Ligand-Gated Channels

- Introduction to GABA Receptors
- References for GABA Receptors

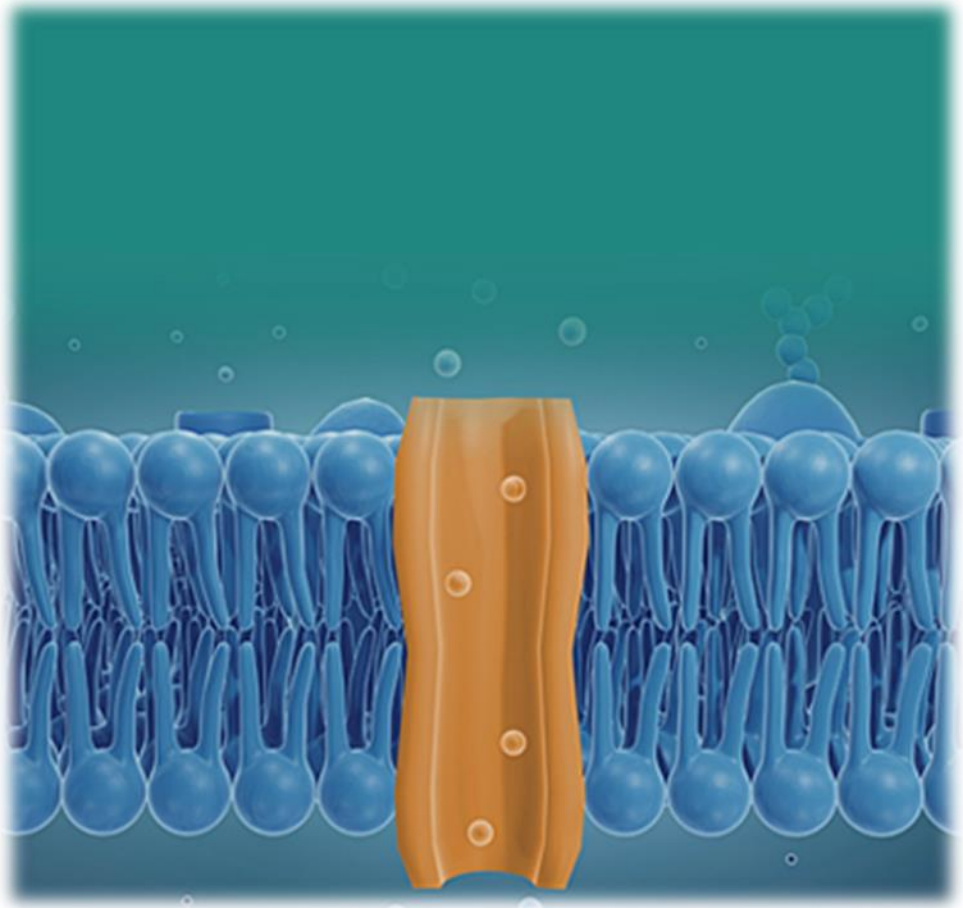
  GABAA α1/β3/γ2
  GABAA α2/β3/γ2
  GABAA α3/β3/γ2
  GABAA α4/β3/γ2
  GABAA α5/β3/γ2
  GABAA α6/β3/γ2

- Introduction to Nicotinic Acetylcholine Receptors
- References for Nicotinic Acetylcholine Receptors

  nAChR α1/β1/δ/ε
  nAChR α3/β4
  nAChR α4/β2
  nAChR α7/ric3

Please press the "Back" button to return to the previous menu

BACK



Click Channel Type to Access Validation Data:

- Introduction to Glycine Receptors
- References for Glycine Receptors

  GlyRA1

- Introduction to Glutamate Receptors
- References for Glutamate Receptors

  GluR6

Introduction to GABA Receptors

BACK

$GABA_A$ receptors are anion channels opened by γ-aminobutyric acid (GABA). Based on their amino acid sequences they belong to the same superfamily as the glycine receptors, anionic glutamate receptors, nicotinic acetylcholine receptors, and the $5\text{-}HT_3$ receptor [1]. Each functional GABA channel molecule contains five subunits. There are at least 19 distinct but related $GABA_A$ receptor subunits in mammals. Alternative exon splicing of the pre-mRNA forms even more heterogeneity.

The five subunit $GABA_A$ receptors in the central nervous system produce combinations of α and β subunits plus one or more of the γ, δ, θ, π or ε subunit types [2]. The majority contain only the αβγ combination. In addition there are three known ρ subunits that occur mainly in the retina and which appear to assemble separately from all others.

$GABA_A$ receptors possess a large and varied set of modulatory sites where ligands modify the response to GABA [3]. Selective ligands at these sites differentiate a series of subtypes for the $GABA_A$ receptor.

The modulatory benzodiazepine site offers distinct pharmacology for the definition of subtypes of the $GABA_A$ receptors. The majority of $GABA_A$ receptors carry some form of the benzodiazepine site. Benzodiazepines generally enhance the action of GABA, but some ligands act in the opposite direction at the benzodiazepine site.

There are many other distinct modulatory sites that have been found on $GABA_A$ receptors [4,5,6], some ligands show a preference for particular subunit isoforms or subunit combinations.

References for GABA Receptors

BACK

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Introduction to Nicotinic Acetylcholine Receptors

BACK

Nicotinic acetylcholine receptors (nAChRs) are multi-subunit transmembrane neurotransmitter receptors that are classified in the 'Cys-loop' superfamily [1,2]. They are found in the central and peripheral nervous systems, as well as the non-neuronal cholenergic system [3,4]. nAChRs contain five subunits, assemble in both heteromeric and homomeric pentamers and at least sixteen subunits in mammals have been found [5].

nAChRs mediate ionotropic effects of the neurotransmitter acetylcholine. nAChRs are involved in excitatory neurotransmission at the neuromuscular junction, in the autonomic nervous system, and at particular synapses in the brain and spinal cord [1,3].

Expression of certain nAChRs in brain and muscle is developmentally regulated [6,7,8]. Some nAChRs are expressed in a variety of other tissues and cell types as well as sensory organs [4]. nAChRs may serve serve a modulatory function in these cells [9,10].

nAChR subunits are homologous, sharing the same topographical features as subunits of other 'Cys-loop' receptors such as GABA and 5-HT receptors, ionotropic glycine, and some glutamate-gated Cl^- channels. These features include four transmembrane segments, a large extracellular N-terminal domain, a cytoplasmic loop of variable length, and a short extracellular C-terminus [7].

Autonomic neurons express α3β4* where asterisk denotes they may include other subunits in this case including α5, β2 and α7 nAChRs. Brain and spinal cord neurons express various combinations of subunits, with α4β2* and α7 nAChRs predominating.

References for nAChR's

1) Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev*, **89** (1): 73-120. [PMID:19126755]

2) Sine SM, Engel AG. (2006) Recent advances in Cys-loop receptor structure and function. Nature, 440 (7083): 448-55. [PMID:16554804]

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BACK

Introduction to Glycine Receptors

BACK

The glycine receptor Cl^- channel (GlyR) mediates inhibitory synaptic transmission in reflex circuits of the spinal cord. Glycine was found to be an inhibitory neurotransmitter from an analysis of its distribution in the spinal cord [1]. Glycine activates a strychnine-sensitive Cl^- conductance in spinal cord neurons [2-4]. The α1 GlyR subunit was cloned in 1987 [5] and due to its homology with the nAChRs it is part of the Cys-loop family of ligand-gated ion channel receptors. GlyR's are also found pre-synaptically, where they modulate neurotransmitter release.

GlyRs are pentameric assemblies around an ion-conducting pore. Similar to other Cys-loop receptor subunits, each GlyR subunit has a large extracellular amino-terminal domain that includes the ligand binding sites.

Amino acid agonists at GlyRs rank order potency is glycine > β-alanine > taurine. The only non-peptidic agonist identified so far is ivermectin [14]. GlyRs are antagonised by strychnine in a potent and specific manner. Picrotoxin distinguishes between α homomeric and αβ heteromeric GlyRs [6]. Much current therapeutic research is focused on the development of GlyR-specific drugs for movement disorders and chronic inflammatory pain.

References for Glycine Receptors

BACK

1) Aprison MH, Werman R. (1965) The distribution of glycine in cat spinal cord and roots. *Life Sci*, **4** (21): 2075-83. [PMID:5866625]

2) Callister RJ, Graham BA. (2010) Early history of glycine receptor biology in Mammalian spinal cord circuits. *Front Mol Neurosci*, **3**: 13. [PMID:20577630]

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Introduction to Glutamate Receptors

BACK

The ionotropic glutamate receptors include NMDA, AMPA, and kainate receptor classes, named originally according to their agonist [1-3]. Receptors are comprised of homo- or hetero-oligomeric subunits assembled into cation-selective tetramers.

GluR6 is a kainite receptor now more commonly known as the GluK2 receptor. Kainate receptors can be expressed as homomers or heterotetramers of GluK1, GluK2 or GluK3 subunits. [4-6]). Kainate receptors require extracellular Na^+ and Cl^- for their activation [7-8].

References for Glutamate Receptors

BACK

1) Dingledine R, Borges K, Bowie D, Traynelis SF. (1999) The glutamate receptor ion channels. *Pharmacol Rev*, **51** (1): 7-61. [PMID:10049997]

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hGABAA α1/β3/γ2 (CYL3053)

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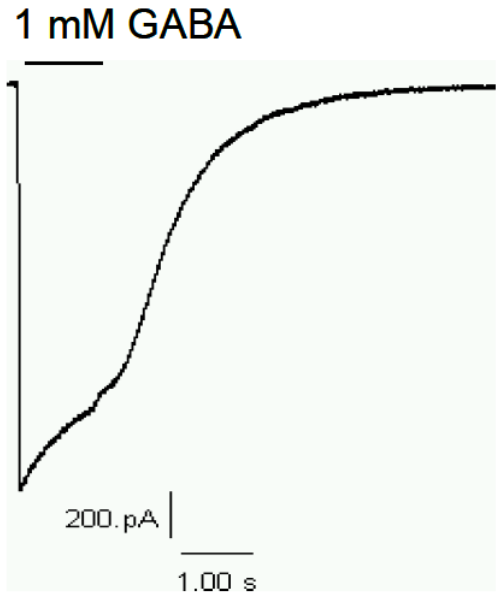

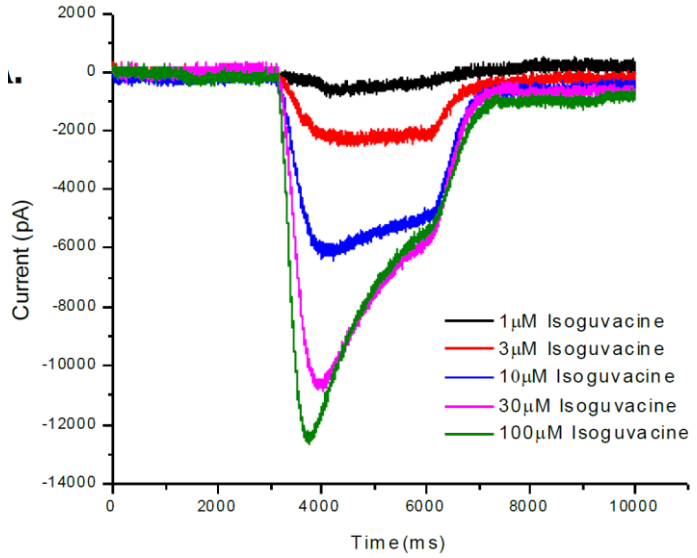

hGABAA α1/β3/γ2 Current Activation by Agonist: **Left:** Typical current trace upon addition of 1 mM GABA. (Manual Patch Clamp Data) **Right**: Example of typical GABAA α1 currents activated by increasing concentrations of isoguavacine, a GABA receptor agonist, applied for 3 s at increasing doses from 1uM to 100uM. (IonFluxHT Data)

hGABAA α1/β3/γ2

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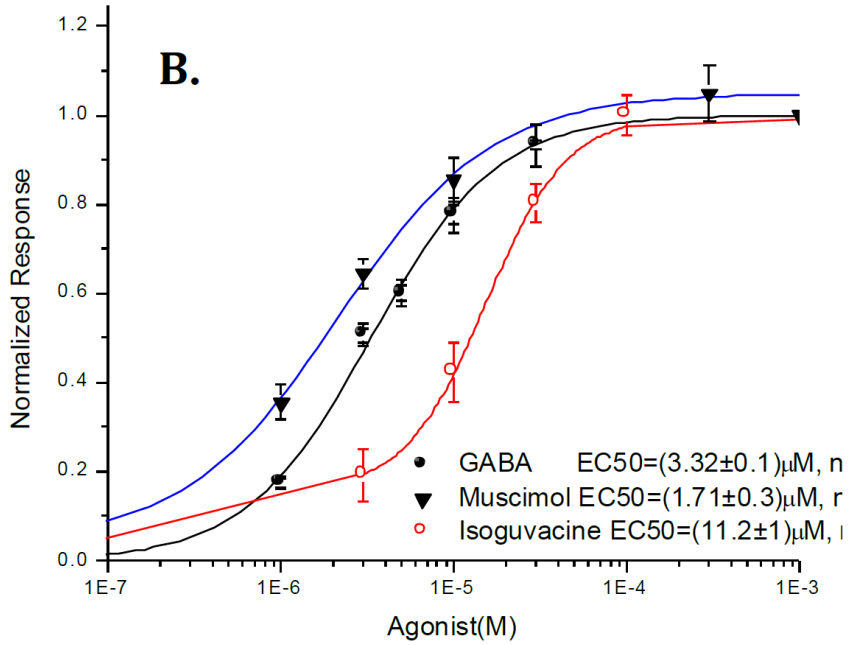

hGABAA α1/β3/γ2 Current Activation by Agonist: Comparison of dose-response curves for three agonists: GABA, isoguavacine, and muscimol. Hill fits yield EC50 values of 3.3, 11.2, and 1.7 μM respectively, in good agreement with literature. (IonFlux HT Data)

hGABAA α1/β3/γ2

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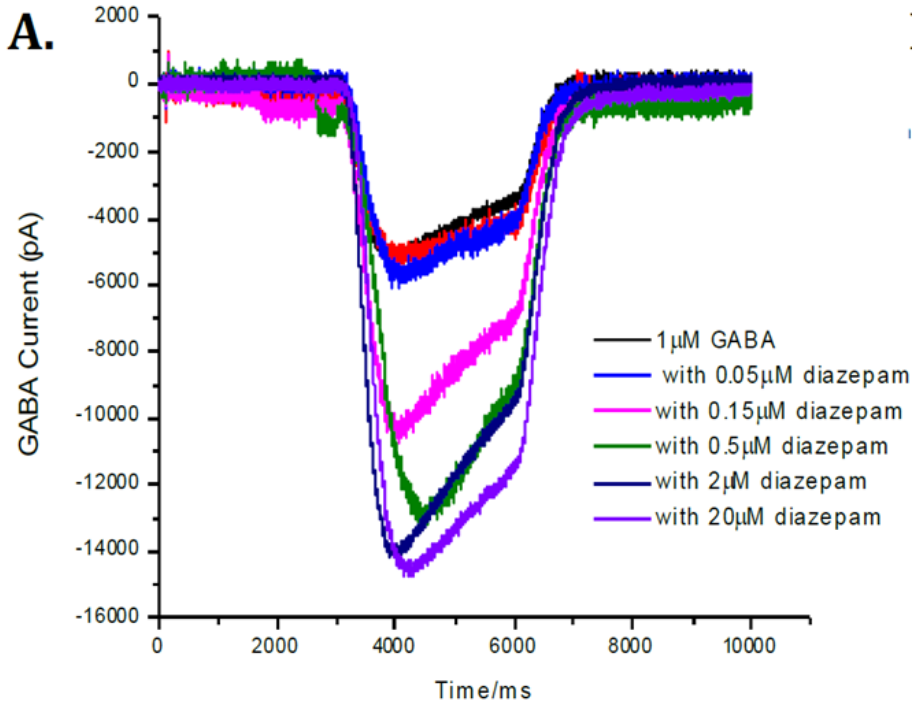

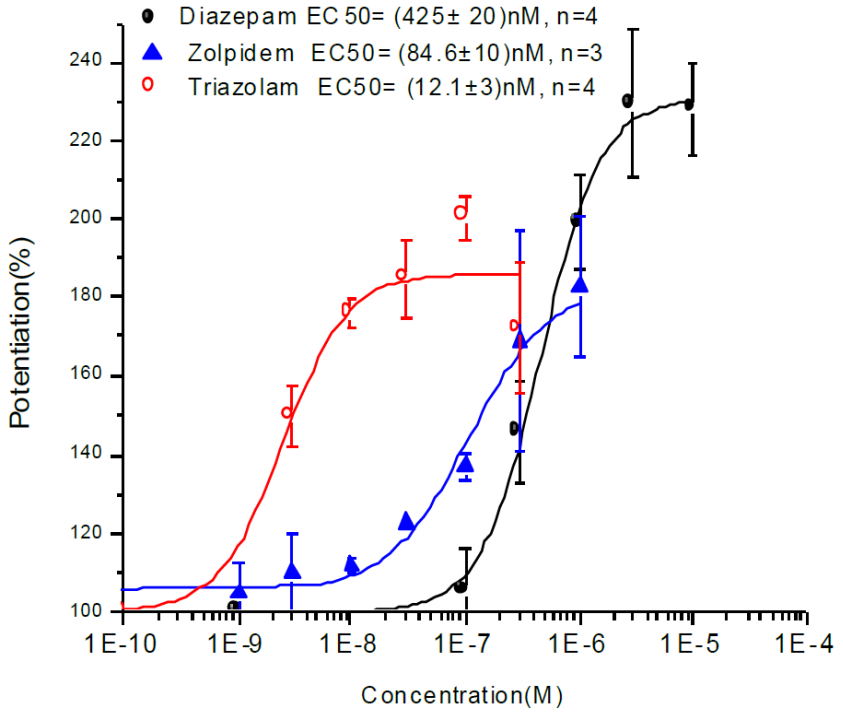

hGABAA α1/β3/γ2 Positive Allosteric Modulator Response: **Left:** Response of a cell ensemble exposed to 1 μM GABA (EC_{20}) with pre-incubated and co-applied with increasing diazepam concentrations. **Right:** Dose response analysis yielded EC_{50} of diazepam 425nM (n=4, hill slope= 1.28, R2= 0.977). The reported literature EC_{50} value is ~160nM. EC_{50} of zolpidem was 84.6nM (n=3, hill slope= 1.25 R2=0.967). The reported literature EC_{50} values range from 70nM to 150nM. EC_{50} of triazolam was 12.1nM (n=4, hill slope =1.41, R2=0.970). The reported literature EC_{50} values range from 22nM to 45nM (IonFlux HT Data).

hGABAA α1/β3/γ2

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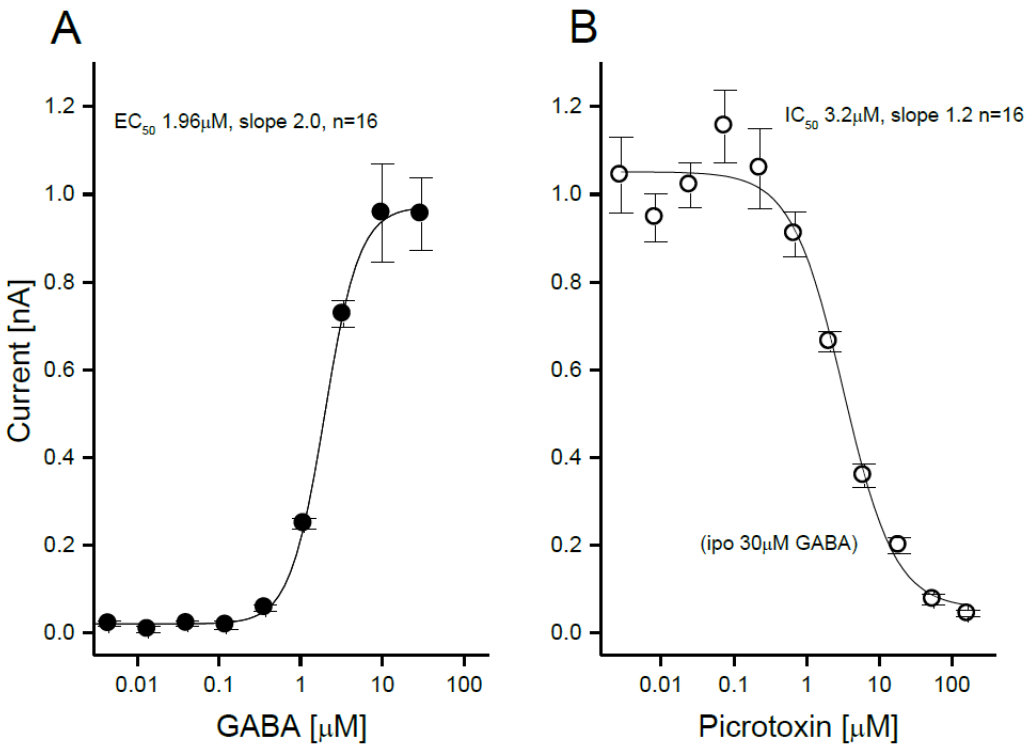

hGABAA α1/β3/γ2 Agonist and Antagonist Dose Response Curves: Activation by GABA **(left)** and inhibition by Picrotoxin **(right)** of the 30 µM GABA response (IonWorks Quattro Data).

hGABAA α1/β3/γ2

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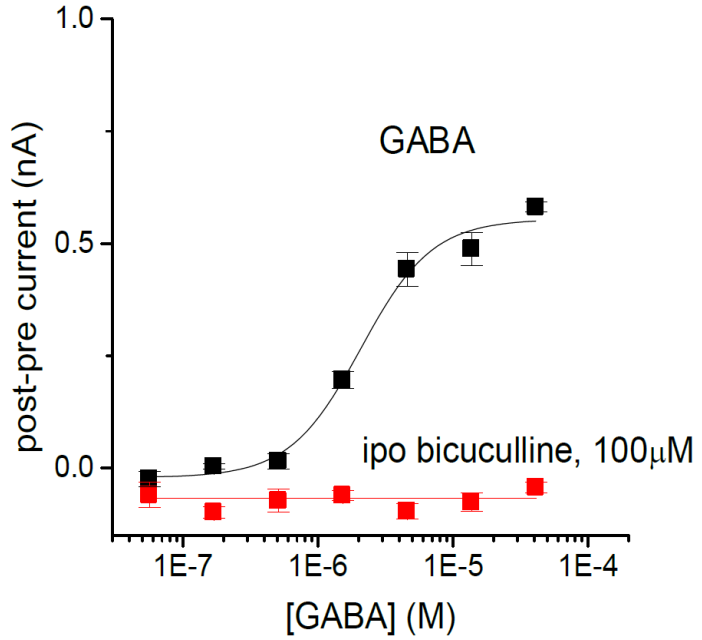

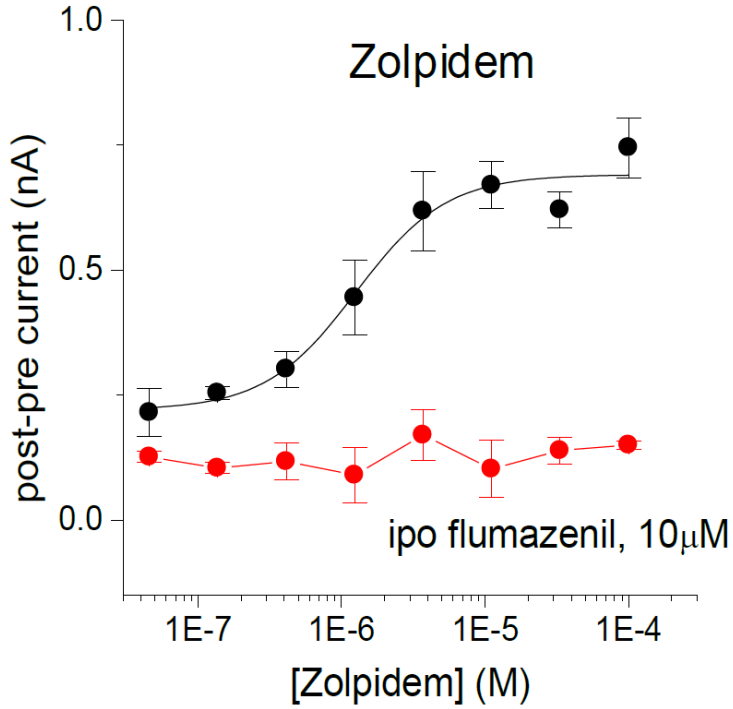

hGABAA α1/β3/γ2 Bicucculine Inhibition of GABA responses, Potentiation of GABA Response by Zolpidem and Block by Flumazenil: **Left:** GABA concentration response with a mean EC_{50} value of 1.96 μM (n=16) and a slope (k) of 2.0. Bicucculine, a known GABA antagonist, abolished (>98% block at 11 μM) responses to 10 μM GABA. It was also further demonstrated that a single dose (100 μM) of bicucculine completely inhibited the currents across a full GABA dose response curve. **Right:** In addition it was shown that the benzodiazepine zolpidem potentiated the 1 μM GABA signal with an approximate EC_{50} of 2 μM. This positive modulation was blocked by 10 μM flumazenil (IonWorks Quattro Data).

hGABAA α1/β3/γ2

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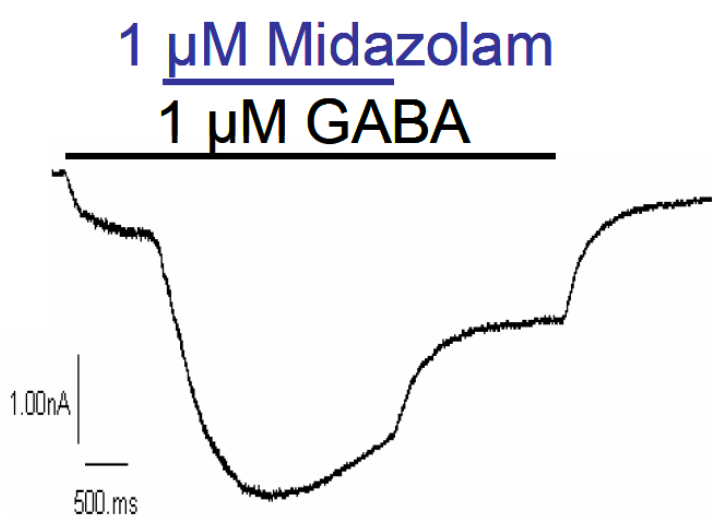

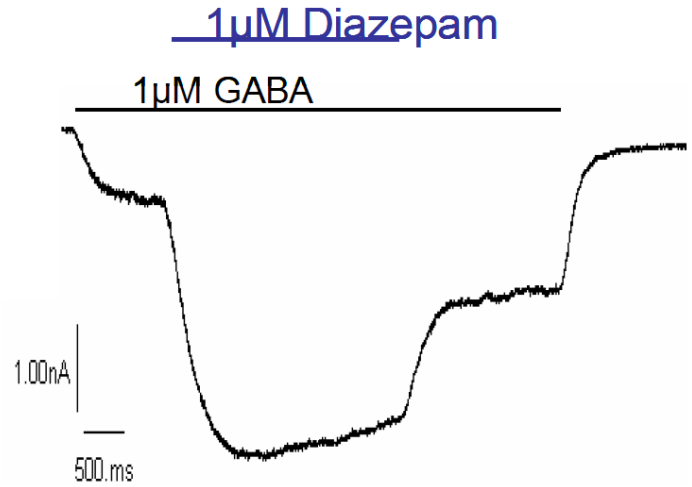

Potentiation of hGABAA α1/β3/γ2 Currents by the Benzodiazepines Midazolam and Diazepam: In order to verify the co-expression of the γ2 subunit, the benzodiazepine midazolam **(left)** was tested on the currents evoked by GABA (1µM, EC20) using conventional patch clamp. 1 µM midazolam augmented this GABA response by 462 ± 88% (mean ± SEM). The 1 µM GABA response was also augmented by diazepam **(right)** (Manual Patch Clamp Data).

hGABAA α1/β3/γ2

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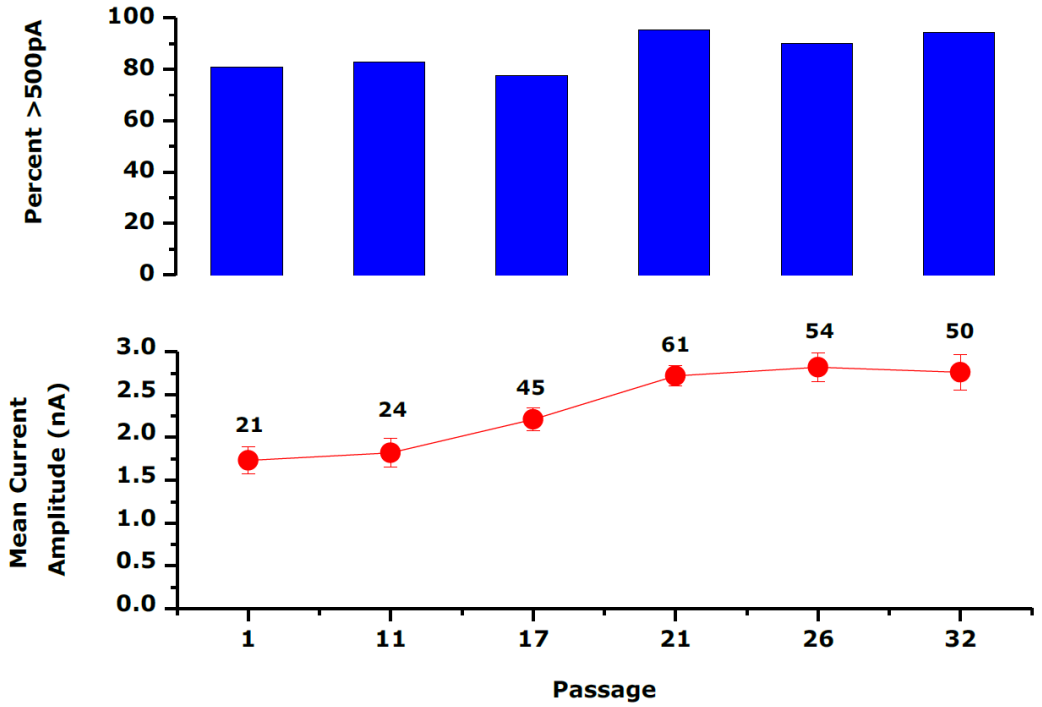
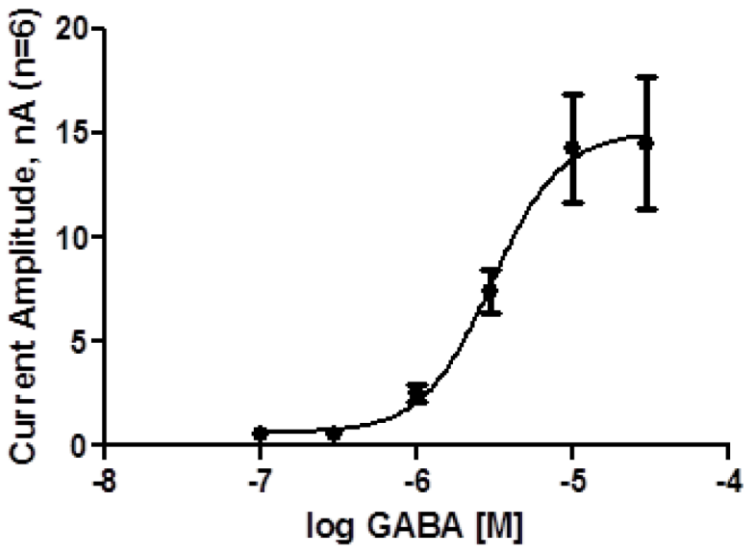


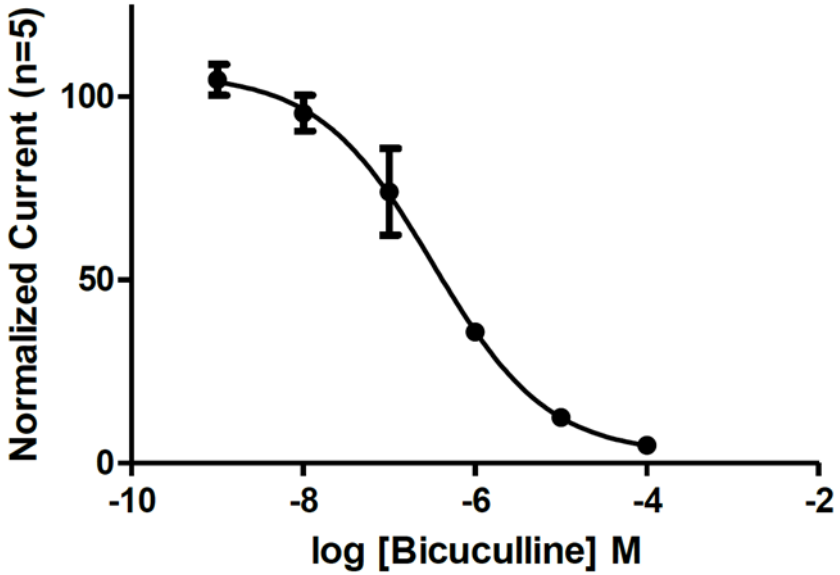
hGABAA α1/β3/γ2 Stability of Expression Over Passage: The upper panel shows the percentage of cells expressing a mean peak current >500 pA at 0 mV at cell passages 1, 11, 17, 21, 26 and 32. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (IonWorks HT Data).

hGABAA α2/β3/γ2 (CYL3072)

BACK



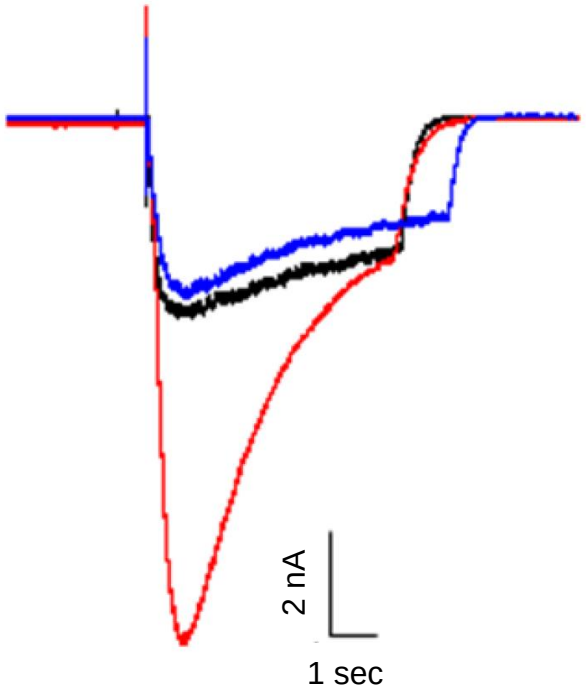
Passage	EC50 (µM)	EC20 (µM)	EC80 (µM)
11	3.71 ± 1.26	2.27 ± 0.70	6.57 ± 3.84
20	3.06 ± 1.12	1.51 ± 0.78	6.19 ± 1.77
25	3.31 ± 2.00	1.54 ± 0.96	8.59 ± 3.84
32	2.80 ± 1.21	1.17 ± 0.71	7.11 ± 2.98

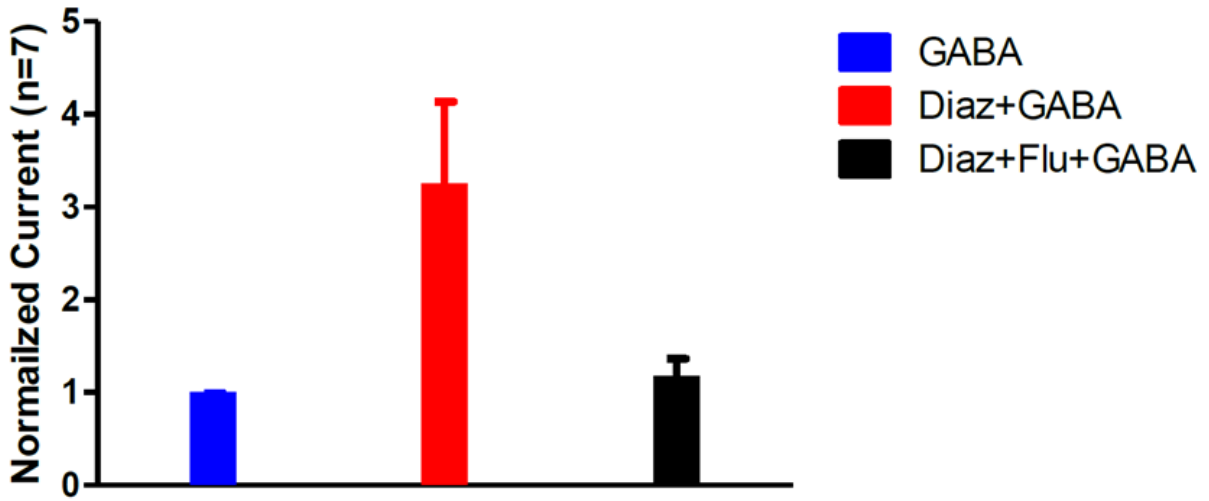


hGABAA α2/β3/γ2 Agonist and Antagonist Dose Response Curves: **Left:** GABA concentration-response curve for hGABA$_A$ α2/β3/γ2-HEK cells at passage 20. **Middle:** Assays designed to detect novel allosteric modulators often employ protocols where the test compound is added with a fixed concentration of GABA. The table shows the reproducibility of the GABA EC_{50}, EC_{20} and EC_{80} values (mean ± SD) with increasing passage number. Each point indicates the mean (±) response for 6 cells. We obtained an EC_{50} of 3.06 µM and a Hill slope of 1.96 **Right:** Bicuculline inhibition of response to addition of GABA at the EC80 concentration. Each point indicates the response of 5 cells. We obtained an IC_{50} of 0.324 µM and a Hill slope of -0.652. (PatchXpress Data).

hGABAA α2/β3/γ2

BACK

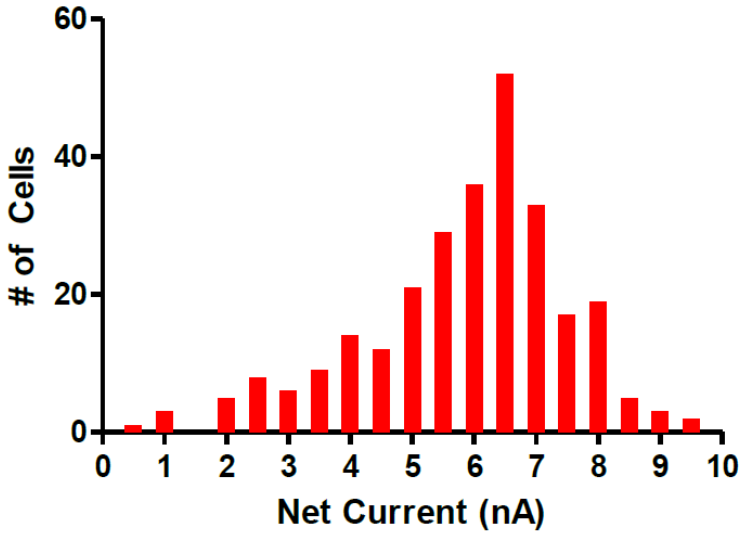


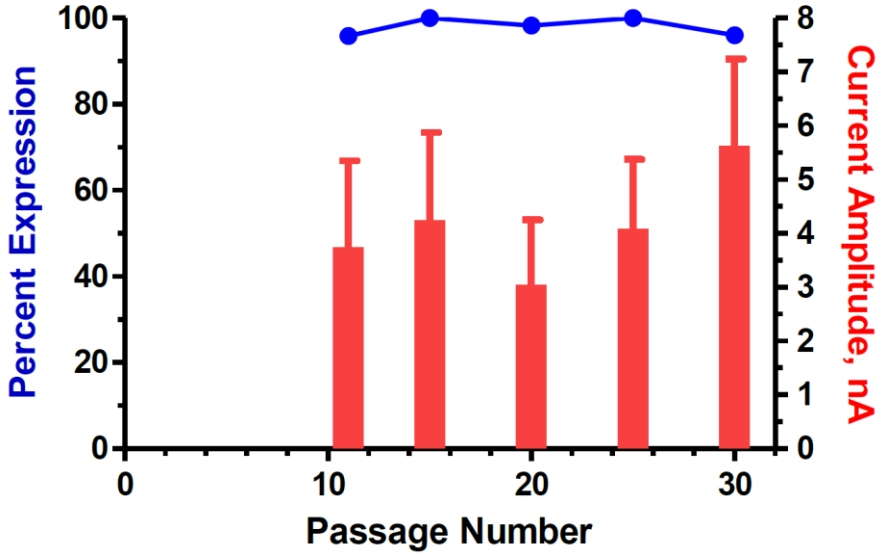


hGABAA α2/β3/γ2 Effect of Diazepam and Flumazenil: **Left:** The response of a single representative cell is shown. The blue trace shows the response to the addition of an EC_{20} concentration of GABA. After washout, 1μM diazepam was added alone and had no effect (not shown). After a 2 min incubation with 1μM diazepam, the response to the addition of an EC_{20} concentration of GABA+diazepam (red trace) was now enhanced. The effect of 1μM diazepam was completely blocked by 10 μM flumazenil (black trace), indicating the effect is mediated through the benzodiazepine binding site on the GABA A α2/β3/γ2 receptor. **Right:** The combined data for seven cells are shown graphically in the bar chart below (PatchXpress Data).

hGABAA α2/β3/γ2 (CYL3072)

BACK





hGABAA α2/β3/γ2 Current Amplitudes and Stability over Passages: **Left:** Frequency distribution of current amplitude obtained in single-hole mode. The $GABA_A$ α2/β3/γ2 current was defined as the peak current post GABA (100μM) addition minus the peak current pre GABA addition with a minimum acceptable current of 500pA. **Right:** Stability of expression and current amplitude. The blue line shows the percentage of cells expressing a mean peak inward current >0.5 nA at –40 mV at cell passages 11, 15, 20, 25 and 30. The red bars show the mean current amplitude (mean ± SD) for 73-269 cells per experiment (IonWorks Quattro Data).

hGABAA α3/β3/γ2 (CYL3068)

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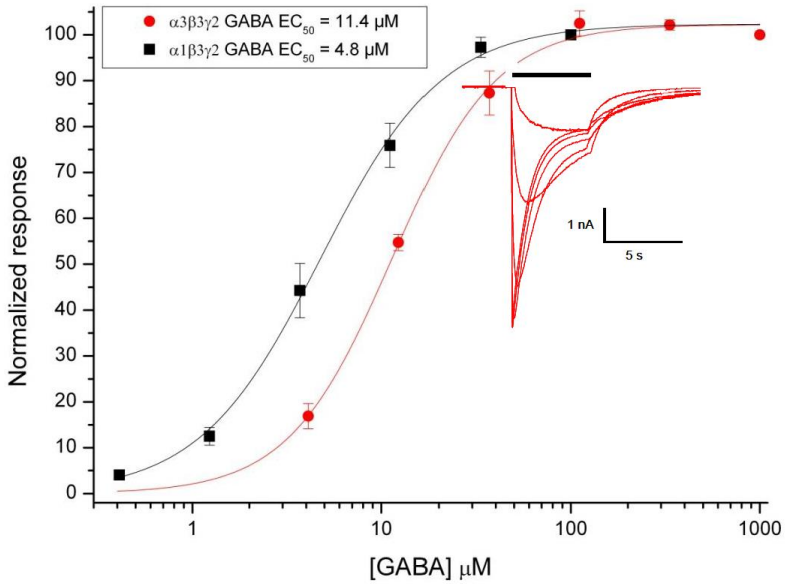
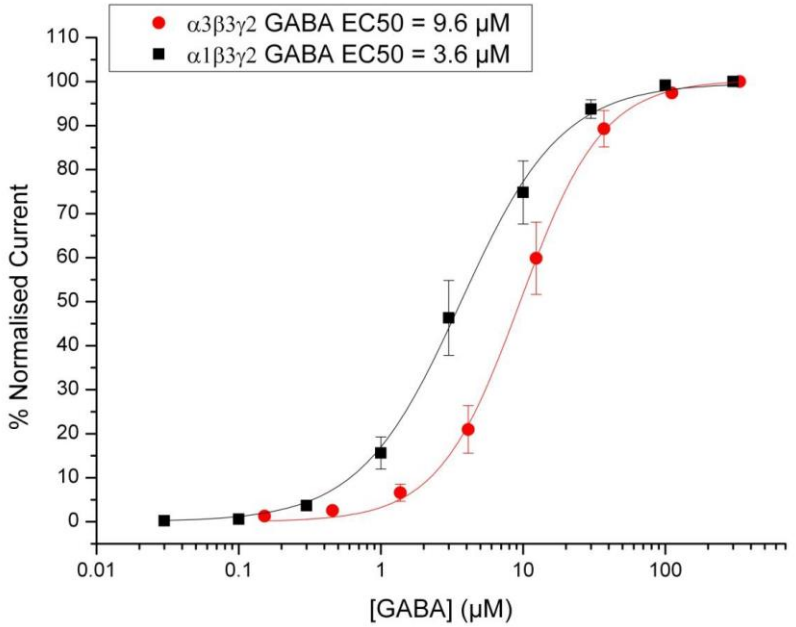
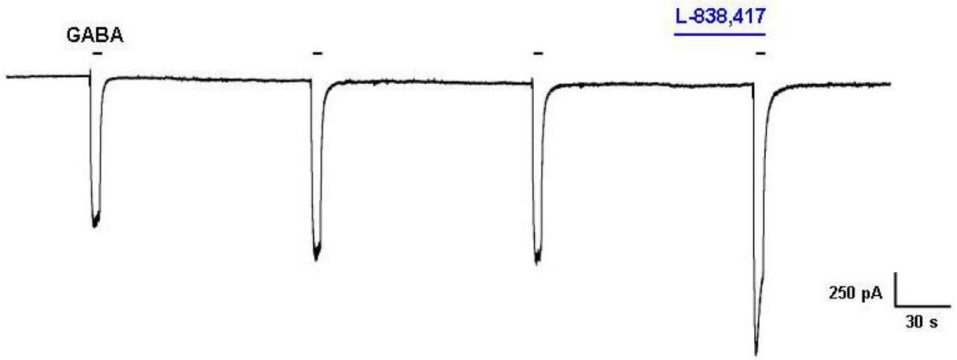


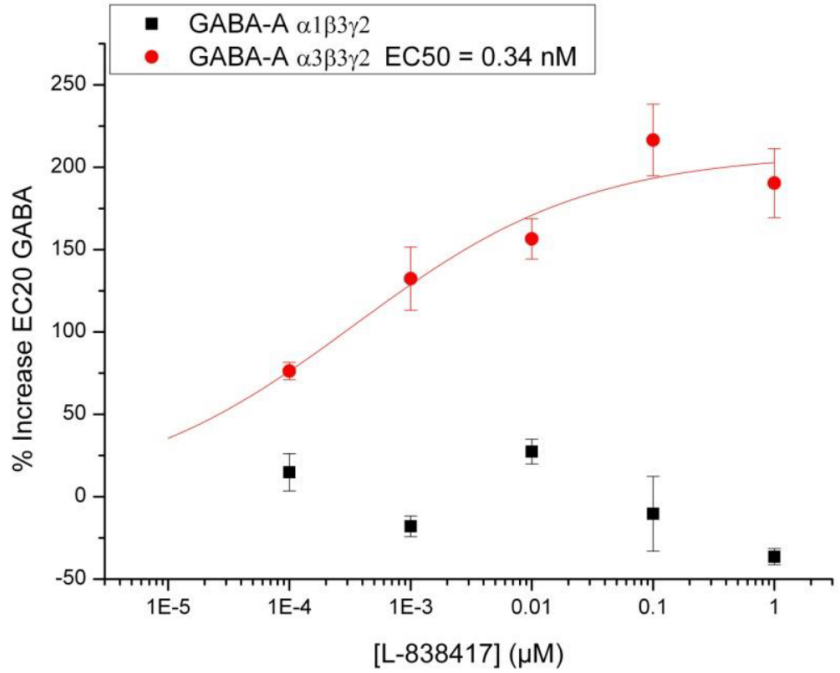


hGABAA α3/β3/γ2 Dose-Response Curve Compared to GABA α1 on Two Different Automated Electrophysiology Platforms: **Left:** GABA concentration-response curves for α1 (black squares) and α3 (red circles). **Left Inset:** Example of typical GABA A α3 currents activated by increasing concentrations of GABA applied for 5 s as indicated by the bar (PatchXpress Data). **Right:** GABA concentration-response curves for α3 (red circles) and α1 (black squares) (IonWorks Quattro Data).

hGABAA α3/β3/γ2

BACK

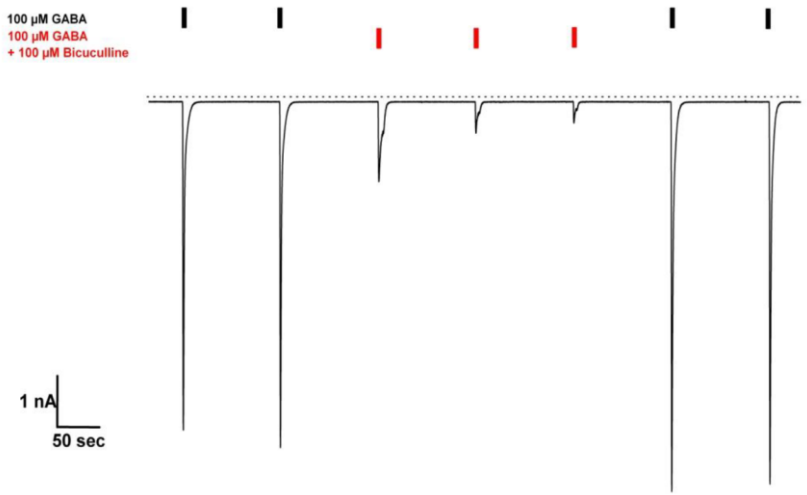


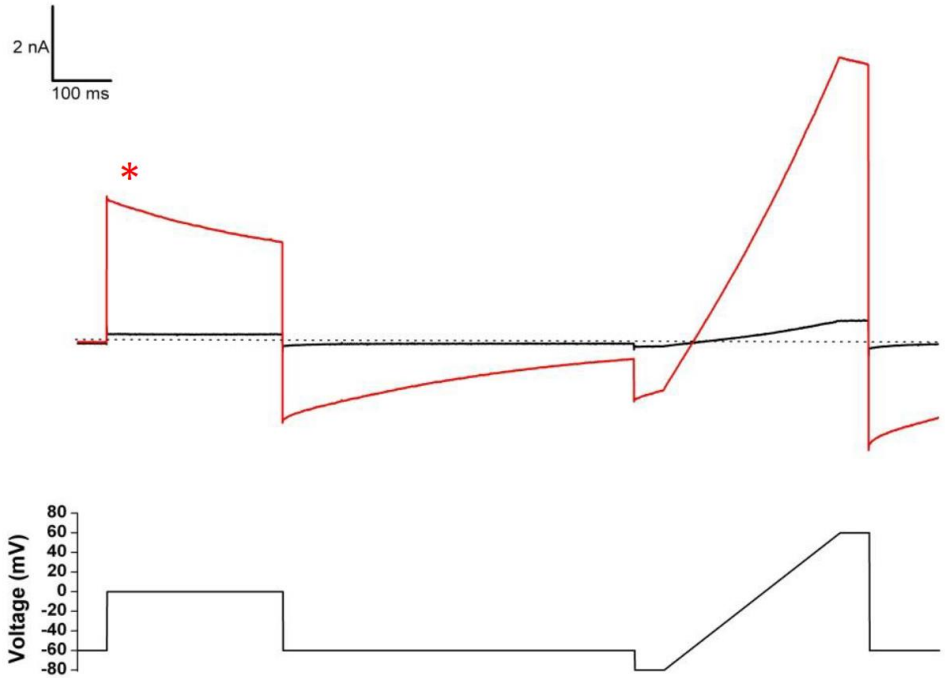


hGABAA α3/β3/γ2 Effect of L-838,417 on Currents Evoked by Sub-Maximal GABA evoked responses: **Left:** The cell was voltage-clamped at a holding potential of -40 mV. Repeated doses of EC_{20} GABA (4 μM) were applied for 5 seconds (black bar). GABA was washed off between doses. The cell was then incubated for 2 minutes in 100 nM L-838,417 followed by co-application of 100 nM L-838,417 (blue bar) and EC_{20} GABA (PatchXpress Data). **Right:** Effect of L-838,417 on α1 and α3 currents evoked by a sub-maximal (EC_{20}) concentration (4 μM) of GABA (IonWorks Quattro Data).

hGABAA α3/β3/γ2

BACK

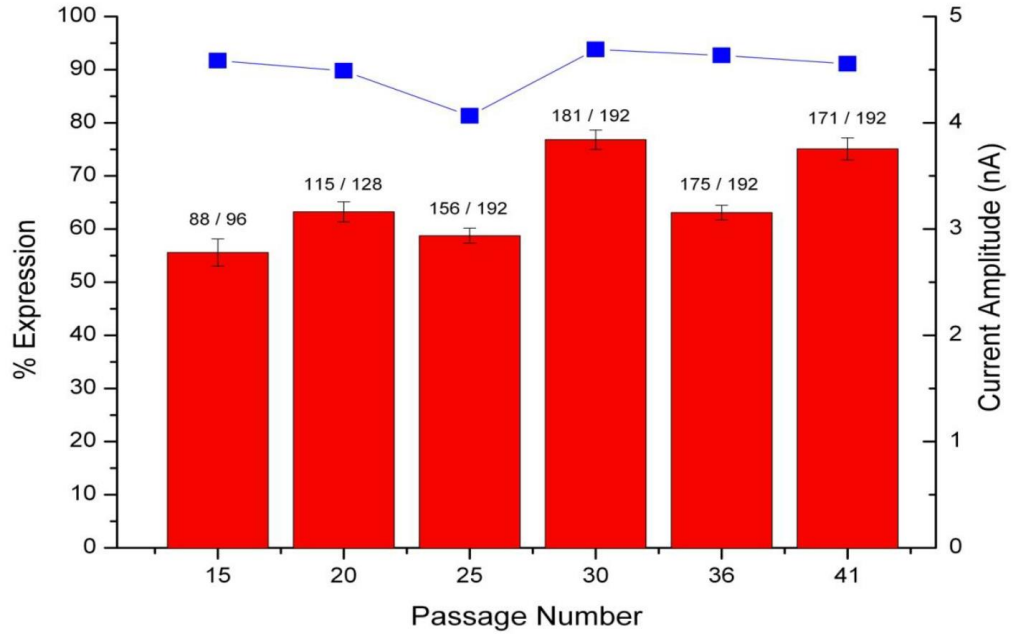




hGABAA α3/β3/γ2 Effect of Bicuculline on Currents Evoked by 100 µM GABA: **Left:** Inward currents were first evoked by brief (5 s) applications of GABA (black bars) from a holding potential of -40 mV. GABA evoked inward currents in the presence of 100 µM bicuculline (red bars) were much reduced in amplitude and readily reversible upon removal of the antagonist. Zero current level is shown by the black dotted line (Manual Patch Clamp Data). **Right:** Typical current traces (upper panel) evoked before (black trace) and after (red trace) the application of 300 µM GABA. The zero current level is shown by the black dotted line. Voltage protocol is shown in the lower panel. (IonWorks Quattro Data).

hGABAA α3/β3/γ2

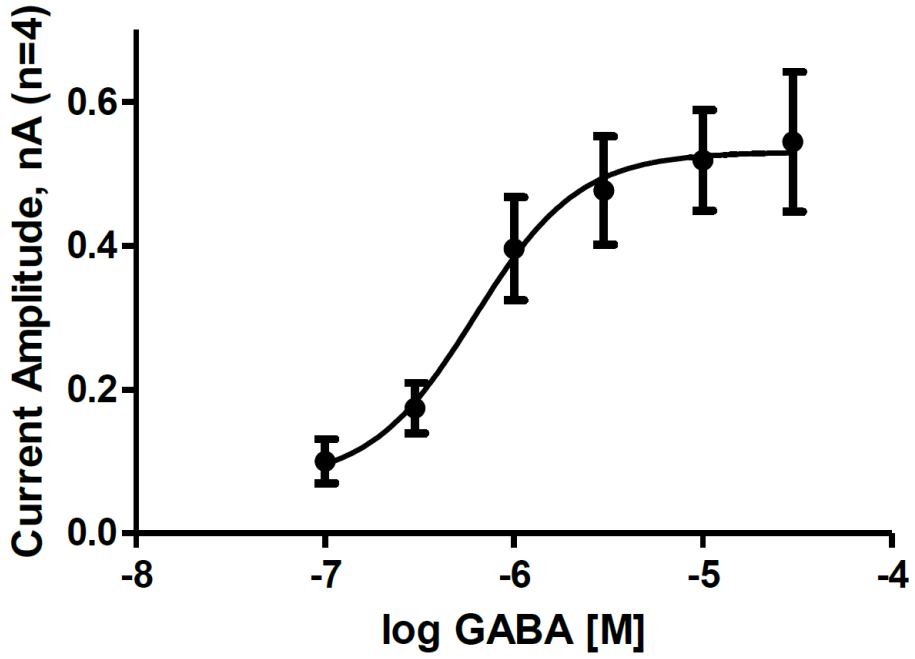
BACK

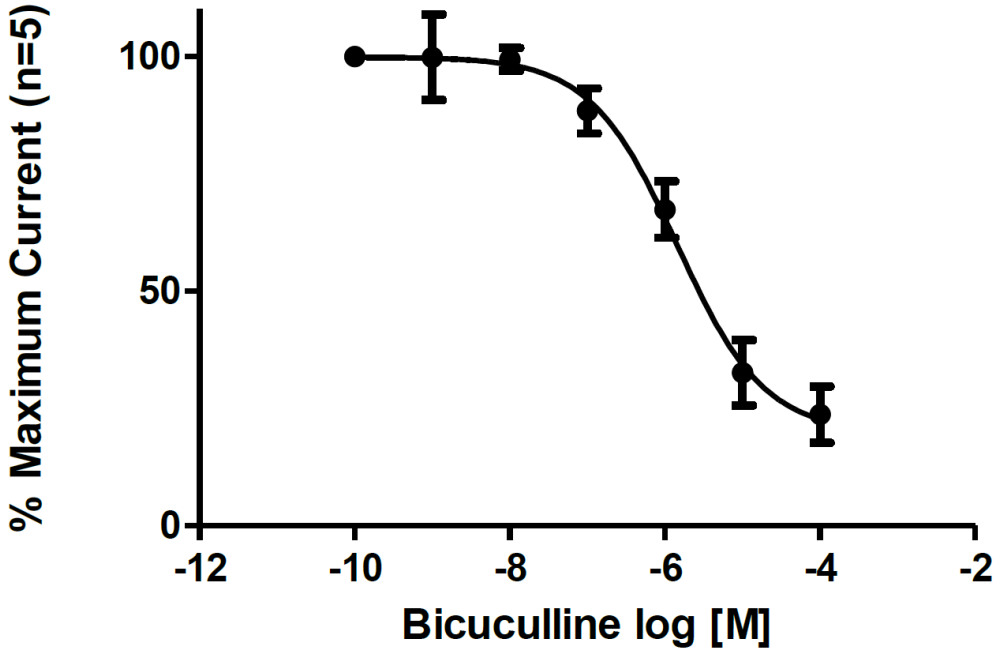


hGABAA α3/β3/γ2 Stability of Expression over Passage: The blue squares show the percentage of cells expressing a mean peak current >200 pA at 0 mV at cell passages 15, 20, 25, 30, 36 and 41. The corresponding red columns show mean current amplitude (mean SEM) the number of cells is shown relative to total cell availability (IonWorks Quattro Data).

hGABAA α4/β3/γ2 (CYL3085)

BACK





hGABAA α4/β3/γ2 Agonist and Antagonist Dose Response Curves: **Left:** GABA concentration-response curve at passage 25. Each point indicates the mean (±) response for 5 cells. We obtained an EC_{50} of 0.840 ± 0.616 µM and a Hill slope of 1.57. **Right**: Bicuculline inhibition of response to addition of the EC80 concentration of GABA. Each point indicates the response of 5 cells. We obtained an IC_{50} of 1.44 µM and a Hill slope of -0.775 (PatchXpress Data).

hGABAA α4/β3/γ2

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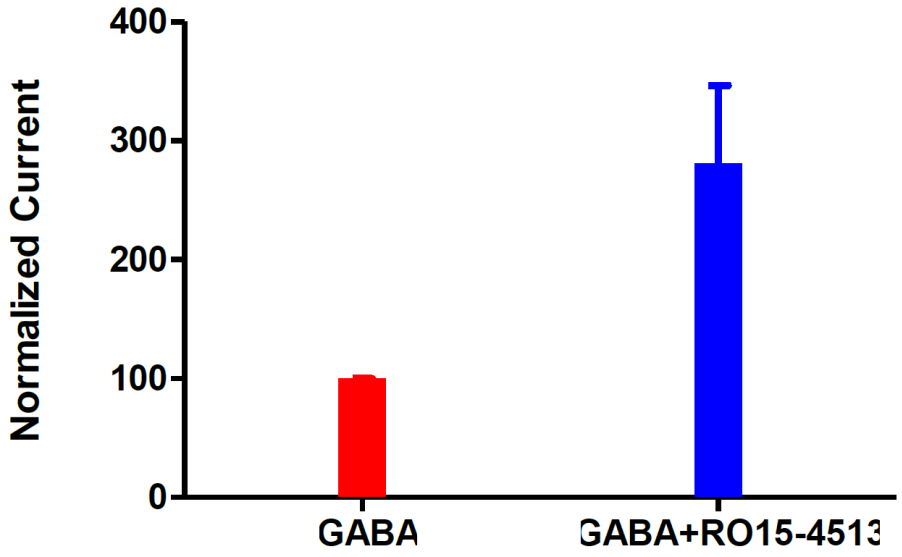
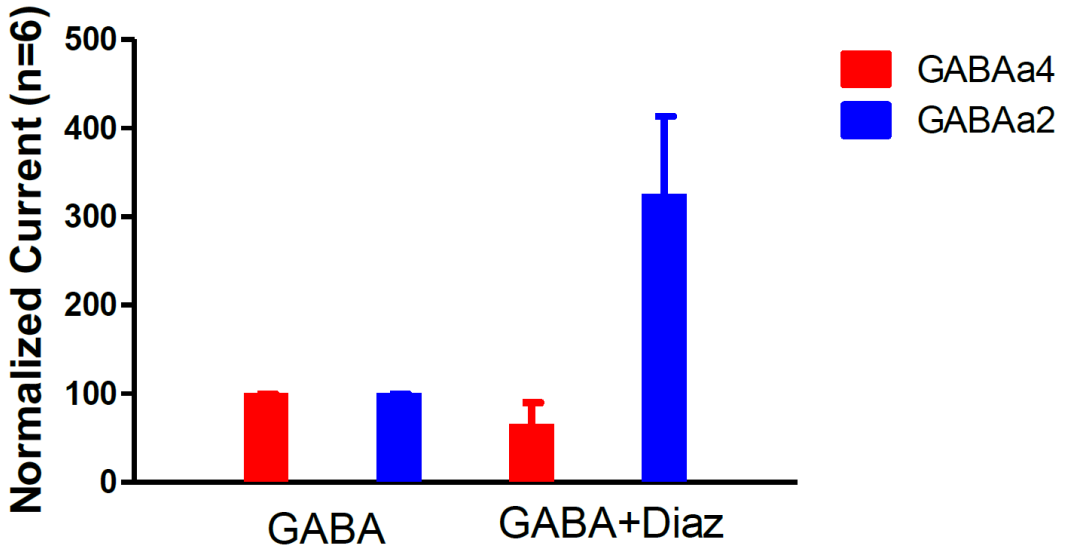
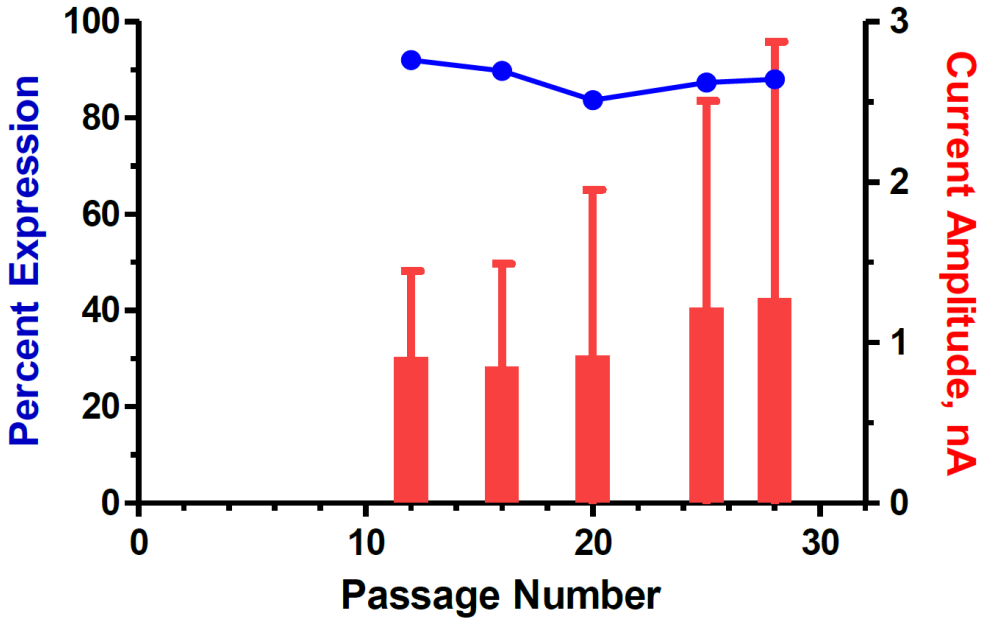




hGABAA α4/β3/γ2 Effect of the Benzodiazapine Site Allosteric Modulators on Currents: **Left:** The positive modifier effect of RO 15-4513 on the hGABAA α4/β2/γ2 current. Here the hGABAA α4/β2/γ2 cells were pre-treated with 100 nM of RO 15-4513 followed by an EC_{20} concentration of GABA + 100 nM RO 15-4513. The GABA induced current was increased approximately 2.5-fold by 100 nM RO 15-4513. **Right**: The effect of 1 µM diazepam on the normalized GABA (EC_{20}) induced current (n=6 cells; red bars) and the hGABAA α2/β3/γ2 (n=5; blue bars). 1 µM diazepam increased the EC_{20} GABA-induced current in the hGABAA α2/β3/γ2 cells, but did not show a positive modulating effect on the hGABAA α4/β3/γ2 current. (PatchXpress Data). **Reference:** Knoflach F et al. (1996). Pharmacological modulation of the diazepam-insensitive recombinant γ-aminobutyric acidA receptors α4β2γ2 and α6β2γ2. Molecular Pharmacology, 50:1253-1261.

hGABAA α4/β3/γ2

BACK



hGABAA α4/β3/γ2 Stability of Expression over Passage: The blue line shows the percentage of cells expressing a mean peak inward current >0.25 nA at –40 mV at cell passages 11, 15, 20, 25 and 28. The red bars show the mean current amplitude (mean ± SD) for 73-330 cells per experiment. (IonWorks HT Data).

hGABAA α5/β3/γ2 (CYL3073)

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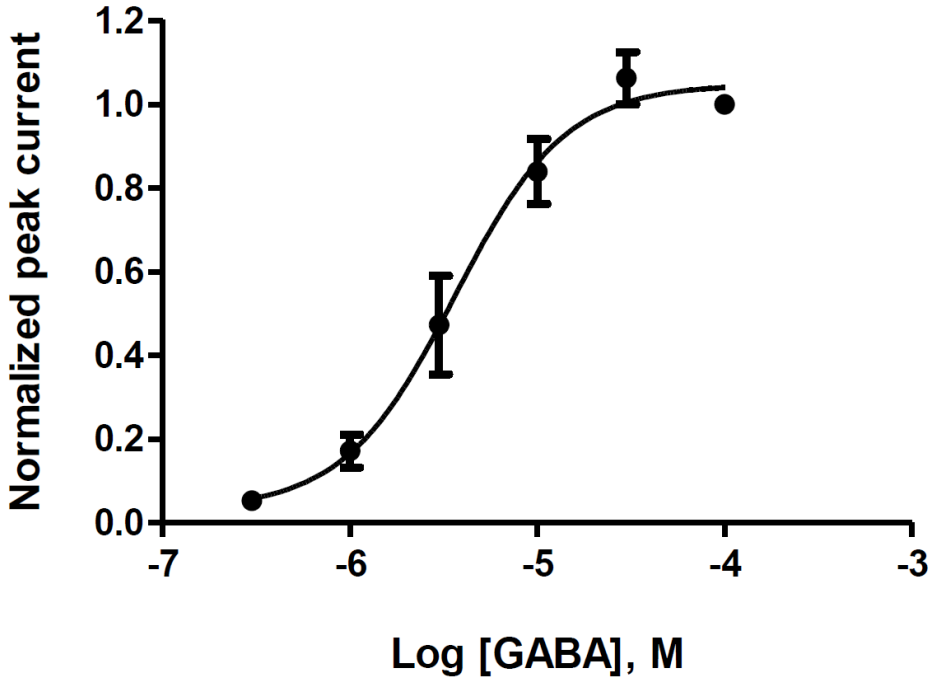
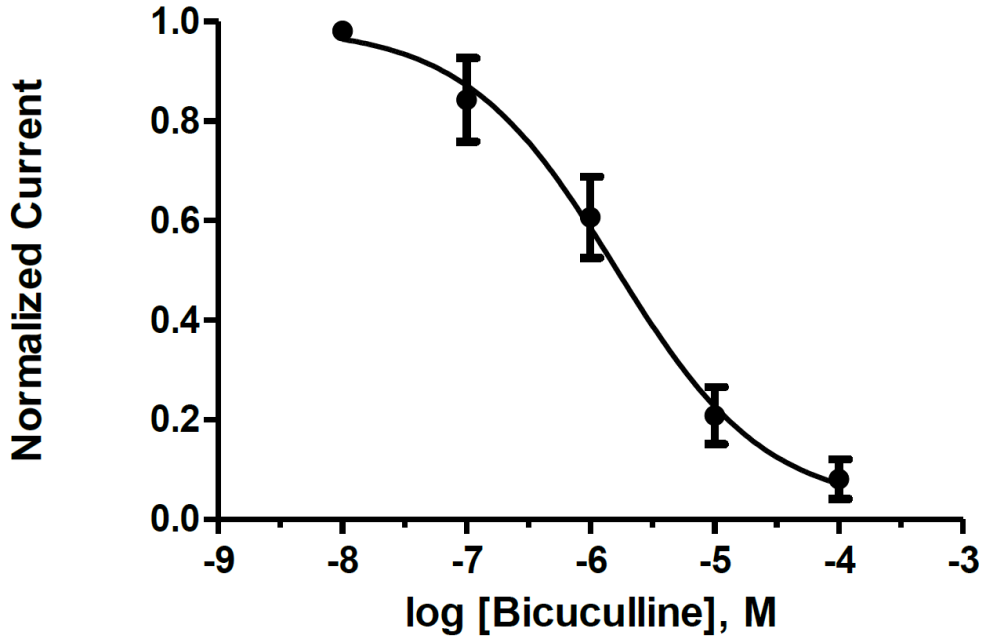
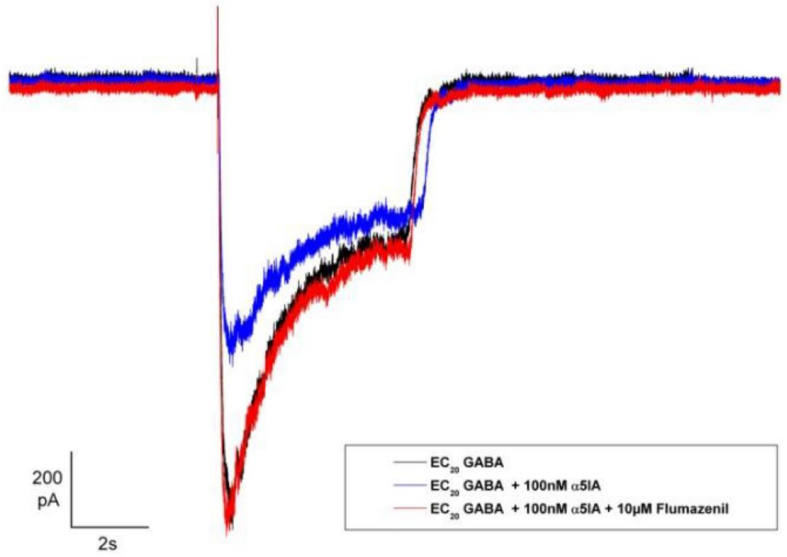


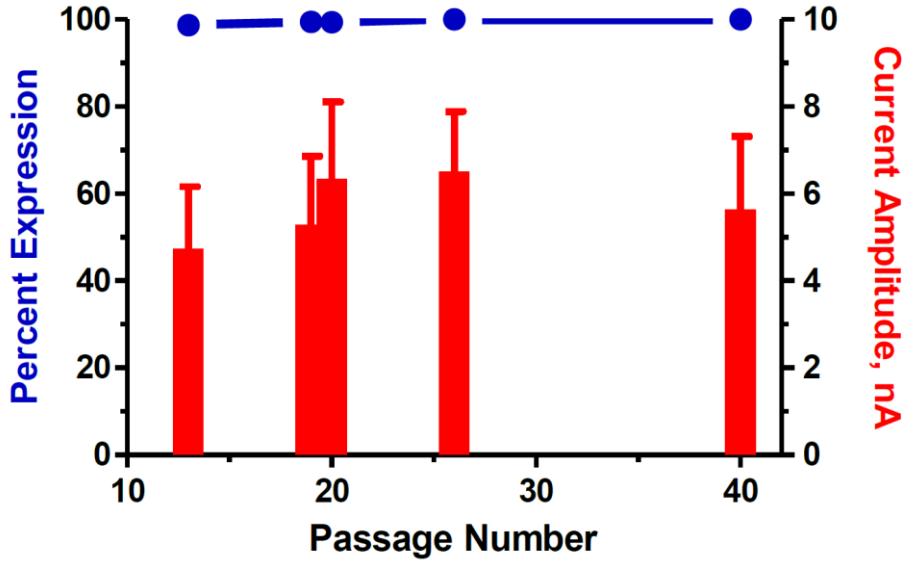


hGABAA α5/β3/γ2 Agonist and Antagonist Dose Response Curves: **Left:** GABA concentration-response curve. Each point indicates the mean (±SEM) response for 3 cells. We obtained an EC_{50} of 3.6 μM and a Hill slope of 1.0. **Right**: Bicuculline inhibition of response to addition of 100 μM GABA. Each point indicates the mean (±SEM) response of 3 cells. We obtained an IC50 of 1.6 μM and a Hill slope of -0.72 (PatchXpress Data).

hGABAA α5/β3/γ2

BACK





hGABAA α5/β3/γ2 Effect of α5IA, Stability of Expression over Passage: **Left:** The black trace shows the response to addition of an EC_{20} of GABA. After washout, 100 nM α5IA was added alone and had no effect (not shown). After a 2 min incubation with 100 nM α5IA, the response to addition of an EC_{20} of GABA (blue trace) was now inhibited. In five cells, 100 nM α5IA reduced the EC_{20} GABA current to 60.9 ±8.5% of control. Following washout, the response to the EC_{20} of GABA completely recovered (red trace). The effect of 100 nM α5IA was completely blocked by 10 μM flumazenil, indicating the effect is mediated through the benzodiazepine binding site on the GABA A α5/β3/γ2 receptor (PatchXpress Data). **Right**: The blue line shows the percentage of cells expressing a mean peak inward current >0.5 nA at –40 mV at cell passages 13, 19, 20, 26 and 40. The red bars show the mean current amplitude (mean ± SD) for 73-351 cells per experiment (IonWorks HT Data).

hGABAA α6/β3/γ2 (CYL3086)

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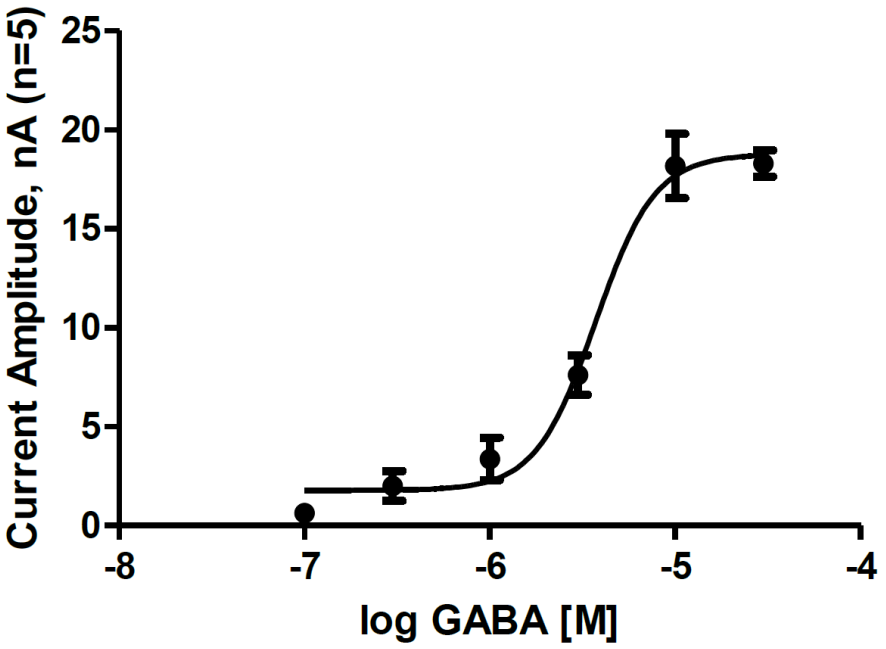

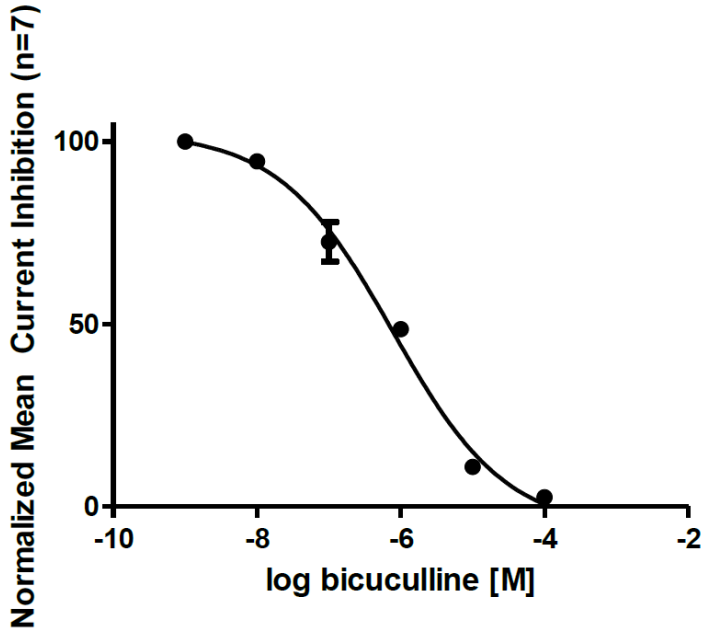

hGABAA α6/β3/γ2 Agonist and Antagonist Dose Response Curves: **Left:** hGABAA α6/β3/γ2-HEK cells at passage 15 were treated with increasing concentrations of GABA and the response measured. Each point indicates the mean (±SD) response for 5 cells. We obtained an EC_{50} of 3.72 µM and a Hill slope of.2.71 **Right**: The block by bicuculline of the EC_{80} GABA induced current was measured. Each point indicates the response of 7 cells. We obtained an IC_{50} of 0.823 µM and a Hill slope of -0.55. (PatchXpress Data).

hGABAA α6/β3/γ2

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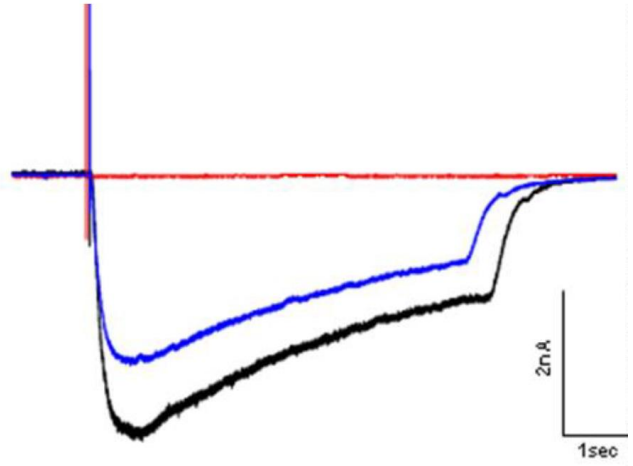

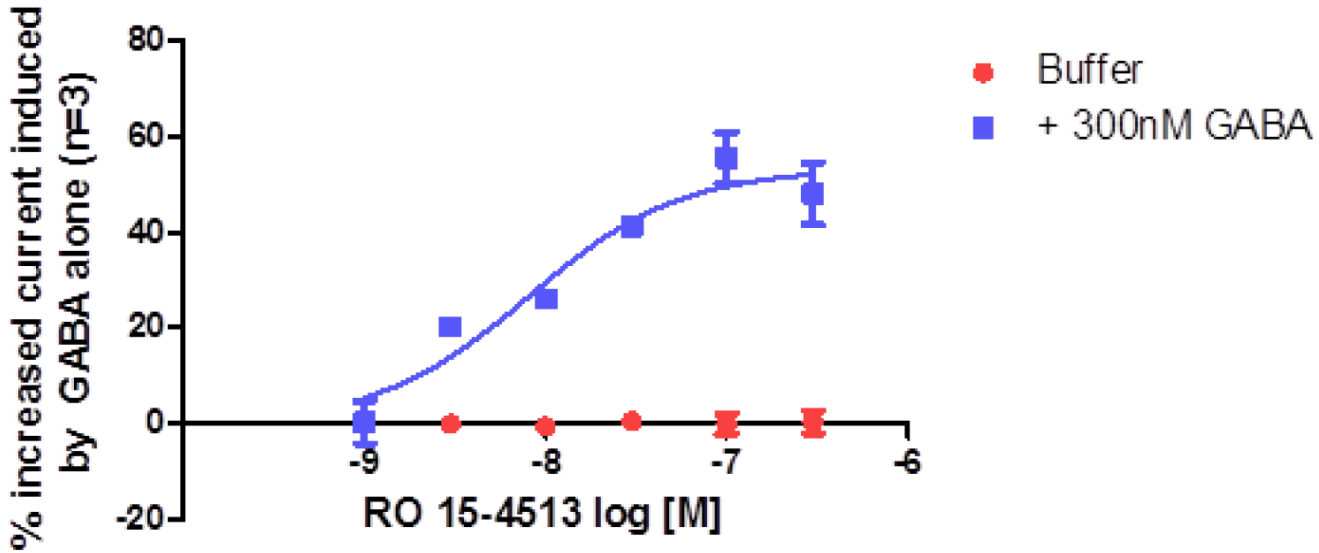

hGABAA α6/β3/γ2 Effect of the Benzodiazapine Site Allosteric Modulators on Currents: **Left:** The positive modifier effect of RO 15-4513 on currents. Cells treated with the EC_{20} concentration of GABA (blue trace). After washout, 0.3 μM RO 15-4513 was added alone and had no effect (red trace). After a 2 min incubation with 0.3 μM RO 15-4513, the response to the addition of an EC_{20} concentration of GABA+RO 15-4513 (black trace) was enhanced. **Right**: Concentration-dependent enhancement in the GABA (EC_{20}) induced current by RO 15-4513. The normalized GABA-induced current was increased approximately 60% by the highest concentrations of RO 15-4513. The EC_{50} for RO 15-4513 was 8.12 nM and the Hill slope of the curve was 1.04. A second partial agonist modifier, bretanzenil, also increased the GABA-induced current at a concentration of 100 μM (data not shown). (PatchXpress Data). **References:** Knoflach F et al. (1996). Pharmacological modulation of the diazepam-insensitive recombinant γ-aminobutyric acidA receptors α4β2γ2 and α6β2γ2. Molecular Pharmacology, 50:1253-1261. Hadingham KL et. al.(1996) Cloning of cDNAs encoding the human γ-aminobutyric acid Type A receptor α6 subunit and characterization of the pharmacology of α6 containing receptors. Molecular Pharmacology 49: 253-259.

hGABAA α6/β3/γ2

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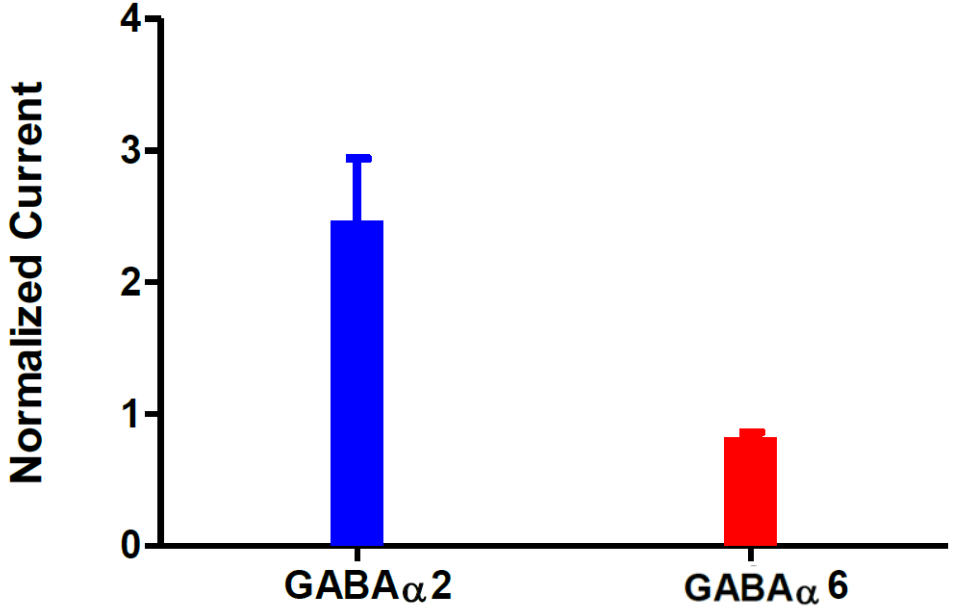

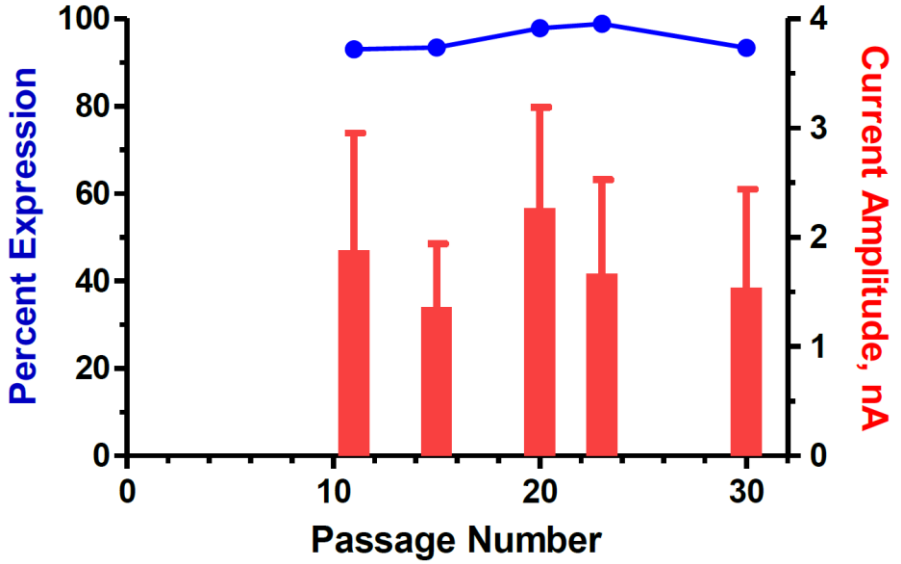

The benzodiazepine site allosteric modulator diazepam does not modulate the hGABAA α6/β3/γ2 current, Stability of Expression over Passage: **Left:** we compared the effect of 10 μM diazepam on the GABA (EC20) induced current in HEK cells expressing either the hGABAA α2/β3/γ2 current (n=7 cells) or on the hGABAA α6/β3/γ2 current (n=5 cells). Diazepam increased the EC20 GABA-induced current in the hGABAA α2/β3/γ2 cells (blue bar) approximately 2.5 fold over the normalized current, but did not show a positive modifier effect on the hGABAA α6/β3/γ2 current (red bar). (PatchXpress Data) **Right**: The blue line shows the percentage of cells expressing a mean peak inward current >0.25 nA at –40 mV at cell passages 11, 15, 20, 23 and 30. The red bars show the mean current amplitude (mean ± SD) for 67-169 cells per experiment. (IonWorks Quattro Data). **References:** Knoflach F et al. (1996). Pharmacological modulation of the diazepam-insensitive recombinant γ-aminobutyric acidA receptors α4β2γ2 and α6β2γ2. Molecular Pharmacology, 50:1253-1261. Hadingham KL et. al.(1996) Cloning of cDNAs encoding the human γ-aminobutyric acid Type A receptor α6 subunit and characterization of the pharmacology of α6 containing receptors. Molecular Pharmacology 49: 253-259.

nAChR α1/β1/δ/ε (CYL3052)

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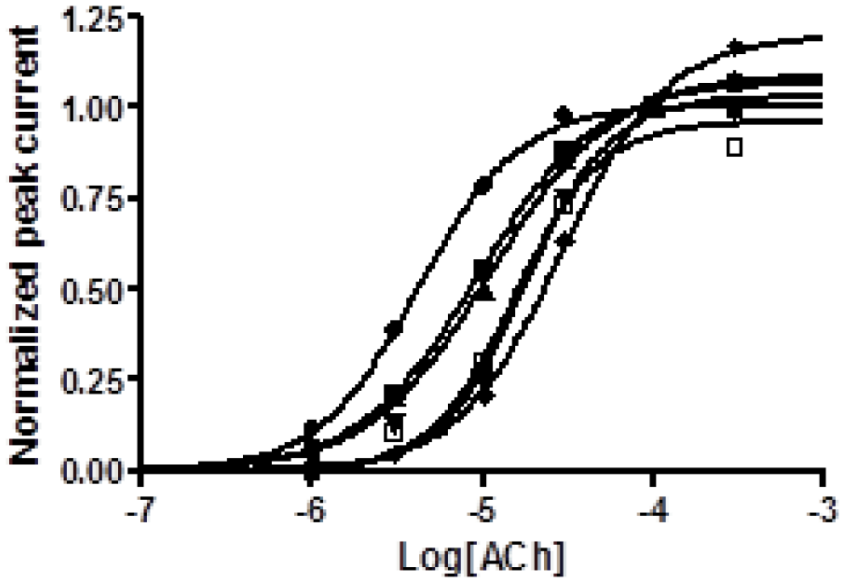

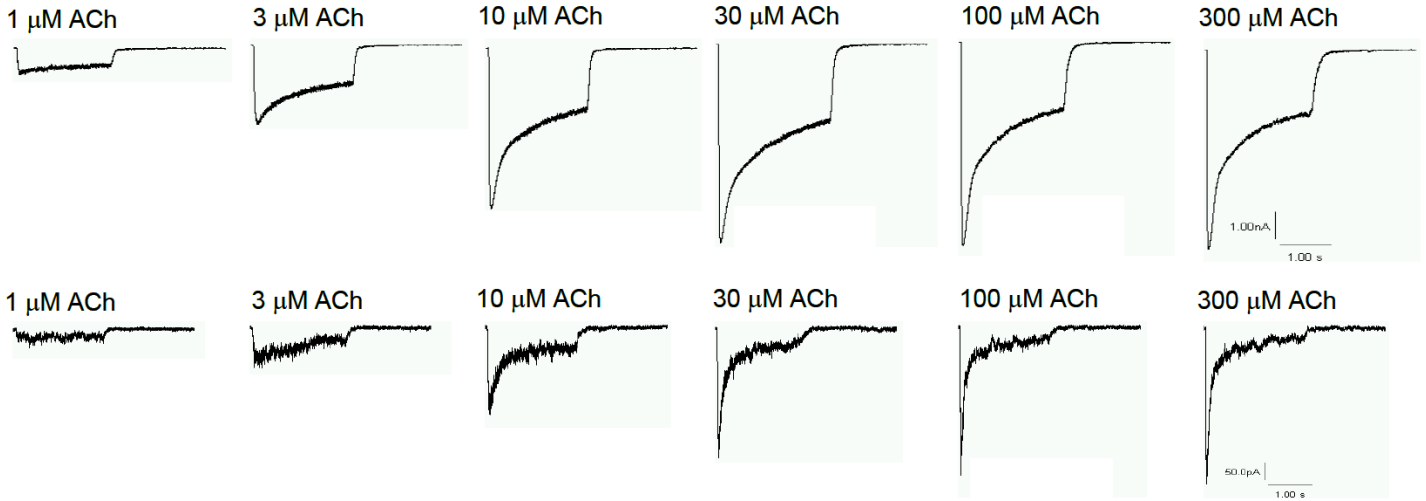

Concentration-Response Curves and Representative Current Records for Activation of hnAChR α1/β1/δ/ε currents: **Left:** Concentration-response curves for ACh-evoked currents. The abscissa shows the log concentration of ACh (M) and the ordinate the peak current amplitude normalized to the 100 µM evoked current amplitude value. Each point represents the single value from each tested cells (6 cells tested). From the 3 parameter logistic equation the pEC_{50} values range from 5.4 to 4.5 (mean ± SEM = 4.92 ± 0.12).**Right**: ACh-induced currents recorded from two different cells, activation was by increasing concentrations of agonist applied for 2 s every 1 minute. The cells are representative of the different degrees of desensitization that was observed during the experiments. (Manual Patch Clamp Data).

nAChR α1/β1/δ/ε

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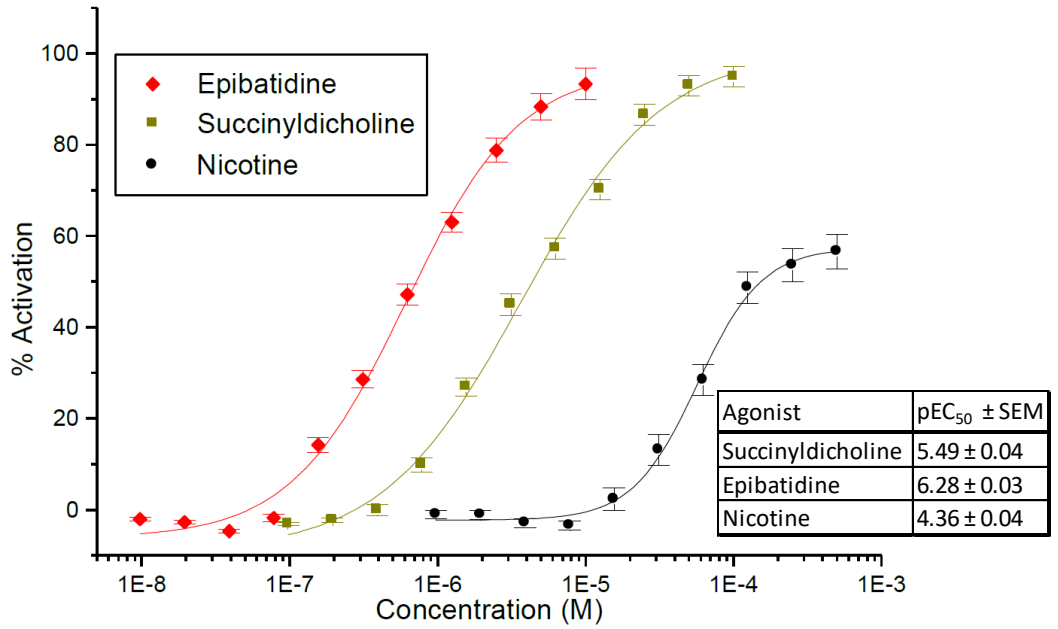
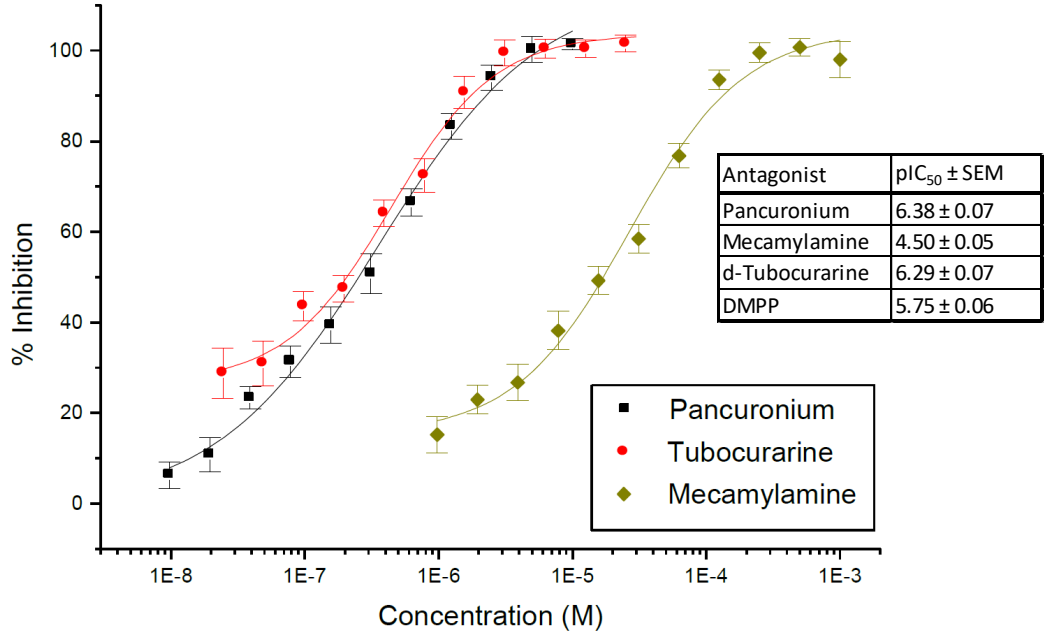


Agonist	pEC_{50} ± SEM
Succinyldicholine	5.49 ± 0.04
Epibatidine	6.28 ± 0.03
Nicotine	4.36 ± 0.04



Antagonist	pIC_{50} ± SEM
Pancuronium	6.38 ± 0.07
Mecamylamine	4.50 ± 0.05
d-Tubocurarine	6.29 ± 0.07
DMPP	5.75 ± 0.06

hnAChR α1/β1/δ/ε Agonist and Antagonist Dose Response Curves: **Left:** To assess agonist pharmacology, three agonists were tested. pEC_{50} values are shown in the inset Table **Right**: Representative antagonist dose response curves (median ± SEM) (FLIPR Calcium Assay Data).

nAChR α1/β1/δ/ε

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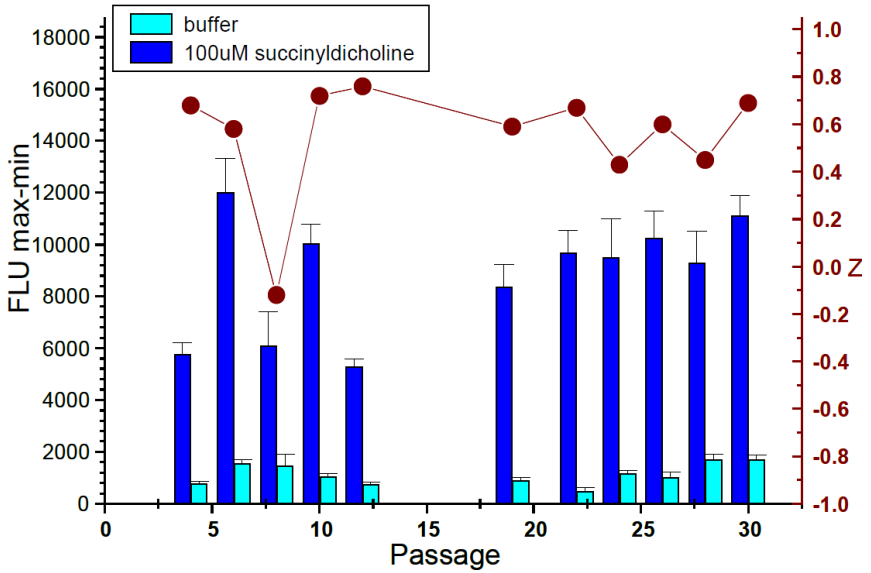

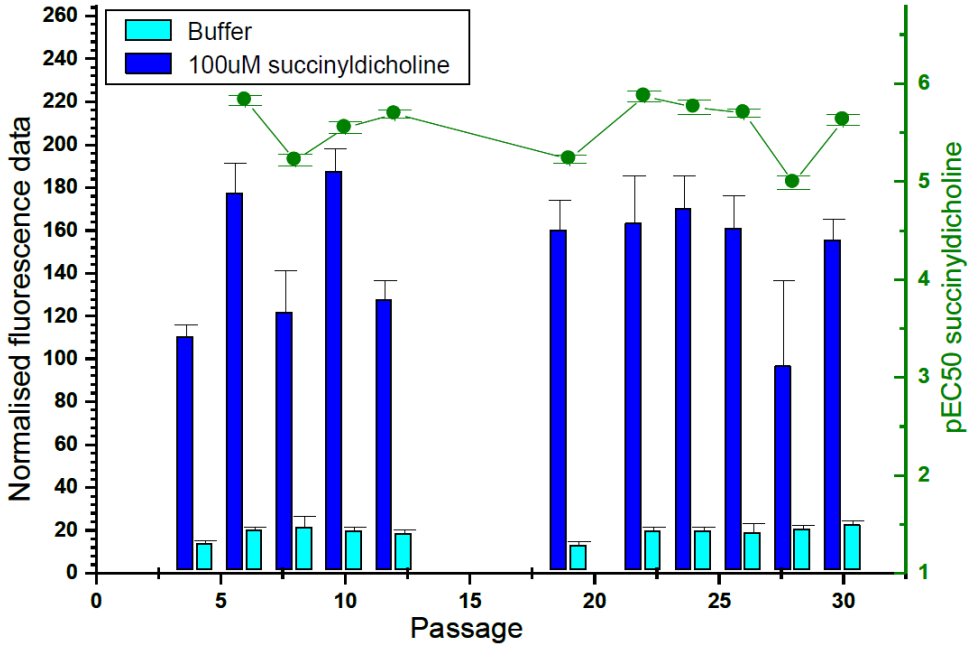

<u>hnAChR α1/β1/δ/ε Stability of the FLIPR fluorescence Assay Over Cell Passage:</u> **Left:** The FLIPR fluorescence signal and Z-prime over cell passage. The fluorescence signal window calculated for each well was the post-compound response minus the basal fluorescence for that well (n=16). **Right**: Stability of the FLIPR normalized fluorescence signal and succinyldicholine pEC50 over cell passage (FLIPR Calcium Assay Data).

nAChR α3/β4 (CYL3057)

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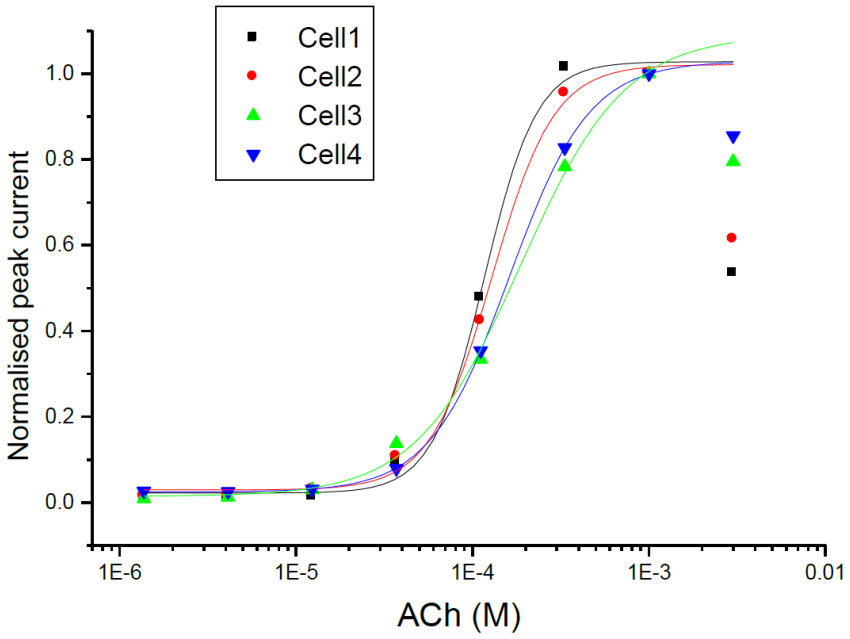

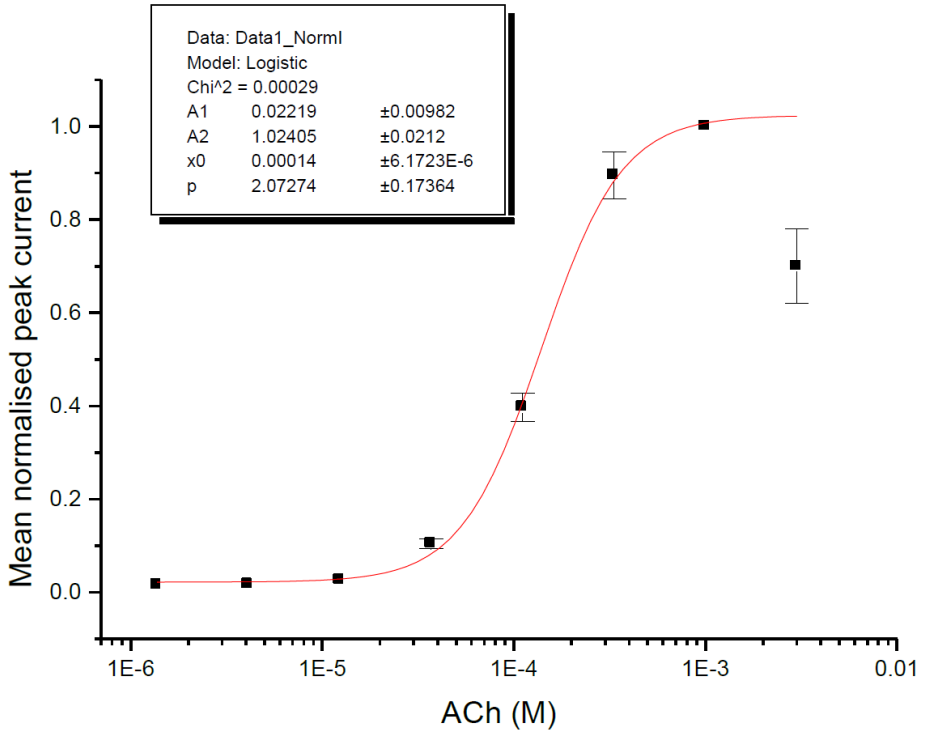

hnAChR α3/β4 Agonist Activation: **Left:** Concentration-response curves for ACh-evoked currents. The x-axis shows the log concentration of ACh (M) and the y-axis the peak current amplitude normalized to the 1 mM evoked current amplitude value. Each point represents the single value from each tested cell (4 cells tested). **Right**: Mean data. EC_{50} value = 140 ± 6.2 µM (mean ± SEM, n=4) (Manual Patch Clamp Data).

nAChR α3/β4

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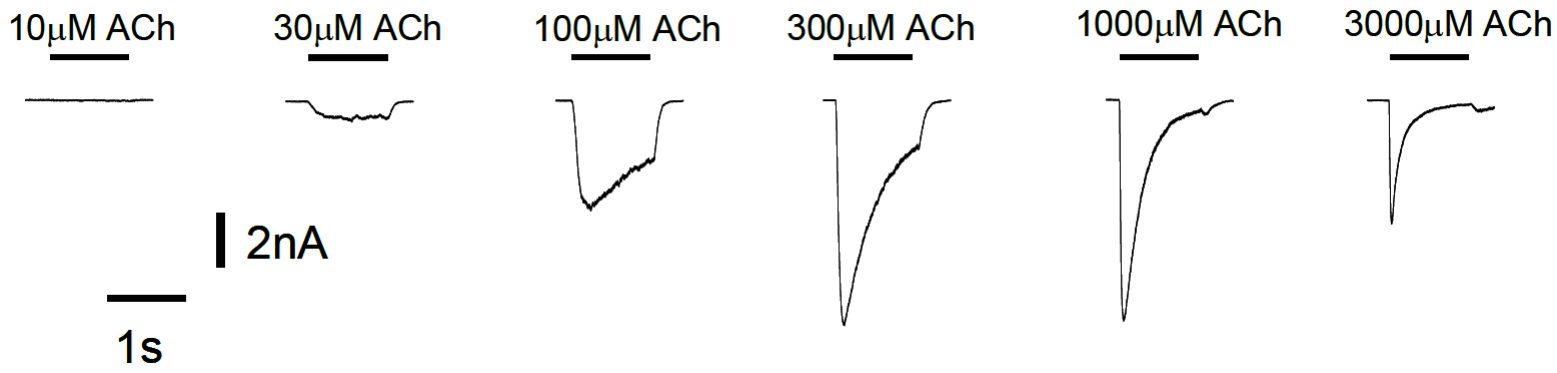

hnAChR α3/β4 Raw Data Currents: ACh-induced currents recorded from a typical cell. Activation was by increasing concentrations of agonist (10-3000 μM) applied for 1 s. This cell was representative of the degree of desensitization that was observed at concentrations of ACh >1 mM during experiments. (Manual Patch Clamp Data).

nAChR α3/β4

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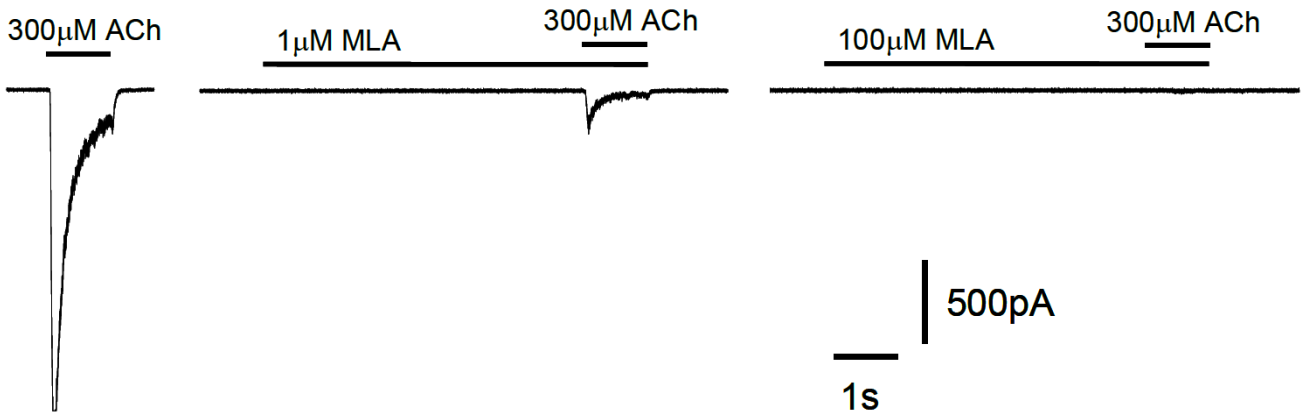
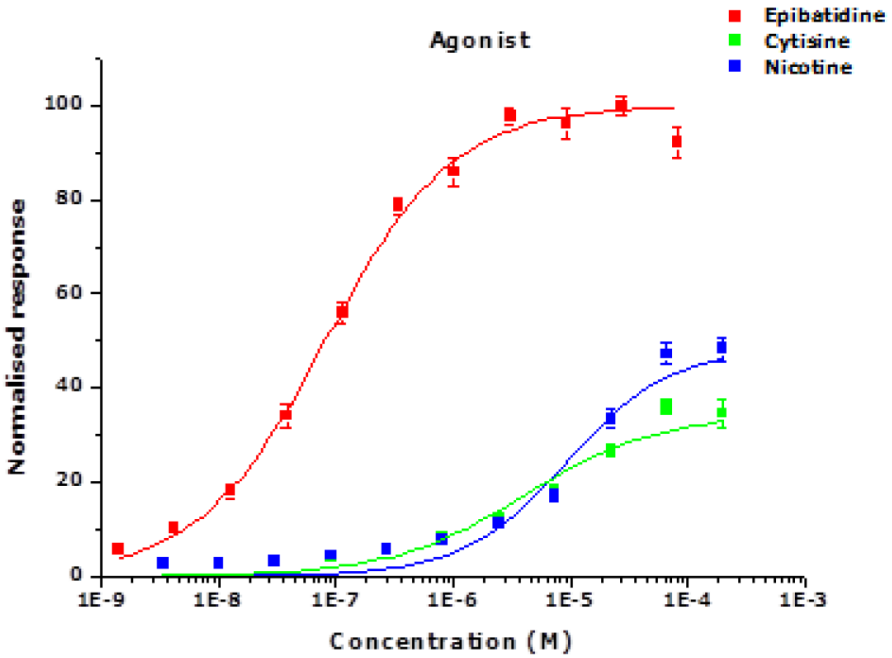
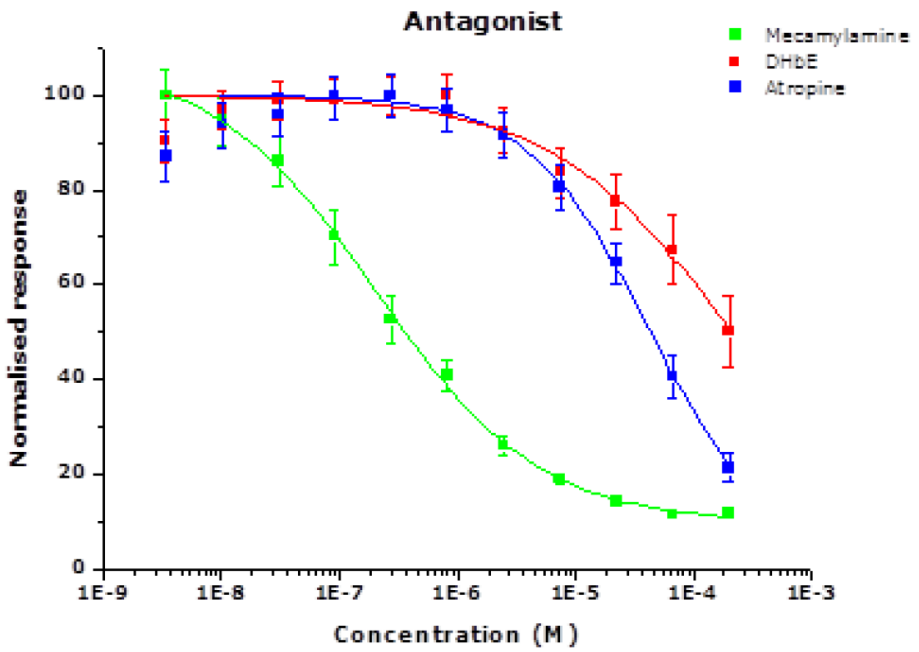


hnAChR α3/β4 Methyllycaconitine (MLA) Pharmacology: The nAChR α3/β4 current elicited by 300 μM ACh was completely blocked by 100 μM MLA and to a lesser degree by 1 μM MLA. Following a pre-application of 300 μM ACh, cells were perfused with MLA (1 μM or 100 μM) for 5 s followed by a 1 s co-application of 300 μM ACh. (Manual Patch Clamp Data).

nAChR α3/β4



Agonist	hnAChR α3/β4-HEK293 EC_{50} ± SEM
Epibatidine	81.4 ± 7.39 nM
Nicotine	9.16 ± 1.77 µM
Cytisine	4.16 ± 0.94 µM



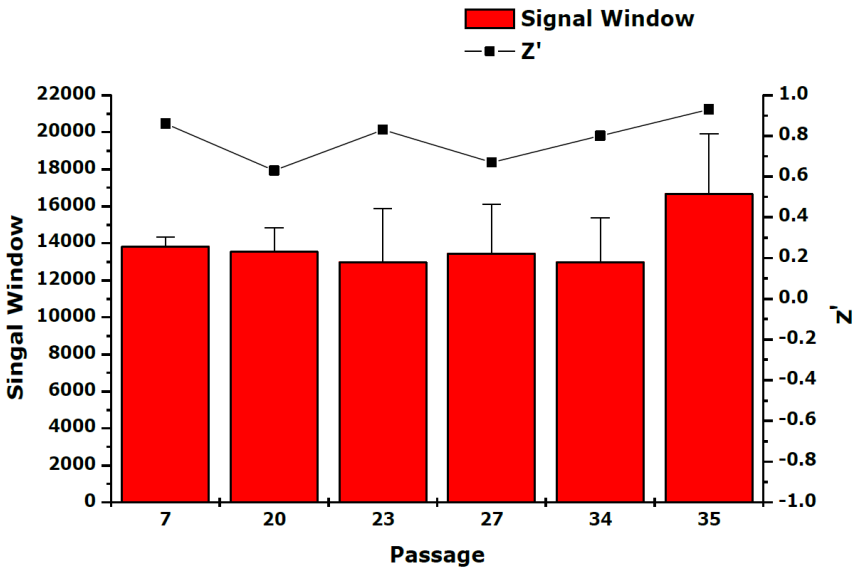
Antagonist	hnAChR α3/β4-HEK293 IC_{50} ± SEM
Mecamylamine	481 ± 83.6 nM
DHbE	210 ± 50.0 µM
Atropine	40.0 ± 6.22 µM

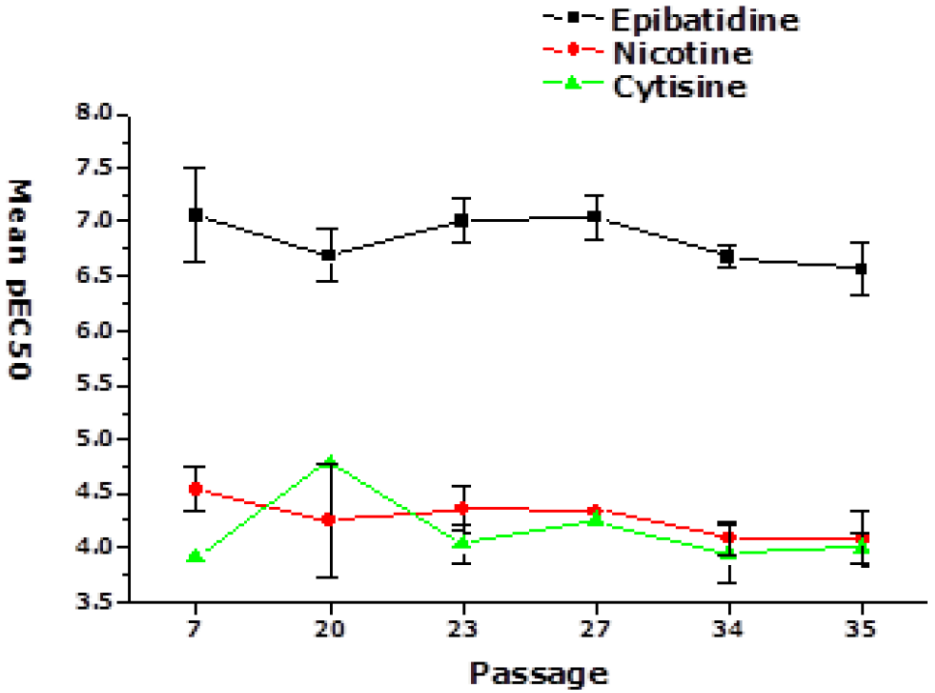
BACK

hnAChR α3/β4 Agonist and Antagonist Fluorescent Calcium Assay: **Left:** To assess agonist pharmacology, three compounds were tested (Figure 3). EC50 values are shown in the Table. The values obtained are in close agreement with published data for [Ca2+]i flux experiments (Stauderman et al., 1998). **Right**: To assess antagonist pharmacology, the ability of three compounds to block the epibatidine response at its EC80 concentration on the day of assay (6-10 µM) was tested (Figure 4). IC50 values for the block of the epibatidine response are shown in the Table. (FLIPR Calcium Assay Data).

nAChR α3/β4

BACK

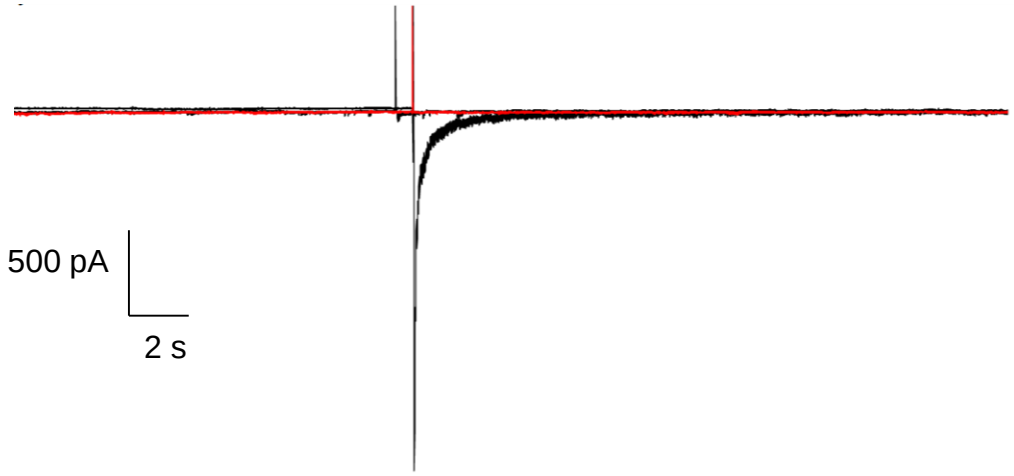


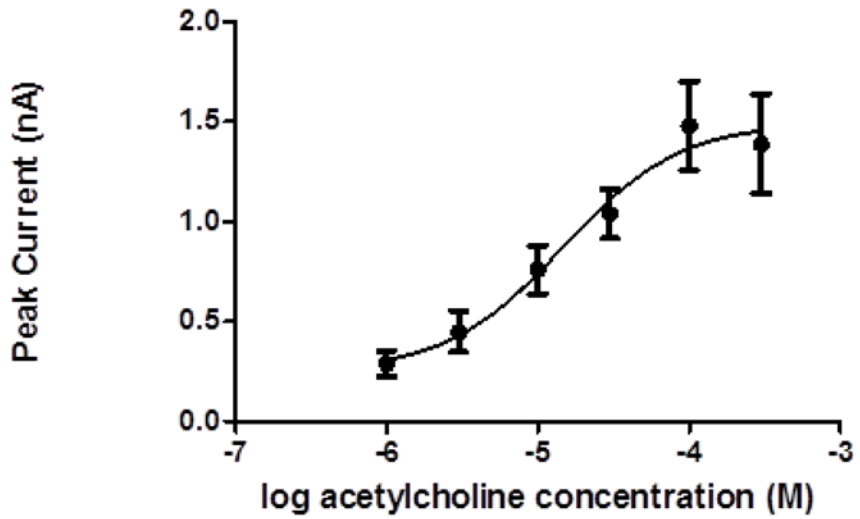


hnAChR α3/β4 Stability of Fluorescent Calcium Assay over Cell Passages: **Left:** Stability of the FLIPR fluorescence signal and Z-prime over passage. The fluorescence signal window calculated for each well was the post-compound response minus the basal fluorescence for that well. **Right:** Stability of agonist pEC_{50} values over passage. (FLIPR Calcium Assay Data).

nAChR α4/β2 (CYL3106)

BACK

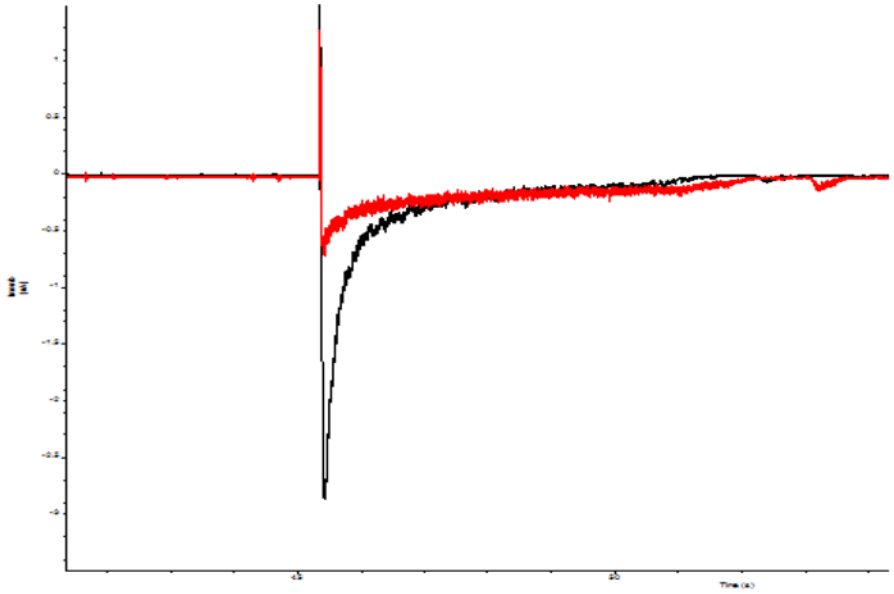


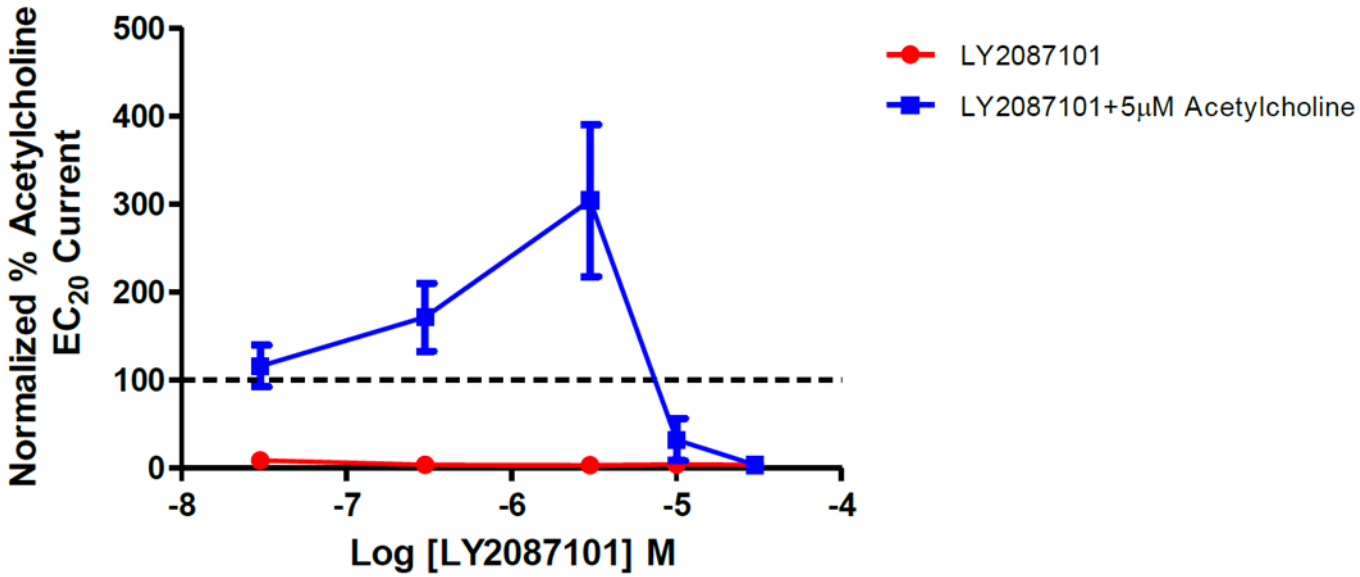


hnAChR α4/β2 Agonist Activation: **Left:** The black trace shows approximately 2 nA of current induced by the application of 100 µM acetylcholine. The red trace shows the same cell after washout of the acetylcholine followed by a 3 minute preincubation with 20 µM mecamylamine and then stimulation of the current by the co-application of 20 µM mecamylamine + 100 µM acetylcholine. Treatment of the cell with mecamylamine blocked the current elicted by the application of acetylcholine (PatchXpress Data).
Right: The addition of acetylcholine elicits an inward current in a concentration-dependent manner. Peak inward current was approximately 1.5 nA and we found an EC_{50} of 14.7 µM with a Hill slope coefficient of 1.12 (PatchXpress Data).

nAChR α4/β2

BACK





hnAChR α4/β2 Positive Allosteric Modulation of the Acetylcholine Elicited Current: **Left:** The red trace represents the current elicited by the addition of 5 µM acetylcholine. Following washout and a three minute incubation with 3 µM LY2087171, the black trace shows the current that results from the addition of 5 µM acetylcholine + 3 µM LY2087171. In this cell the addition of LY2087101 positively modulated the acetylcholine-induced current approximately three fold (PatchXpress Data). **Right:** Currents induced by the approximate EC20 concentration of acetylcholine (5 µM) are represented by the dotted line at 100% on the Y-axis. Pre-incubation of the cells with increasing amounts of LY2087101 (6) for three minutes (red line) followed by the addition of 5 µM acetylcholine + LY2087101 (blue line) resulted in the positive modulation of the current induced by acetylcholine in a concentration-dependent manner. At 10 and 30µM LY2087101 inhibited the current elicited by acetylcholine (PatchXpress Data).

nAChR α4/β2

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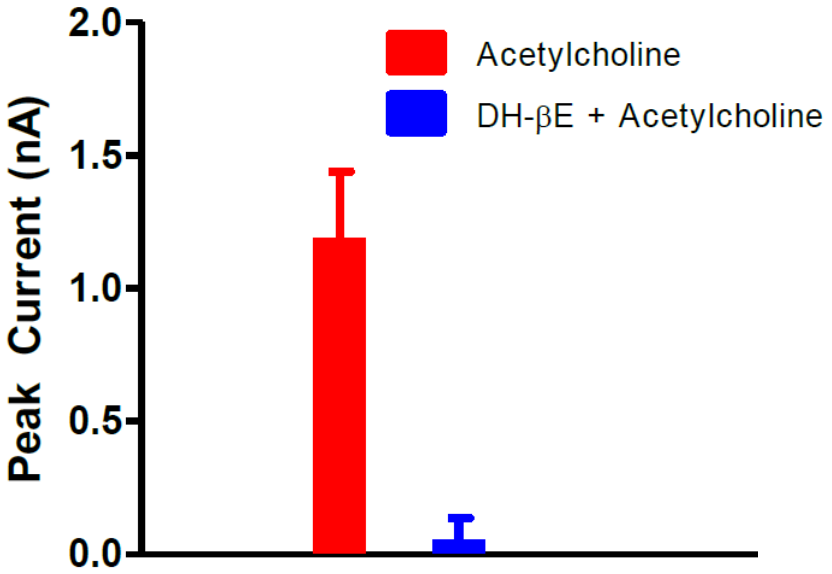
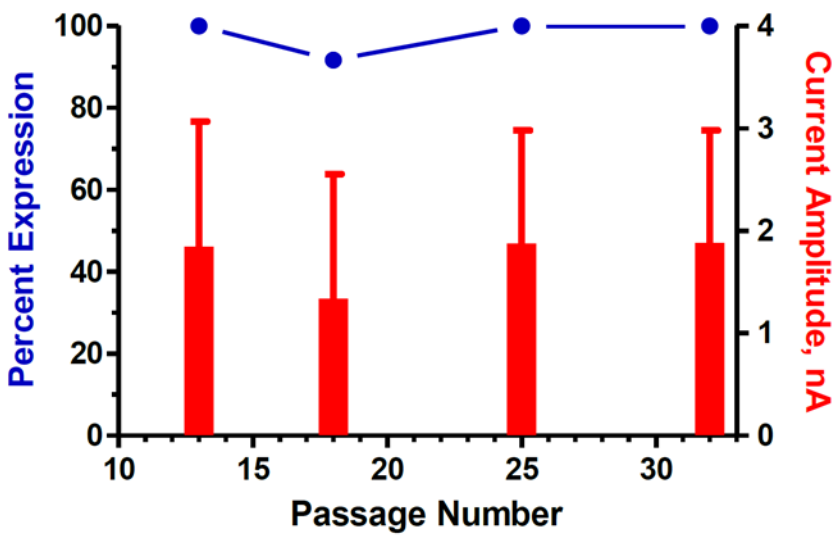
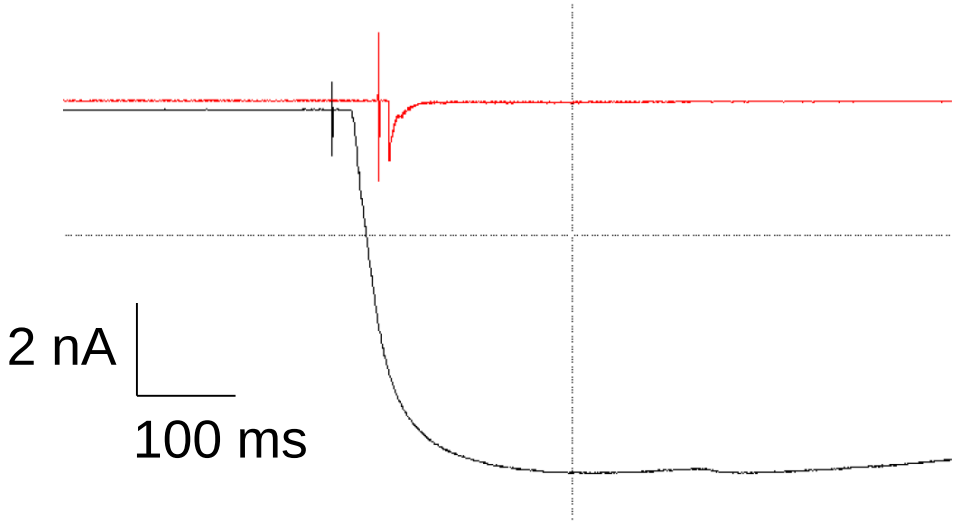


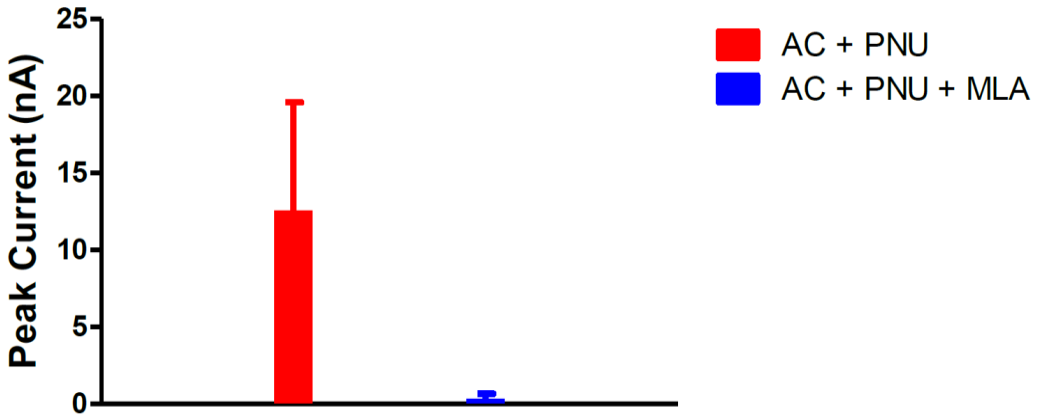


hnAChR α4/β2 Block of Acetylcholine Induced Current by Dihydro-β-erythroidine, Stability of Expression over Passage: **Left:** The red bar represents the current elicited by 100 µM acetylcholine. Following washout and a four minute preincubation with 10 µM dihydro-β-erythroidine (DH-βE), the inhibition of the current induced by the addition of 100 µM acetylcholine in the presence of 10 µM DH-βE is represented by the blue bar. These data are in agreement with previously published data (PatchXpress Data). **Right:** The stability of expression was determined on the PatchXpress® by the addition of 100µM acetylcholine at a holding potential of -60 mV. The blue line shows the percentage of cells expressing a mean peak inward current >500pA at cell passages 13, 18, 25 and 32. The red bars show the mean peak current amplitude (mean ± SD) for 7-14 cells per experiment. Functional expression of the hnAChR α4/β2 currents was > 91% through 32 passages and the mean current amplitude was > 1.33nA. (PatchXpress Data).

nAChR α7/ric3 (CYL3097)

BACK





hnAChR α7/ric3 Allosteric Modulation of Acetylcholine Induced Current, Blockade by Methyllycaconitine: **Left:** The red trace shows approximately 800 pA of current induced by the application of 300 µM acetylcholine alone. The black trace shows the same cell after washout of the acetylcholine followed by a 3 minute preincubation with 5 µM PNU-120596 and then stimulation of the current by the co-application of 5 µM PNU-120596 + 300 µM acetylcholine. There was approximately 8.5 nA of a persistent inward current induced by the co-application of 5 µM PNU-120596 + 300 µM acetylcholine. (PatchXpress Data). **Right:** Currents induced by 300 µM acetylcholine (AC) and 5 µM PNU-120596 (PNU) (red bars) were blocked by the selective nAChR α7 blocker 10 µM methyllycaconitine (MLA) (blue bars). (PatchXpress Data).

nAChR α7/ric3

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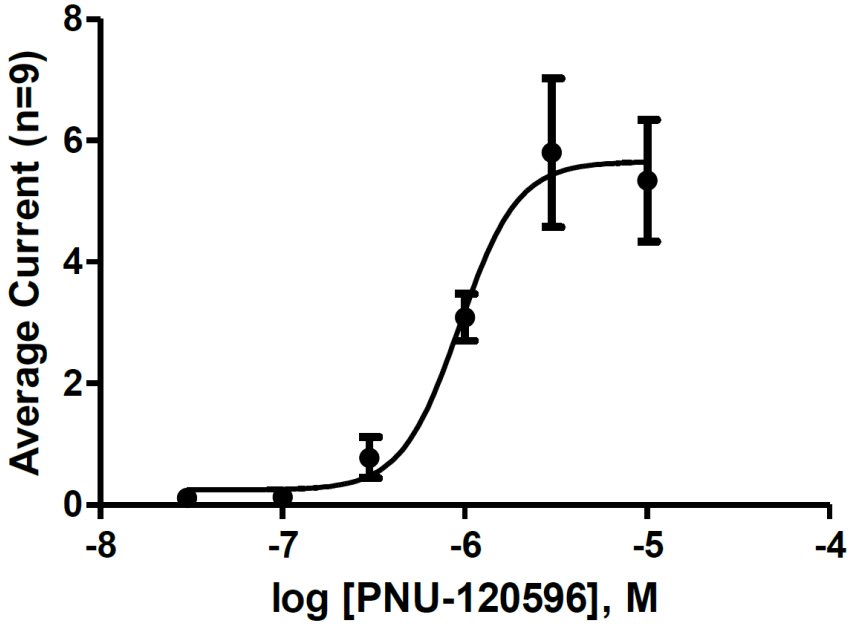

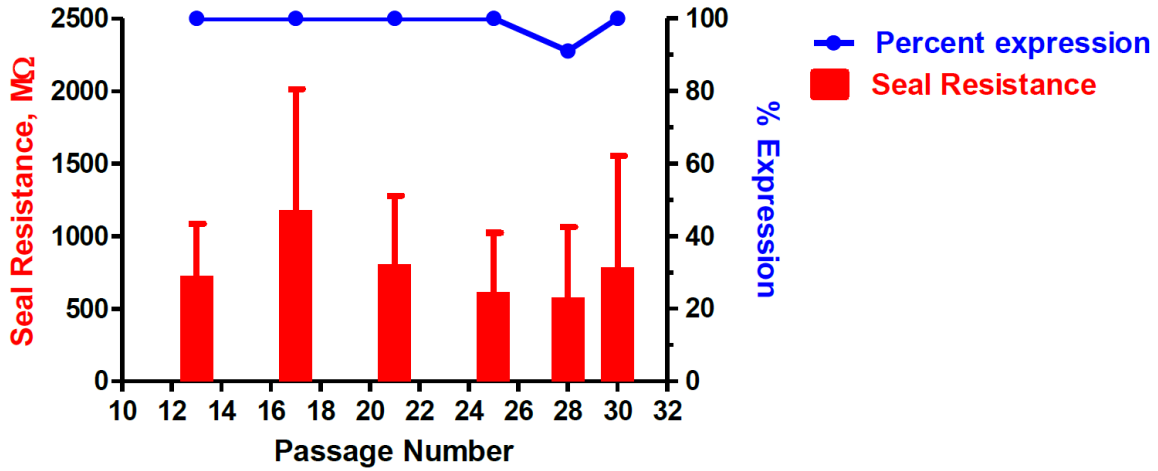

hnAChR α7/ric3 Allosteric Modulation of Acetylcholine Induced Current, Stability of Expression and Seal Resistance over Passage: **Left:** The partial allosteric modulator PNU-120595 increases the current activated by the addition of 300 µM acetylcholine in a concentration-dependent manner. The peak inward current was approximately 6 nA and we found an EC_{50} of 926 nM and a Hill slope of 2.73. **Right:** Stability studies were performed determined by preincubating the cells with 5 µM PNU-120596 for 3 minutes followed by the addition of 300 µM acetylcholine + 5 µM PNU-120596 at a holding potential of -60 mV. The blue line shows the percentage of cells expressing a mean peak inward current >4 nA at cell passages 13, 17, 21, 25, 28 and 30. The red bars show the mean seal resistance (mean ± SD) for 8-14 cells per experiment. Functional expression of the hnAChR α7//ric3 currents was > 91% through 30 passages and the mean seal resistance was > 575 MΩ. (PatchXpress Data).

nAChR α7/ric3

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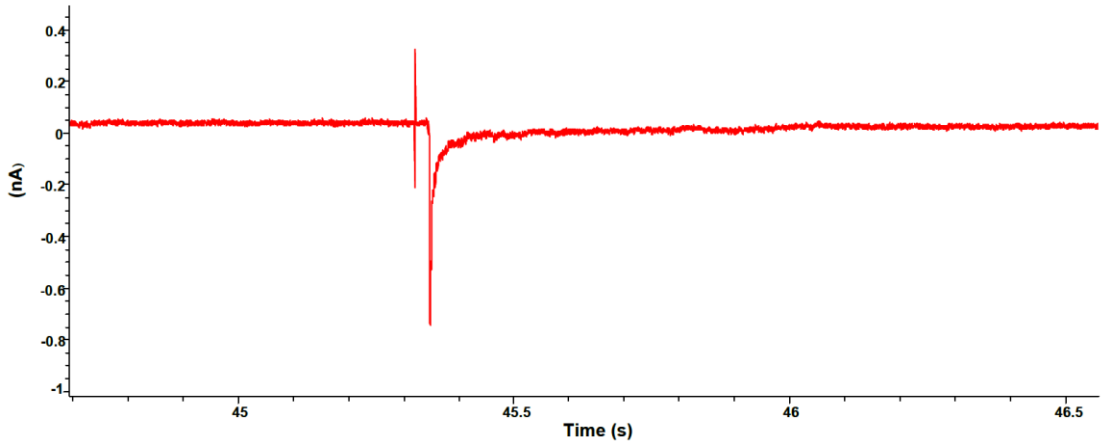

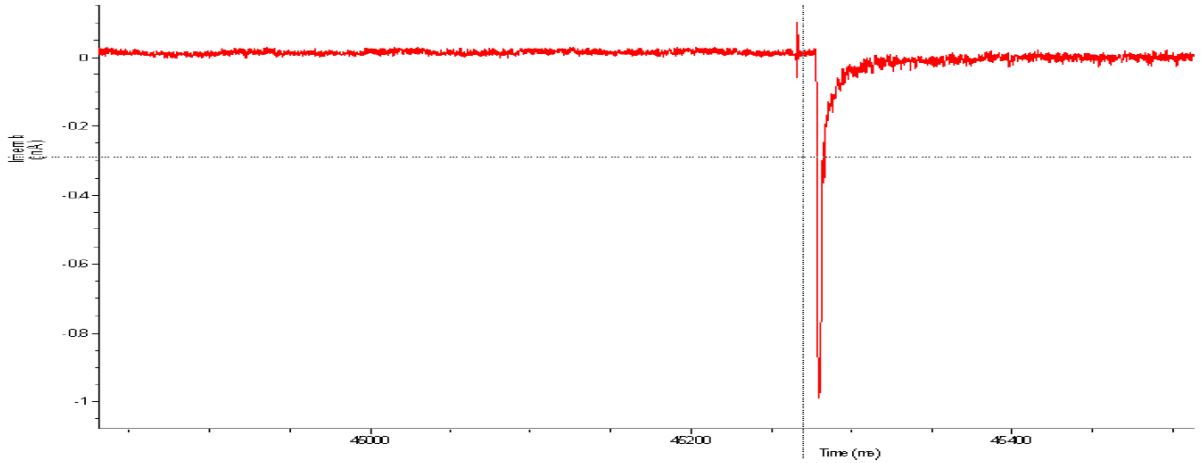

hnAChR α7/ric3 Other Agonist Activation: **Left:** The response to epibatidine. The cells were treated with 100 µM epibatidine, the cell responded with an inward current of approximately 700 pA. **Right:** The second figure shows the response to AR-R 17779. Here this cell responded to a challenge with 5 µM AR-R 17779 with an inward current of approximately 950pA (PatchXpress Data).

nAChR α7/ric3

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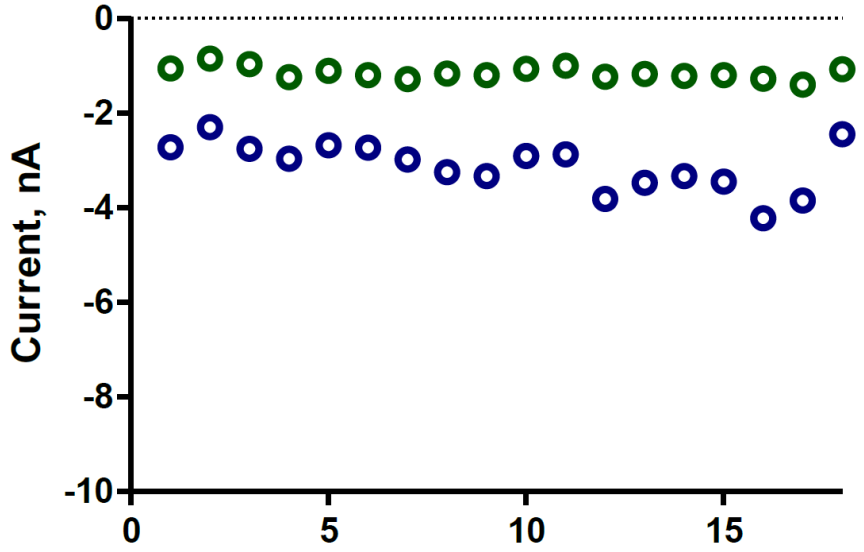
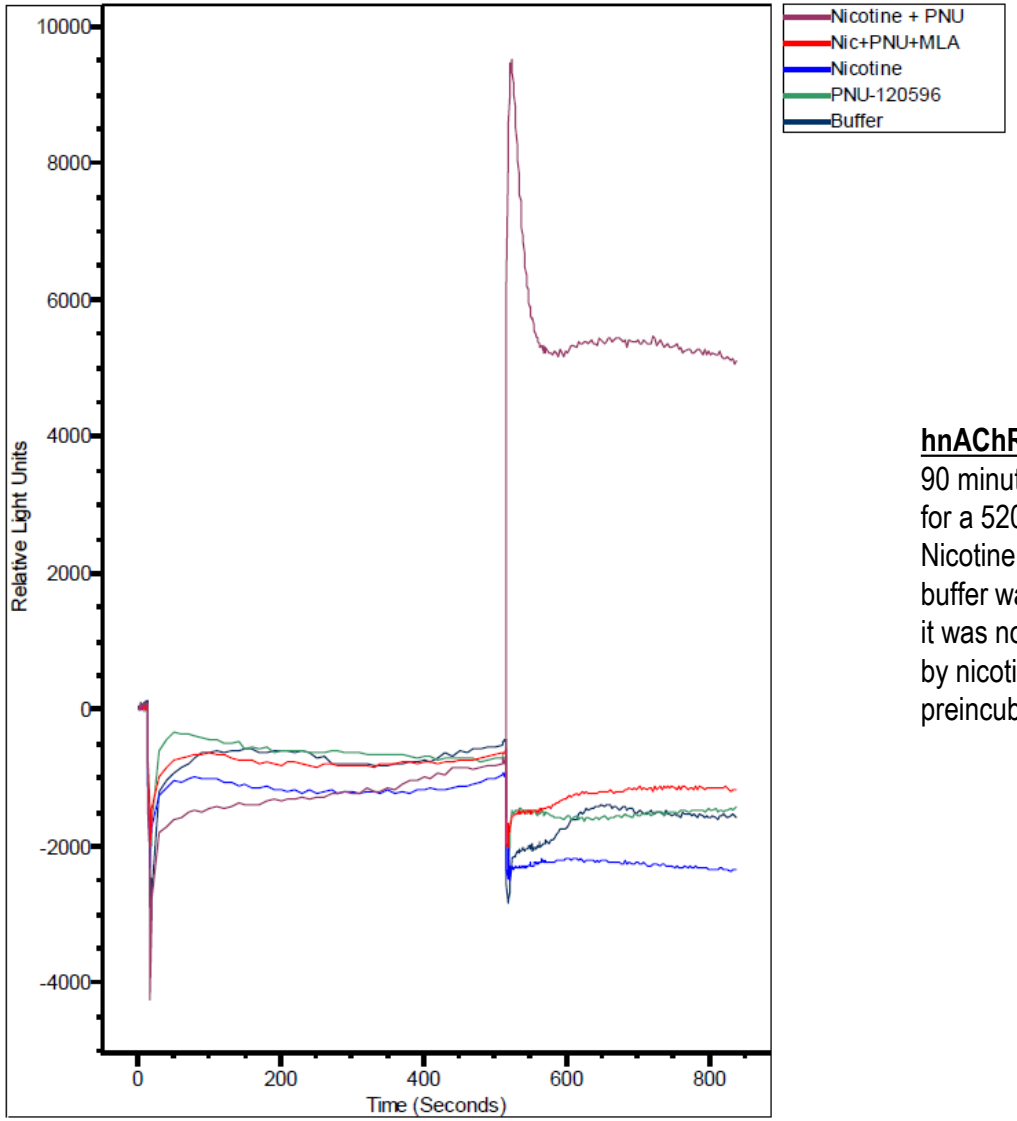


hnAChR α7/ric3 Currents Measured on Port-A-Patch: **Left:** To examine the reproducibility of currents elicited by repeated application of agonists or modulators, cells were held at -80 mV, and currents elicited by application of 300 µM acetylcholine with 5 µM PNU-120596 were elicited every 2 minutes. Each current trace was elicited by adding 5 µM PNU-120596 for 500 ms, then adding 300 µM acetylcholine with 5 µM PNU-120596 for 100 ms, then washing with external solution for 6 seconds to allow the current to recover toward the baseline. The wash with external solution continued for an additional minute, then PNU-120596 was added for 30 seconds before eliciting the next current. Traces one and 18 are superimposed in the figure, and the red arrow points to trace 18. **Right:** The peak inward current amplitude, and the current integral, recorded for 38 minutes in the whole-cell configuration are shown in the green and blue symbols, respectively. (Port-a-Patch Data).

nAChR α7/ric3



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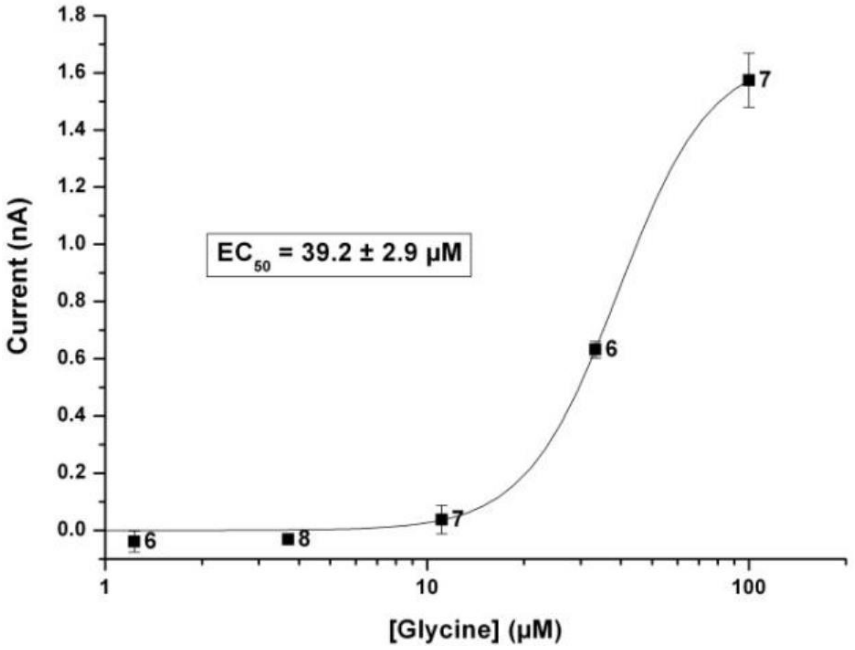
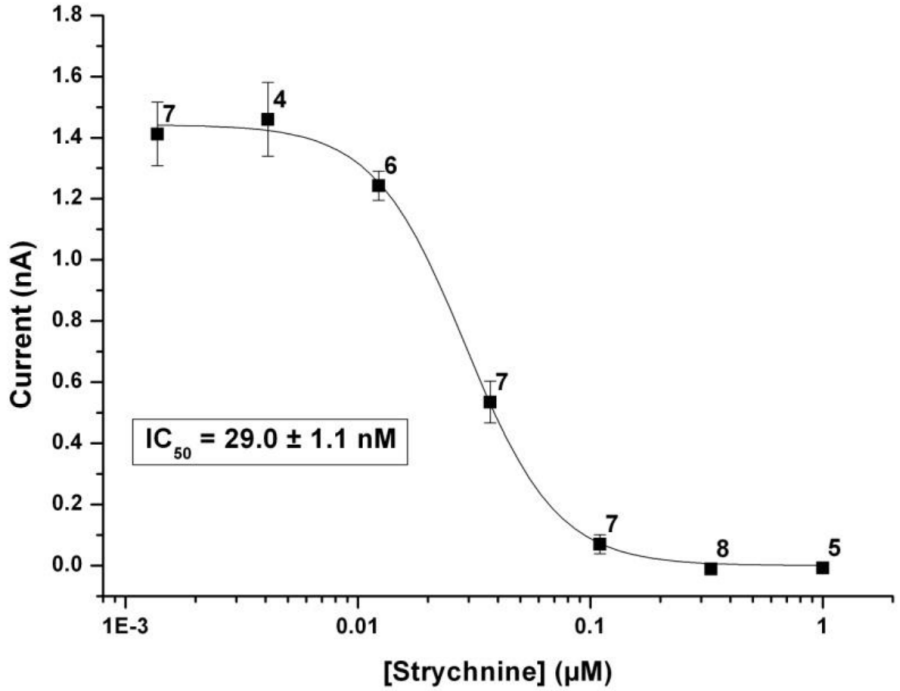
hnAChR α7/ric3 Calcium Flux Data: Cells were pre-loaded with the calcium indicator dye Fluo-8 (Alexa) at 30□C for 90 minutes. Following two wash cycles the cells were placed onto the instrument and the test compounds were added for a 520 seconds preincubation at the following final concentrations: methyllycaconitine = 10μM; PNU120-596 = 5μM. Nicotine was added at 520 seconds at final concentration of 100μM and data collected as graphed below. The external buffer was used as described in (7). The addition of nicotine alone (blue trace) either did not stimulate calcium influx or it was not detectable under these conditions. Preincubation with the positive allosteric modulator PNU-120596 followed by nicotine addition (maroon trace) did result in a large, sustained influx of calcium. This calcium flux was blocked by preincubation with the selective nAChR α7 blocker methyllcaconitine (red trace). (FLIPR^{TETRA} Data).

GlyRA1 (CYL3056)

BACK





hGlyRA1 Agonist and Antagonist Dose Response Curves: **Left:** Increasing concentrations of glycine were added to separate patch-plate wells and the evoked outward current at 0 mV was measured. Glycine dose-dependently increased the outward current with an EC_{50} of 39 µM. **Right:** Various concentrations of strychnine were incubated for 100 s and then in the presence of 100 µM glycine. Strychnine dose-dependently inhibited glycine evoked outward currents with an IC50 of 29 nM. (IonWorks Quattro Data).

GlyRA1

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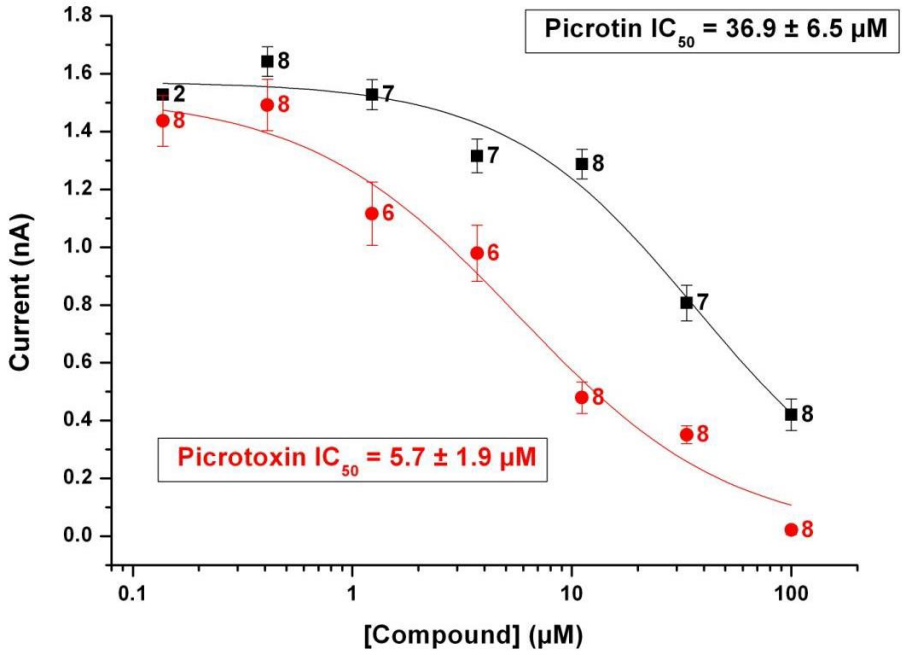
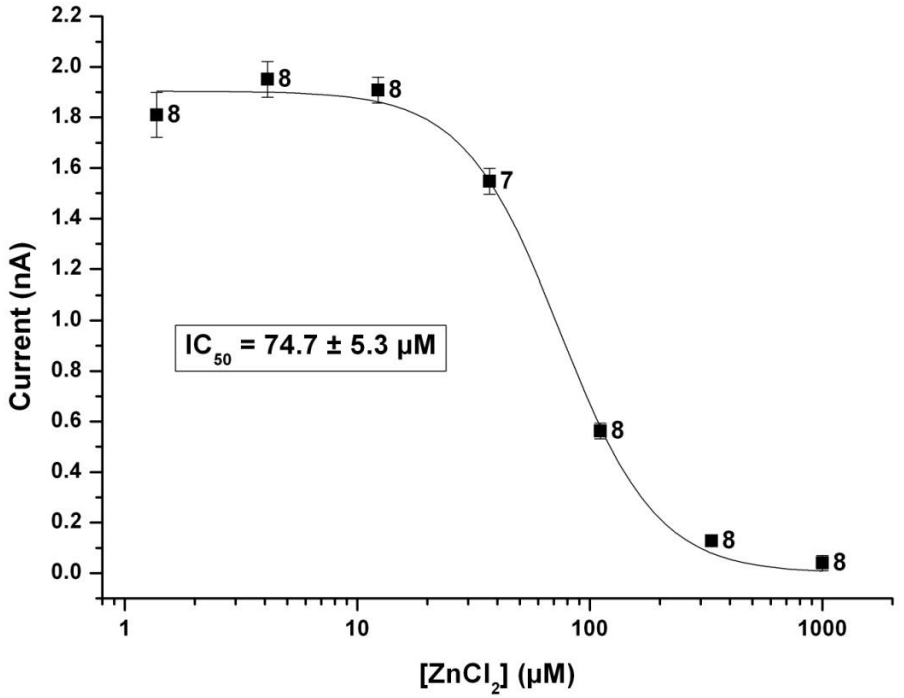
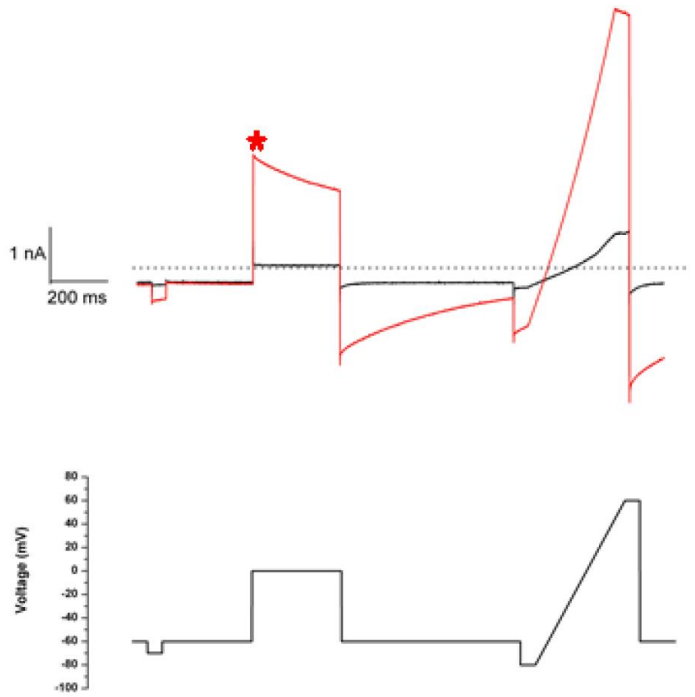


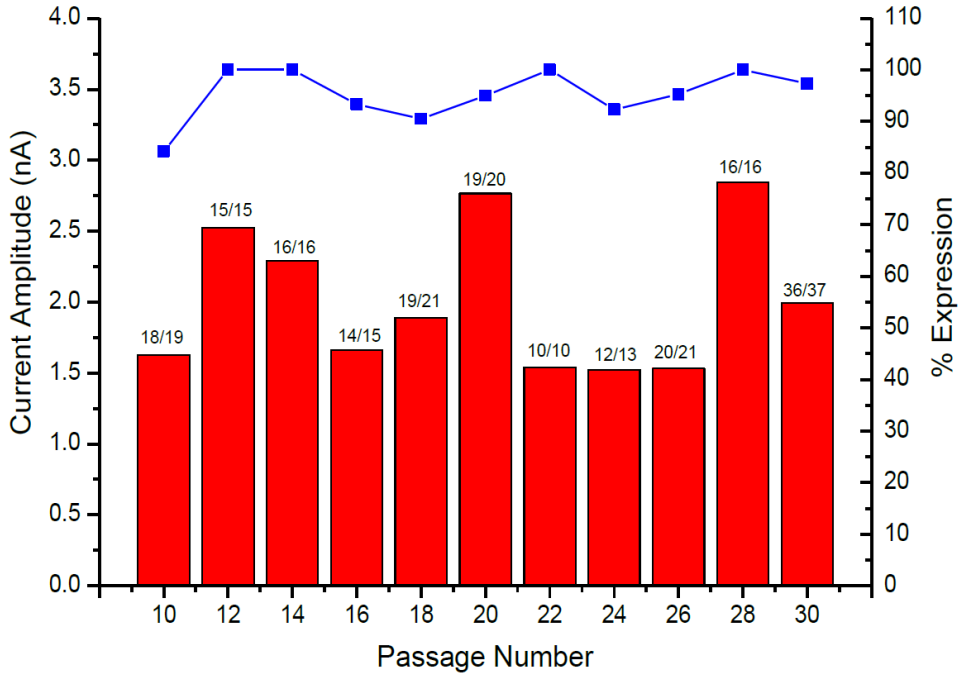


hGlyRA1 Effect of Picrotoxin and Picrotin on Glycine-evoked Currents, Effect of Zn^{2+} on Glycine-evoked Currents: **Left:** Various concentrations of compound were incubated for 100 s and then in the presence of 100 µM glycine. Picrotoxin and picrotin dose-dependently inhibited glycine evoked outward currents with IC_{50} values of 5.7 µM and 36.9 µM respectively. **Right:** Various concentrations of Zn^{2+} were incubated for 100 s and then in the presence of 100 µM glycine. Zn^{2+} dose-dependently inhibited glycine evoked outward currents with an estimated IC_{50} value of 74.7 µM. Between 1 and 10 µM Zn^{2+} the mean outward current evoked by 100 µM glycine was >1.8 nA whereas in the absence of Zn^{2+} the mean current was around 1.6 nA. (IonWorks Quattro Data).

GlyRA1

BACK

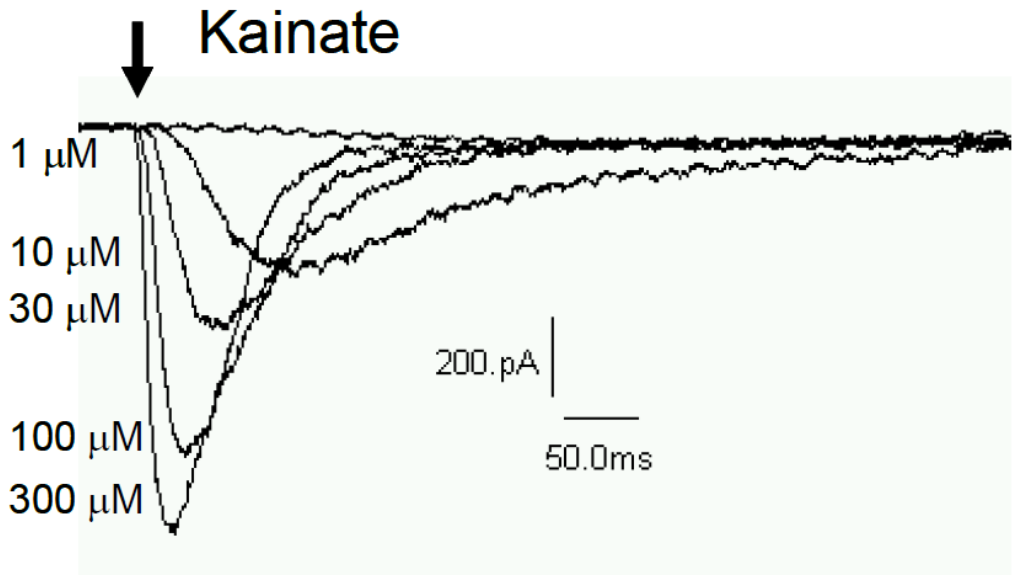


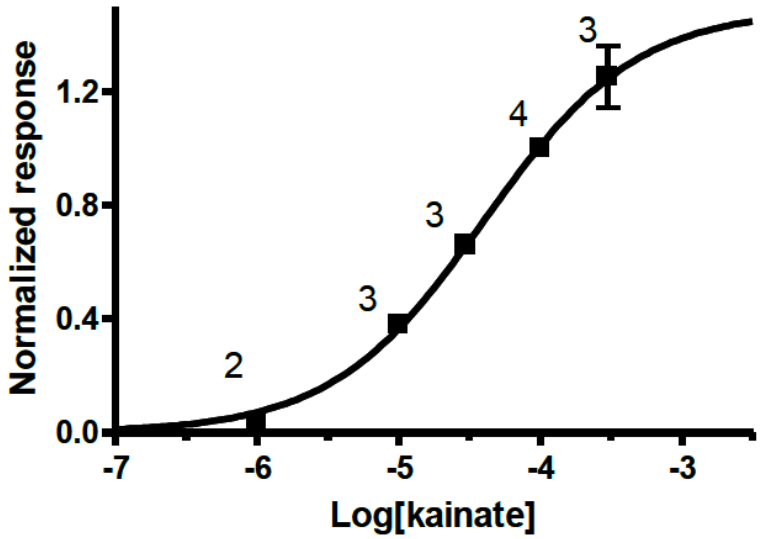


hGlyRA1 Glycine Evoked Currents on IonWorks HT, Stability of Expression over Passage: **Left:** Typical current traces evoked before (black trace) and after (red trace) the application of 300 µM Glycine. The zero current level is shown by the black dotted line. Voltage protocol shown below current trace. **Right:** The blue squares show the percentage of cells expressing mean current amplitude of ≥ 500 pA at 0 mV in response to 300 µM glycine at cell passage 10 through to 30. The lower panel shows the mean current amplitude (red bars) and adjacent to these the number of these cells (number of cells expressing >500 pA / number of cells sealing >50 MΩ). The maximum n-number was 24 cells for passages 10 through to 28 and 48 cells at passage 30 (IonWorks HT Data).

GluR6 (CYL3049)

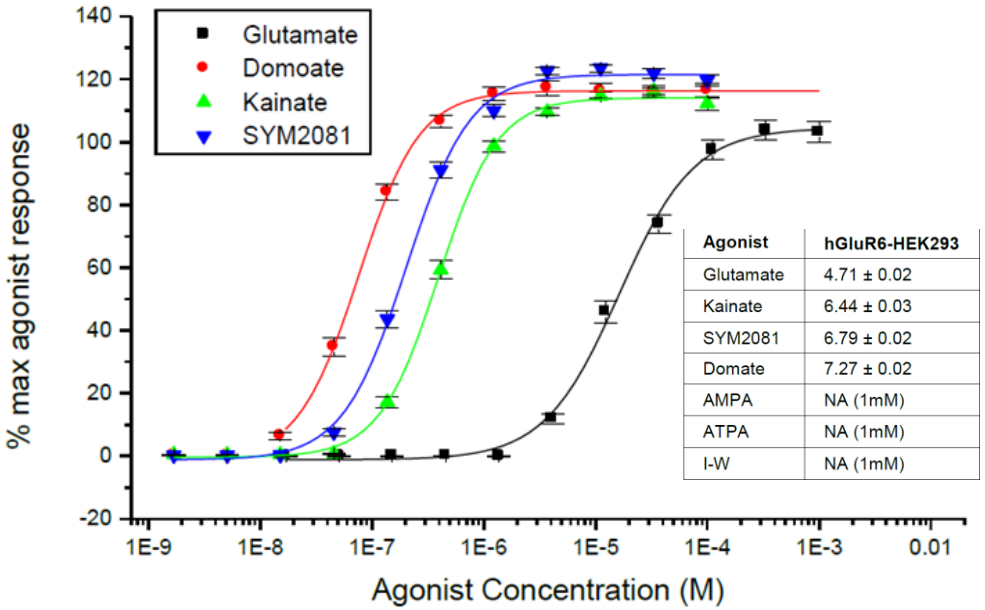
BACK



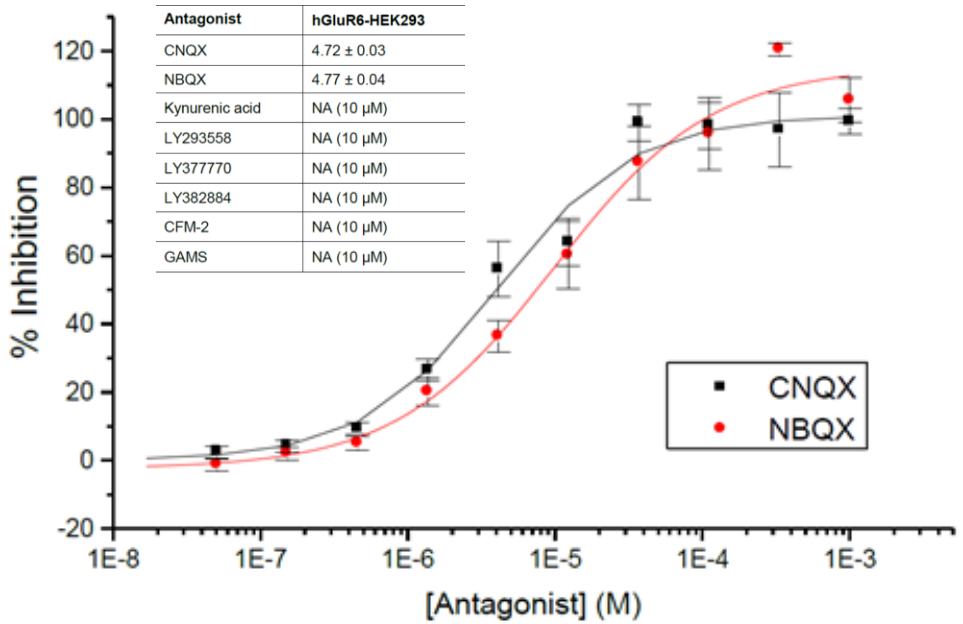


Raw Kainate Currents and Agonist Activation: **(Left)** Kainate-induced hGluR6 currents recorded from a single cell, activated by increasing concentrations of agonist applied for 1s starting as indicated by the arrow. (**Right)** Concentration-response curve for kainate-evoked currents. The x-axis shows the log concentration of kainate (M) and the y-axis the current amplitude normalized to the 100 μM evoked current amplitude value. Each point represents the mean value from the indicated number of determinations (different cells). From the four parameter logistic equation the pEC50 value was 4.4 (EC50 = 39.8 μM) and the Hill slope 0.8 (Manual Patch-Clamp Data).

GluR6



Agonist	hGluR6-HEK293
Glutamate	4.71 ± 0.02
Kainate	6.44 ± 0.03
SYM2081	6.79 ± 0.02
Domate	7.27 ± 0.02
AMPA	NA (1mM)
ATPA	NA (1mM)
I-W	NA (1mM)



Antagonist	hGluR6-HEK293
CNQX	4.72 ± 0.03
NBQX	4.77 ± 0.04
Kynurenic acid	NA (10 µM)
LY293558	NA (10 µM)
LY377770	NA (10 µM)
LY382884	NA (10 µM)
CFM-2	NA (10 µM)
GAMS	NA (10 µM)

BACK

Agonist and Antagonist Pharmacology for GluR6: **(Left)** To assess agonist pharmacology, four agonists with reported activity at hGluR6 were tested alongside three that were reported to be inactive against hGluR6 but active against hGRIK1. **(Right)** Data in Table 1 demonstrates that the agonist pharmacology is consistent with expression of the hGluR6 channel. The standard antagonists CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and NBQX (3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline) both blocked the response to 100 mM glutamate with a pIC50 value of 4.7 (FLIPR Data).