Automated patch clamping for screening and profiling in drug discovery and risk assessment

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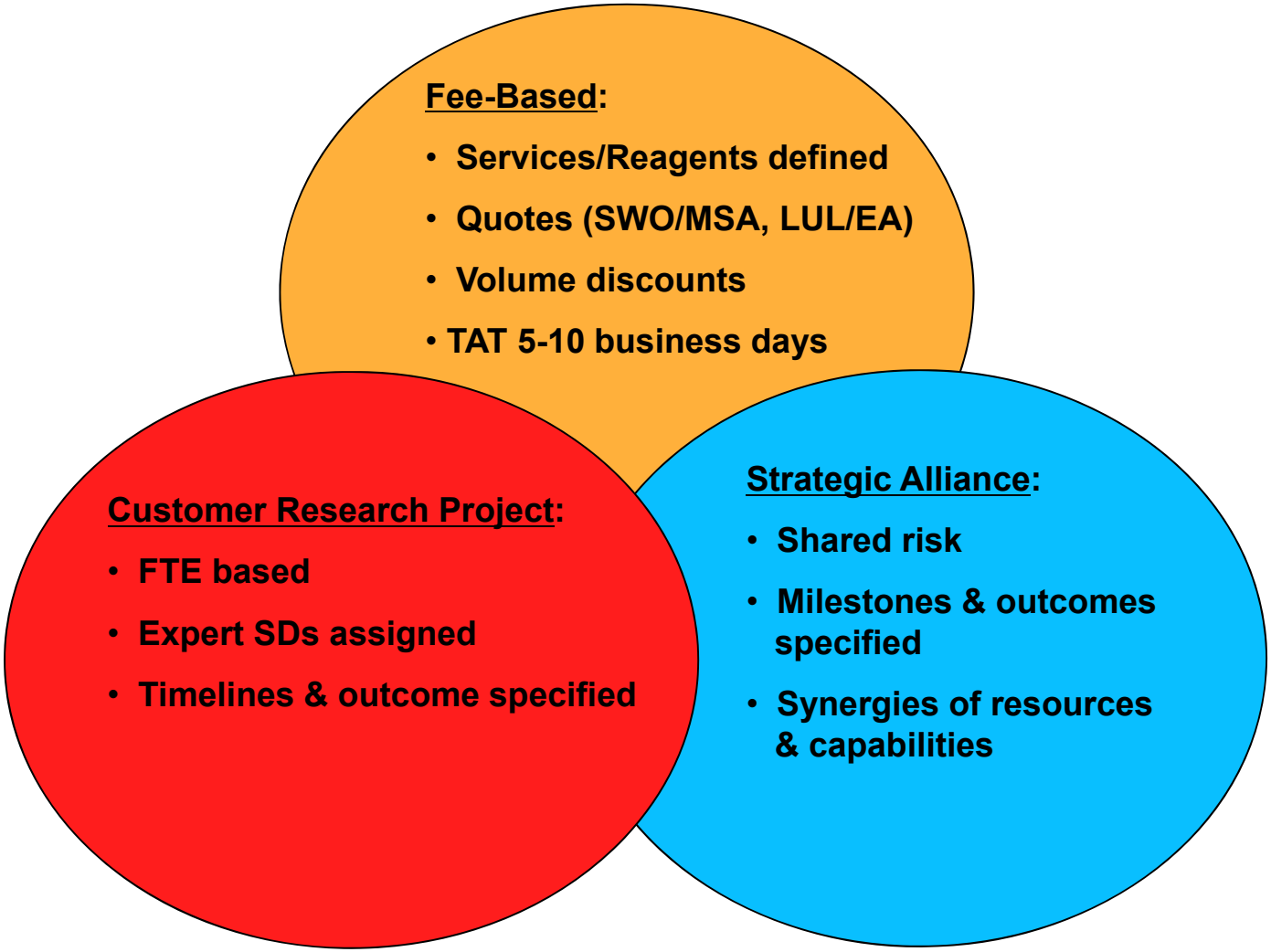
Overview

- ChanTest in Brief
- IonWorks Barracuda (IWB) Platform
  - Advantages over other platforms
  - Assay validation examples:
    - Nav1.7 validation for screening
    - $GABA_A$ validation for profiling
    - Cardiac Channel Panel for risk assessment
- Summary

ChanTest at a Glance

- Founded 1998; introduced hERG patch clamp safety assay
- Invented hERG- and Chan-Lite trafficking inhibition assays
- **Highly Trained and Motivated Personnel**
- **~300,000 compounds tested for 350+ clients**
- Successful audits by FDA & 200+ sponsors
- RCA with FDA to improve cardiotox predictivity
- Industry's most complete Ion Channel portfolio
- Recombinant cell lines, SC-derived human cells, primary cells
- Extensive GPCR targets, strong Transporter program
- Named "**most trusted fee-for-service provider**" 2006 – 2012
  (HTStec Ion Channel Trends Survey)

Client Interaction Models



Discovery Services

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Assay Development	*Screening*	*Lead Optimization*
Cell lines development & Characterization **Custom Assay Development for Primary and Secondary Screening**	**Assay Ready Cryo-preserved Cells** **Primary &Secondary Screening Services** **Hit Triage and Hit to Lead Chemistry**	***In vitro* Potency and Efficacy** **- Mechanism of Action** **- Selectivity** **- GPCR Target Panels** **Safety Testing** **- hERG Screening Service** **- Disease Channel Panels** **Lead Optimization Chemistry**

Choose single projects or an entire program

Acknowledgements

ScreenPatch Team

Yuri Kuryshev

Hung Lee

Mike Fraifogl

Cory Stebal

Channel Pharmacology

Glenn E. Kirsch

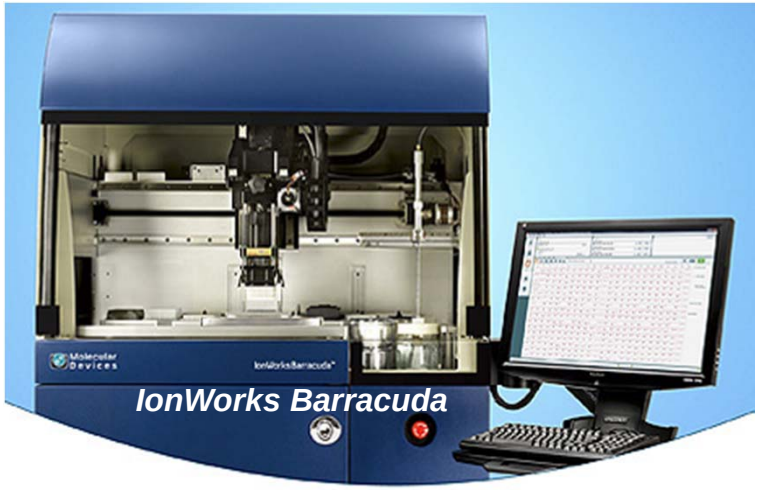
CEO/CSO

Arthur "Buzz" Brown

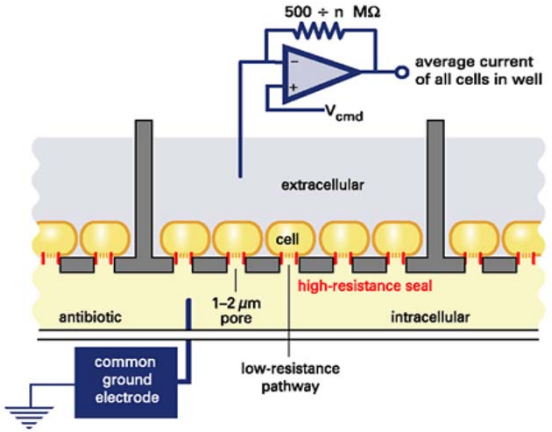
Compound Management

Peter Hawryluk

IonWorks Barracuda Platform



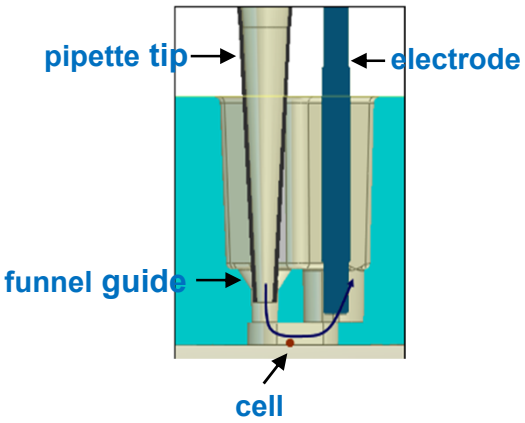
Planar Patch Method



Cells delivered to electrode

- Population Patch Clamp (PPC) and single hole (SH) recording modes (384 well format)
- Controls single cell membrane potential and measures ionic currents in single-cell or cell population (≤ 64 cells/well)
- 384-channel pipettor, integrated 384-channel electronic head
- Features continuous voltage-clamp current measurement with rapid solution addition for fast-desensitizing, ligand-gated channels

Flow-through design



Advantages of IWB Platform

Characteristic	E-Phys Platform		
	IWB	IWQ	QPatch
Continuous voltage clamp	**Yes**	**No**	**Yes**
Seal Resistance	> 300 MΩ	< 50 MΩ	Gigaseal
Parallel recording for all 384 wells	**Yes**	**No**	N/A
Possibility of cross-contamination	**No**	**Yes**	**Yes**
Multiple voltage protocols/experiment	**Yes**	**No**	**Yes**
Automated prep of compound plates	Yes ***(standard)***	Yes ***(standard)***	Yes ***(optional)***
Screening capabilities (“Targeted HTS”)	**Yes**	**Yes**	No
Manual prep of compound plates	Yes *(optional)*	Yes *(optional)*	Yes *(standard)*
Multiple TA additions	**Yes** *(optional)*	No	**Yes** *(standard)*
Cost/data point	Moderate	Moderate	High

Assay Development Process

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Cell Line Development & Validation	Assay Development, Optimization & Validation	Assay Automation
~3 months	*3 weeks*	*2 weeks*
Expression confirmation: ▪ Western blot ▪ RT-PCR ▪ Functional cell-based assay **Cell line optimization:** ▪ Induction conditions ▪ Monitor functional ▪ expression over 10-12 passages **Functional Testing:** ▪ Basic stimulus/response detection ▪ Test basic functional characteristics ▪ Reference compounds pharmacology	▪ Optimize recording conditions, voltage protocols, intra- & extra-cellular solution (to slow run-down or increase current) ▪ Adjust stimulus parameters (e.g., ligand addition, voltage steps) ▪ Stimulus/response testing ▪ Optimize Cell density ▪ Optimize SW ▪ Optimize ZPrime ▪ DMSO Tolerance ▪ Signal Stability ▪ Plate Uniformity ▪ Intra- and Inter-day variability ▪ Reference compound pharmacology ▪ Minimum Significance Ratio (MSR) study	▪ Optimize liquid and plate handling ▪ Mock Run ▪ 3 Days Uniformity ▪ 3 Days MSR tests ▪ Pilot Screen (10 plates; 3200 cpds)

Assay Acceptance Criteria - IWB

Well-level

Parameter	Value
Seal Resistance (R_{seal})	> 100 MΩ*
Current Amplitude	> 0.2 nA
R_{seal} Stability	< 50% decrease
Current Stability	< 30% decrease
Voltage Clamp Quality	Visual control

R_{Seal} >300 MΩ *for Na_v, K_v and Ca_v channels

Assay Acceptance Criteria -IWB

Plate-level

Parameter	Value
Z' Factor	≥ 0.4
CV for MAX Control	≤ 20%
CV for MID Control	≤ 20%
Signal Window (SW)	≥ 2
IC_{50} for reference antagonists	≤ 0.5 log*
Voltage Offset (mV)	< 10 mV**
Current Stability	< 30% decrease
Success rate (percent valid wells)	≥ 90%

** from historical mean*
*** measured as I-V curve shift*

Assays available on IWB Platform

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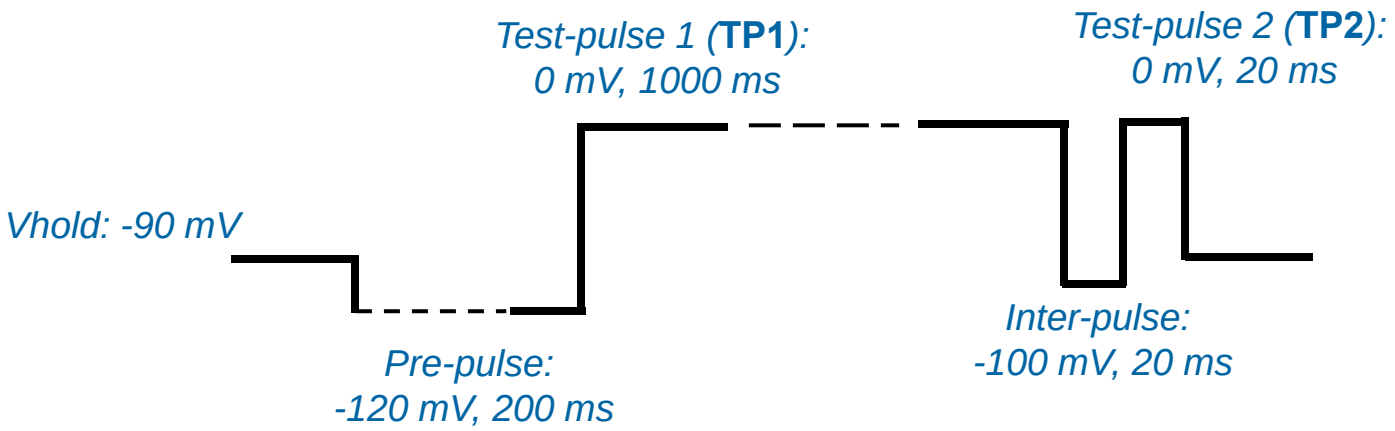
	Ligand Gated Channels	Voltage Gated Ca_v Channels	Voltage Gated K_v channels	Voltage Gated Na_v channels
1	5-HT3A	Cav1.2 (β2/cardiac L-type)	hERG	Nav1.1
2	ASIC-1 (ASIC-1a)	Cav2.1 (β4 P/Q type)	Kir2.1	Nav1.2
3	$GABA_A$ ($\alpha_1\beta_3\gamma_2$)	Cav2.2 (β3/$\alpha_2\delta$ N-type)	Kv1.1	Nav1.3
4	$GABA_A$ ($\alpha_2\beta_3\gamma_2$)	Cav3.2 (T-type)	Kv1.3	Nav1.4
5	$GABA_A$ ($\alpha_3\beta_3\gamma_2$)		Kv1.4	Nav1.5
6	$GABA_A$ ($\alpha_4\beta_3\gamma_2$)		Kv1.5	Nav1.6
7	$GABA_A$ ($\alpha_5\beta_3\gamma_2$)		Kv2.1	Nav1.7
8	HCN2		Kv3.2	Nav1.8
9			Kv3.4	
10			Kv4.3/KChiP2.2	
11			KvLQT1/minK	

Nav1.7 Assay Protocol on IWB

- Cells:
  - CHO cells stably transfected with SCN9A ion channel cDNA *(**ChanTest cell line Cat.# CT6003**)*
  - Plated 40 - 56 hours before experiments

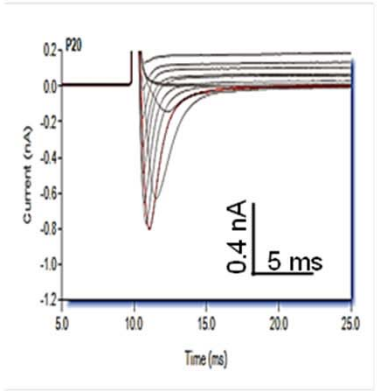
- Voltage Protocol:



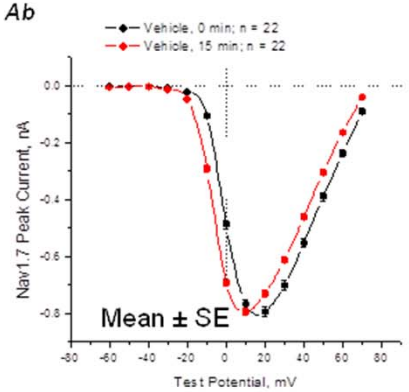
Nav1.7 Biophysical Characteristics: IWB (PPC) vs. MPC

A. IonWorks Barracuda

Aa



Ab



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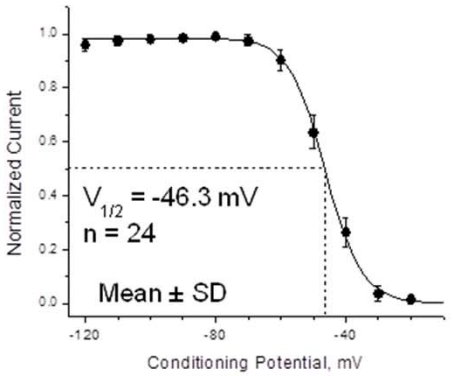
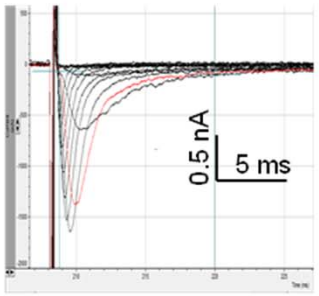
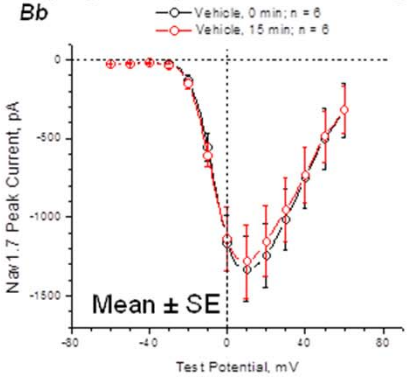


B. Manual Patch Clamp [Axopatch 200B]

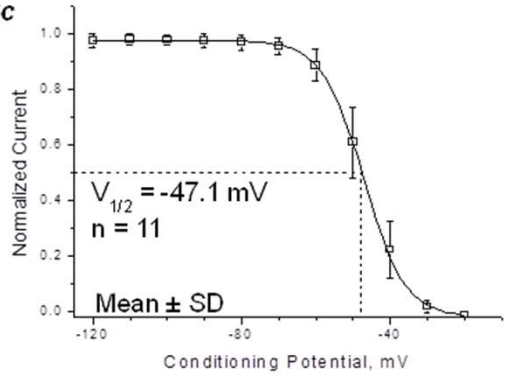
Ba



Bb



Bc

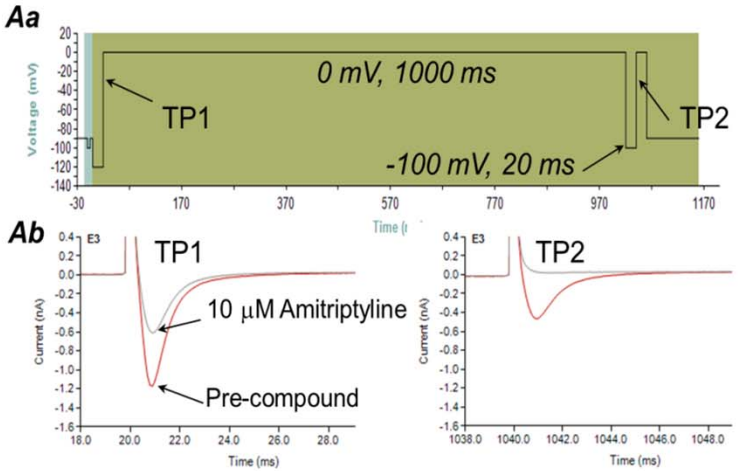


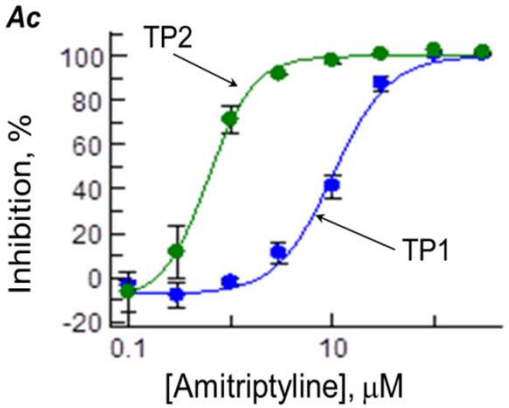
Voltage protocol:

- ***holding potential -90 mV.***
- ***1-second conditioning potential from -120 mV to 50 mV***
- ***10 mV increment followed by a 20-ms test potential to 0 mV***

Inhibition with Amitriptyline: IWB (PPC) vs. MPC

A. IWB

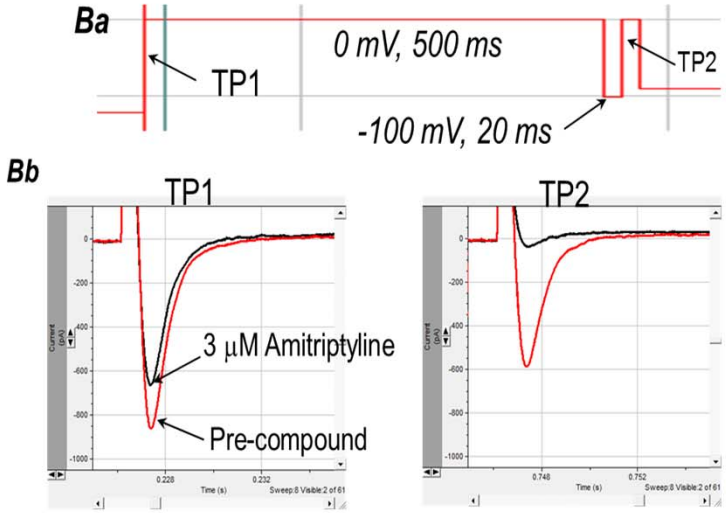


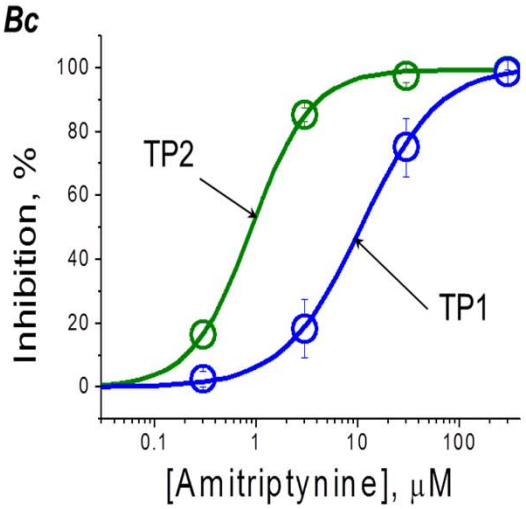


IC_{50} (µM)

TP1 10.01
TP2 0.59

B. MPC [Axopatch 200B]





IC_{50} (µM)

TP1 10.80
TP2 0.89

Reference Compounds Potency: IWB (PPC) vs. MPC

Compound	IWB (IC_{50}, μM)*		MPC (IC_{50}, μM)**	
	TP1 (tonic block)	TP2 (phasic block)	TP1 (tonic block)	TP2 (phasic block)
Amitriptyline	10.01	0.59	10.80	0.89
Mexiletine	121.94	30.36	116.25	31.46
Flecainide	40.92	16.26	25.07	16.19
Lamotrigine	>300	19.82	631.24	60.15
Carbamazepine	245.70	110.78	659.10	106.30

***Mean value [8-point CRC (4 replicas/conc.)3 independent exp. (1 exp./day)]**
****Mean value [1-2 days/compound; 4-5 CRC (5-8 replicas/conc.)]**

Summary of Uniformity Study

Day	Plate	Mean	SD	%CV	Z'	SW	Success Rate (%)
DAY 1	Max 1	111.68	9.92	8.88	0.651	6.95	88.0
	Max 2	99.79	8.56	8.58			89.3
	Mid 1	57.27	7.22	12.61			83.6
	Mid 2	62.46	7.32	11.71			87.0
	Min 1	6.51	2.20	33.74			91.9
	Min 2	7.04	2.31	32.75			91.1
DAY 2	Max 1	91.22	9.94	10.90	0.566	4.85	88.8
	Max 2	95.36	10.05	10.54			91.9
	Mid 1	47.75	7.08	14.82			89.8
	Mid 2	57.45	8.46	15.04			93.3
	Min 1	5.85	2.38	40.80			89.3
	Min 2	9.23	2.48	26.85			92.7
DAY 3	Max 1	94.89	11.47	12.08	0.586	5.14	82.0
	Max 2	100.38	9.29	9.25			83.3
	Mid 1	49.51	8.57	17.20			85.7
	Mid 2	61.14	8.71	14.25			84.9
	Min 1	6.56	2.85	43.46			79.9
	Min 2	5.70	1.23	21.56			85.9

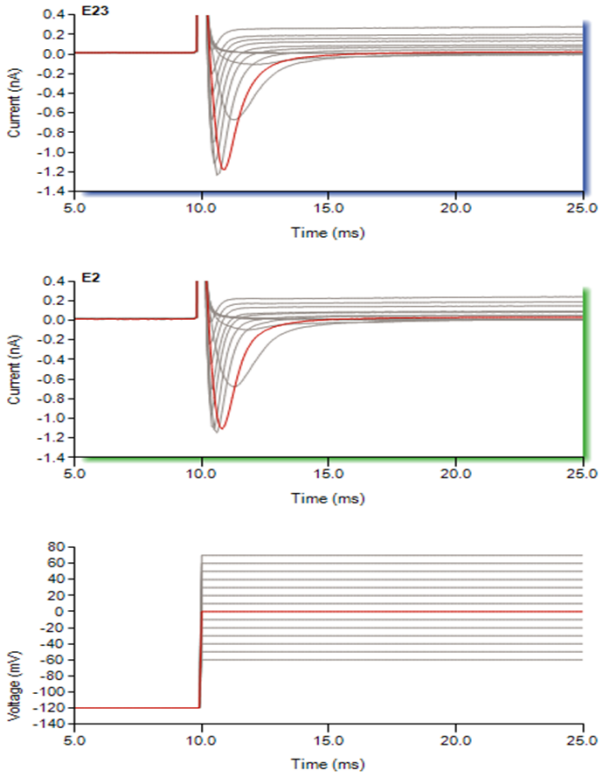
Max signal *(Vehicle)*; Mid Signal *(150 μM Mexiletine)*; Min Signal *(1000 μM Mexiletine)*

Mexiletine Uniformity at TP1 and TP2

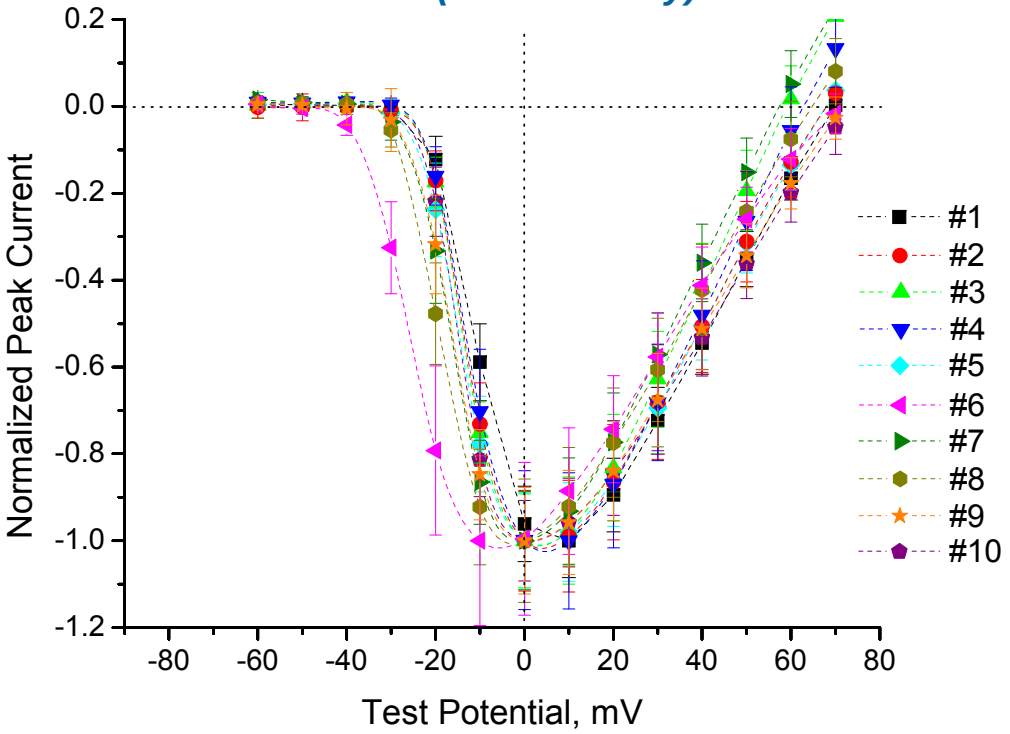
Experiment ID	Z'		Mexiletine IC50 (μM)		Success Rate (%)
	T1	T2	T1	T2	
Day 1 #1	0.63	0.63	98.5	23.1	83.9
Day 1 #2	0.71	0.82	163.2	28.2	90.1
Day 1 #3	0.67	0.68	73.2	9.5	88.8
Day 1 #4	0.67	0.71	128.0	19.7	90.1
Day 1 #5	0.67	0.51	67.6	16.1	79.7
Day 1 #6	0.56	0.43	120.3	13.3	80.7
Day 1 #7	0.71	0.68	123.8	16.6	89.6
Day 1 #8	0.51	0.58	136.0	20.3	96.6
Day 1 #9	0.67	0.67	159.1	36.2	94.8
Day 1 #10	0.66	0.68	246.6	37.6	96.1
Day 2 #1	0.81	0.78	105.0	43.8	96.4
Day 3 #1	0.76	0.79	162.4	24.1	95.3
Mean	**0.67**	**0.66**	**132.0**	**24.0**	**90.2**
SD	**0.08**	**0.11**	**48.1**	**10.5**	**6.1**
Range	**0.51 - 0.81**	**0.51 - 0.82**	**67.6 - 246.6**	**9.5 -43.8**	**79.7 - 96.6**

Daily Stability of Voltage Potentials

A. Representative Nav1.7 current traces



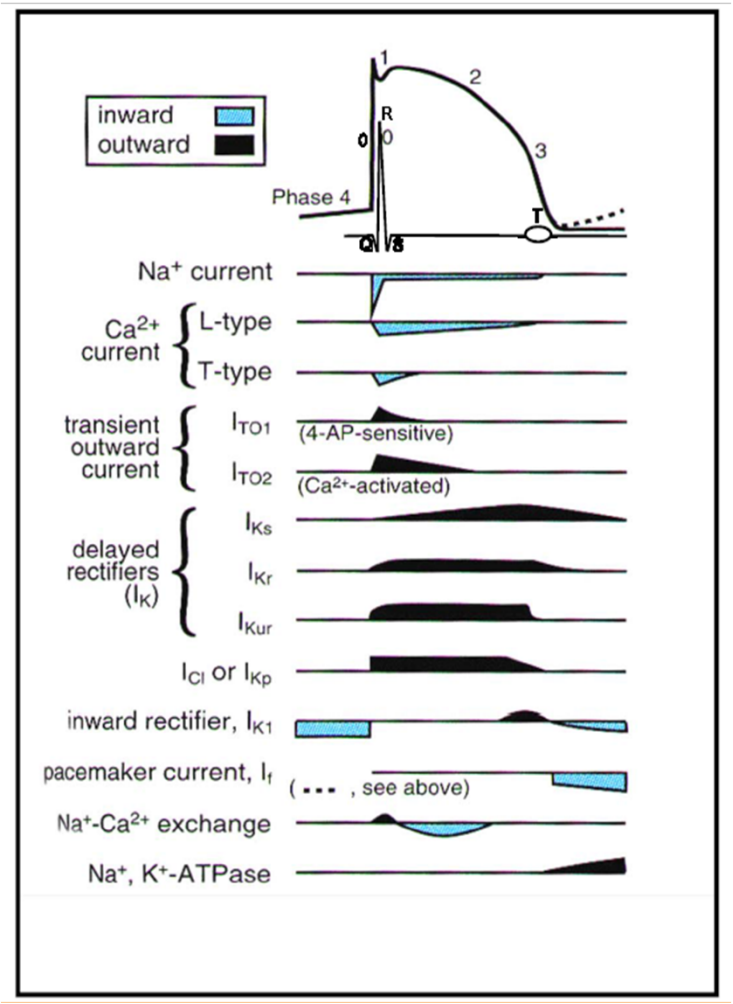
B. I-V curves for 10 independent experiments (the same day)



Data presented as Mean ± SD (n = 26 – 32).

It is Not Just About hERG

Cardiac Channel Panel ™	Action Potential Phase
Nav1.5 (I_{Na})	0,2
Cav 1.2/β2, α2δ, (L-type)	2
Cav 3.2 (T-type)	1
Kv4.3 (I_{TO1})	1
KvLQT1/minK (I_{KS})	2-3
hERG (I_{Kr})	2-3
Kv1.5 (I_{Kur})	2-3
Kir2.1 (I_{K1})	4
HCN2 (pacemaker, I_f)	4
HCN4 (pacemaker, I_f)	4
Kir3.1/3.4 ($I_{K,Ach}$)	4
Kir6.2/SUR2A ($I_{K,Ach}$)	4
NCX1 (Na-Ca exchange)	2



Cardiac Channel Panel: Cav1.2/β2/α2δ1 Protocol on IWB: Uniformity of Nifedipine and Verapamil Inhibition

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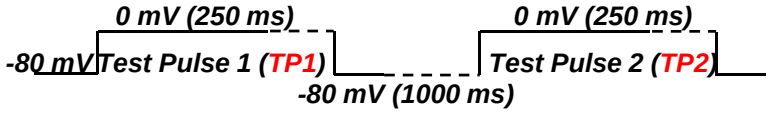
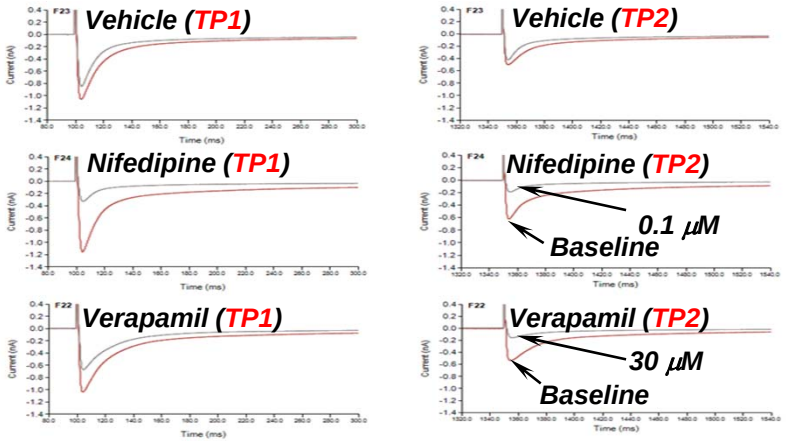
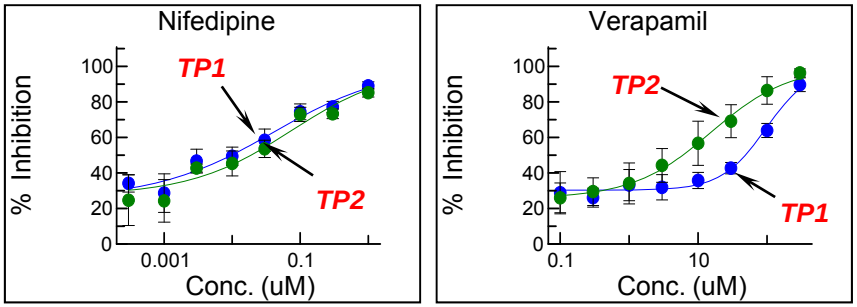
A. Voltage Protocol



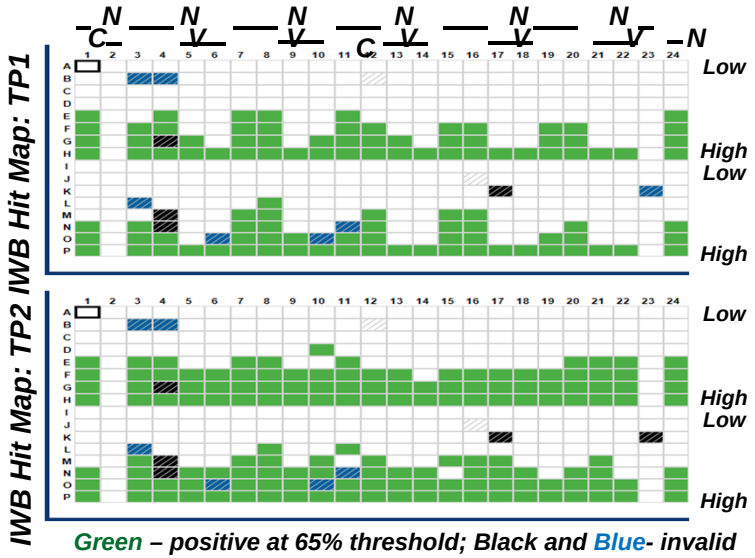
B. Representative Current Traces



C. Representative Dose-Response Curves



D. Hit Map



Green – positive at 65% threshold; Black and Blue- invalid
C – Vehicle Control, N – Nifedipine, V - Verapamil

E. Intra-plate Uniformity

Nifedipine Block

Column	IC_{50} (μM)	
	TP1	TP2
3, 4	0.044	0.074
7, 8	0.028	0.035
11,12	0.045	0.067
15,16	0.035	0.043
19, 20	0.036	0.058
Mean	0.038	0.055
SD	0.007	0.016
Range	0.028-0.045	0.035-0.074

Verapamil Block

Column	IC_{50} (μM)	
	TP1	TP2
5, 6	96.8	15.4
9, 10	81.7	13.3
13, 14	115.4	14.4
17, 18	81.5	14.0
21, 22	118.1	19.1
Mean	98.7	15.2
SD	17.6	2.3
Range	81.5-118.1	13.3-19.1

Example of Cardiac Panel Report

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Program Code	Study Submission ID	IC_{50}, μM											
		Cav1.2		Nav1.5		hERG	Kv1.5		Kv4.3/KChIP2.2		LQT1/ minK	Kir2.1	HCN2
		TP1	TP2	TP1	TP2	Peak	Peak	300-ms	Peak	25-ms	2000-ms	500-ms	2000-ms
1	CPD_00845_02_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
2	CPD_00854_02_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
3	CPD_00872_01_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
4	CPD_00905_01_A	>30	24.73	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
5	CPD_00822_03_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
6	CPD_00745_03_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
7	CPD_00801_02_A	26.03	12.43	>30	>30	>30	23.57	17.05	>30	29.35	>30	>30	>30
8	CPD_00763_02_A	>30	>30	>30	>30	>30	>30	23.66	>30	>30	>30	>30	>30
9	CPD_00449_01_A	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
10	CPD_00524_01_A	>30	>30	>30	>30	>30	>30	25.16	>30	14.92	>30	>30	>30
PC	Nifedipine	0.050	0.055										
PC	Lidocaine			1035.92	12.92								
PC	E-4031					0.032							
PC	4-AP						1173.74	97.92					
PC	Flecainide								61.13	6.57			
PC	Chromanol										3.14		
PC	Ba^{2+}											7.96	
PC	ZT-7288												11.35

Summary

- Using IonWorks Barracuda (IWB) platform we have developed 31 robust electrophysiology assays for screening and profiling compounds against voltage-gated and ligand-gated ion channels
- All assays were validated using strict acceptance criteria at the well and plate level
- For voltage-gated channels assay protocols has been configured to assess both tonic and use dependent block
- Assays validated on IWB platform can be used at early stage of drug discovery process for rapid cardiovascular safety risk assessment

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Thank You for your attention!

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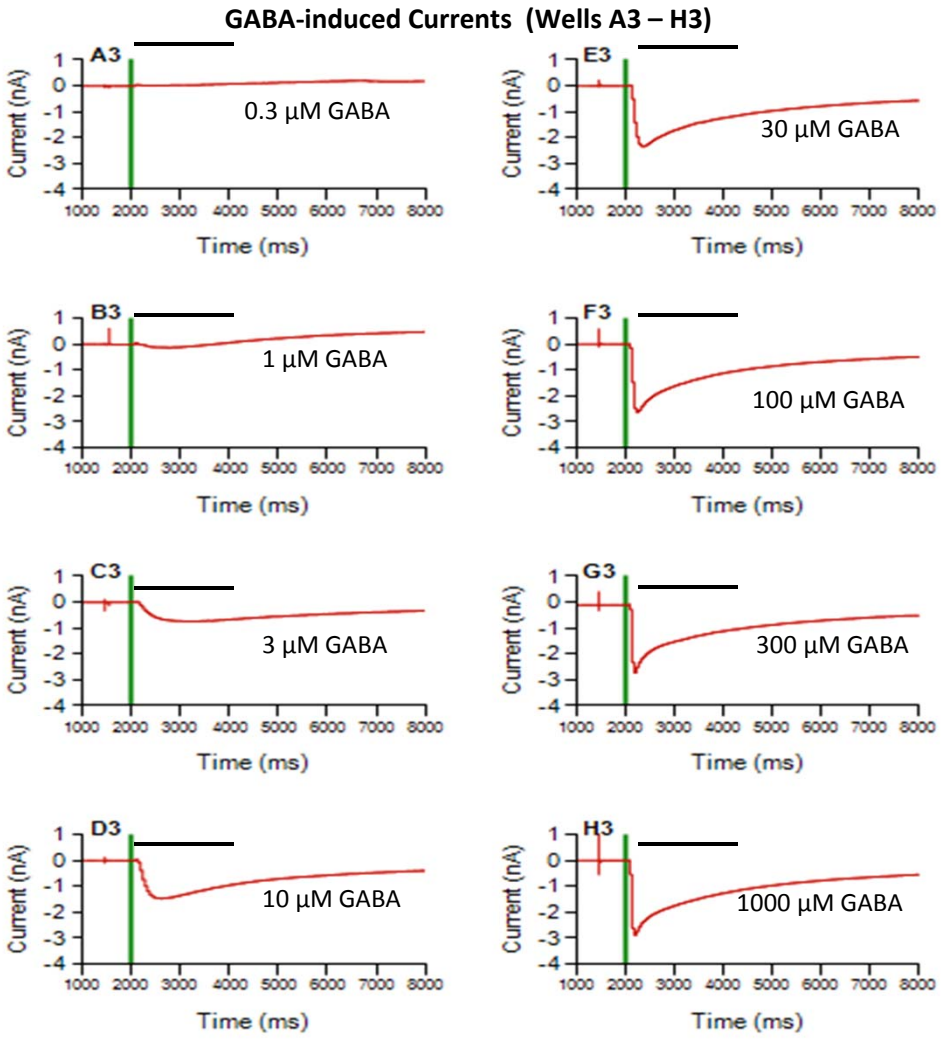
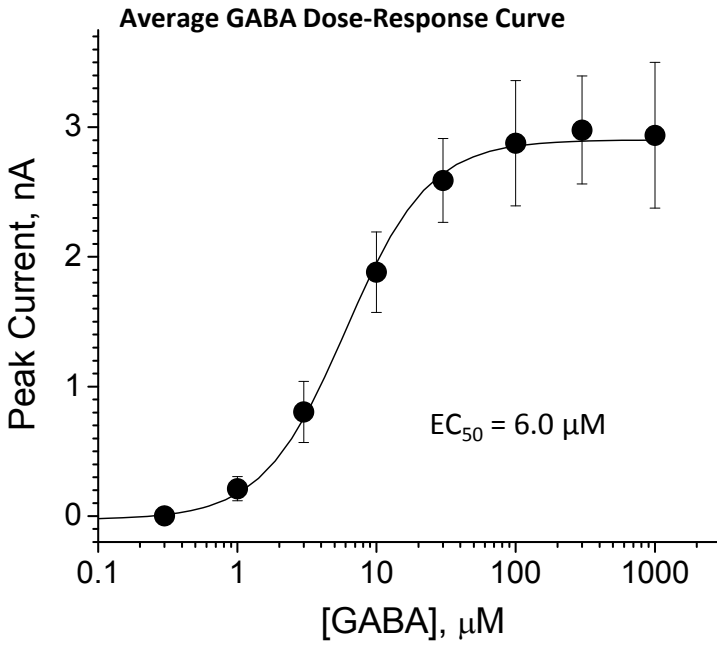
Tel 216 584-0531

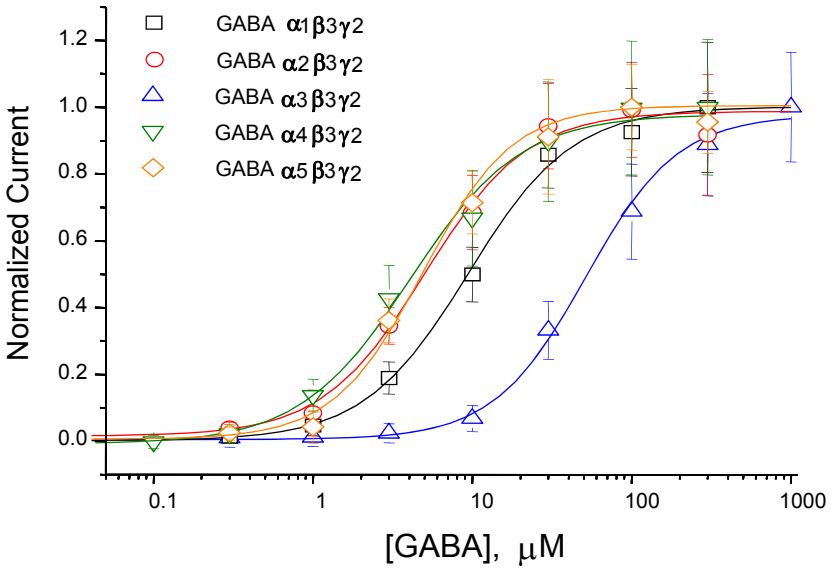
Backup

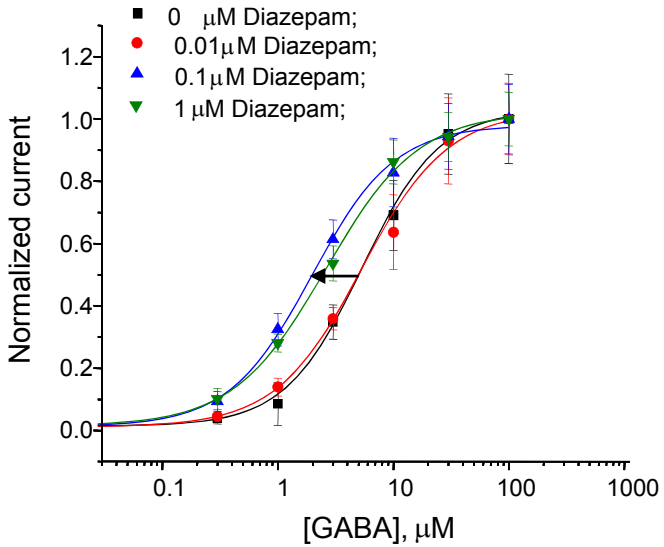
$GABA_A$ $\alpha_2\beta_3\gamma_2$–HEK: GABA Concentration-Response





$GABA_A(\alpha_x\beta_3\gamma_2)$ Profiling: Agonist and PAM Modes

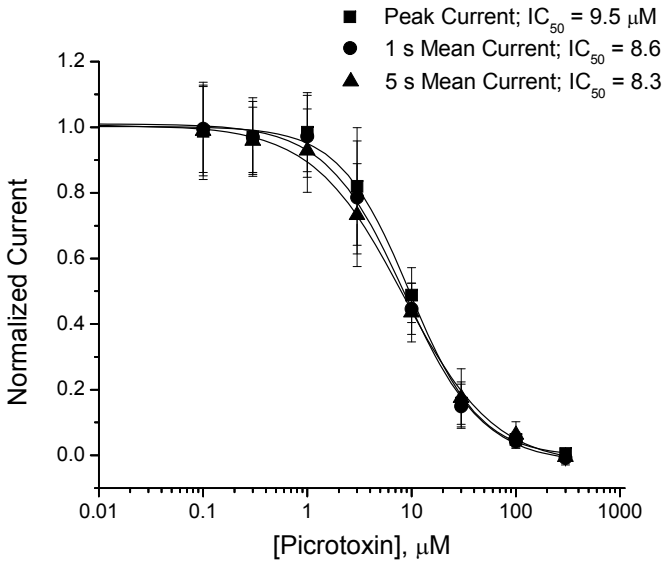


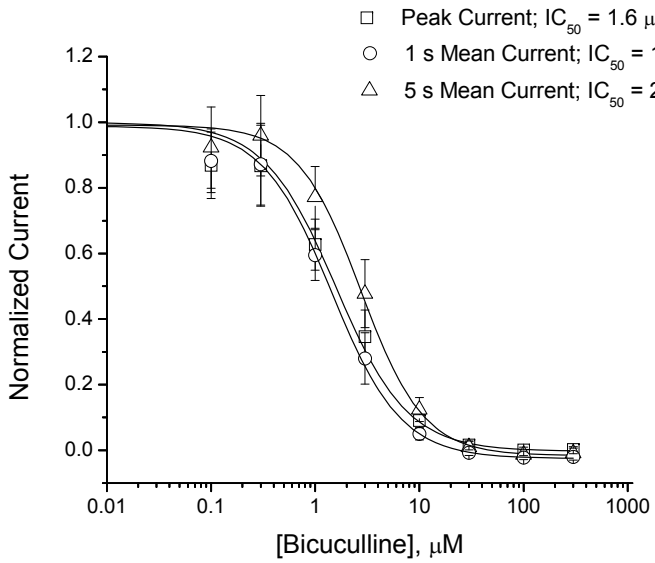


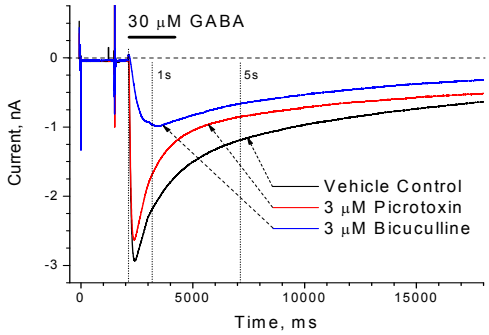
$GABA_A$ Receptor	EC_{50} (μM)			
	Mean	SD	N	Publish Values
α1/β3/γ2	12.7	4.2	4	8.3
α2/β3/γ2	5.4	0.7	4	
α3/β3/γ2	47.1	20.3	4	34.7
α4/β3/γ2	4.1	0.3	4	3.9
α5/β3/γ2	4.7	0.8	3	

Diazepam (μM)	GABA EC_{50} (μM)
0	5.4
0.01	5.3
0.1	2.0
10	2.6

$GABA_A$ ($\alpha_2\beta_3\gamma_2$) Profiling: Antagonist Mode







Superimposition of representative traces of the GABA-induced current