
sb ION CHANNELS
A division of SB Drug Discovery
Ion Channel Retreat
Vancouver, June 2014

Background

- A division of SB Drug Discovery
- Located in Glasgow, Scotland
- Established in 1994
- 25 researchers

Overview

- SB Drug Discovery specializes in protein expression, cell line development and assay services
- Developed > 100 custom cell lines expressing ion channels, GPCRs, antibodies etc
- Developed Nav1.1 – Nav1.8 sodium channel panel
- Highly experienced electrophysiology team
- SB Ion Channels Division set up in 2013
    - Off the shelf ion channel cell lines
    - Custom ion channel cell line development
    - Ion channel screening services

SB Ion Channels

- Parallel processing systems put in place to enable efficient development of multiple ion channel cell lines simultaneously
- Last 12 months: 40 ion channel cell lines developed
- Currently offering over 50 ion channel targets
- Further 6 targets in development

Voltage gated channels

Sodium Channels
Nav1.1
Nav1.2
Nav1.3
Nav1.4
Nav1.5
Nav1.6
Nav1.7
Nav1.8
Nav1.7 monkey
Nav1.7 dog
Nav1.7 rat

Potassium Channels	
Kv2.1	Kir2.1
Kv2.1 / 9.3	Kir2.2
Kv2.2	Kir2.4
Kv3.1	SLACK
Kv3.2	TREK-1
Kv7.2	TRESK
Kv7.2/7.3	

Other Channels
Ano1
Ano2

Ligand gated channels

Purinergic Channels	TRP Channels				Other Channels
P2X1	TRPA1	TRPV1	TRPM2	TRPC1	NMDA
P2X2	TRPA1 monkey	TRPV2	TRPM3	TRPC3	
P2X3	TRPA1 sheep	TRPV3	TRPM4	TRPC4	
P2X4		TRPV4	TRPM5	TRPC5	
P2X5		TRPV5	TRPM8	TRPC6	
P2X6		TRPV6		TRPC7	
P2X7		TRPV1 rat			
P2X7 mouse		TRPV1 dog (transient)			

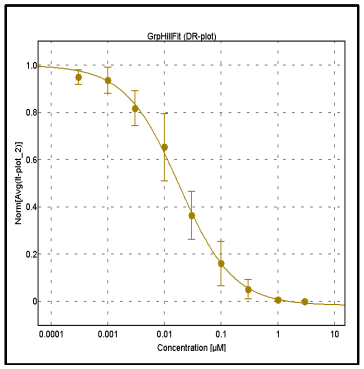
Pain related channels

Pain related channels available at SB			
Nav1.1	TRPV3	TRPC4	Kv7.2/7.3
Nav1.2	TRPV4	TRPC5	TREK-1
Nav1.3	TRPM2	TRPC6	TRESK
Nav1.7	TRPM3	P2X1	SLACK
Nav1.8	TRPM4	P2X2	Ano1
TRPA1	TRPM8	P2X3	NMDA
TRPV1	TRPC1	P2X4	
TRPV2	TRPC3	P2X7	

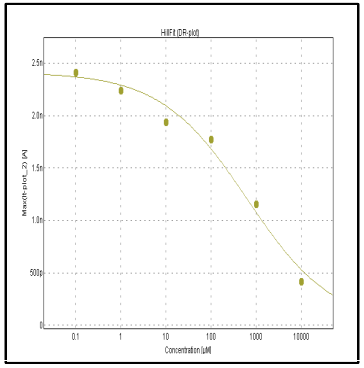
Animal variants		
Dog Nav1.7	Monkey TRPA1	Rat TRPV1
Rat Nav1.7	Sheep TRPA1	Dog TRPV1 (transient)
Monkey Nav1.7		

Validation

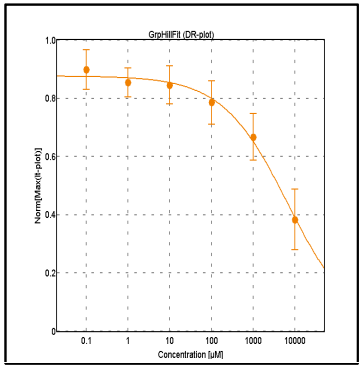
QPatch - voltage gated



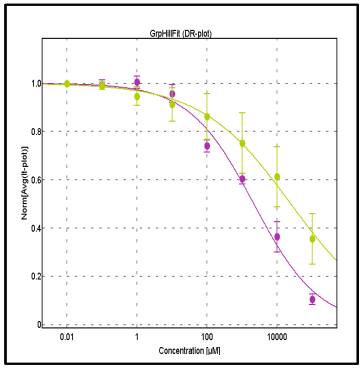
Nav1.1 – Nav1.8



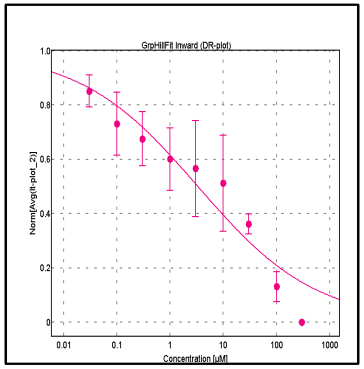
TREK-1



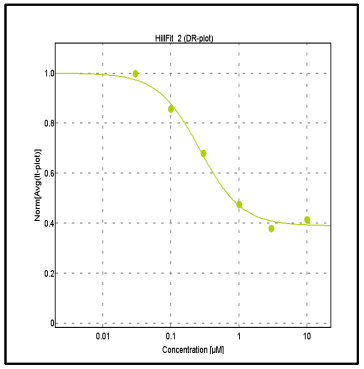
TRESK



Kv3.1



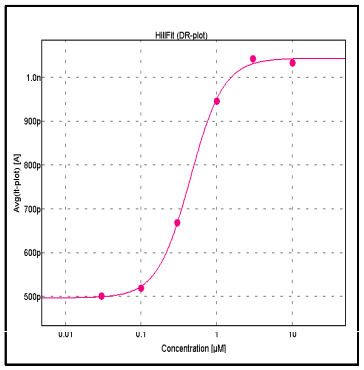
Ano 1



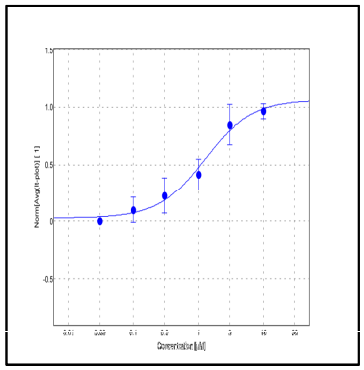
Ano 2

Validation

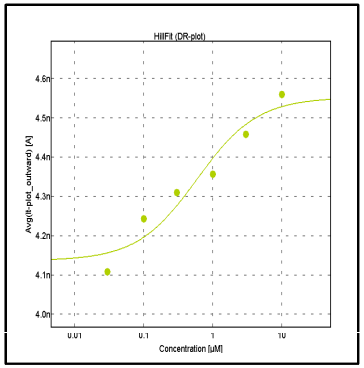
QPatch – ligand gated



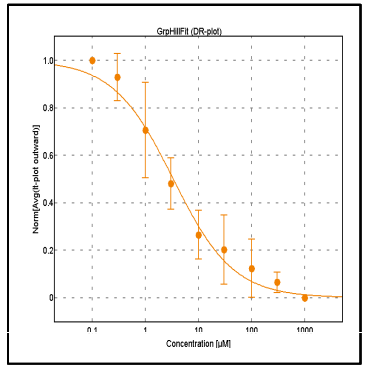
TRPM4



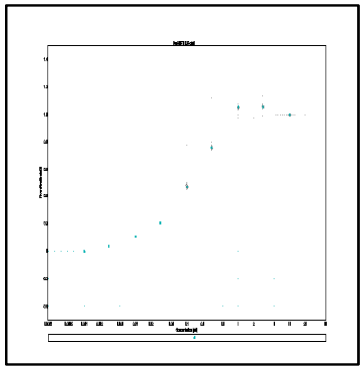
TRPM5



TRPA1



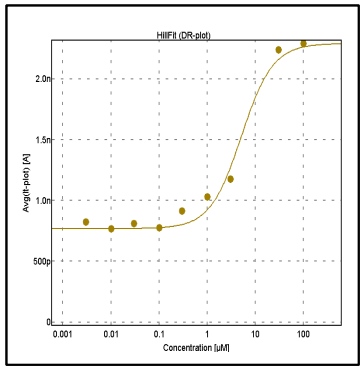
TRPA1 (monkey)



TRPV1

Awaiting data analysis

TRPV3



TRPV4

TRP channels

- 22 TRP channels

TRPA1	TRPA1 (monkey)	TRPA1 (sheep)					
TRPC1	TRPC3	TRPC4	TRPC5	TRPC6	TRPC7		
TRPM2	TRPM3	TRPM4	TRPM5	TRPM8			
TRPV1	TRPV2	TRPV3	TRPV4	TRPV5	TRPV6	TRPV1 (rat)	TRPV1 (dog)

- Customized selectivity panels

TRP select

- Flexstation assay or manual patch clamp assessment

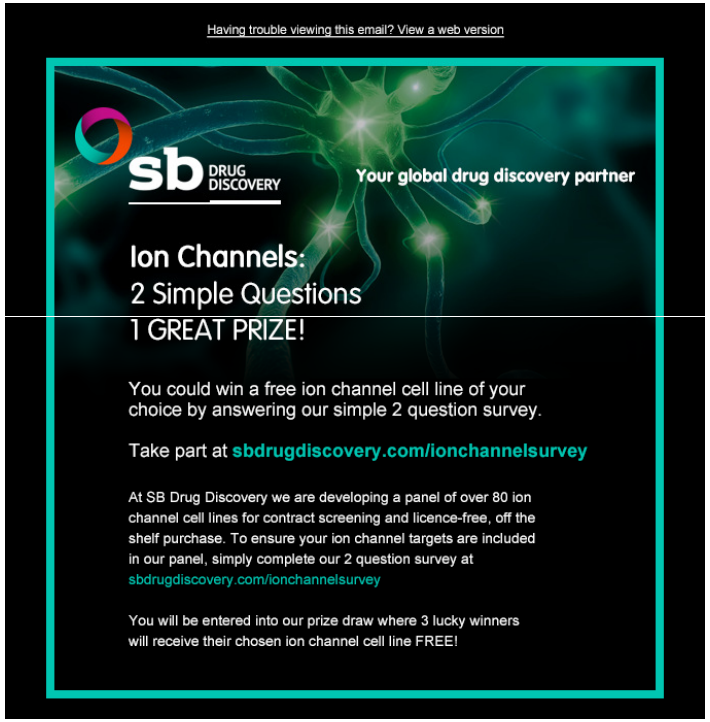
- Ongoing development of single use, assay ready cells for custom selectivity kits

Moving forward

Development of 30 new ion channel cell lines

Targets selected based on client feedback

Optimized cell lines for QPatch & IonWorks



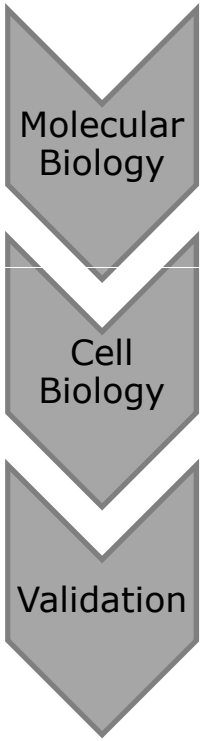

Delivering your stable cell line of choice
sb
CELL LINE
DEVELOPMENT

Custom Cell Line Development

- Conventional cell line development process
- Risk-free development

Milestone 1 →

Deliverable – sequence validated expression plasmid



- Gene synth, subcloning, mutagenesis
- Sequence analysis

- Transfection, clonal selection
- Single cell colony growth

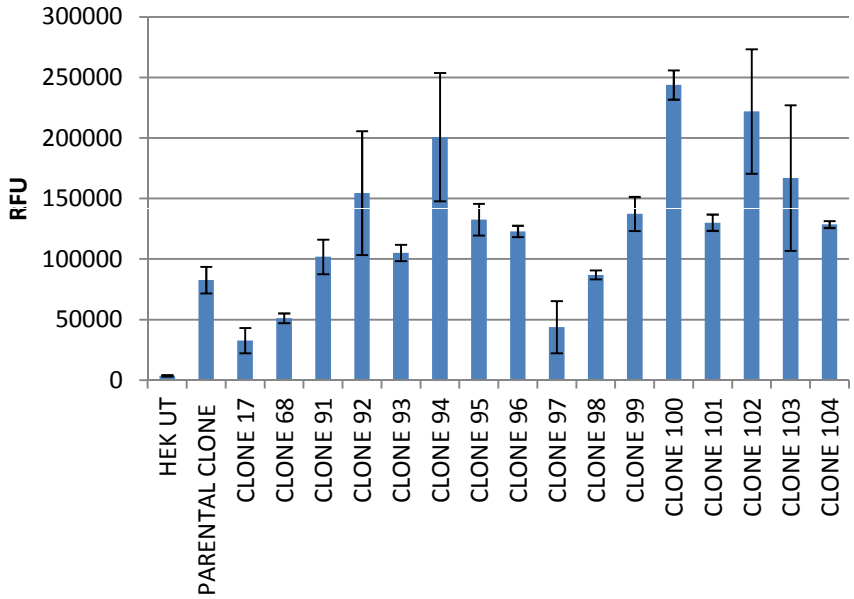
- Colony screening
- Detailed electrophysiology assessment
- Stability, mycoplasma, stock generation

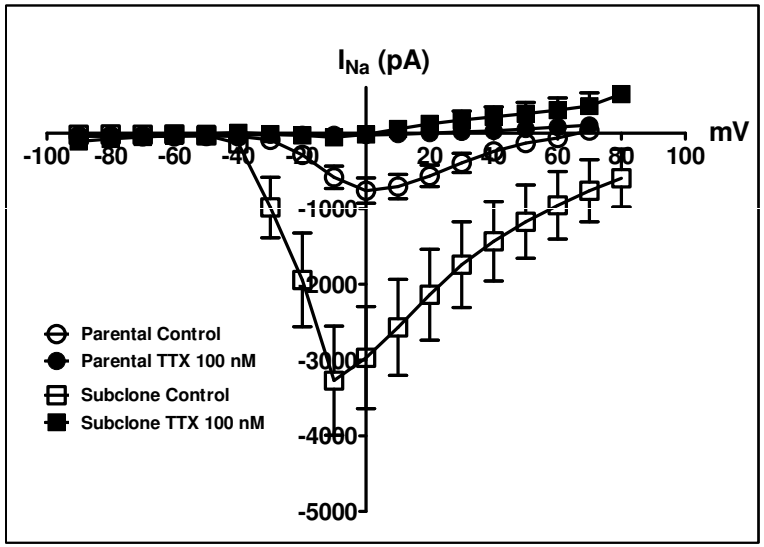
Milestone 2 →

Deliverable – Functionally validated stable cell line (defined criteria)

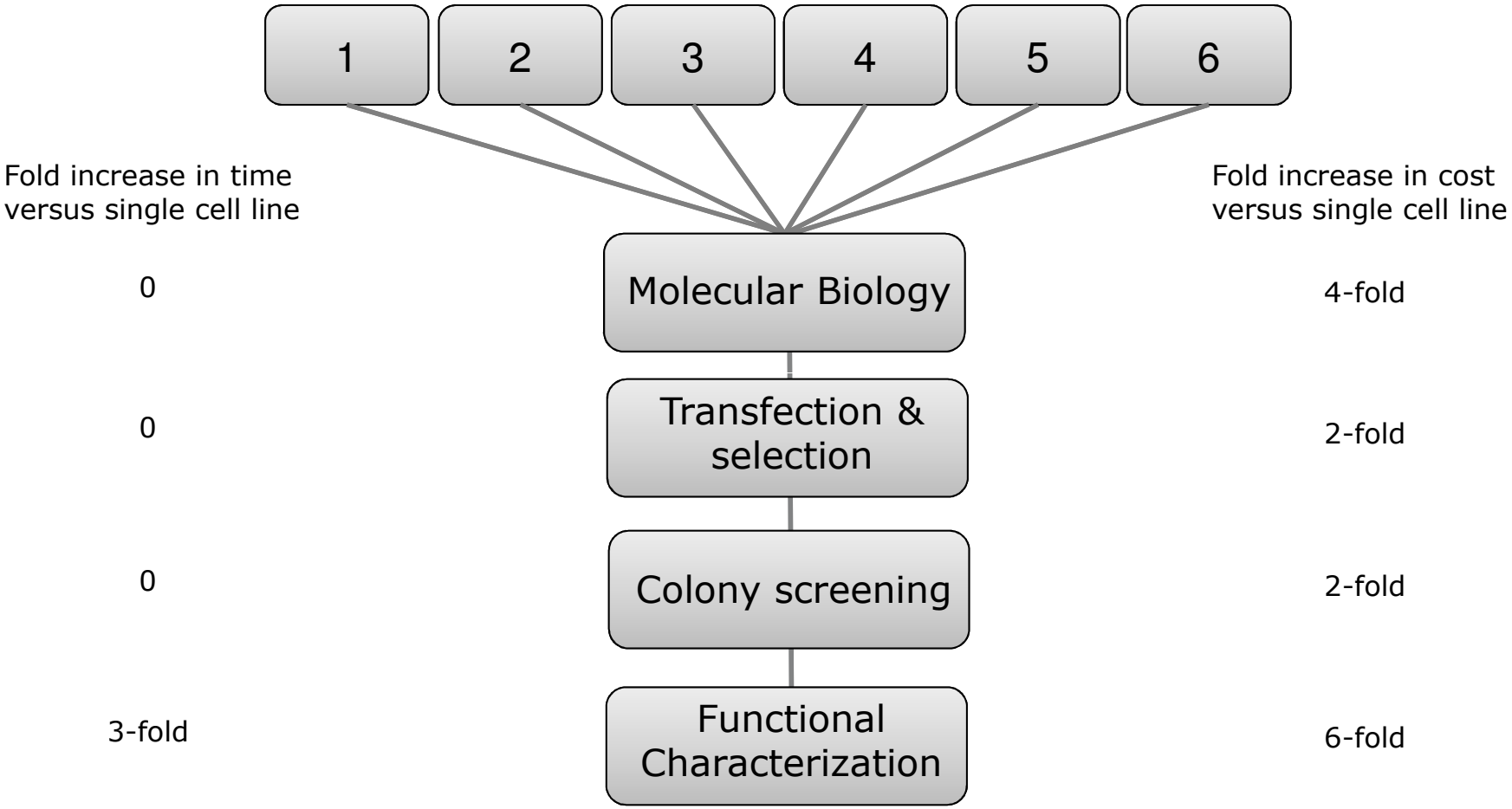
Improving cell lines

Sub-cloning





Parallel Processing



Parallel Processing

Development of multiple cell lines in parallel

- Selectivity panels
- Animal variants
- Disease-related mutants
- Mixed selections

Benefits

- Minimal increase in timelines
- Significant cost savings


Your global drug discovery partner
sb
SCREENING
& ASSAYS

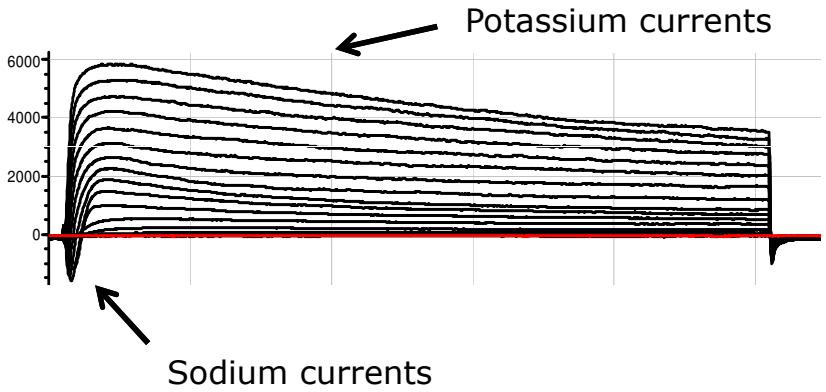
Assay services

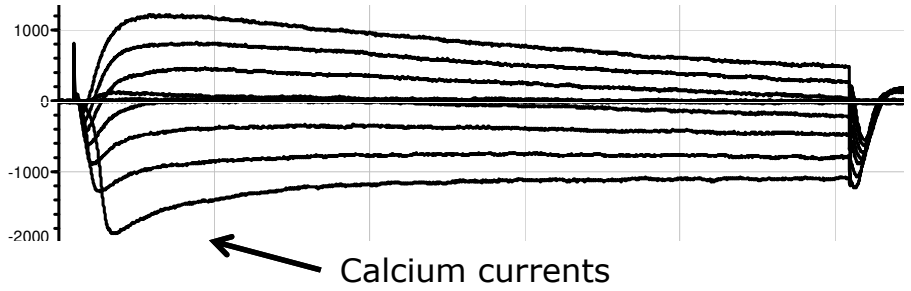
- Flexstation, conventional electrophysiology, IonWorks, QPatch
  - High throughput screening
  - Selectivity profiling
  - Biophysical characterization
  - Lead optimization
- DRG studies
- *In vivo* models of pain

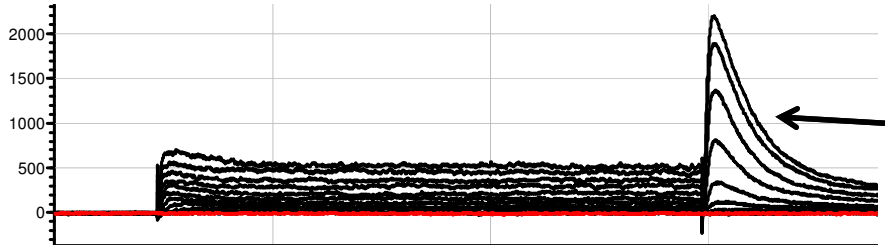
DRG studies

Freshly isolated rat DRG

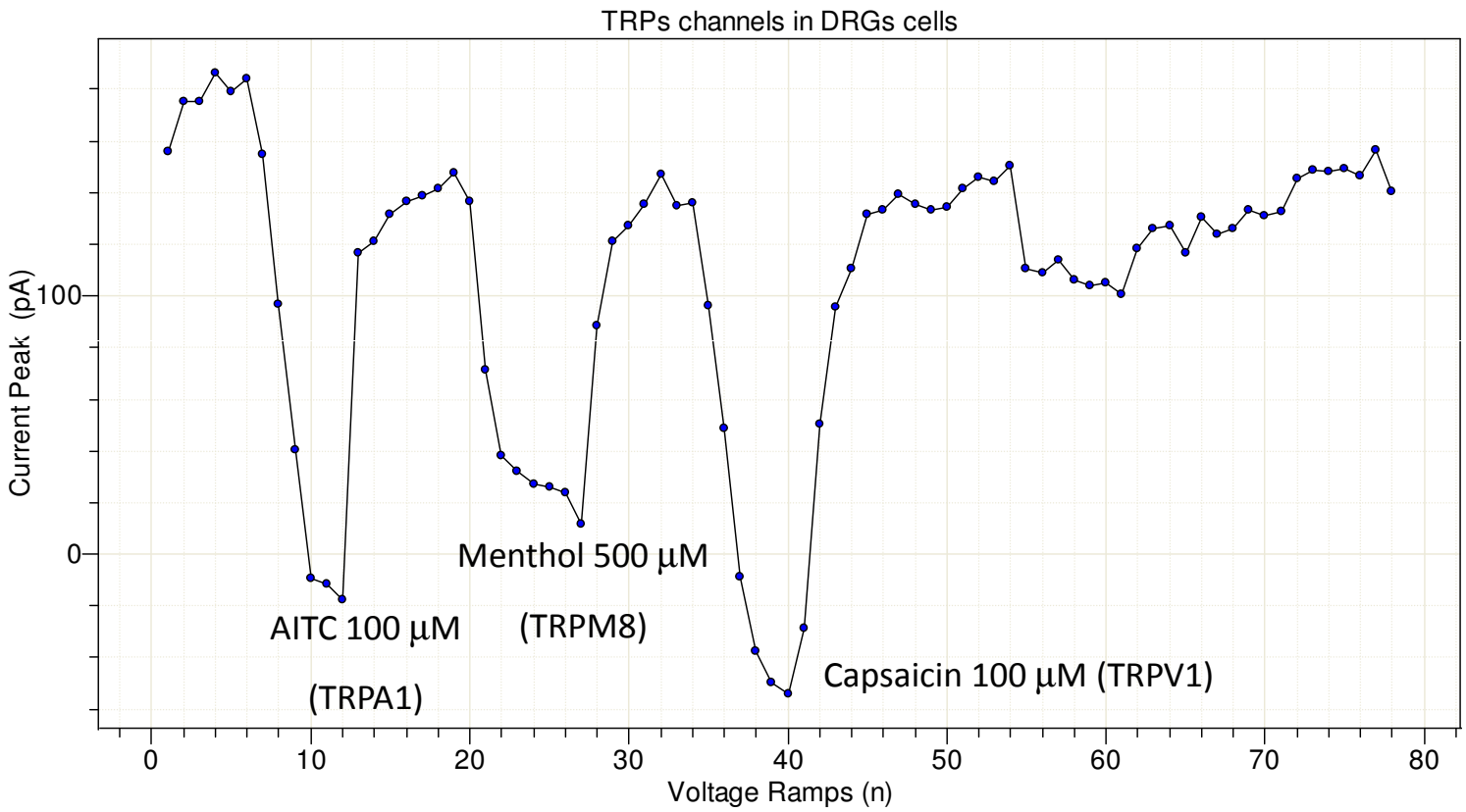
Presence of sodium, calcium, potassium & TRP channels







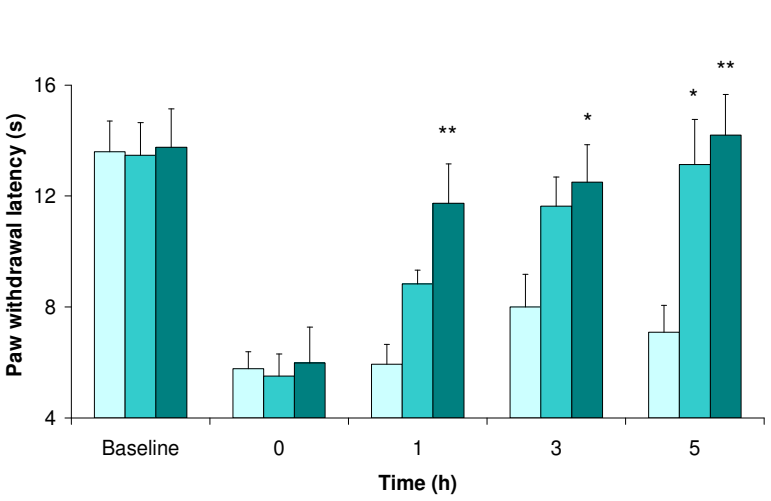
DRG studies

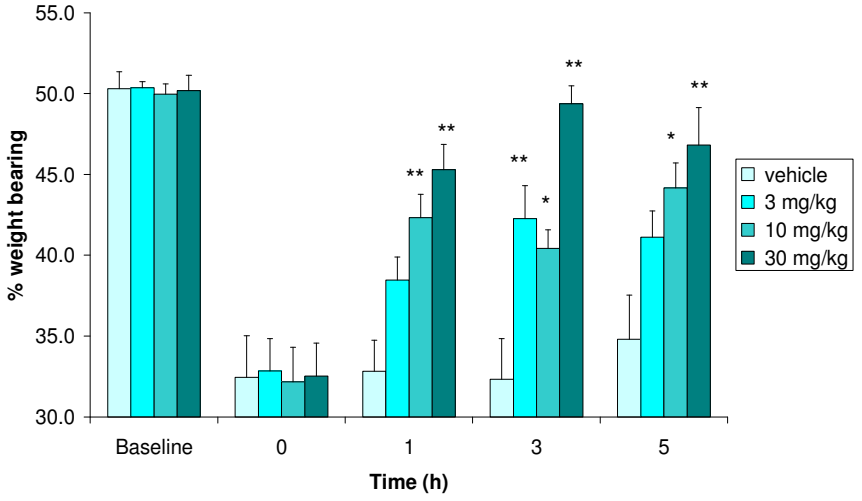

TRPs channels in DRGs cells
Current Peak (pA)
100
0
AITC 100 µM
(TRPA1)
Menthol 500 µM
(TRPM8)
Capsaicin 100 µM (TRPV1)
0
10
20
30
40
50
60
70
80
Voltage Ramps (n)

Proof of concept *in vivo* models of pain

Inflammatory pain (CFA)

- Complete Freund's adjuvant injected into hind paw
- Acute & sub-chronic inflammation at 24hr / 7 days respectively
- Inflammatory cascade causes swelling & pain lasting 1-14 days
- Thermal hyperalgesia (Hargreaves plantar test) & mechanical hypersensitivity (weight bearing) assessed



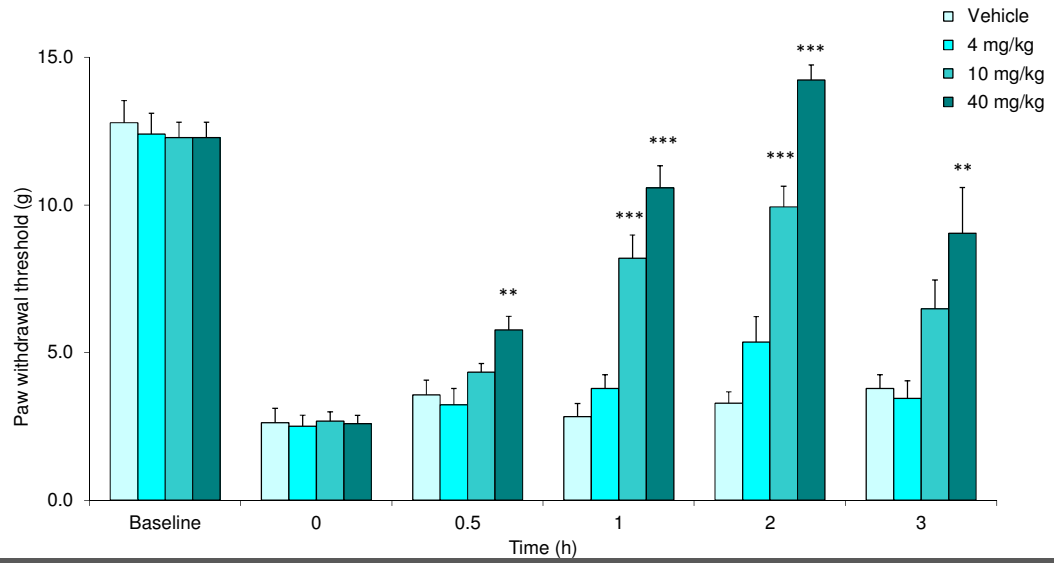


Carrageenan model also available

Proof of concept *in vivo* models of pain

Neuropathic pain (Chung model)

- Tight ligation of the L5 spinal nerve
- Mechanical allodynia measured by paw withdrawal threshold (von Frey filaments)
- Thermal hyperalgesia measured by Hargreaves plantar test

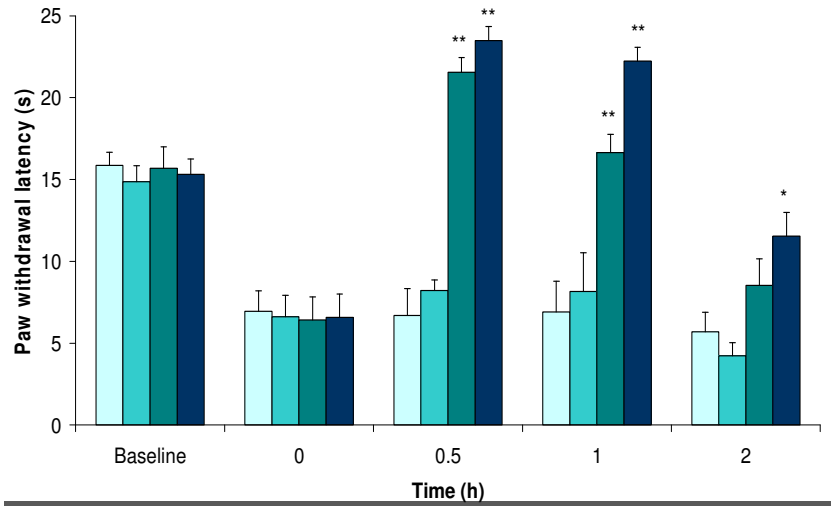


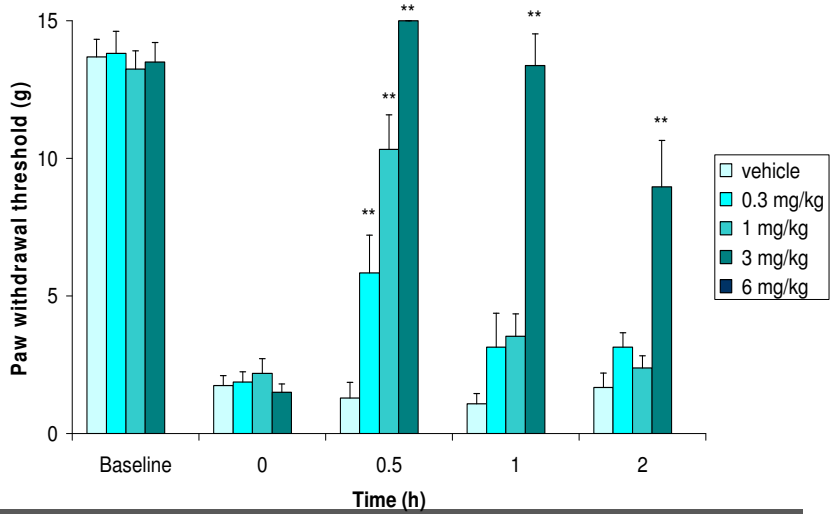
Bennett (CCI) model also available

Proof of concept *in vivo* models of pain

Post-operative pain (Brennan model)

- Incision made in plantar surface of hind paw, plantaris muscle elevated & wound closed
- Causes spontaneous pain, thermal & mechanical thresholds reduced for 4 days
- Thermal hyperalgesia (Hargreaves plantar test) & mechanical allodynia
  paw withdrawal (von Frey filaments) assessed





Proof of concept *in vivo* models of pain

Acute nociception tests

- Measure response thresholds to high intensity stimuli
- Hotplate & tailflick tests in response to thermal stimulus

in vivo expertise

- *In vivo* specialists are SB Drug Discovery employees
- All work carried out at SB Drug Discovery's site
- Scientists possess the relevant animal licences
- Each specialist has >10 years *in vivo* experience
- SB standard protocols or client specified methods
- All models are validated and ready to run

Summary

Extensive panel of off-the-shelf ion channel cell lines

Largest commercial source of TRP channels

Risk-free ion channel cell line development

High throughput screening, profiling & biophysical characterization

DRG studies & *in vivo* models of pain

Enjoy!