PharmaCore Labs

Discovery Services

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What we do

- Preclinical Cardiac Safety Assessment (details in pages 4-8)

  In vitro: CiPA, hERG, Nav1.5, Cav1.2
  Ex vivo: Purkinje fiber AP, isolated heart ECG, arrhythmia model
  In vivo: Dog telemetry ECG

- Neuroscience (details in pages 9-15)

  In vitro: Ion channel research in cell lines, isolated DRG and iPS cells
  Ex vivo: Brain slice electrophysiology
  In vivo: Animal models

Why choose PharmaCore Labs

About us:

- Founded in 2013 with headquarters in San Diego, California.
- Main laboratories based in Suzhou and Haimen, China.
- State-of-the-art facility including *in vivo*, *in vitro* labs, cell culture room, animal housing, molecular biology and chemistry labs.
- Total of 120 clients till 2022.

What we offer:

- High quality (experienced staff; two internal QC review processes; free of charge for any unsatisfied result).
- Competitive price (details in pages 16-18; matching any offer from the competitor).
- Rapid Turnaround time (details in page).

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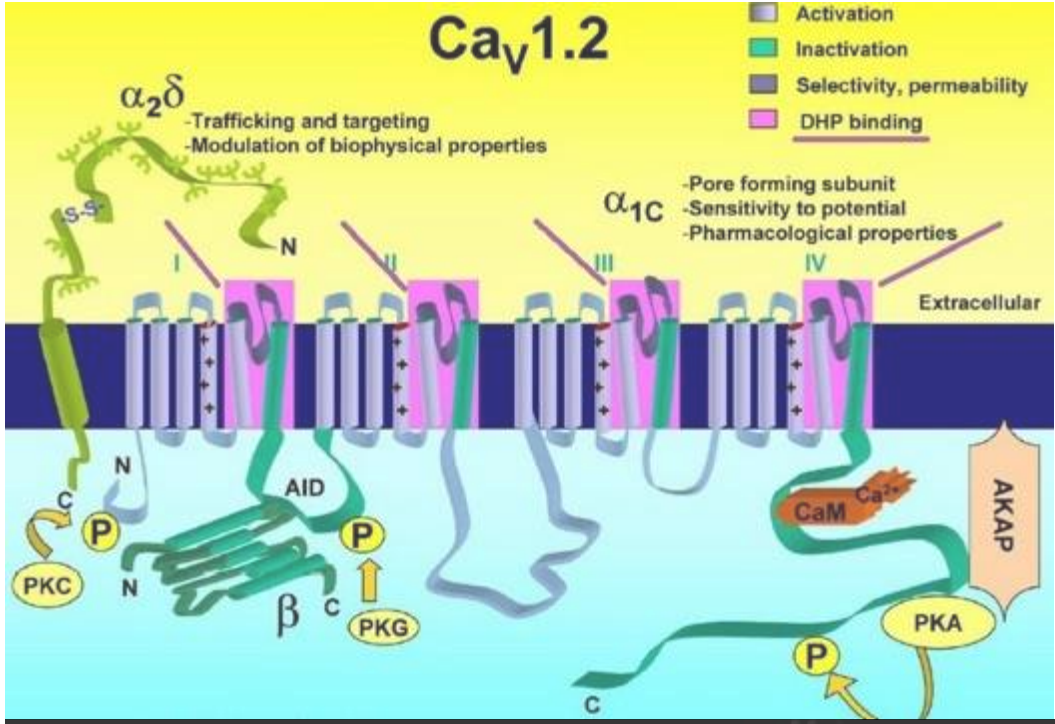
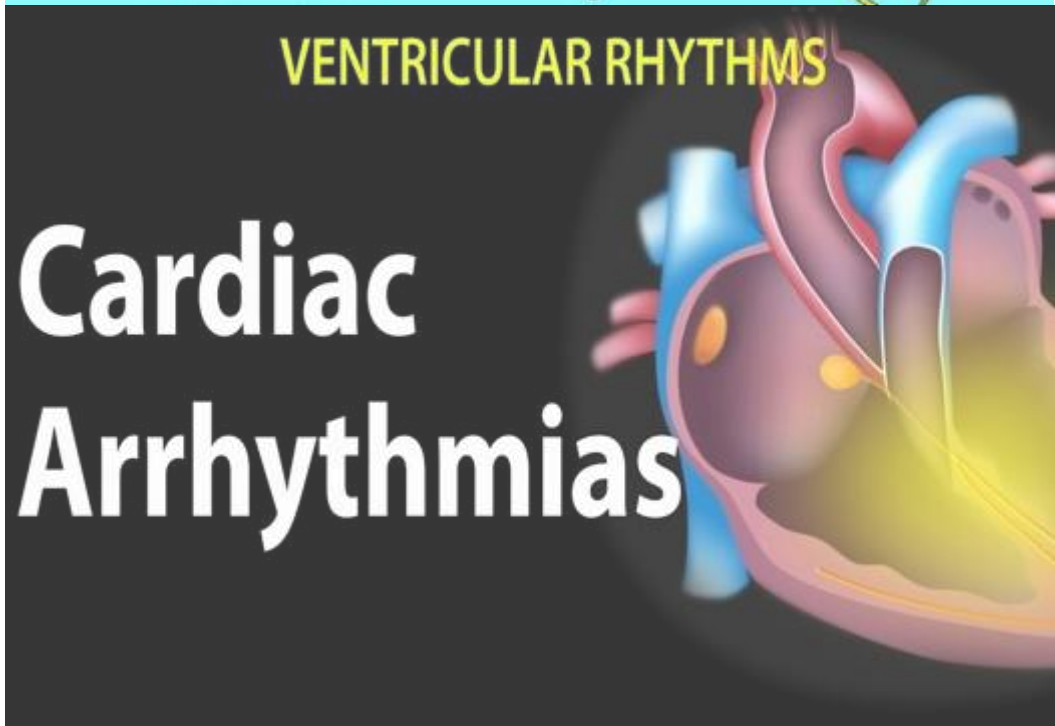
Cardiac Safety Assessment





In Vitro

Stable cell lines

- CiPA IC cardiac safety assessment
- hERG, Cav1.2, Nav1.5 and KvLQT1 panel screening
- iPS CM recording

Fresh isolated cell

- Fresh isolated myocytes manual patch clamp
- Calcium imagine and contractility measurement

Ex-in Vivo

Langendorff isolated heart

- QTc assessment
- Left ventricular contractility test
- Cardiac safety risk assessment

Purkinje fiber/ventricular muscle

- Action potential recording
- Use-dependent and triangulation study
- Cardiac safety assessment

In Vivo

Telemetry

- ECG, blood pressure, temperature recording

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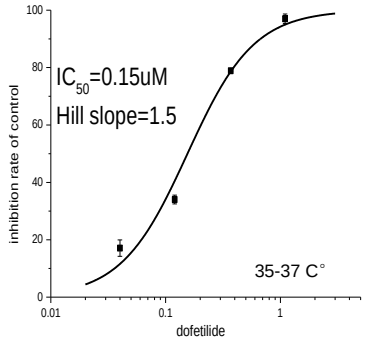
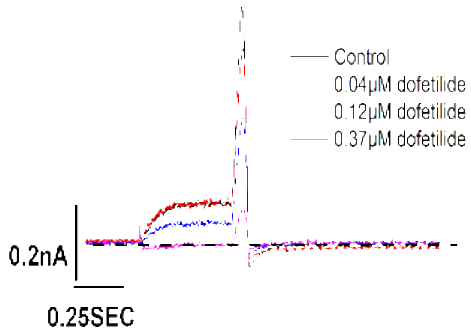
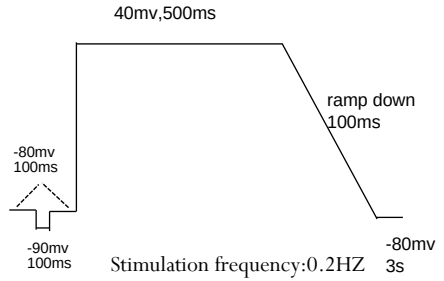
CiPA IC cardiac safety assessment

09.18.2019

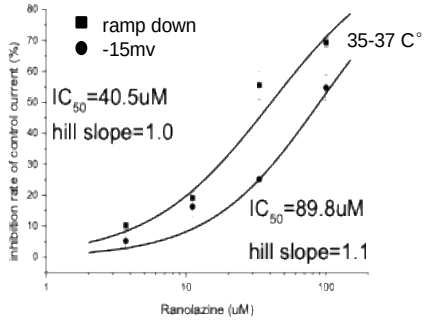
hERG

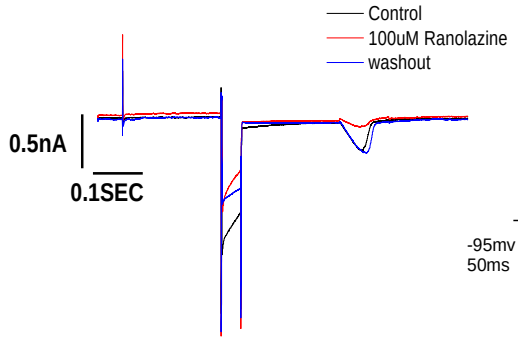


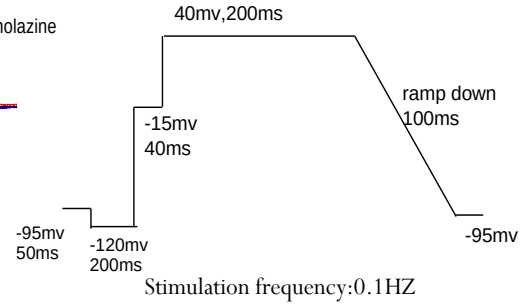




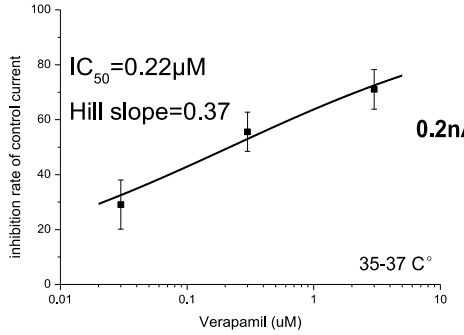
Cav1.2

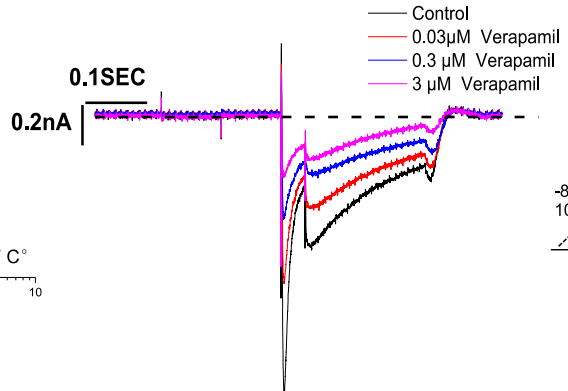


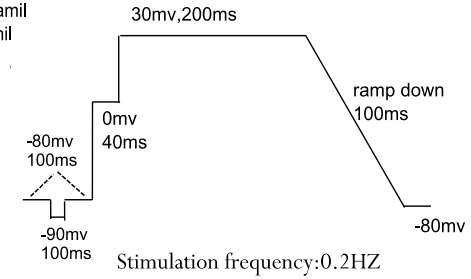




Slow Nav1.7







Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines

CONTEXT OF USE

As it is anticipated that nonclinical ion channel data will play an important role for regulatory decision-making in drug development programs, standardized protocols, methods for data quality assessment, and data analysis plans to quantify drug effects are recommended. The following contains detailed voltage protocol recommendations for hERG, CaV1.2, and NaV1.5 channel studies using patch clamp method to support an evaluation of torsade de pointes risk using the Comprehensive in vitro Proarrhythmia Assay (CiPA). These recommendations are based on current knowledge and are expected to evolve over time. Therefore, the document is time-stamped for version control. We encourage you to verify with the FDA prior to initiating the studies to: 1) ensure that the document you have is up-to-date; 2) clarify which protocol(s) to test for a specific drug; and 3) address additional questions.

Note that drug effects on additional cardiac ionic currents may be requested by the review Division on a case-by-case basis to address cardiac safety concerns. With the exception of the hERG protocol, protocols to study other ionic currents are not routinely requested by the review division.

ION CHANNEL PROTOCOLS TO ASSESS IC_{50}

Drug block of ion channels is sensitive to voltage protocol, recording temperature, and additional experimental factors. Therefore, standardized protocols are recommended for each ionic current to allow for data interpretation and data quality assessment. Data quality here is defined by cell health, recording quality, and stability of ionic current measured for the duration of individual experiments. Cell health and recording quality are defined by passive membrane properties including holding current and input resistance measured at rest or holding potential. Because most ionic currents measured in whole cell configuration exhibit time- and/or activity-dependent change in characteristics following whole cell formation, baseline current stability in control solution must be recorded and achieved prior to drug application for accurate assessment of drug effects. Recording temperature should be done at physiological temperature (~37°C) or as close to physiological temperature as possible unless stated otherwise.

HERG current protocol

Data derived from this protocol are used to understand the relationship between drug potency on affecting hERG current and therapeutic exposure level. Seal resistance should be ≥1GΩ. This voltage protocol is approximately 5 s in duration, and is to be repeated every 5 s. The voltage "ramp down" phase is 100 ms in duration, from +40 mV to -80 mV (hence a voltage change of -1.2 V/s). The small hyperpolarizing voltage pulse from -80 to -90 mV is used to calculate input resistance according to Ohm's law. Quality of the recorded cell and ongoing experiment integrity should be reflected in stable holding current (associated with the -80 mV step just prior to the depolarizing voltage step) and input resistance. If high seal resistance is obtained, then holding current and input resistance may be used as indicators of cell health and are expected to remain stable following initial whole cell dialysis period for the remaining duration of the experiment.

The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 5 KCl, 1MgCl2*6H2O, 1 CaCl2*H2O, 12.5 dextrose; pH adjusted to 7.4 with 5 M NaOH; ~280 mOsM.

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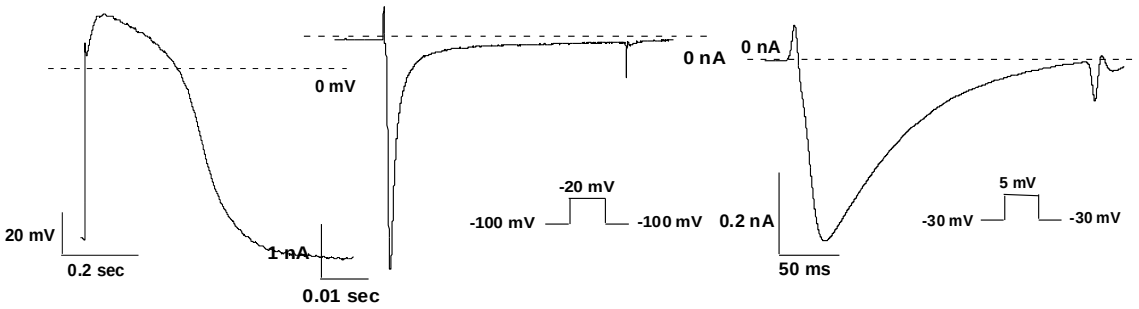
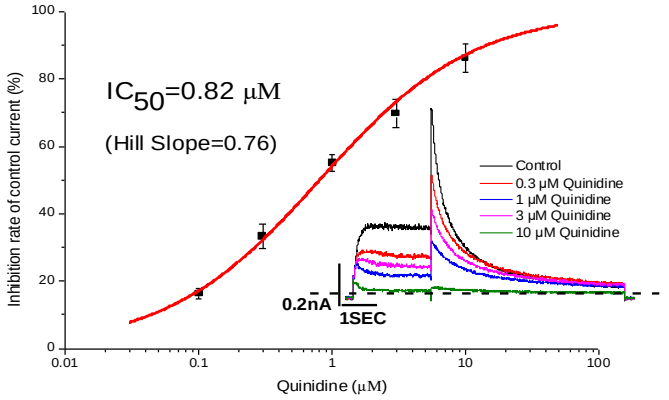
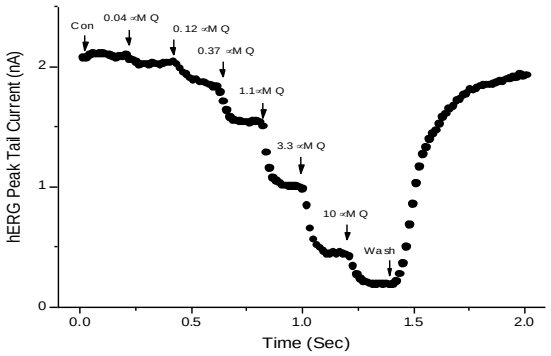
In vitro cardiac ion channel assays

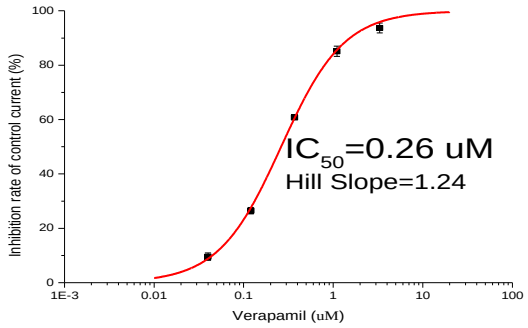


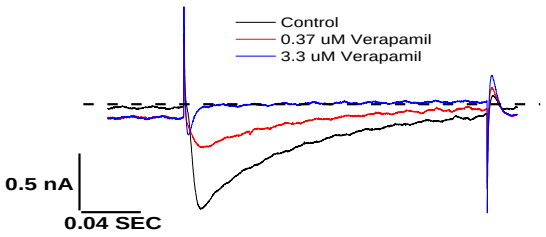
iPS CM



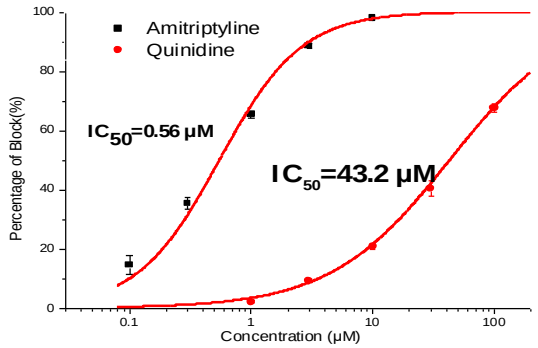


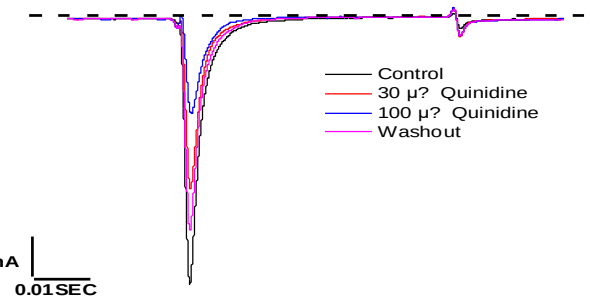
hERG





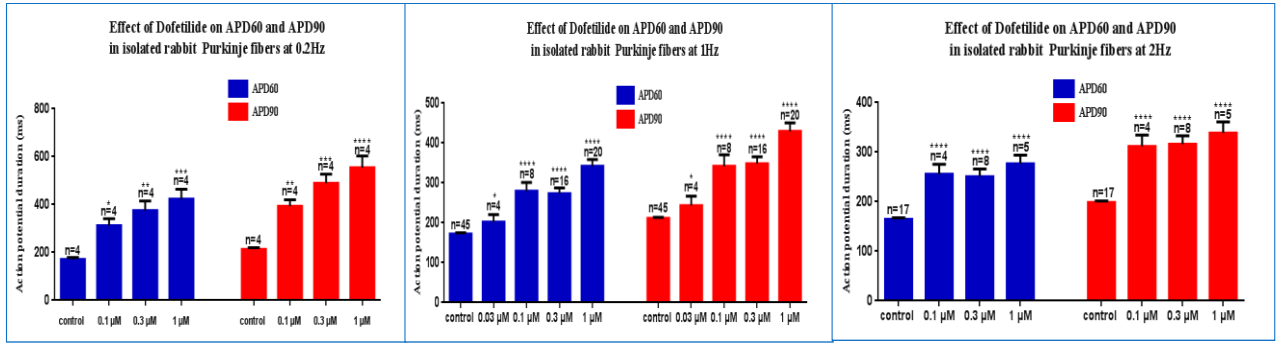
Cav1.2

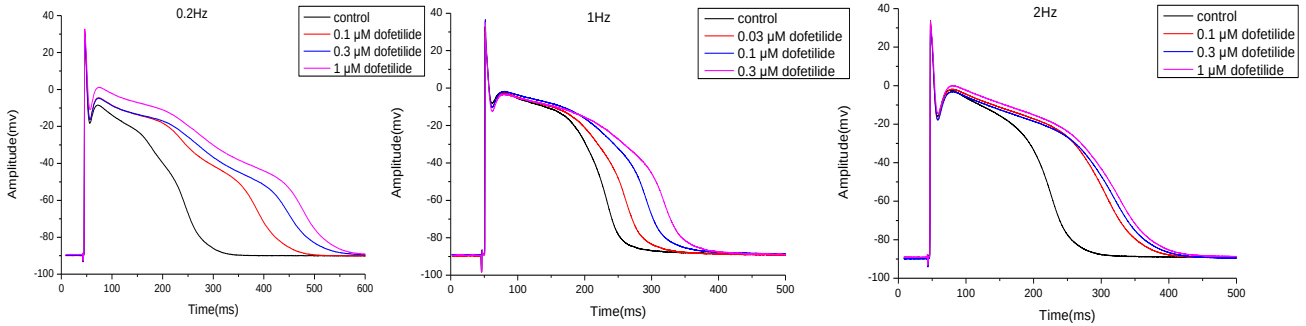




Nav1.5

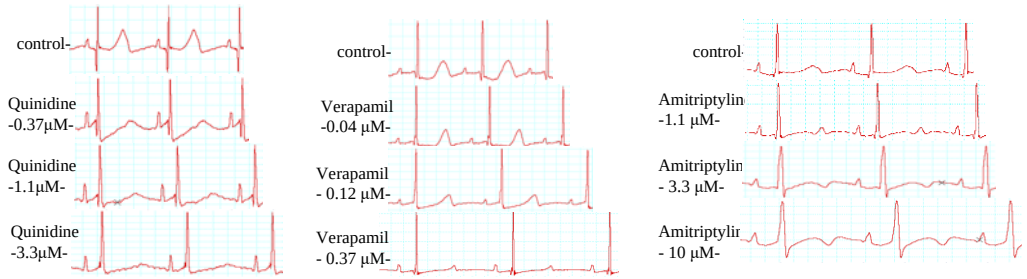
Ex vivo cardiac safety follow-up experiments





- Reverse-use dependence of prolongation APD refers to a decrease in lengthening AP duration at higher heart rate.
- Dofetilide significantly prolonged APD in rabbit heart Purkinje fiber. The APD prolongation by Dofetilide also shows very strong reverse use-dependency.
- 0.3 μM Dofetilide induce EAD at the lower stimulus rate of 0.2 Hz.

Compound	Concentration	RR (%)	HR (%)	PR (%)	QRS (%)	QT (%)	QTc (%)
Quinidine (n=3) hERG Cav1.2 Nav1.5	0.37 μM	3.0±2.6	-2.7±2.5	2.4±2.8	10.6±3.7	20.3±10.0	18.3±9.5
	1.1 μM	14.6±3.1	-12.6±2.4	30.9±12.6	12.6±8.2	38.2±9.7**	29.2±9.3*
	3.3 μM	23.6±3.9	-18.9±2.5	32.8±17.6	13.6±6.0	44.5±11.9***	30.0±10.4*
Verapamil (n=2) hERG Cav1.2 Nav1.5	0.04 μM	14.9±11.9	-10.4±8.2	-0.7±5.0	-4.3±2.6	3.7±4.6	-2.7±0.7
	0.12 μM	19.4±13.3	-15.2±9.4	5.8±13.5	-11.2±2.8	11.7±1.9	2.8±7.5
	0.37 μM	36.0±12.8**	-25.8±7.0**	5.4±9.4	-11.3±3.5	15.4±0.7	-0.6±4.0
Amitriptyline (n=1) hERG Cav1.2 Nav1.5	1.1 μM	4.42	-4.2	3.9	12.2	5.4	3.2
	3.3 μM	8.3	-7.7	40.4	17.8	12.8	8.4
	10 μM	20.4	-16.9	49.9	70.0	20.1	9.4



- Patch clamp results showed that both quinidine and verapamil could significantly block HERG, and the IC50 was less than 1 μM, indicate that both drugs has high risk of cardiac safety;
- However, ECG results showed that verapamil had no significant effect on the QTc, while quinidine prolonged QTc significantly;
- Langendorff assay has the ability of comprehensive cardiac safety risk assessment;

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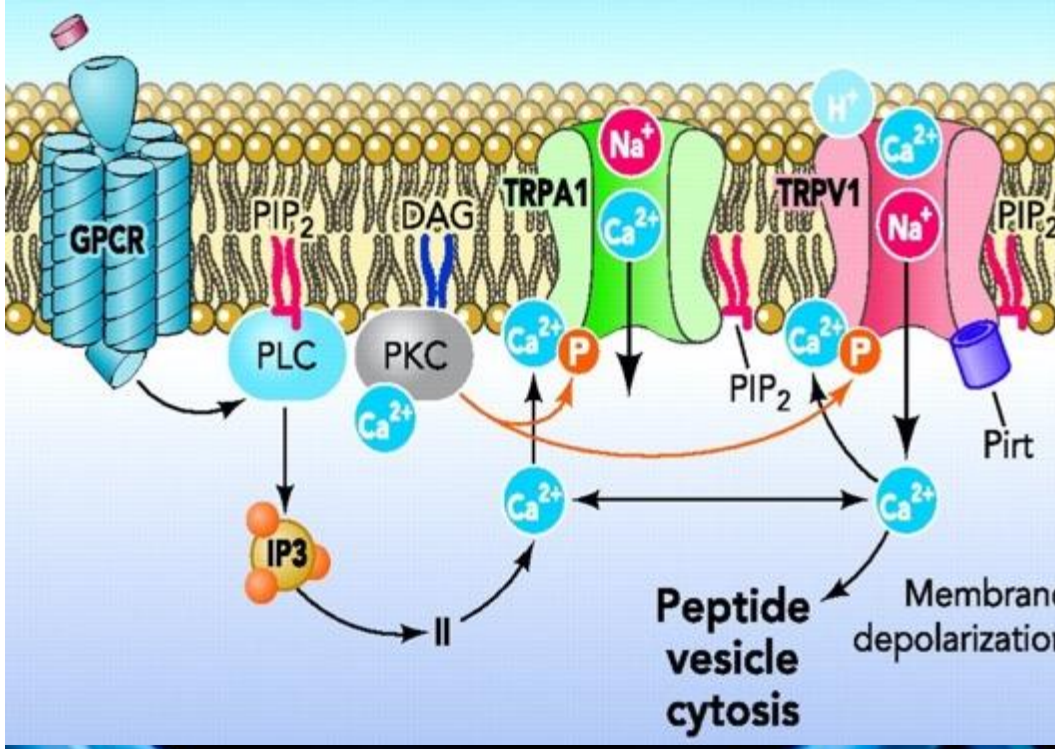
Neuroscience



In Vitro

Stable cell lines/transient expression：

1.Compound screening and IC50 ranking

2. Drug discovery mechanism study

3. Ion channel kinetics analysis

Fresh isolated cells：

1. DRG neurons and cardiac myocytes

2. Ion channel mechanism study

3. Neuronal excitability test

4.Calcium imagine and contractility measurement

Ex-in Vivo

Brain Slice and spinal cord：

1.Electrophysiological detection of spinal cord slices:

2. Field potential detection

3. Neural circuit examination

Langendorff isolated heart：

1.Hemodynamic and electrophysiological functions test

2.Left ventricular contractility test

3. Cardiac disease models

In Vivo

Disease models：

1. Inflammatory pain

2. Visceral pain

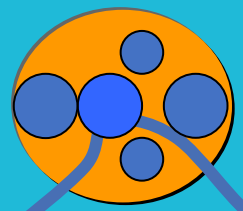
3. Bone cancer pain

4. Depression, itching

Ion channel drug discovery in neuroscience

- DRG ion channels
- Neuron excitability
- Voltage gated IC: Nav, Cav and K channels
- ligand gated IC: TRP, P2X channels, GABA, ASIC channel, NMDA/AMPA

Dorsal Root Ganglion (DRG)



Transmission (Ca^{2+})

Spinal cord

Brain slice

- Electrophysiological detection of spinal cord slices:
- Neuron excitability test (action potential)
- Voltage gated channel detection (sodium, potassium, calcium)
- Field potential detection
- Neural circuit examination

In vivo model

- Inflammatory pain
- Visceral pain
- Bone cancer pain
- Neuralgia

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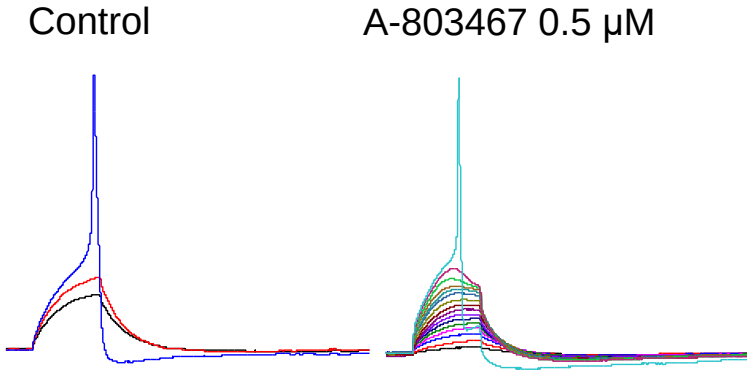
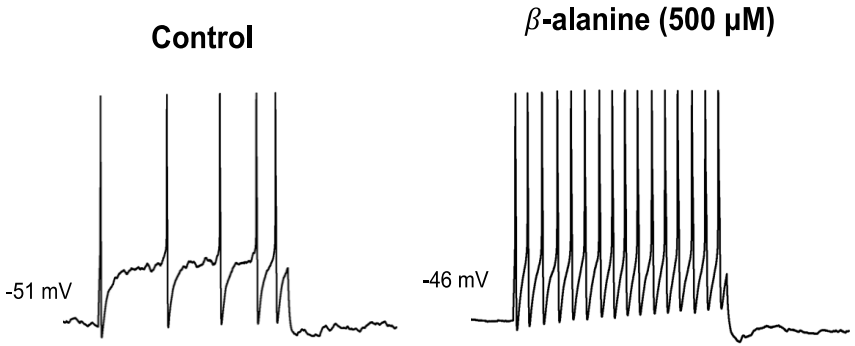
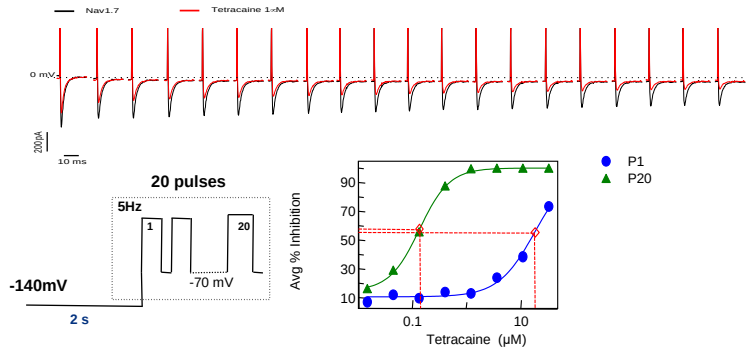
In vitro ion channel assays (voltage gated)



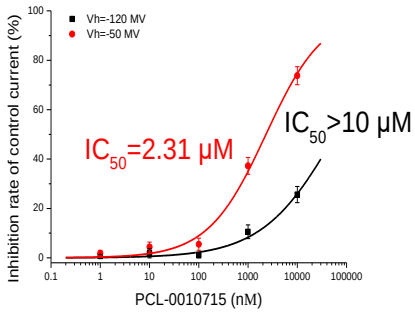
APs (DRG)

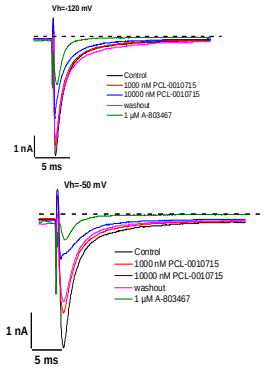


Excitability (DRG)

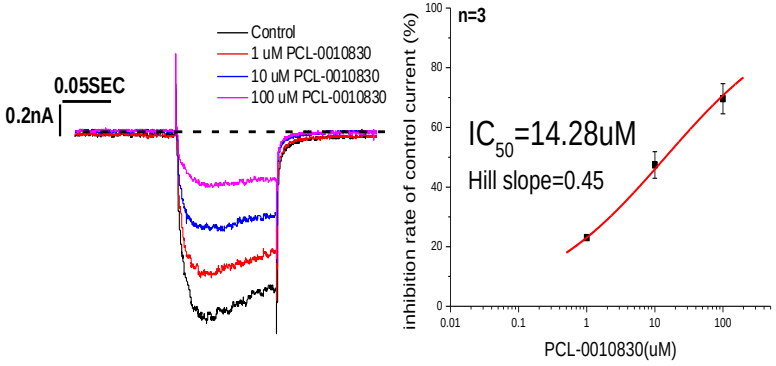


Nav1.7

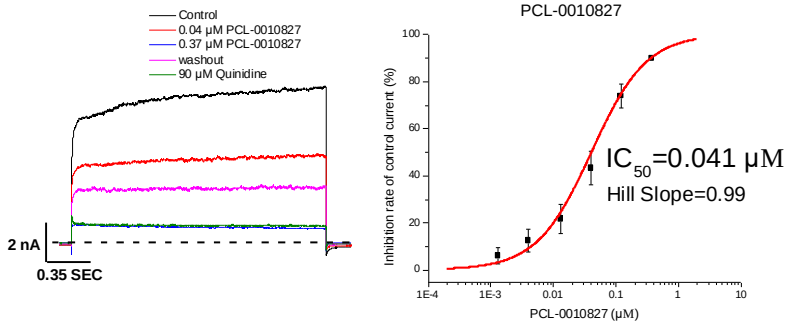




Nav1.8

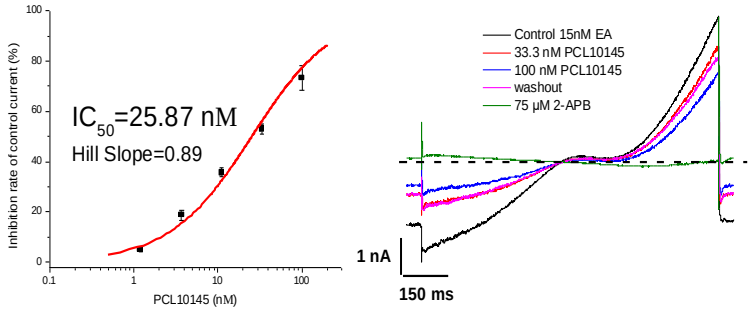


Nav1.9

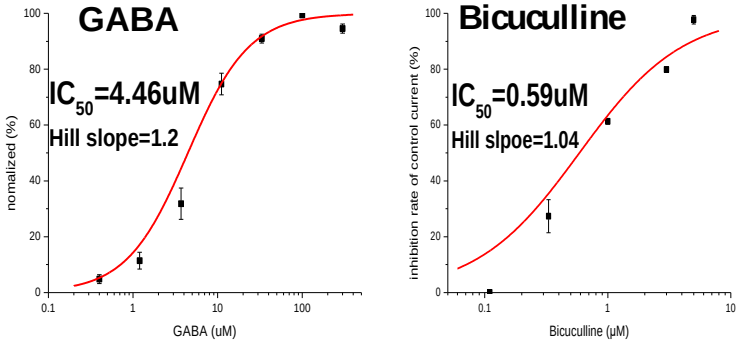


KCNT1

In vitro ion channel assays (ligand gated)

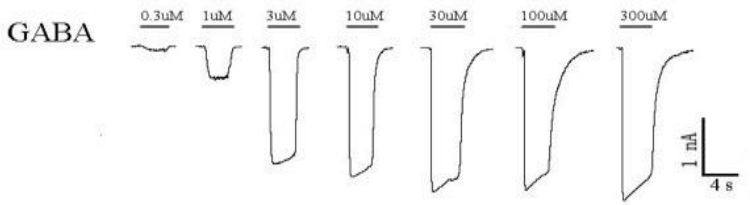


rTRPC5

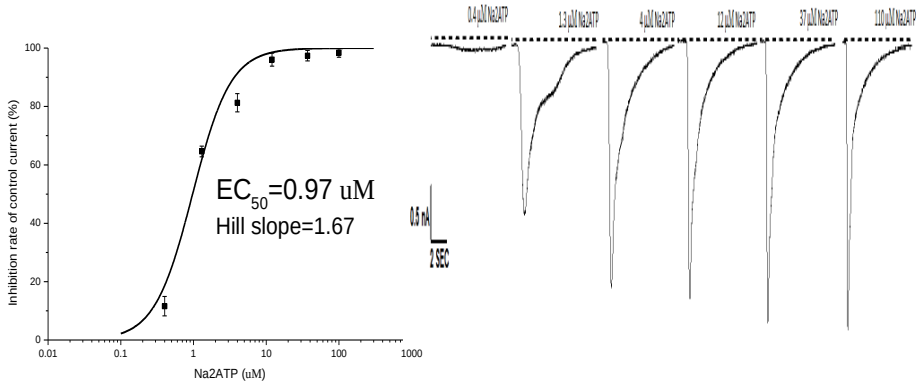


GABA (DRG)

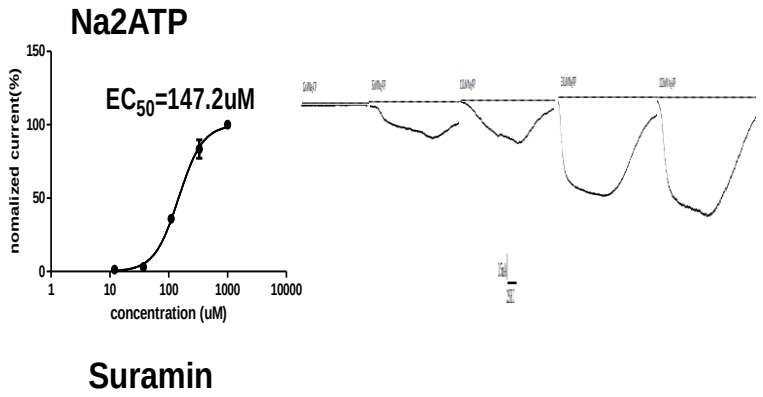
Dose response potentiating effect of GABA



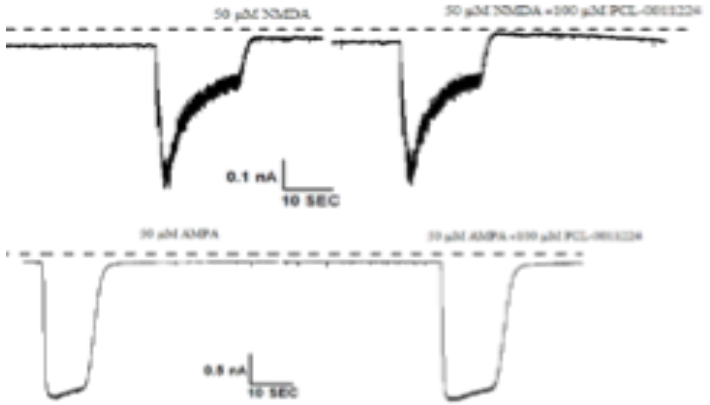
GABA



hP2X3

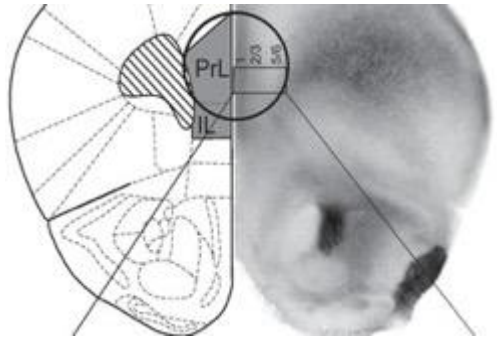


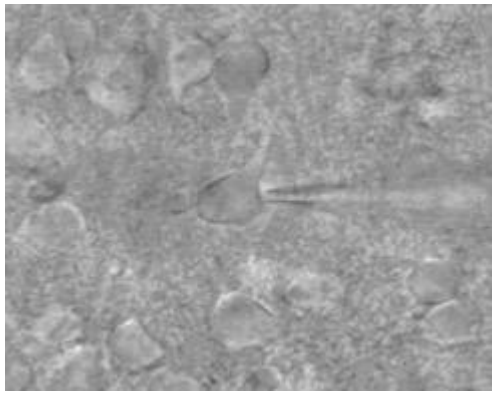
hP2X7

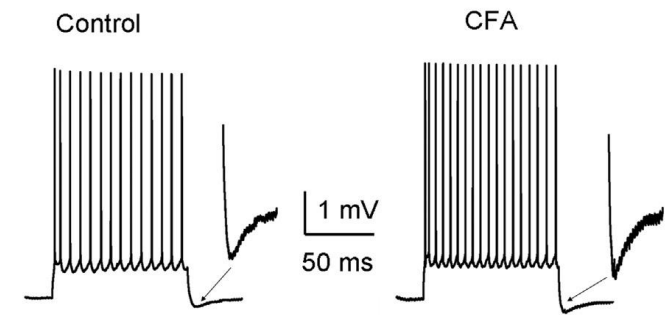


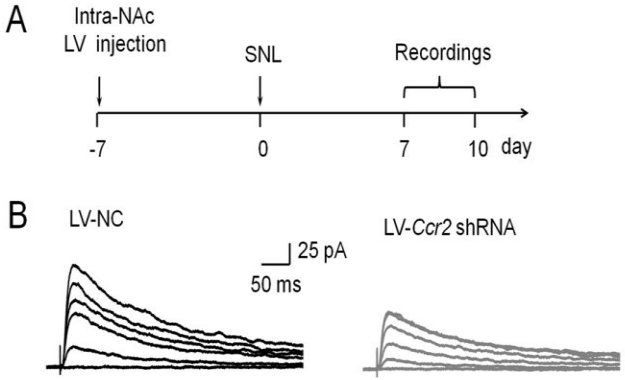
NMDA AMPA (DRG)

Brain slice electrophysiology study

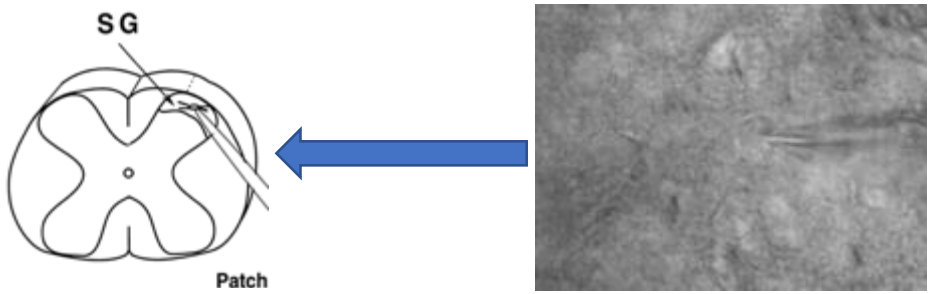


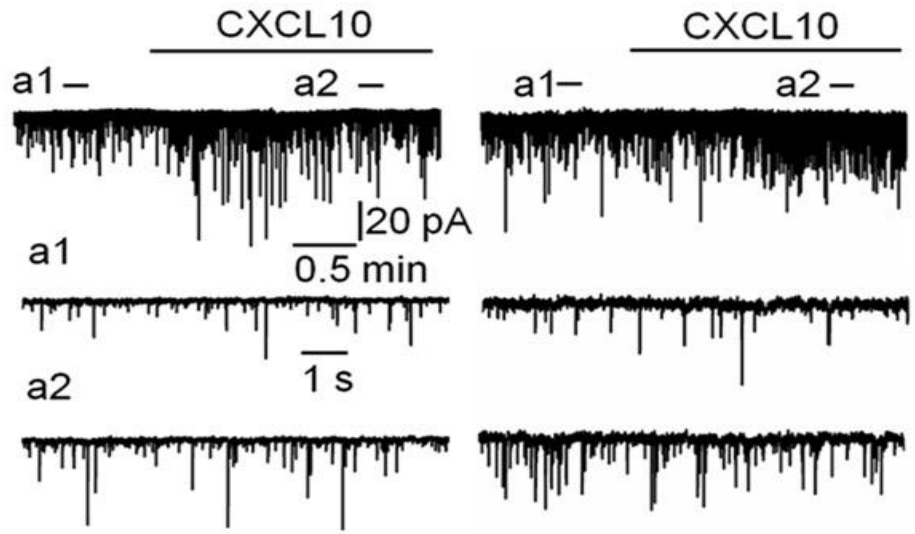






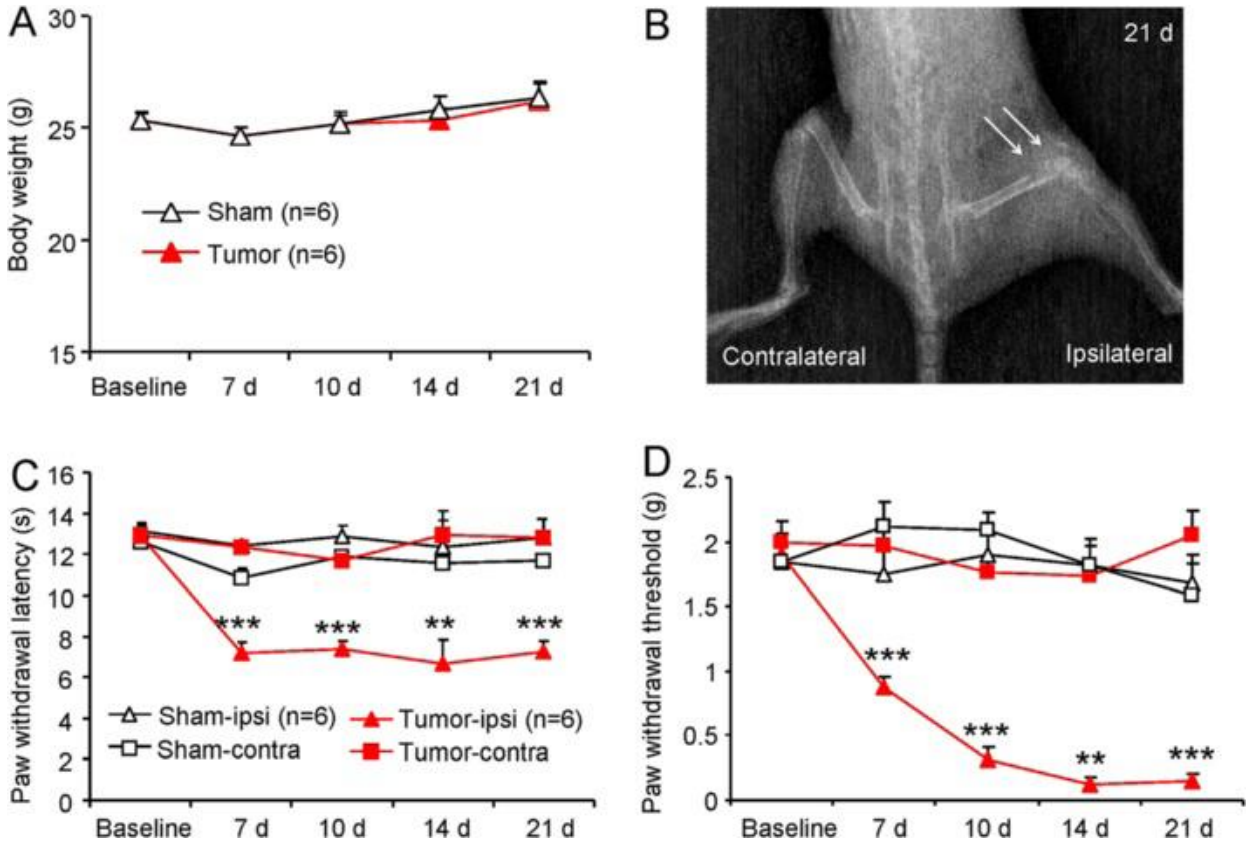
APs recorded on neurons in brain slices (prefrontal cortex) and NMDAR currents induced by electrical stimulation



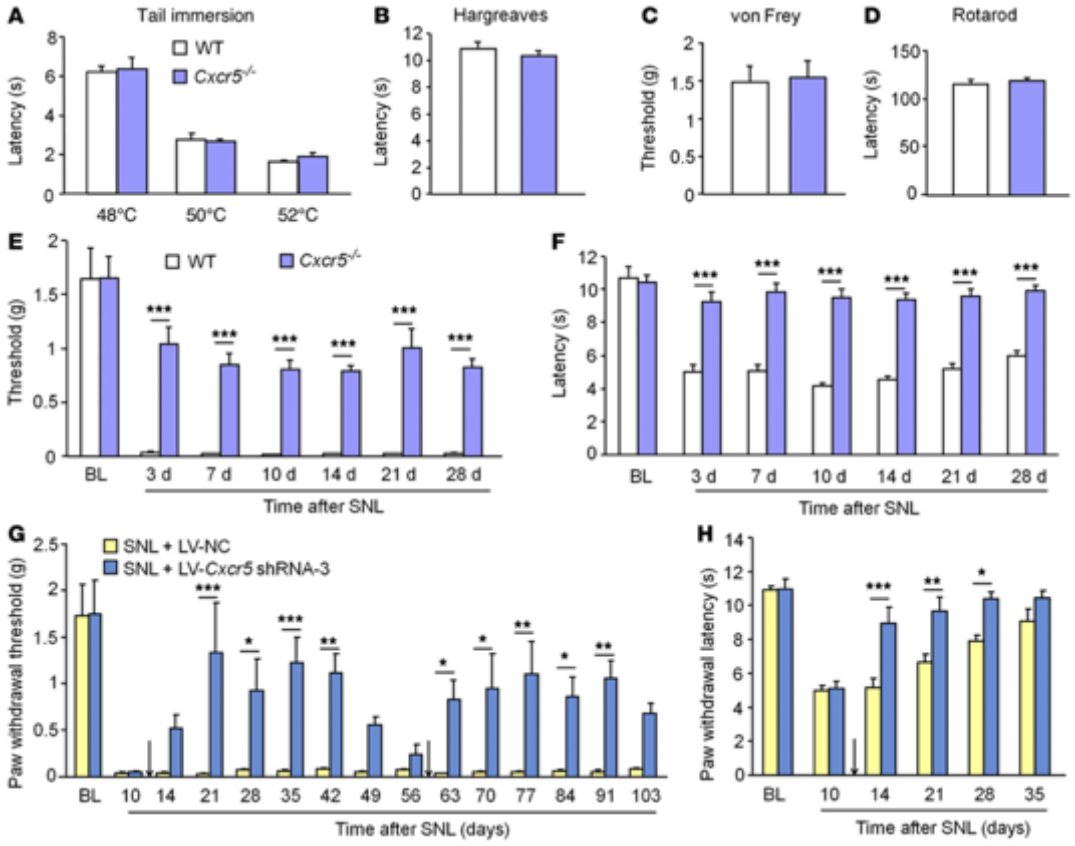


AMPAR mediated micro EPSCs in SG layer neurons of spinal cord slice

Pain animal model



Bone cancer pain in vivo model



Neuralgia in vivo model

PharmaCore Labs assay list and corresponding prices (2022)

Preclinical Cardiac Safety Assessment:

Assay name		Assay details	Price/compound	Comments
CiPA latest guidelines, recommended (Sept. 18, 2019) for IC cardiac safety	**hERG**	n = 4, 5 doses, physiological temperature (35~37 °C)	**$4999**	Stable cell line, Manual patch clamp, Turnaround time: about 20-30 business days after compound delivery, Two concentrations/cell
	Slow Nav1.5		**$5999**	
	hCav1.2		**$5999**	
Ion channel assay for cardiac safety	**hERG**	IC_{50}, n = 3, 5 doses	**$1200**	Stable cell line, Manual patch clamp, Turnaround time: about 2-3 business days after compound delivery
	hNav1.5	IC_{50}, n = 3, 5 doses	**$2500**	
	Cav1.2	IC_{50}, n = 3, 5 doses	**$2860**	

	Cav1.2 (I_{ca-L})	IC_{50}, n = 3, 4 doses	**$2800**	Acutely isolated cardiomyocytes from guinea pig, rat, rabbit, etc., Manual patch clamp, Turnaround time: about 3-5 business days after compound delivery
	Kv4.3 (I_{to})	IC_{50}, n = 3, 4 doses	**$2500**	
	Kv1.5 (I_{Kur})	IC_{50}, n = 3, 4 doses	**$2700**	
	KvLQT1/Mink (I_{Ks})	IC_{50}, n = 3, 4 doses	**$2500**	
	I_{k1}	IC_{50}, n = 3, 4 doses	**$1300**	
Cardiac action potential assay	**Purkinje Fiber**	n = 4, 3 doses, (3 stimulation frequencies, for example, 0.5, 1 and 2 Hz)	**$9999**	Rabbit, Turnaround time: about 12-15 business days after compound delivery
Langendorff heart ECG analysis	**Langendorff isolated perfused heart**	n = 4, 3 doses	**$3999**	Guinea pig, Turnaround time: about 3-5 business days after compound delivery
Telemetry	**Non-invasive telemetry**	n = 6, 3 doses	**$42000**	Dog, non-GLP, Turnaround time: about 60 business days

Neuroscience:

Assay name	Assay details	Price/compound	Comments
hNav1.7	IC_{50}, n = 3, 5 doses	**$2999**	Stable cell line, Manual patch clamp, Turnaround time: about 3-5 business days
hGABAa	EC_{50} or IC_{50} n = 3, 5 doses	**$3299**	
hP2X (P2X3, P2X2/3)	IC_{50} n = 3, 5 doses	**$3299**	
GABA	EC_{50} or IC_{50} n = 3, 5 doses	**$3999**	DRG，neuron, Manual patch clamp, Turnaround time: about 3-5 business days
Nav1.8	IC_{50} n = 3, 5 doses	**$3299**	
Nav1.9, NMDA, AMPA, P2X	IC_{50} n = 3, 5 doses	**$3999**	
AP	EC_{50} or IC_{50} n = 3, 5 doses	**$3999**	
Rat myocytes contraction and Ca transient	2 concentrations, n>3	**$4999**	
Brain slice, Pain model, behave science	Call for detail	**Call for detail**	Rat, mouse

Notes:

1. Test concentrations and data point could be adjusted based on client’s request with final price adjusted accordingly.
2. Turnaround time will be adjusted based on the number of test compounds.
3. Positive control in each assay is included.