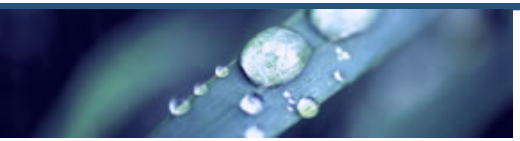

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Validation of a High-throughput, Microfluidic Patch Clamp System for Screening of Nicotinic Compounds

Haiyang (David) Wei, Ph.D.

Eurofins Discovery Services

25/06/2014

Eurofins as a leading partner for the pharmaceutical industry worldwide

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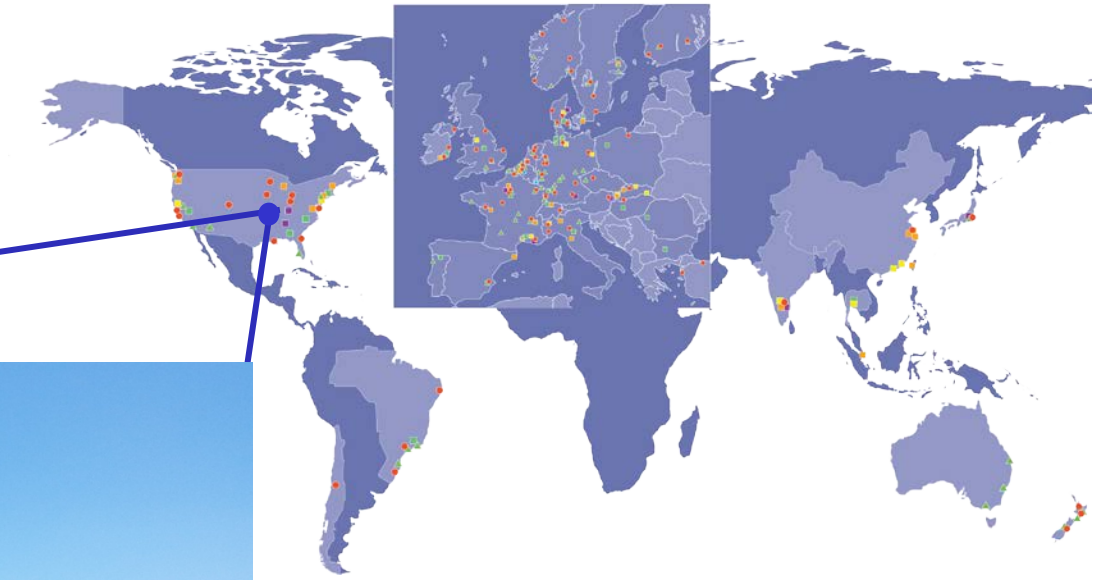
The Eurofins Scientific is the global leader in the pharmaceutical, food, and environmental testing market with 190 labs in 36 countries



St. Charles, MO. USA

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Eurofins Discovery Services – cell-based assay capabilities

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- ✓ Ion channel functional assays
- ✓ Fluorescence and luminescence-based assay development and HT screening
- ✓ Cell membrane production and radioligand binding assays
- ✓ Bulk transfection of cells and custom cell line engineering
- ✓ Signaling pathway assays
- ✓ Custom FlexLab assays

IonFlux HT™

FLIPR^TETRA

IonWorks

PatchXpress

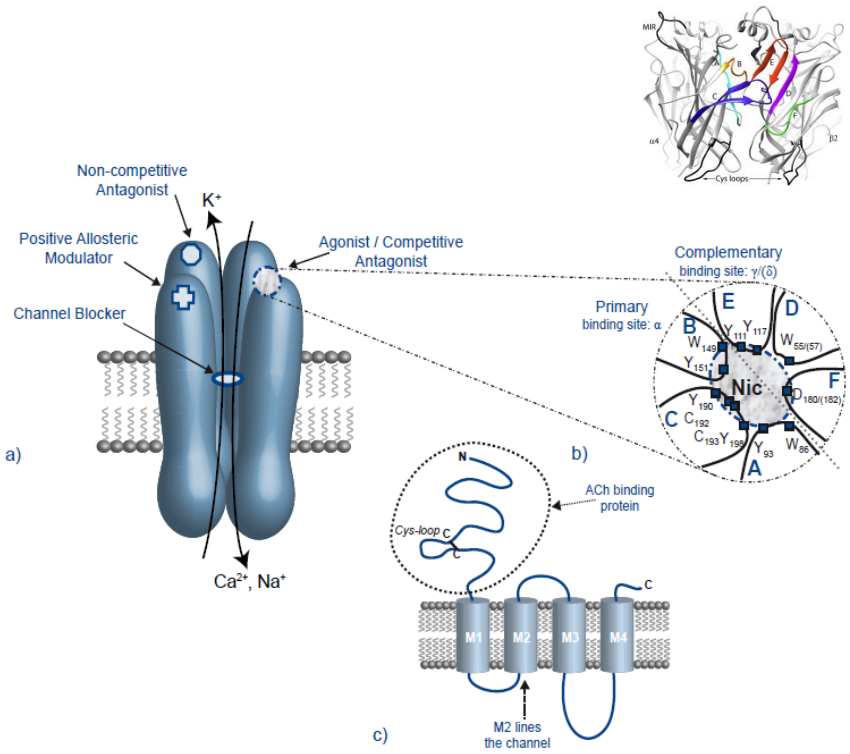
Manual Patch

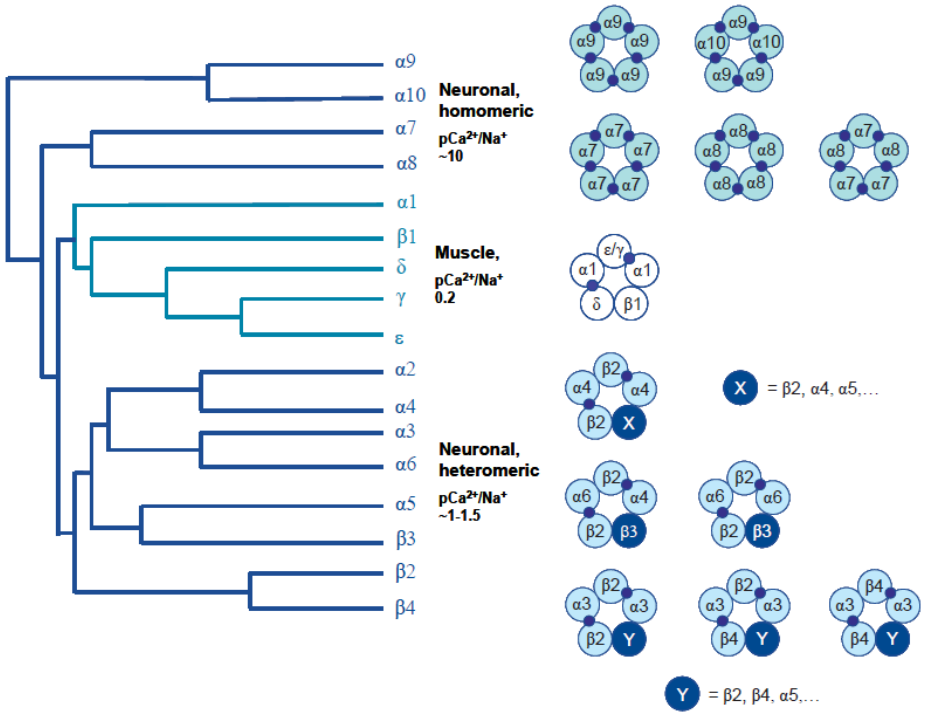
MaxCyte

Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChRs) belong to the ‘‘Cys-loop superfamily’’ of ligand-gated ion channels that includes $GABA_A$, glycine, and 5-HT3 serotonin receptors.

Basic structure of nicotinic acetylcholine receptors





Merging old and new perspectives on nicotinic acetylcholine receptors. Roger L. Papke ; Nicotinic ACh Receptors, Tocris Bioscience

Nicotinic acetylcholine receptors

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- Nicotinic acetylcholine receptors (nAChRs) belong to the ‘‘Cys-loop superfamily’’ of ligand-gated ion channels that includes $GABA_A$, glycine, and 5-HT3 serotonin receptors.
- Eurofins Discovery Services PrecisION® nAChRs cell lines:
  - ✓ α1β1δε (CYL3052)
  - ✓ α3β4 (CYL3057)
  - ✓ α4β2 (CYL3106)
  - ✓ α4α6β2 (CYL3107)
  - ✓ α7 (CYL3097)

Nicotinic acetylcholine receptors

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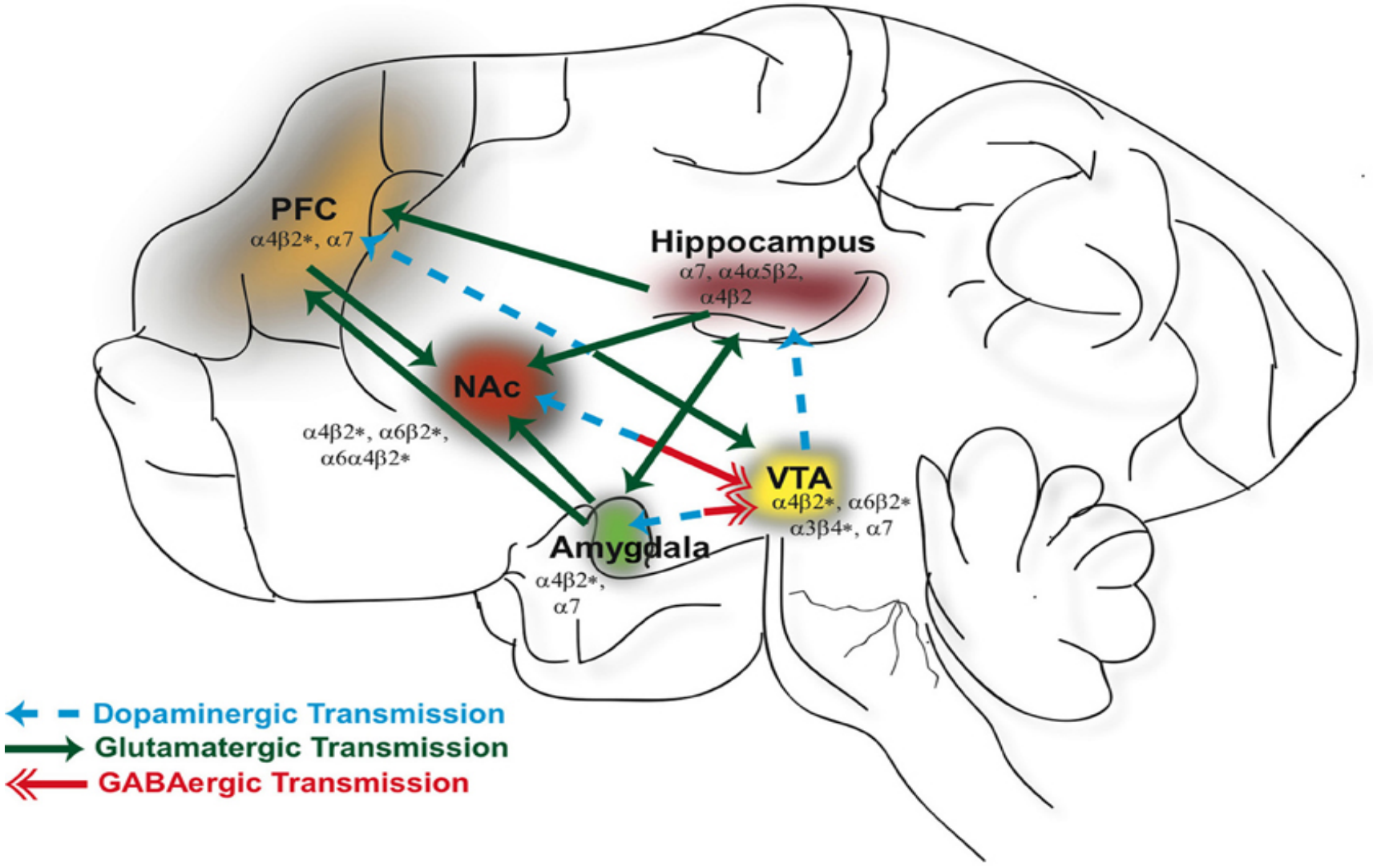

Front. Mol. Neurosci., 03 August 2012 | doi: 10.3389/fnmol.2012.00083

Nicotinic acetylcholine receptors

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- Nicotinic acetylcholine receptors (nAChRs) belong to the ‘‘Cys-loop superfamily’’ of ligand-gated ion channels that includes $GABA_A$, glycine, and 5-HT3 serotonin receptors.
- nAChRs participate in fundamental aspects of synaptic plasticity that are involved in attention, learning, memory, cognition and development.
- Decline, disruption, or alterations of nicotinic cholinergic mechanisms have been implicated in various human pathologies such as schizophrenia, epilepsy, autism, Alzheimer’s disease (AD), Parkinson's disease (PD), major depression and addiction.

IonFlux™ HT for screening of nicotinic compounds

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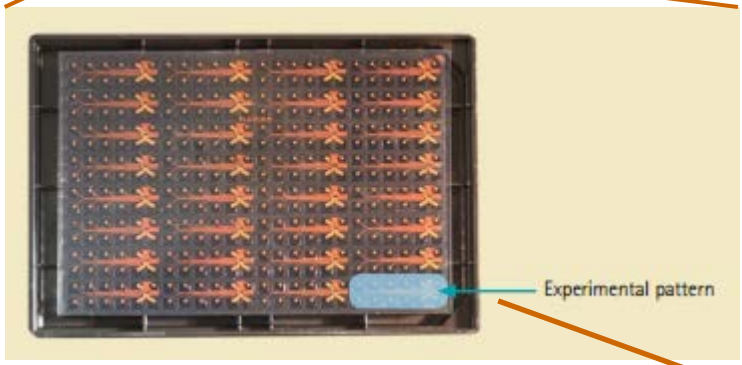

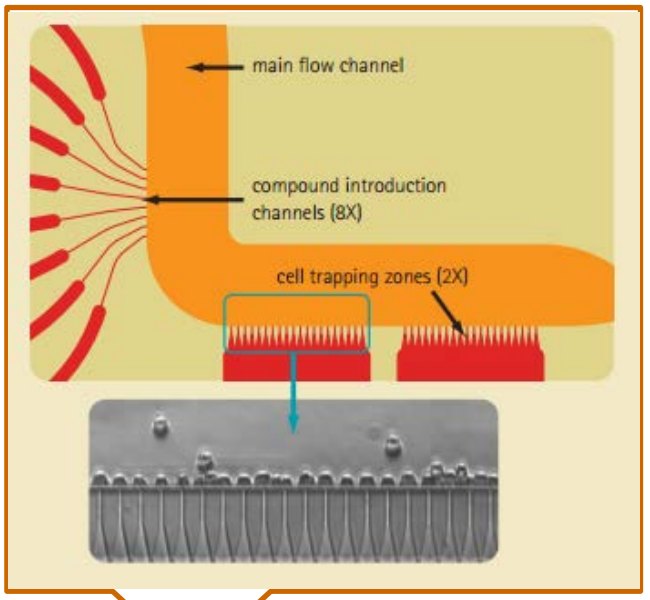

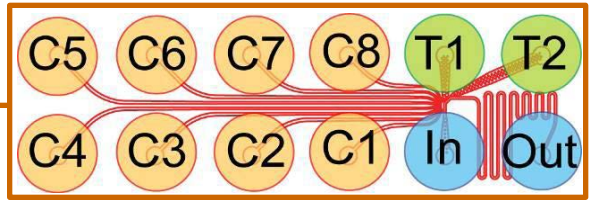

Validation objectives

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nAChRs response characterization

- Recording conditions optimization
- Current stability
- Cross plate uniformity and inter-run precision.
- Pharmacology of agonists, antagonists and positive allosteric modulators (PAMs)
- Binding constants and channel kinetics

Current stability with internal solutions

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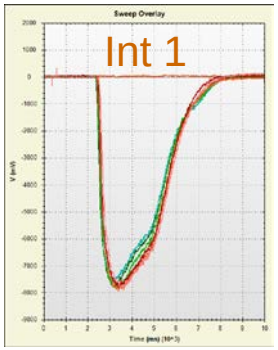
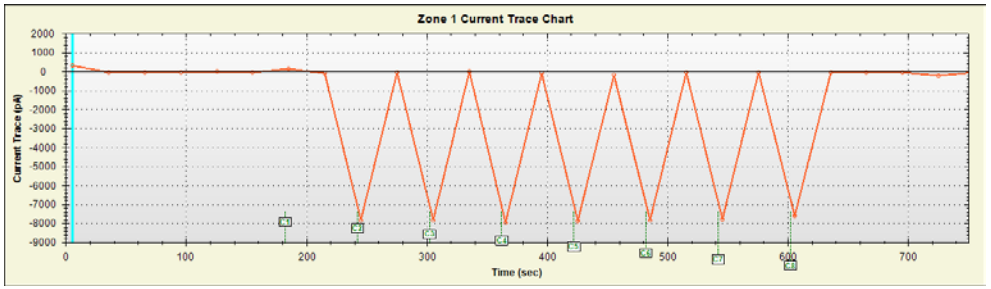
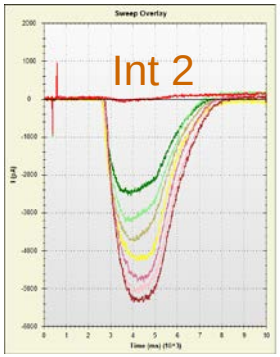
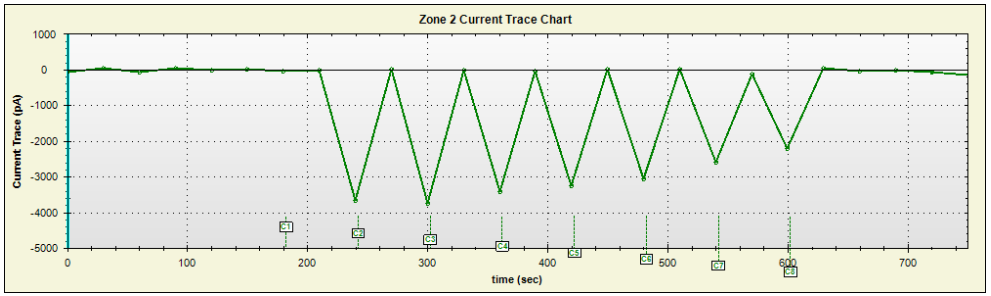


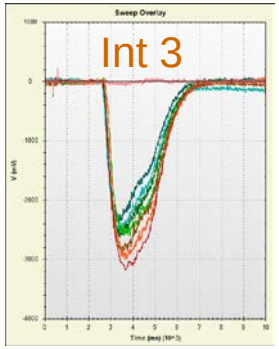


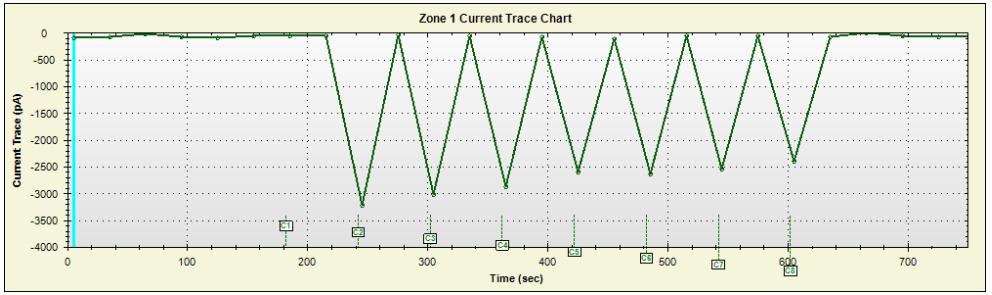
Internal 1(in mM):
110 TRIS-PO_4, 28 TRIS-base, 0.1 $CaCl_2$, 2 $MgCl_2$, 4 Mg-ATP, 11 EGTA, pH 7.3





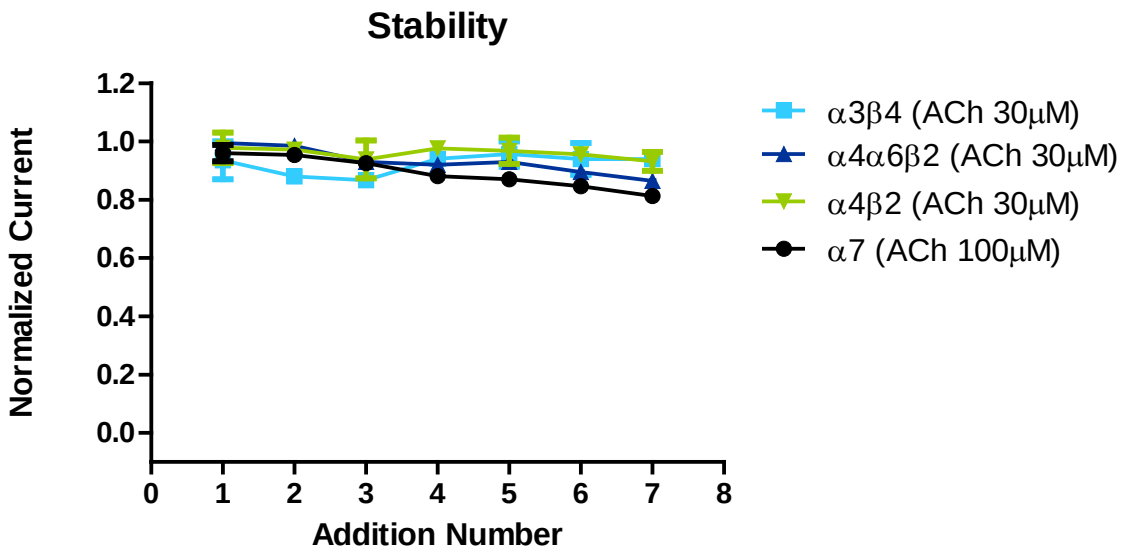
Internal 2 (in mM):
60 KCl, 70KF, 10NaCl, 4 Mg-ATP, 10 HEPES, 11 EGTA, pH 7.3

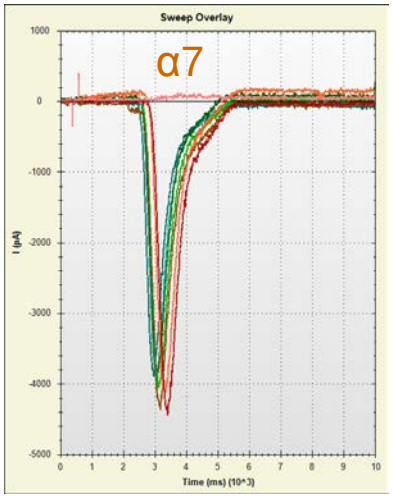


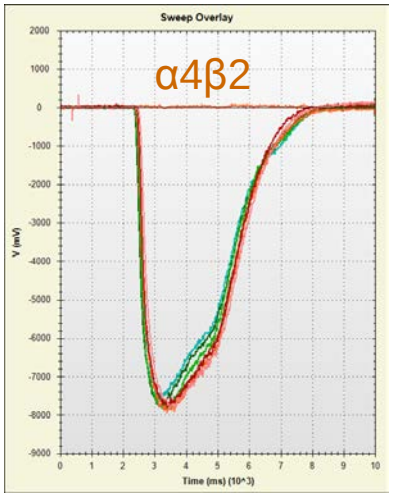


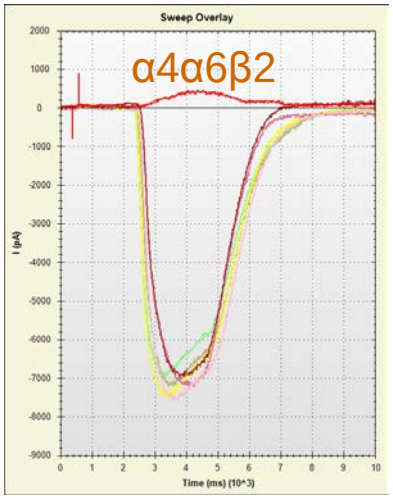
Internal 3 (in mM):
138 Tris base, 2 $MgCl_2$, 0.5 $CaCl_2$, 4 Na-ATP, 11 EGTA, pH 7.2

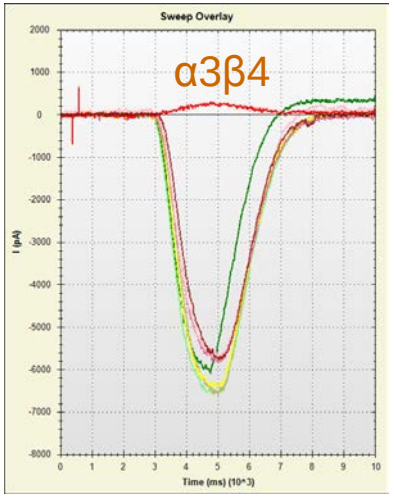
Current stability











Cross plate uniformity and inter-run precision of α4β2 receptors

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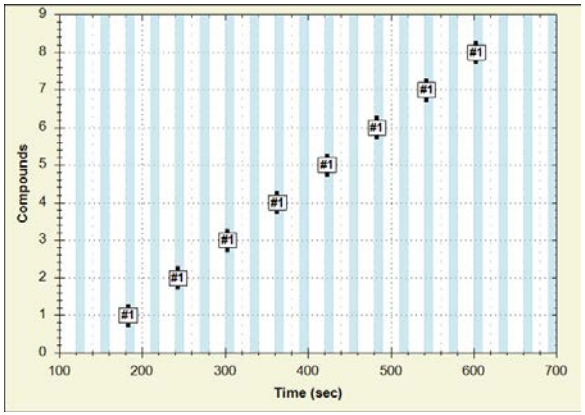

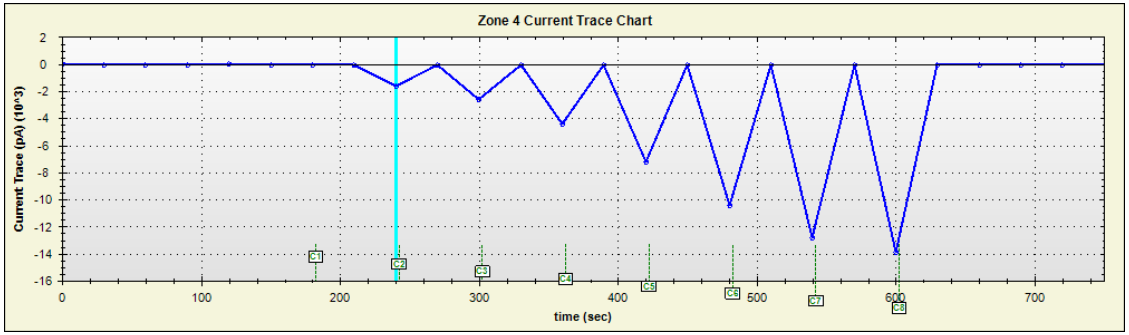

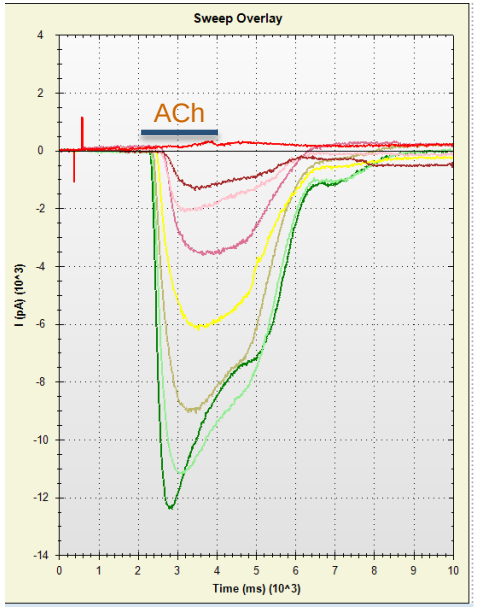

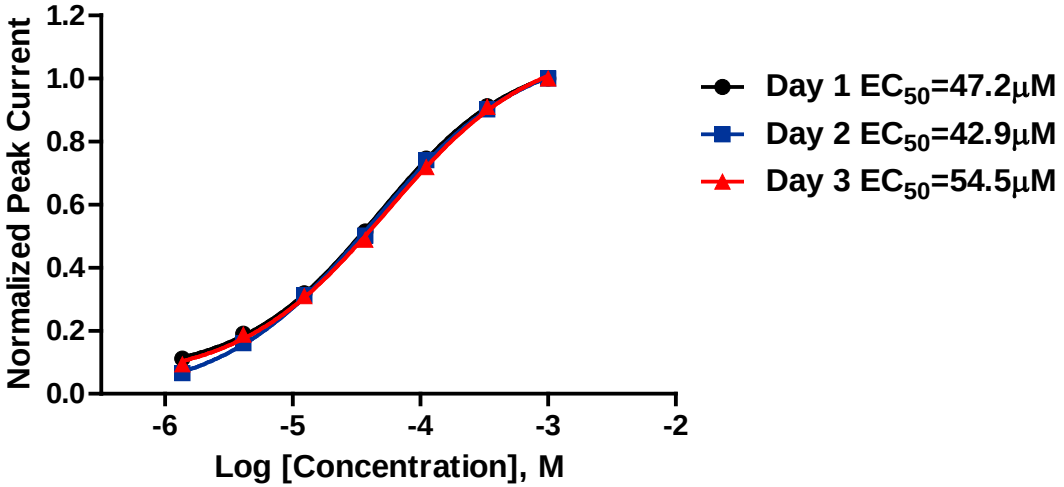

Cross plate uniformity and inter-run precision of nAChRs

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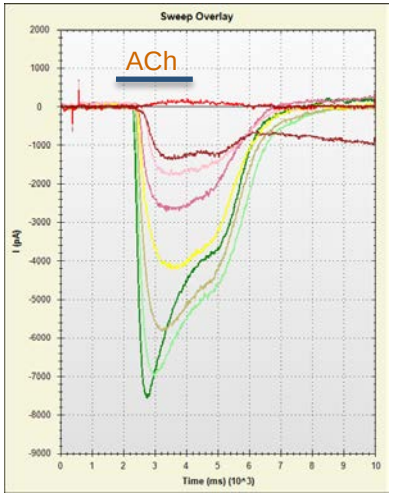
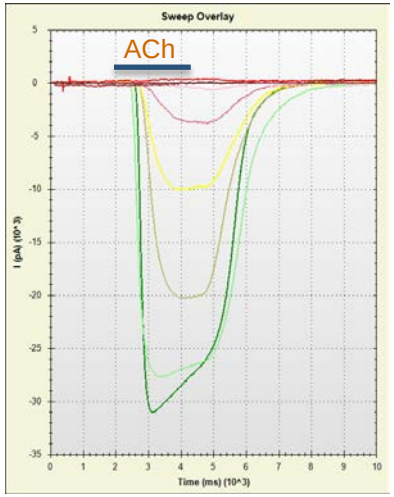
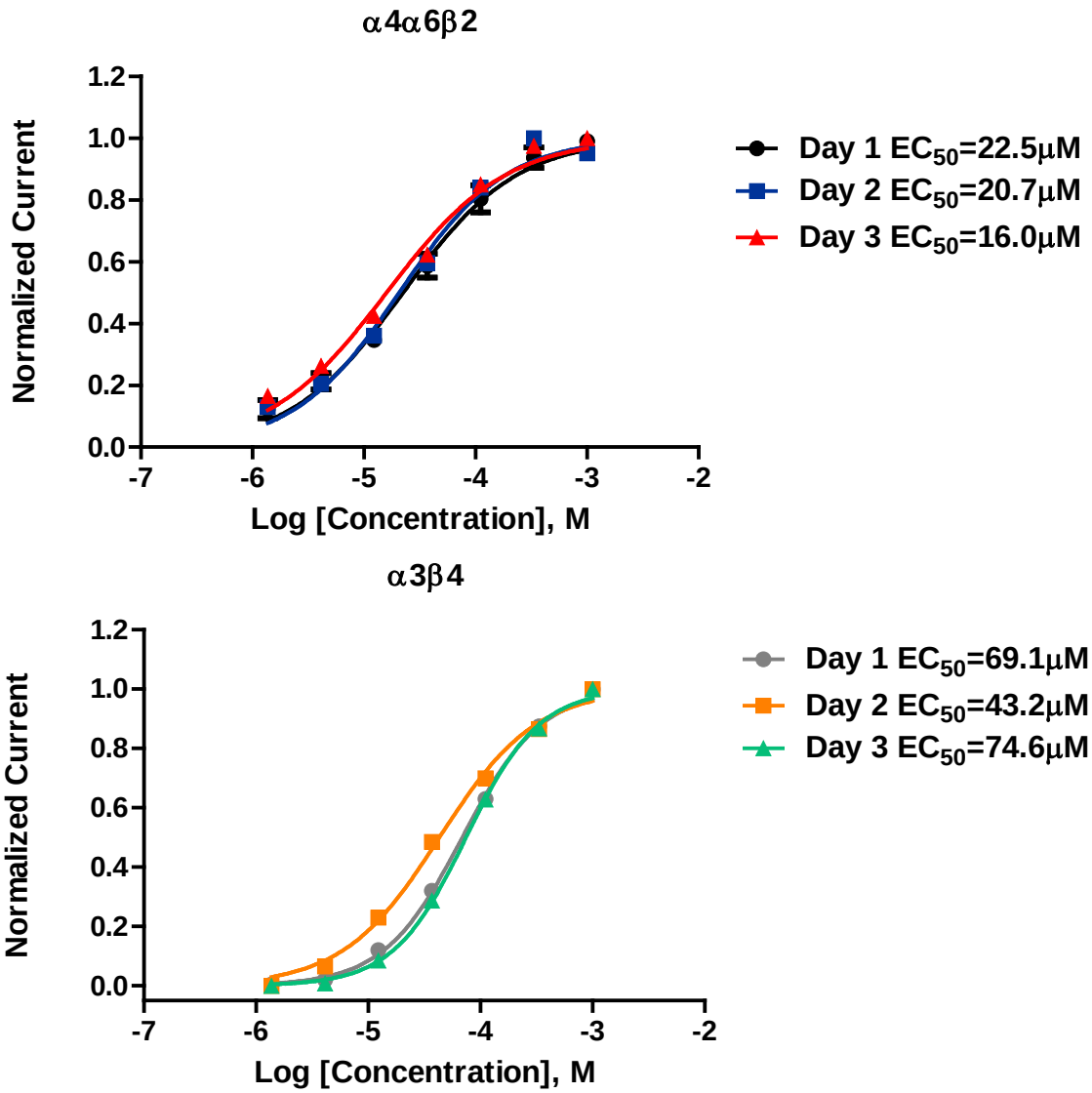
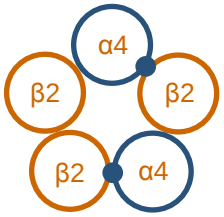






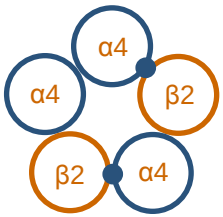
Two stoichiometries of α4β2 receptors

HS



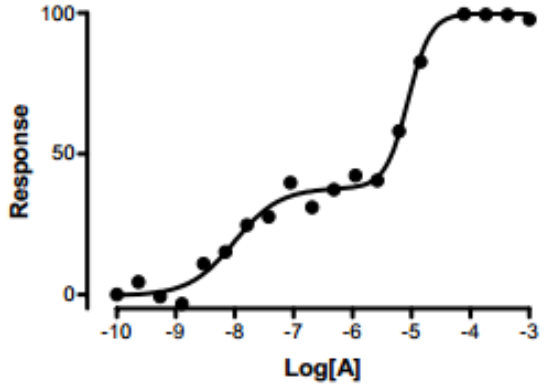
$(\alpha4)_2(\beta2)_3$

LS



$(\alpha4)_3(\beta2)_2$

$$Y=\text{Bottom}+\frac{(\text{Top-Bottom})\cdot\text{Frac}}{1+10^{(\text{LogEC}_{50}1-\text{Log[A]})\cdot n_H 1}}+\frac{(\text{Top-Bottom})\cdot(1-\text{Frac})}{1+10^{(\text{LogEC}_{50}2-\text{Log[A]})\cdot n_H 2}}$$



GraphPad Prism: Fitting Models to Biological Data using Linear and Nonlinear Regression, Harvey Motulsky & Arthur Christopoulos

Two stoichiometries of α4β2 receptors

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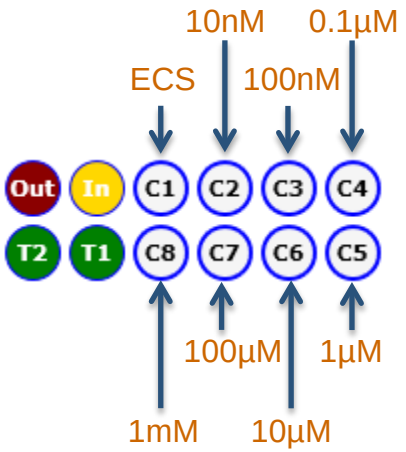
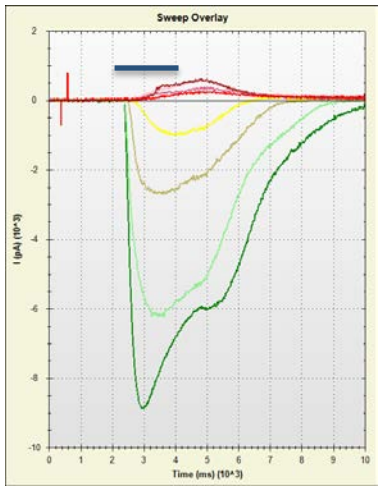
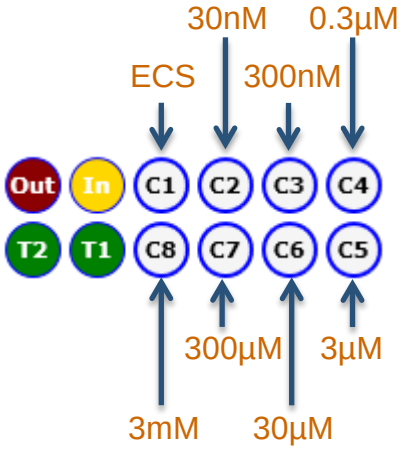
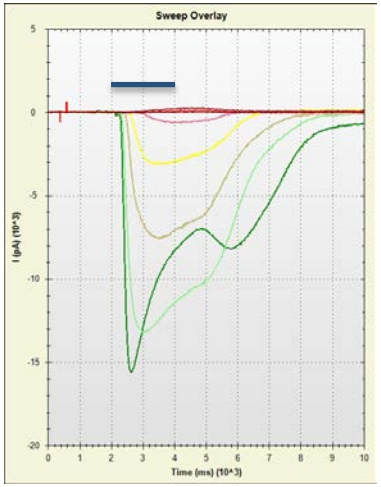
Pattern 1



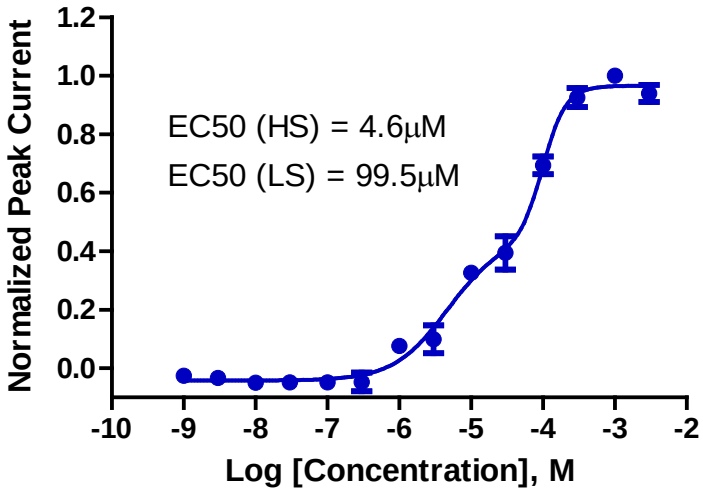


Pattern 2

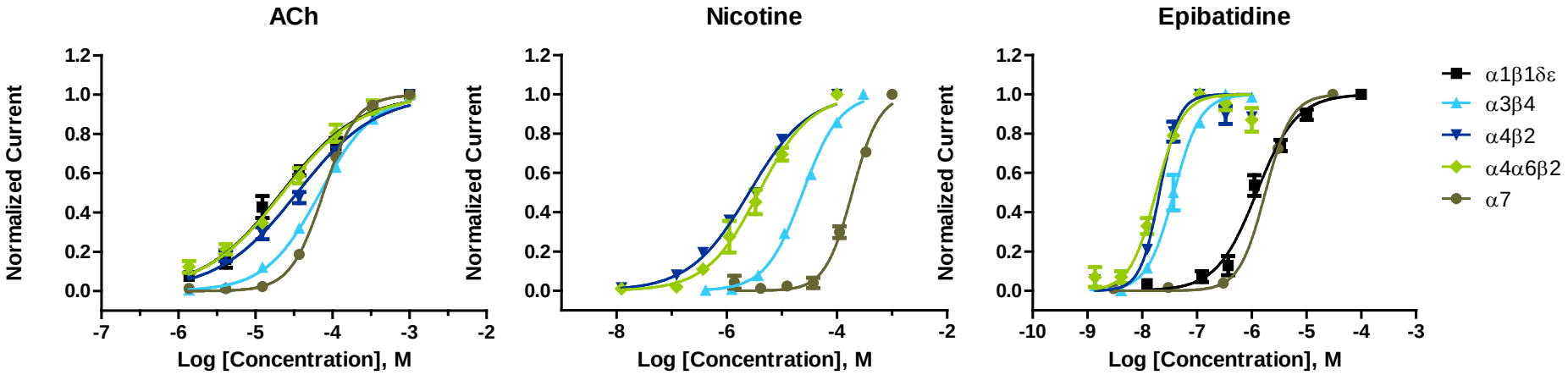


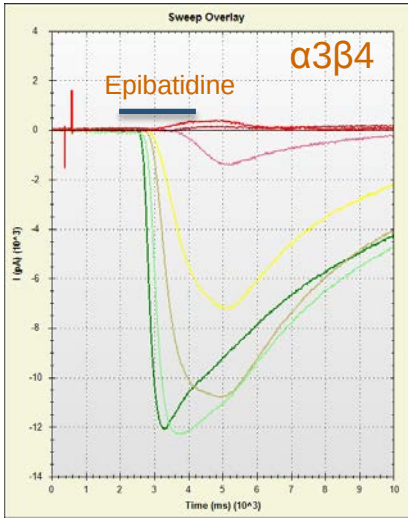


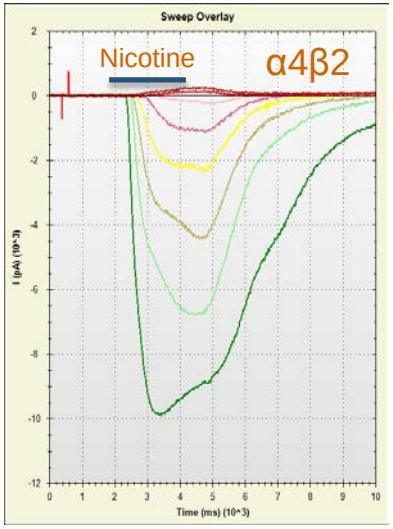
Concentration-response curve for ACh



Pharmacology of nAChRs agonists







nAChRs	Estimated EC_{50} (μM)		
	ACh	Nicotine	Epibatidine
α1β1δε	21.8	-	1.23
α3β4	69.1	23.9	37.8nM
α4β2	34.9	2.54	20.1nM
α4α6β2	22.5	3.79	17.9nM
α7	76.4	188.4	1.77

Pharmacology of nAChRs antagonists

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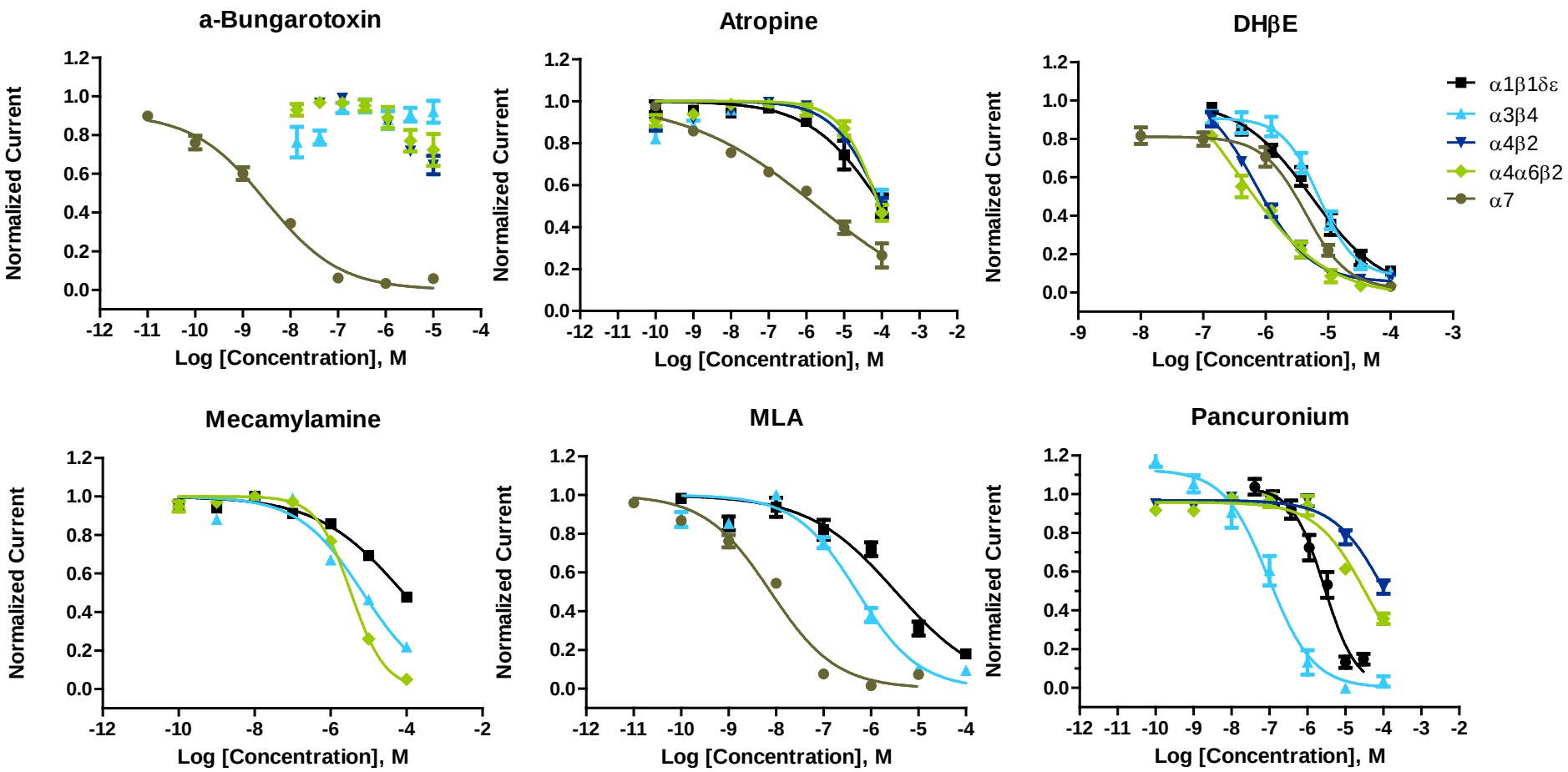

Pharmacology of nAChRs antagonists

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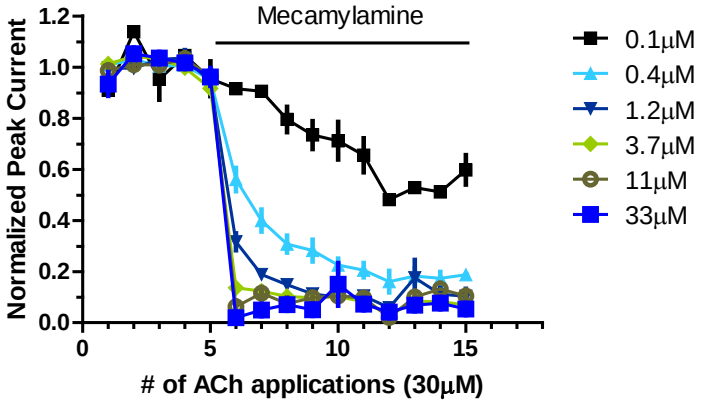
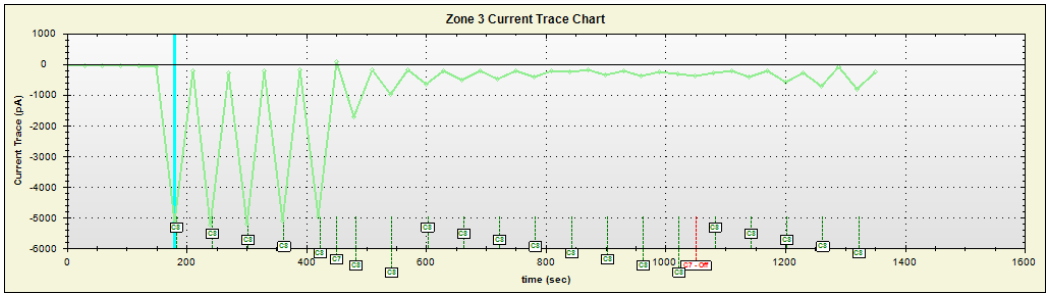
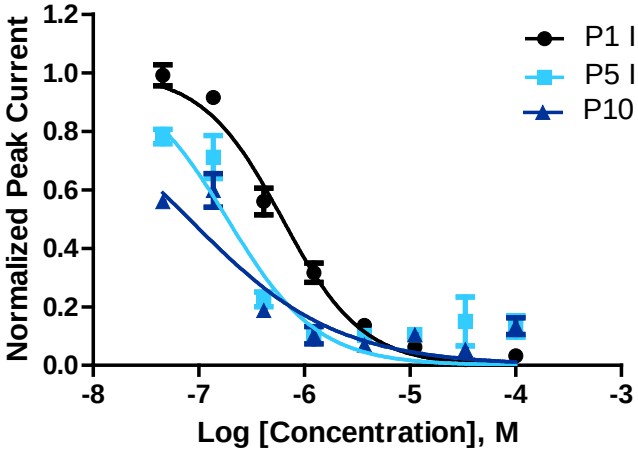
nAChRs	Estimated IC_{50} (μM)					
	α-Bungarotoxin	Atropine	DHβE	Mecamylamine	MLA	Pancuronium
α1β1δε	-	103.6	5.4	82.4	3.3	2.8
α3β4	-	-	7.2	7.0	0.5	96.0nM
α4β2	-	99.0	0.7	-	-	119.7
α4α6β2	-	86.7	0.4	3.4	-	36.9
α7	3.2nM	1.9	4.3	-	7.7nM	-

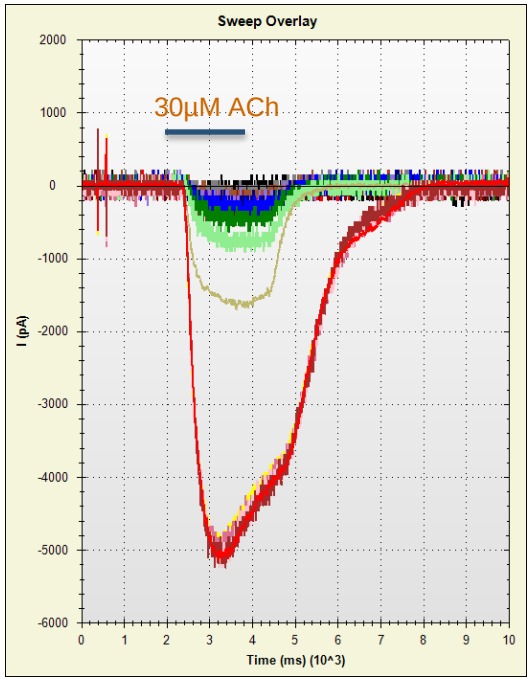
Open channel block of α4β2 receptor



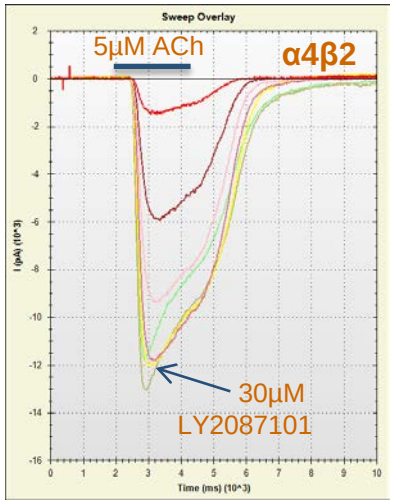
1.2µM Mecamylamine

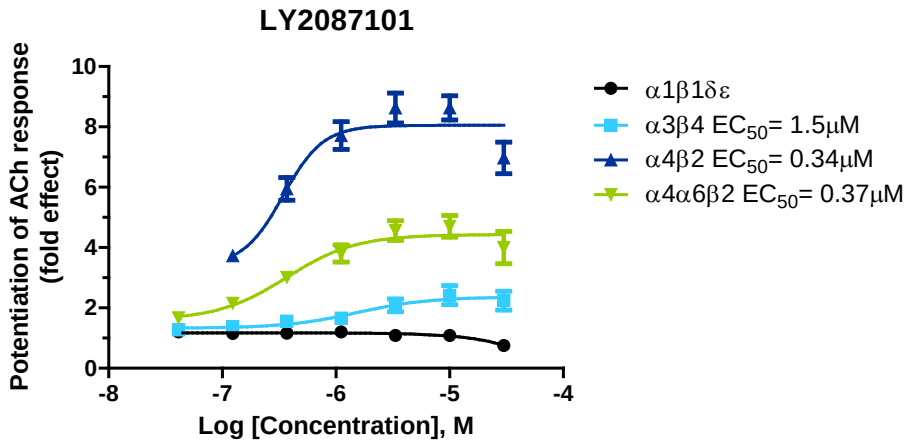


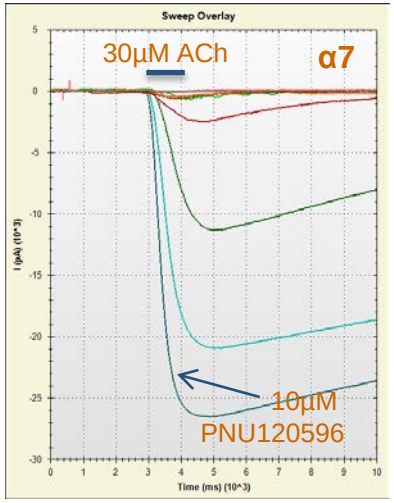


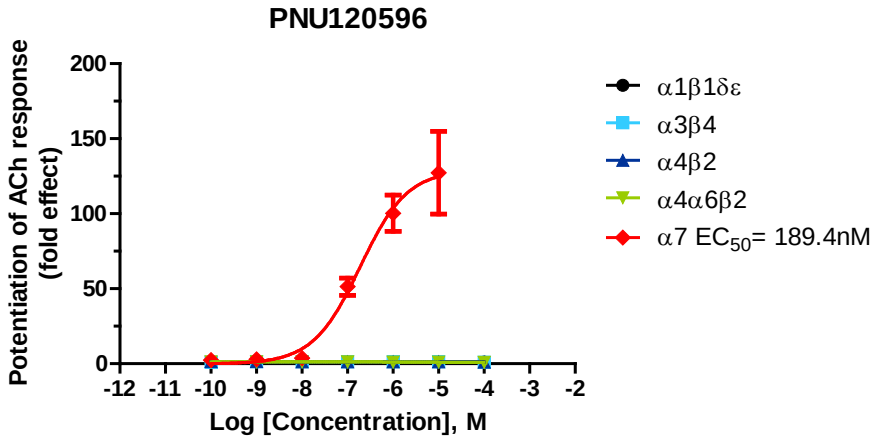


Pharmacology of nAChRs PAMs

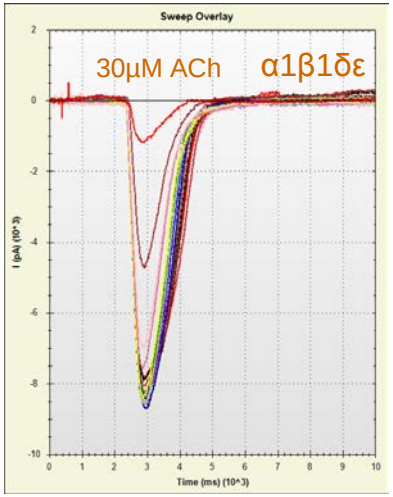


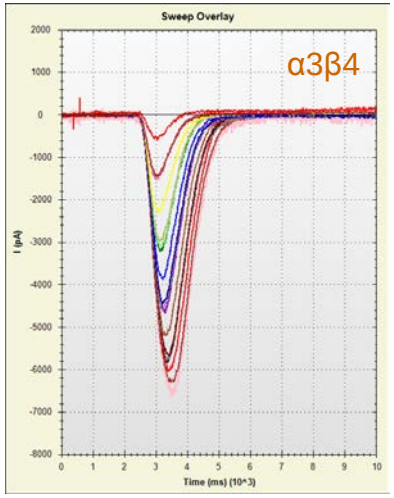


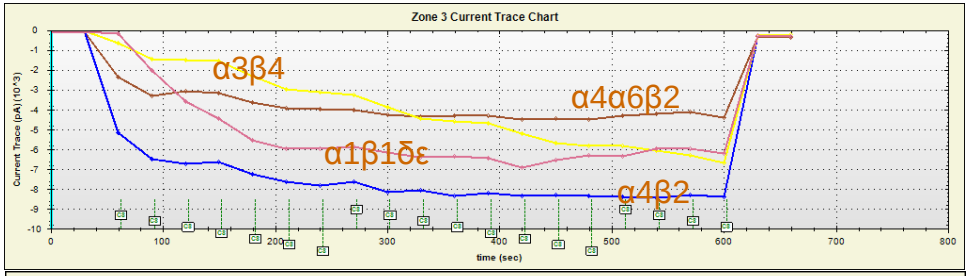


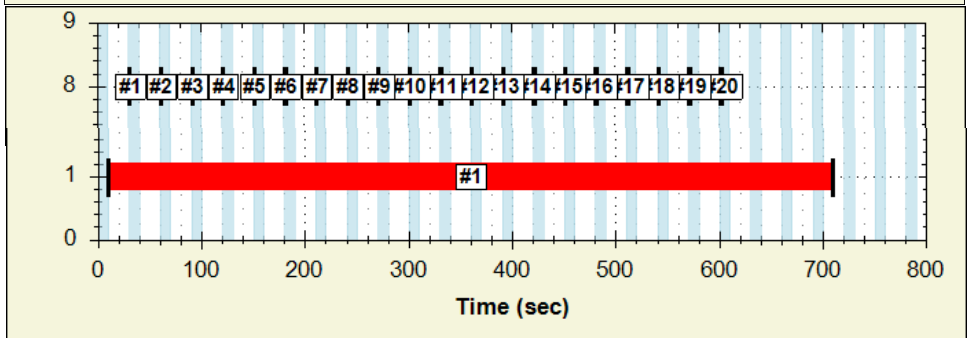


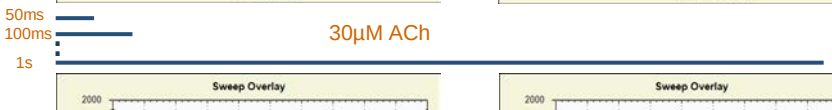
ACh Binding Kinetics

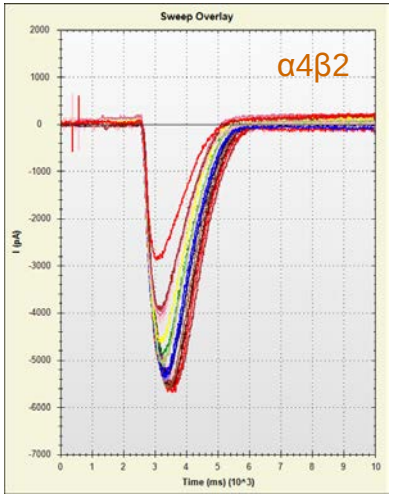


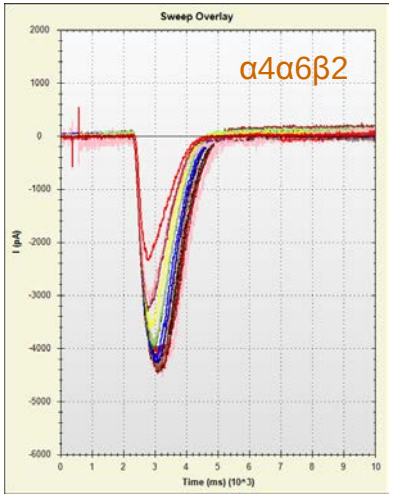


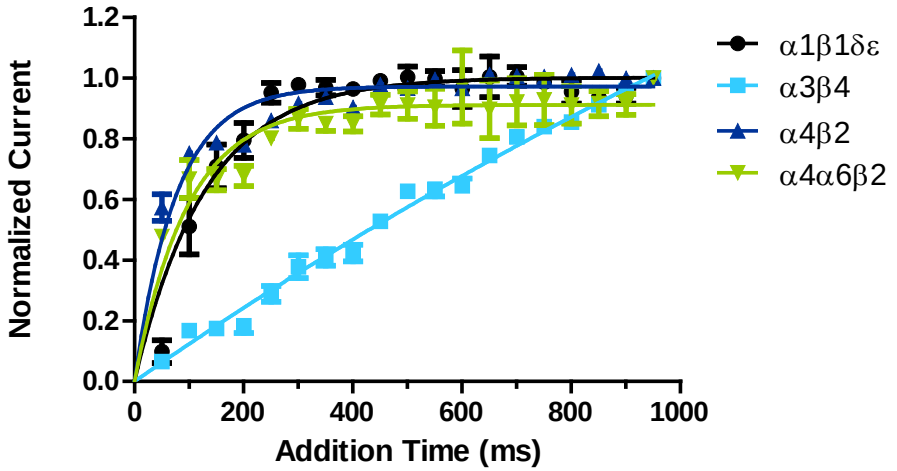












Desensitization characteristics of α4β2 receptor

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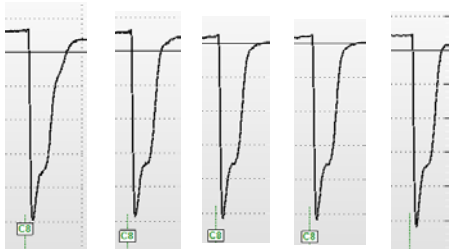
0.05Hz



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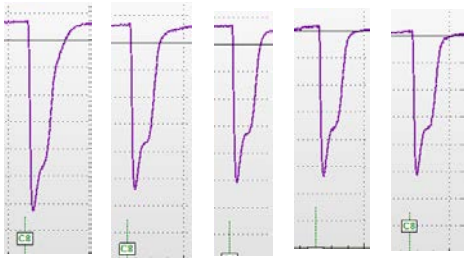

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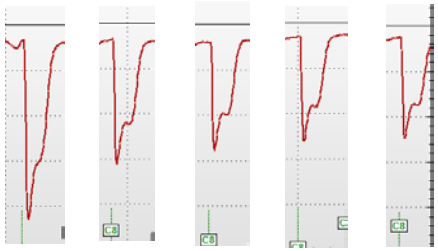

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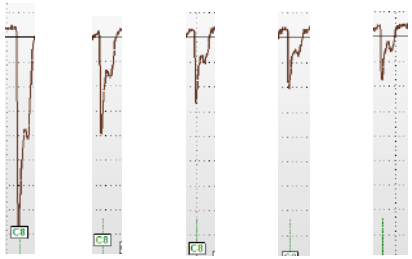

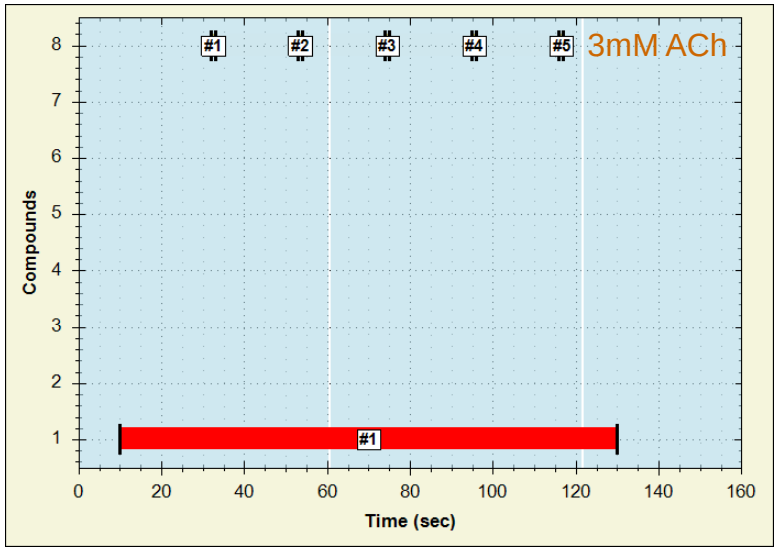

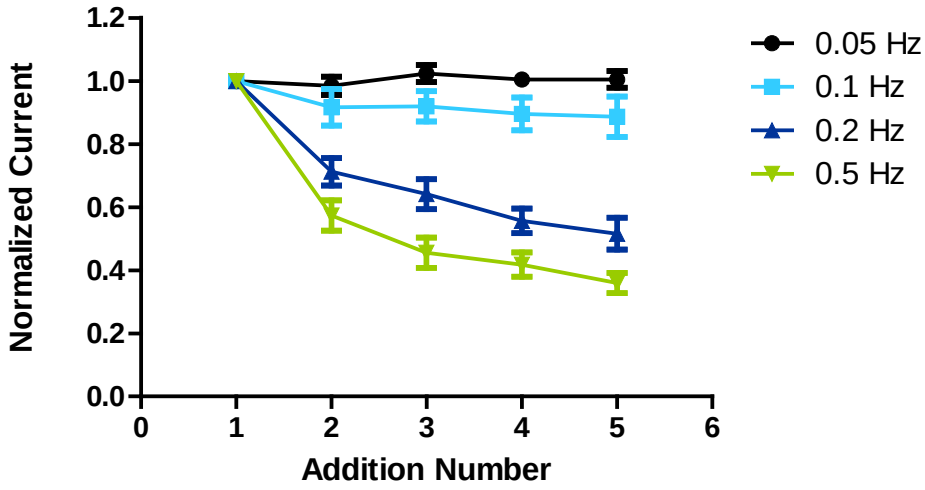

Desensitization characteristics of α4β2 receptor

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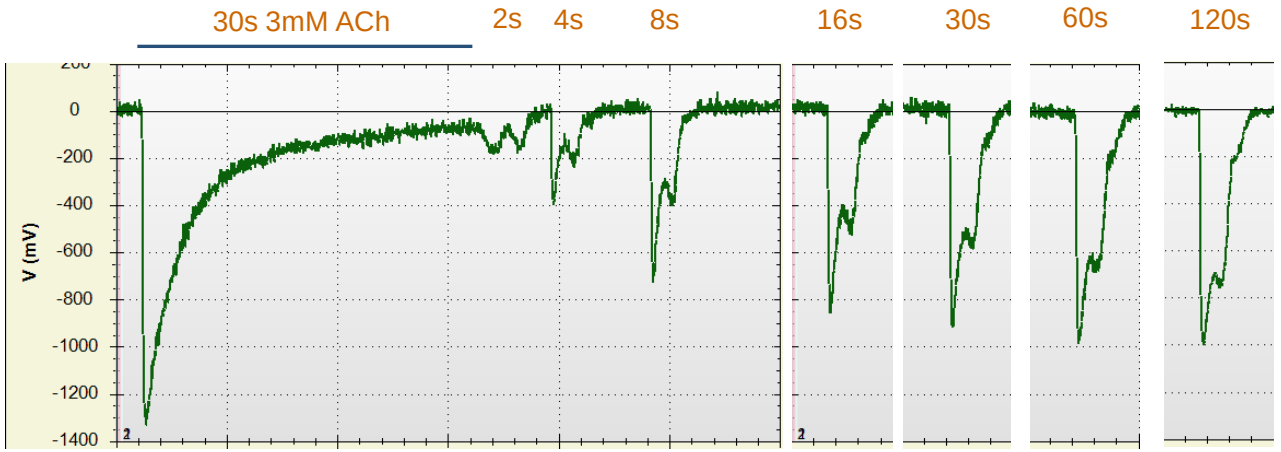

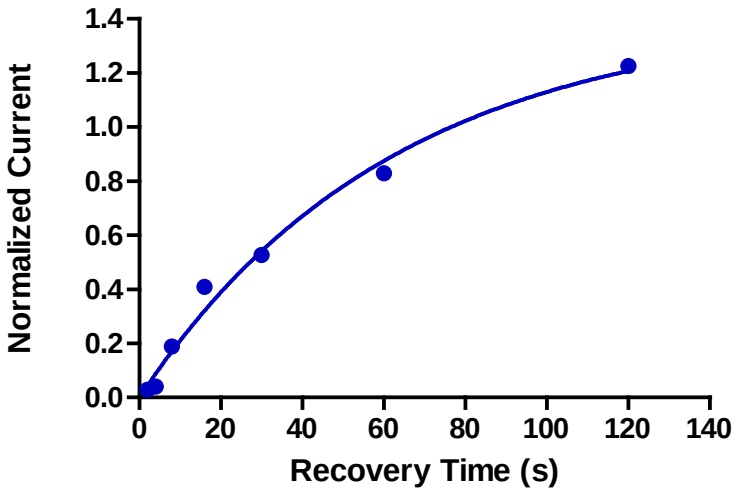

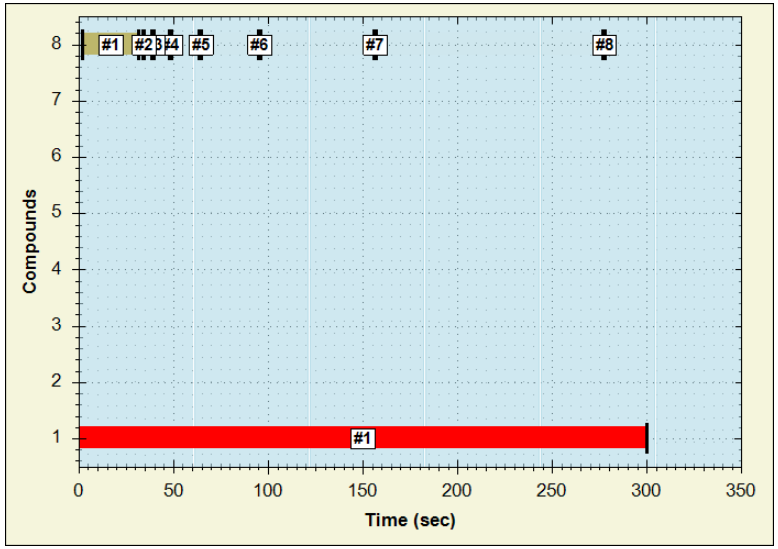

Onset and recovery of inhibition for DHβE on α4β2 receptor

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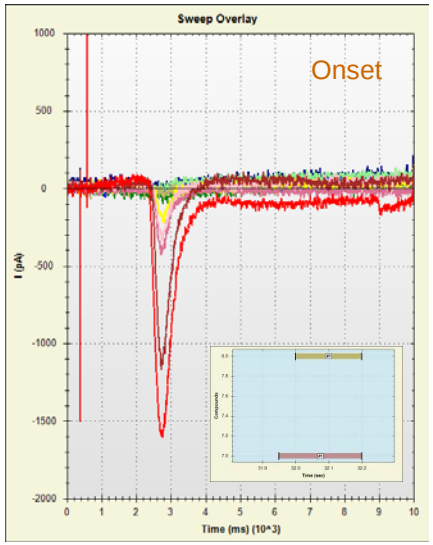
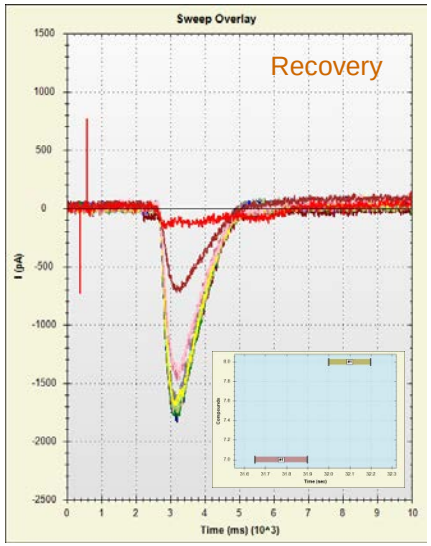
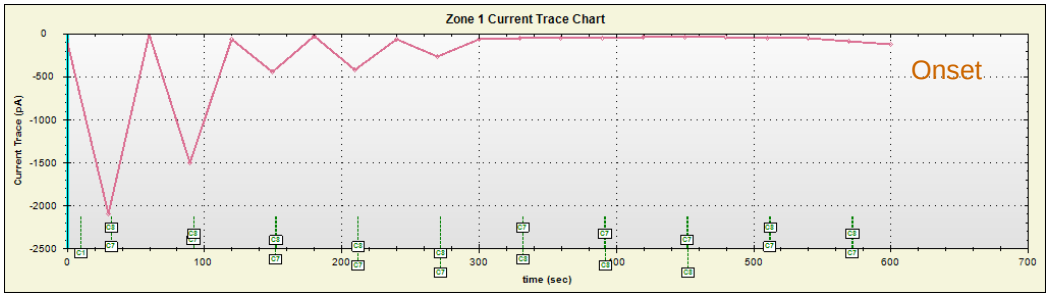
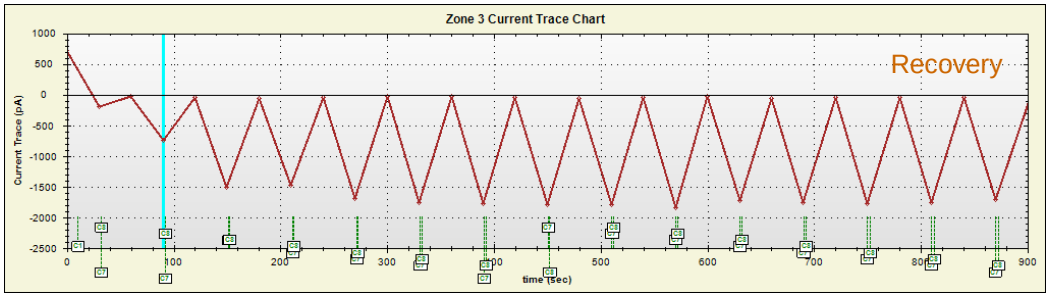
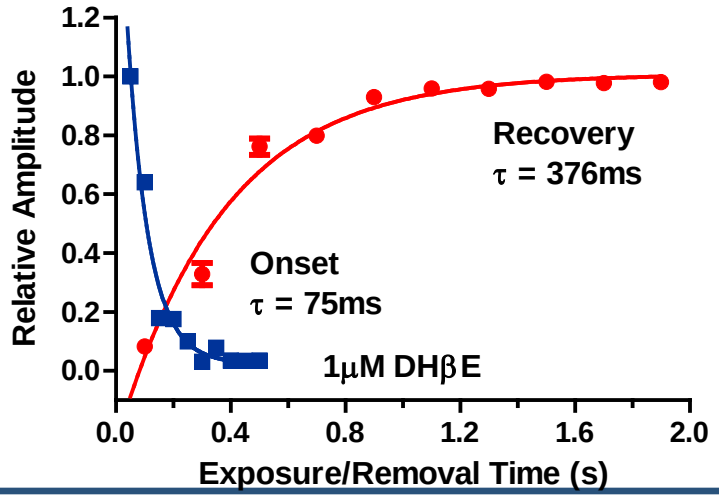








DHβE time course



Validation objectives

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nAChRs response characterization

- ☑ Recording conditions optimization
- ☑ Current stability
- ☑ Cross plate uniformity and inter-run precision.
- ☑ Pharmacology of agonists, antagonists and positive allosteric modulators (PAMs)
- ☑ Binding constants and channel kinetics

Thank you

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