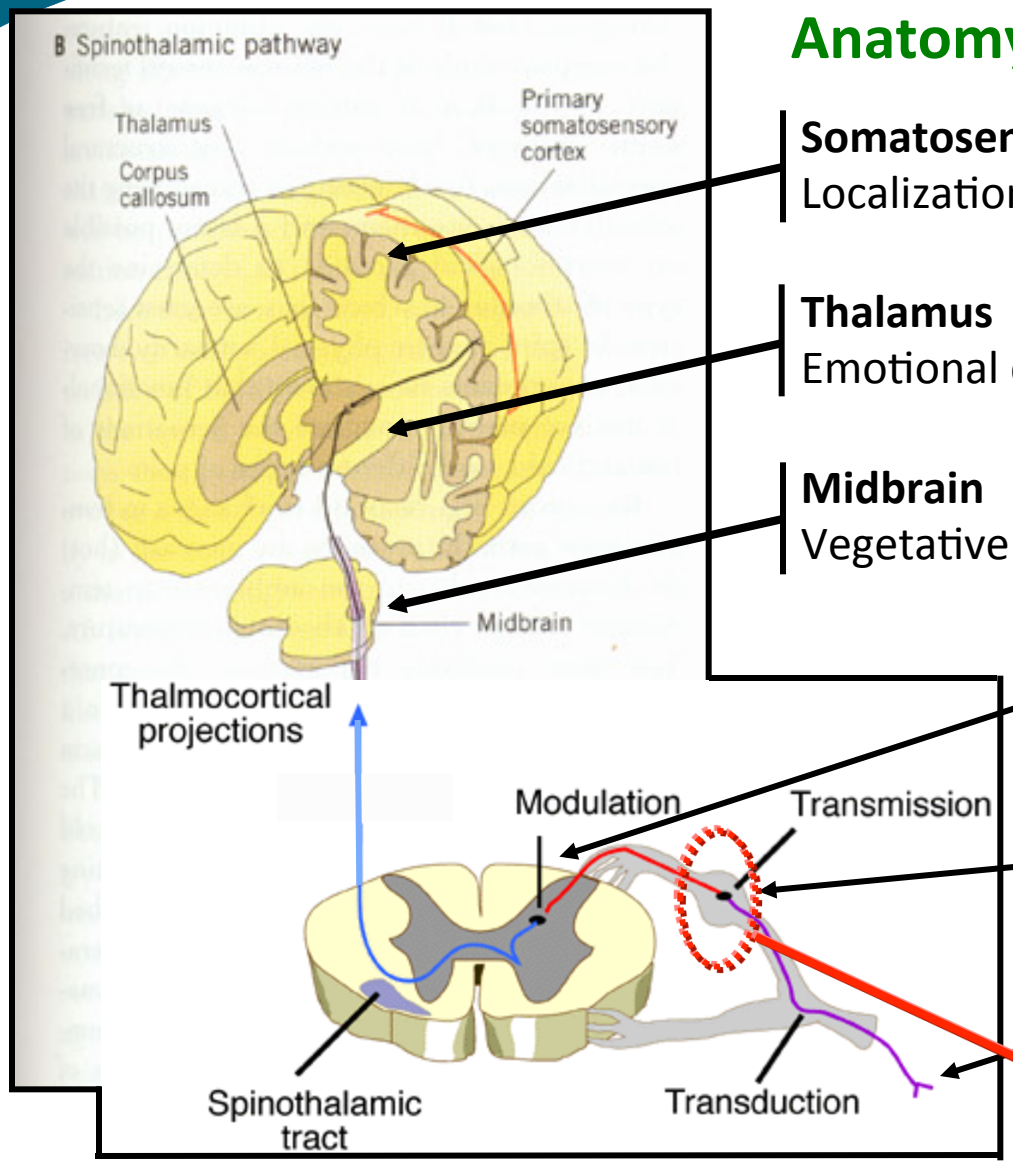
Multimodal

Analgesic

Strategies

Cell-based – Cell line characterization

Anatomy of Pain



Somatosensory cortex
Localization & Consciousness

Thalamus
Emotional quality & Importance

Midbrain
Vegetative response & Descending inhibition

Dorsal horn neurons
Modulation & Reflex response

Peripheral C-fibers -
they transmit the signal

Peripheral nociceptors -
they catch the pain

DRG cultures

In the pain pathway there are different locations where to act…

Cell-based – Cell line characterization

F11 cell line

F11 cell line is derived from the fusion of a mouse embryonic neuroblastoma (N18TG-2) and rat dorsal root ganglion (DRG) neurons. When differentiated in the presence of cAMP, this cell line exhibit many properties of neuronal cells, including action potentials, extensive neurite outgrowth, synthesis of neurotransmitters, expression of neuropeptide receptors and voltage-gated ion channels (Jow et al 2006).

ND7/23 cell line

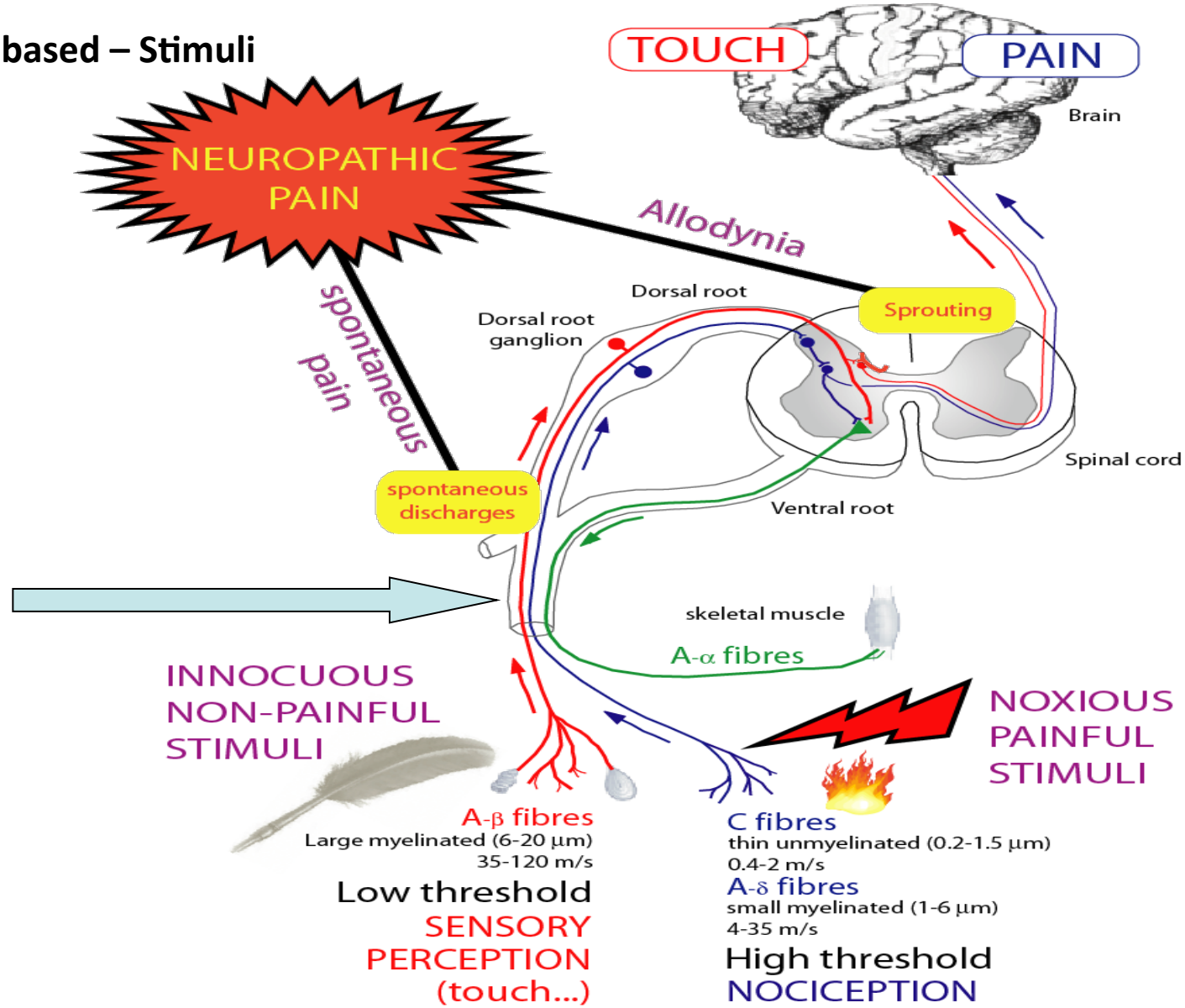
ND7/23 is a neuroblastoma x DRG neuron hybrid cell line that when differentiated shows neuronal-like properties as neurite outgrowth and action potentials (Dunn et al 1991).

DRG primary cultures

Cells from different providers are being studied

Cell-based – Stimuli

Neuronal excitability

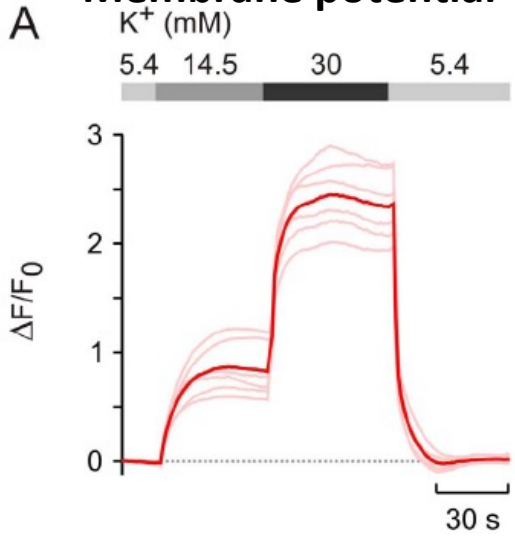


When neurons transmit pain, they become excited by different stimuli, noxius painful stimuli in normal conditions, but also innocuous sensory stimuli can be perceived as painful in hypersensitive conditions such in neuropathic pain …

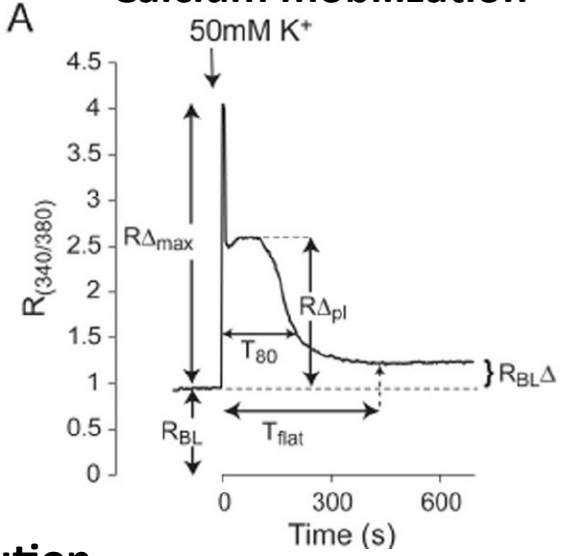
Cell-based – Stimuli

KCl induced neuronal depolarization as a general excitatory stimuli…

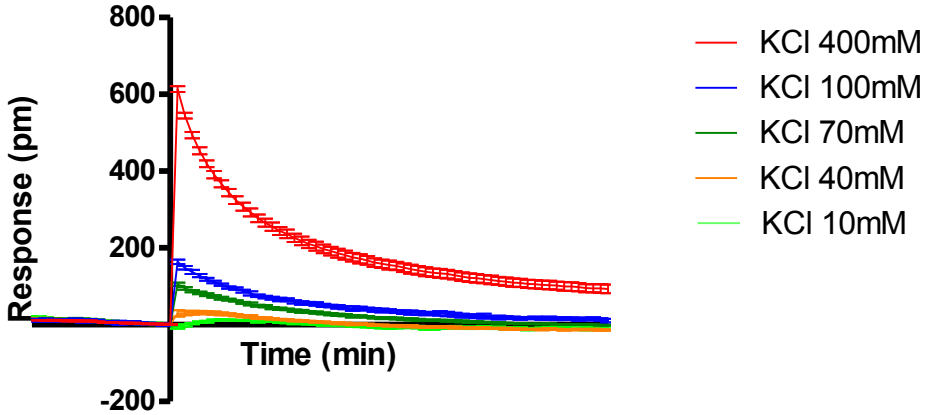
Membrane potential



Calcium mobilization



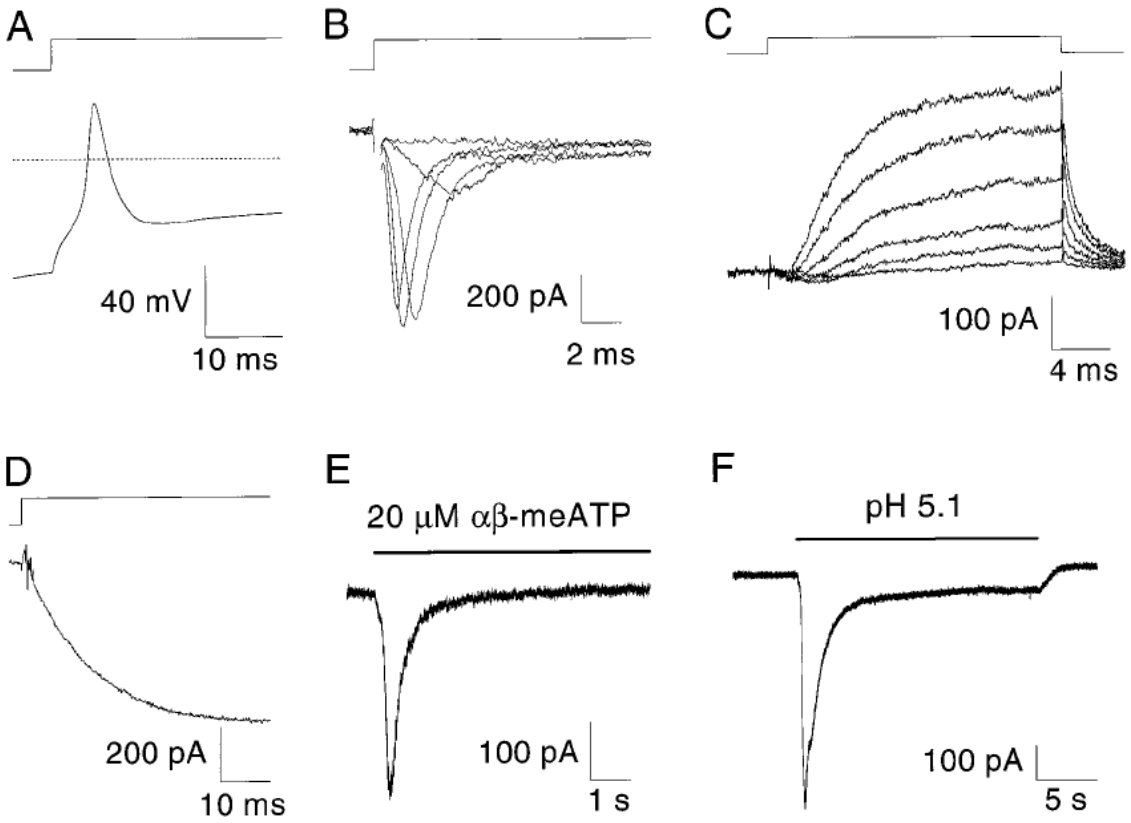
Dynamic mass redistribution



Will these stimuli and readouts mimic physiological responses?

Cell-based – Stimuli

Electrical depolarization as a more physiological excitatory stimuli…



Excitable cells like neurons or neuronal-like cells are best studied using patch-clamp techniques where a single type or multiple types of ion channels can be studied as well as whole cell action potentials. Immortalized dorsal root ganglion cells have been shown to fire action potentials in response to current injection and they elicit both voltage and ligand-gated currents corresponding to channels present in native cells (Raymon et al 1999).

In Vivo-based – Compound selection

A critical issue due to assay low throughput

>=5mg unique structures and Soluble in DMSO → 17,897 → No mixtures → 17,825

OK or Not Tested

- Chem. Stab. (17,794)
- Citotox (14,616)
- hERG (14,418)
- AMES (14,417)

→ 14,417 → Reference Compounds/ Named Cpds → 13,115

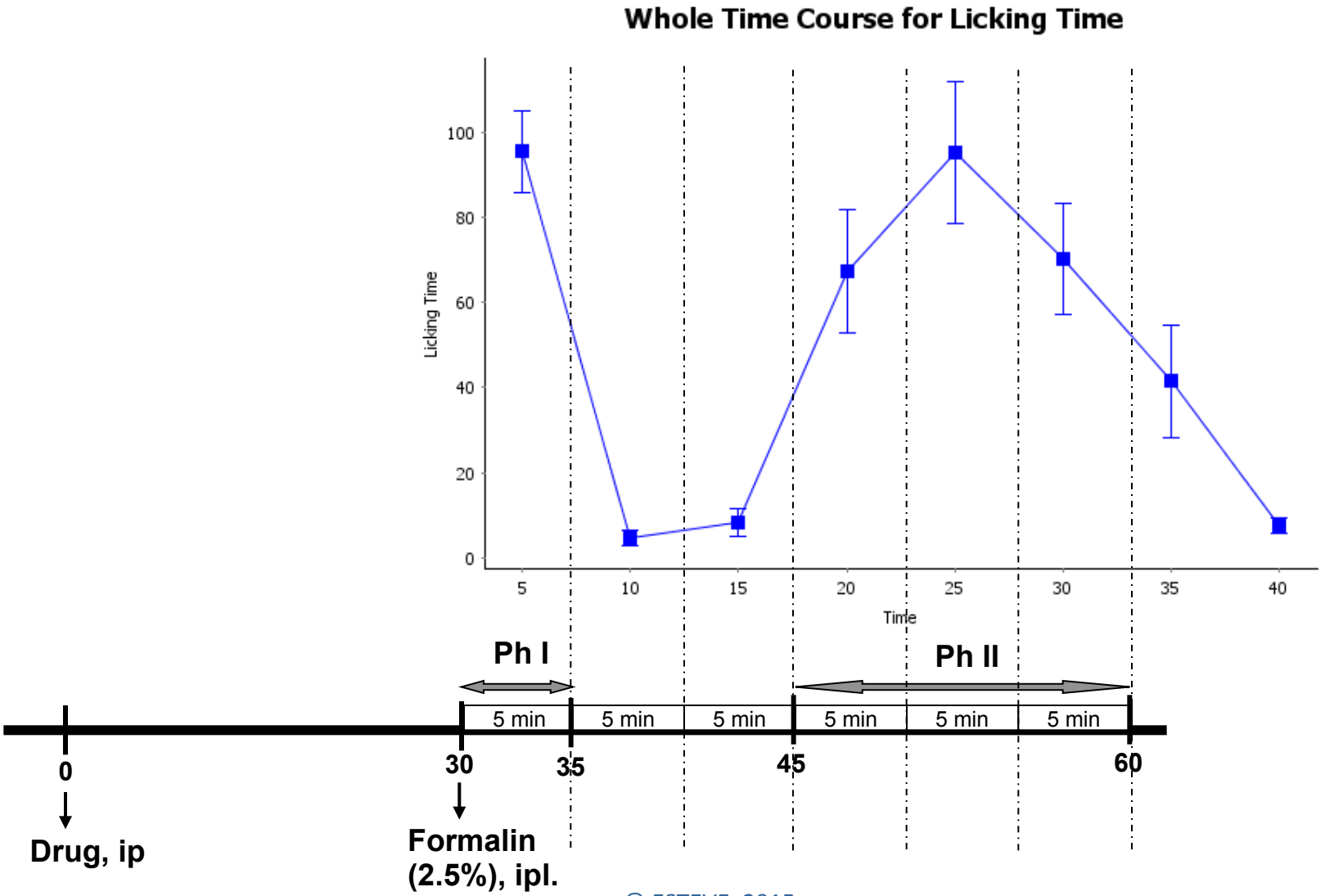
"Orphan target": do not hit any target at the tested concentrations → 7,785 → CYPs determined (and OK at 1µM or 10 µM) → 346

- Reviewed one by one, excluding non interesting cpds (reactive groups,…)
- Diversity
- Less probability of hERG
- Higher number of targets tested

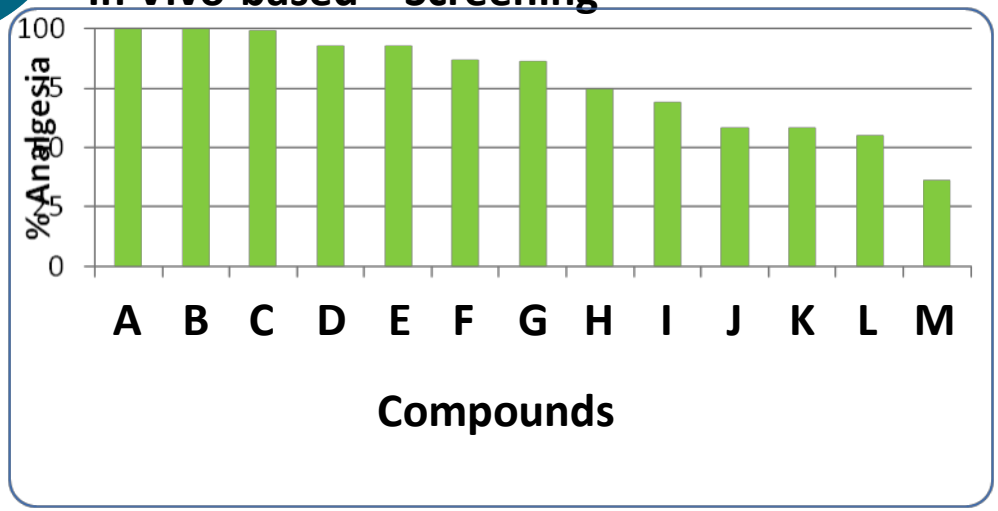
→ ~100 → Formalin test (Phase II) in mice (40 mg/kg, i.p.; N=5)

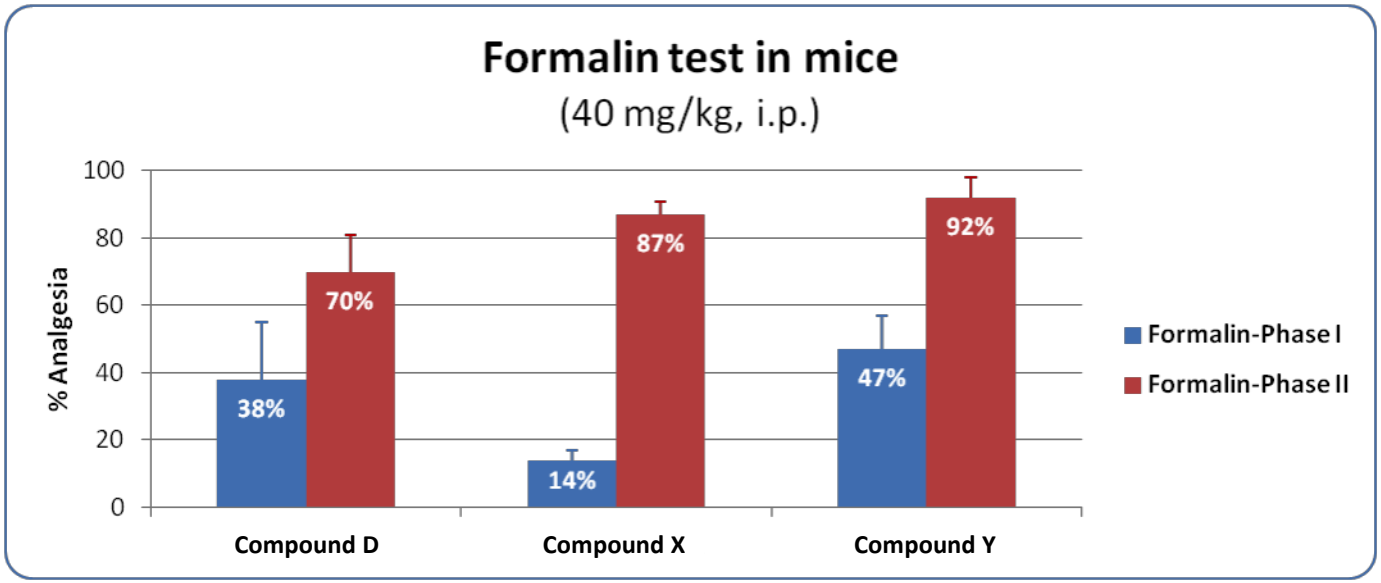
In Vivo-based – Screening

The assay should have a reasonable throughput and be able to detect as many type of analgesics as possible → Formalin test in mice



In Vivo-based – Screening





Initially discovered in screening

New close analogues

In Vivo-based – Screening

Next steps:

Target deconvolution

Similarity prediction software

Similarity Ensemble Approach

Ligand → **Target**

PYTHIA

Discovering your pharmacology profiles

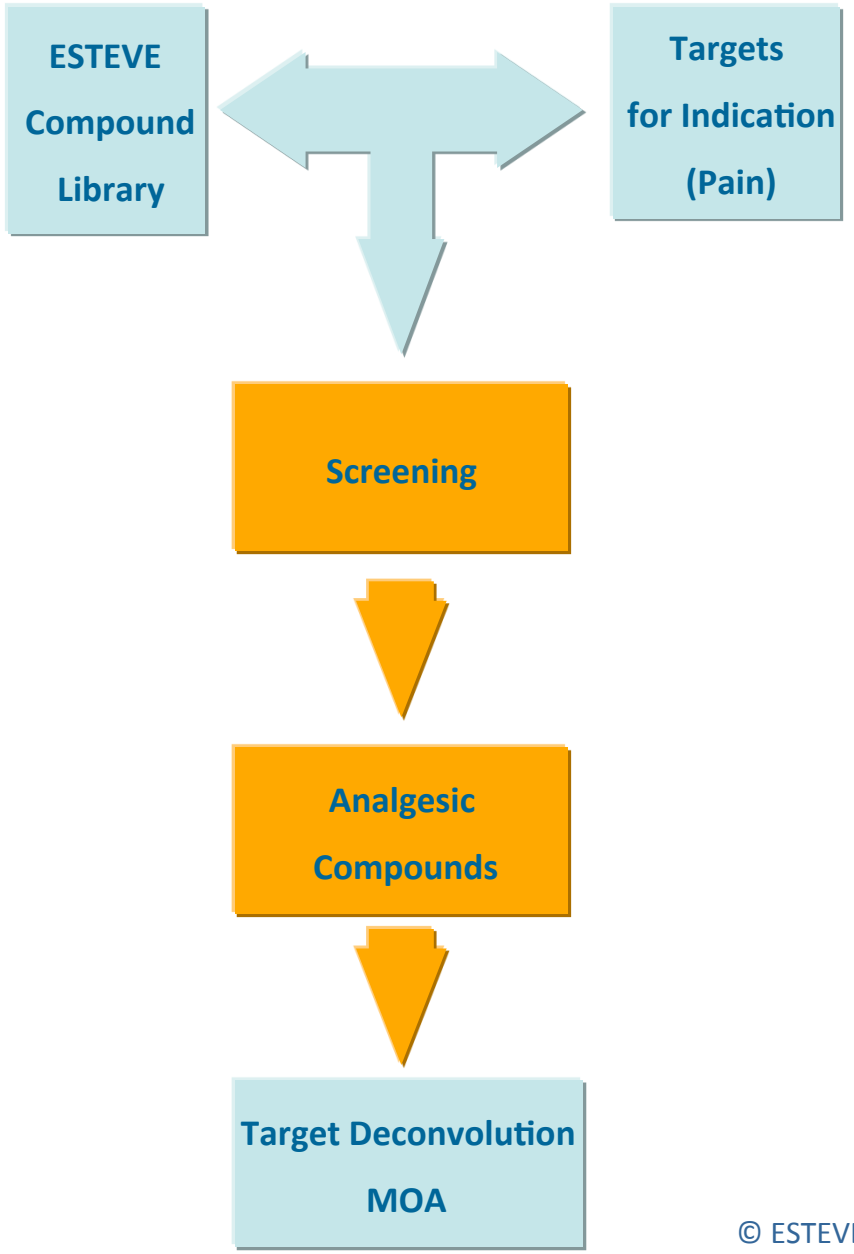
Pythia predicts both therapeutic targets and off-targets for molecules. Medical indications are also suggested.
3,150 targets and 90 disease areas are considered

Selectivity panels

Cerep

eurofins | Cerep Panlabs

Phenotypic screening challenges - Summary



Challenge 1: Which are the best compounds to be tested focused on the indication? Combination of targets → multimodality.

Challenge 2: Which are the best assays and more translational readouts in complex disease/behaviours like pain?

Challenge 3: Which are the best tools for target deconvolution?

Computational

Experimental

Thank you very much for your attention!