

Reproducible activation and pharmacology of $\alpha 1\beta 1\gamma\delta$ nAChR on the SyncroPatch 384

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Cells were obtained from CLS.



Summary

Nicotinic acetylcholine receptors (nAChR) are acetylcholine- (ACh) and nicotine-gated cation permeable ion channels, which mediate fast synaptic transmission at central synapses and neuromuscular junctions. Neuromuscular nAChR form heteromeric proteins composed of four subunits: α , β , γ (or ϵ) and δ . Depending on the developmental stage, the AChR subunit stoichiometry changes from $\alpha 1\beta 1\gamma\delta$ (embryonic) to $\alpha 1\beta 1\epsilon\delta$ (adult)¹. Several inherited and acquired diseases are associated with nAChR dysfunction, most of which lead to impaired neuromuscular transmission and muscle weakness. The acquired autoimmune disease myasthenia gravis (MG) is caused by autoantibodies targeting muscle nAChRs² that disrupts nerve-muscle communication resulting in muscle weakness and fatigue^{3,4}. Inherited diseases called congenital myasthenic syndromes (CMS) are associated with several abnormalities affecting ACh-release, acetylcholinesterase activity, nAChR function and/or nAChR number^{5,6,7}. Treatment has been limited to nonselective, chronic immunosuppressive therapies which have long-term toxicities. More selective and targeted therapies are now under development⁶.

Here we present data collected on the SyncroPatch 384 showing activation and block of nAChR $\alpha 1\beta 1\gamma\delta$ expressed in human TE671 cells with rapid application of ligand or co-application with blockers. We found that ACh activates nAChR $\alpha 1\beta 1\gamma\delta$ receptors with an EC_{50} value similar to those reported in the literature⁸. We recorded highly reproducible currents in response to ACh and obtained IC_{50} values for mecamylamine and α -conotoxin GI that were in good agreement with the literature^{9,10,12,13}.

Results

Nicotinic ACh $\alpha 1\beta 1\gamma\delta$ endogenously expressed in TE671 cells was activated by acetylcholine (ACh) in a concentration-dependent manner (Fig. 1). Fitting the normalized data by applying a Hill equation revealed an EC_{50} of $3.6 \pm 0.5 \mu M$ ($n = 361$), which is in good agreement with values reported in manual patch clamp studies⁸. Success rate for completed experiments was 94%.

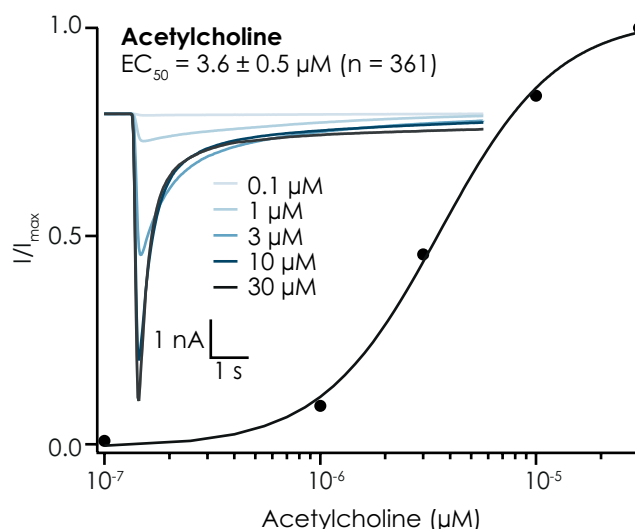


Figure 1: Cumulative activation of nAChR $\alpha 1\beta 1\gamma\delta$ by acetylcholine recorded on the SyncroPatch 384. The concentration response curve for acetylcholine for an average of 361 wells is shown with the average traces for each concentration shown in the inset. Multihole chips (4 holes per well) were used, and nAChR $\alpha 1\beta 1\gamma\delta$ was recorded using a constant holding potential of -80 mV. Five concentrations of ACh (0.1 - 30 μM) were cumulatively added to each well and the average concentration response curve calculated from the plate. Currents from each well were normalized to the maximal response within the well, averaged and fitted with a Hill equation revealing an EC_{50} of $3.6 \pm 0.5 \mu M$ ($n = 361$). Data represent mean $\pm$ SEM.

Application Note

In order to minimize desensitization of the channel and to allow for repetitive activation, nAChA1 β 1 γ δ receptors were activated by brief exposure (0.5 secs) to a small amount of ligand (2 μ l). Using this approach combined with two washes between applications, nAChA1 β 1 γ δ could be repetitively activated by repeated stimulation with 3 μ M ACh and lower concentrations (Fig. 2) allowing inhibition to be studied.

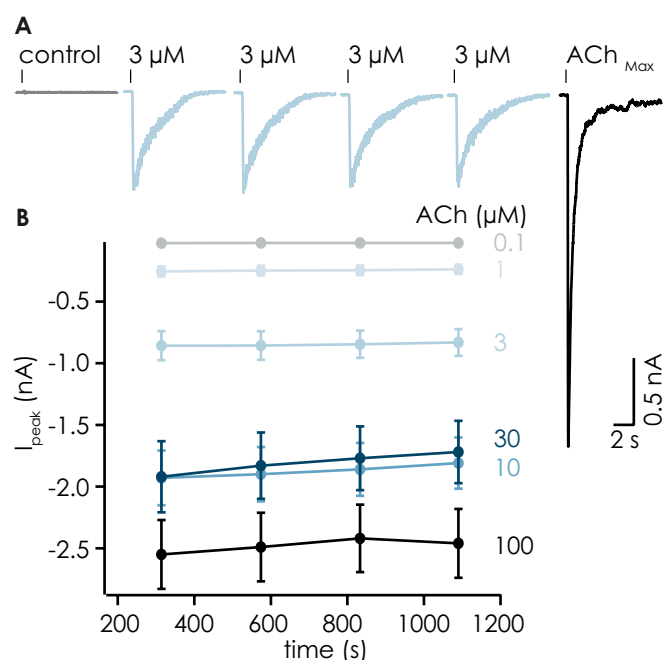


Figure 2: Repetitive activation of nAChR α 1 β 1 γ δ by acetylcholine. Nicotinic AChA1 β 1 γ δ could be repetitively activated by 6 μ M and lower concentrations of ACh at least 4 times in the same well with a peak amplitude consistent with prior applications (<10% change in peak amplitude). **A**, shows raw current traces in response to 3 μ M ACh from an exemplar cell. **B**, the average timecourse of the experiment for all ACh concentrations used is shown. Each ACh concentration was applied 4 times to the same well. Different concentrations varied across the plate.

To record concentration-response curves, each well received 3 applications of 3 μ M ACh followed by co-application of ACh and a single concentration of either mecamylamine hydrochloride or α -conotoxin GI. Mecamylamine is a noncompetitive antagonist targeting all nAChR subtypes¹⁰. Figure 3 shows the concentration response curve for mecamylamine for an average of 181 wells. The IC₅₀ for mecamylamine was calculated to be 2.2 ± 1.1 μ M. For comparison, the effect of mecamylamine isomers were examined on neuronal and muscular nAChR subtypes blocking α 3 β 4 containing nAChR with an IC₅₀ of $\sim$ 0.6 μ M, α 7 containing nAChR with an IC₅₀ of $\sim$ 7 μ M and adult muscular α 1 containing nAChR with an IC₅₀ of 2.2 μ M⁹.

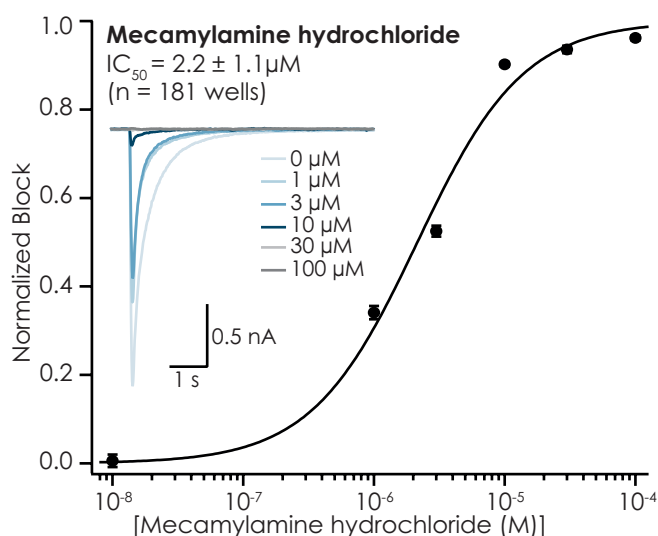


Figure 3: Average concentration response curve for mecamylamine. A single concentration of mecamylamine hydrochloride was co-applied with 3 μ M acetylcholine and the concentration response curve for an average of 181 wells is shown. Average traces in different concentrations of mecamylamine are also shown in the inset. The IC₅₀ for mecamylamine was 2.2 ± 1.1 μ M (n = 181) giving a success rate of 94% for completed experiments.

α 1 (a)-conotoxin GI is a competitive antagonist with an IC₅₀ in the nM range which does not block neuronal nicotinic acetylcholine receptors¹¹. In our experiments, nAChA1 β 1 γ δ activated by 3 μ M ACh was blocked by α -conotoxin GI with an IC₅₀ = 6.8 ± 2.9 nM (n = 171 wells; Fig. 4), which agrees with the range reported for other α -subunits^{12,13}.

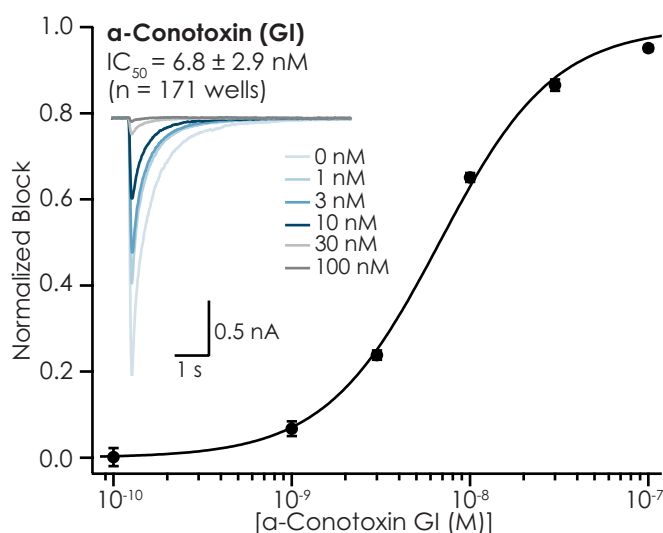
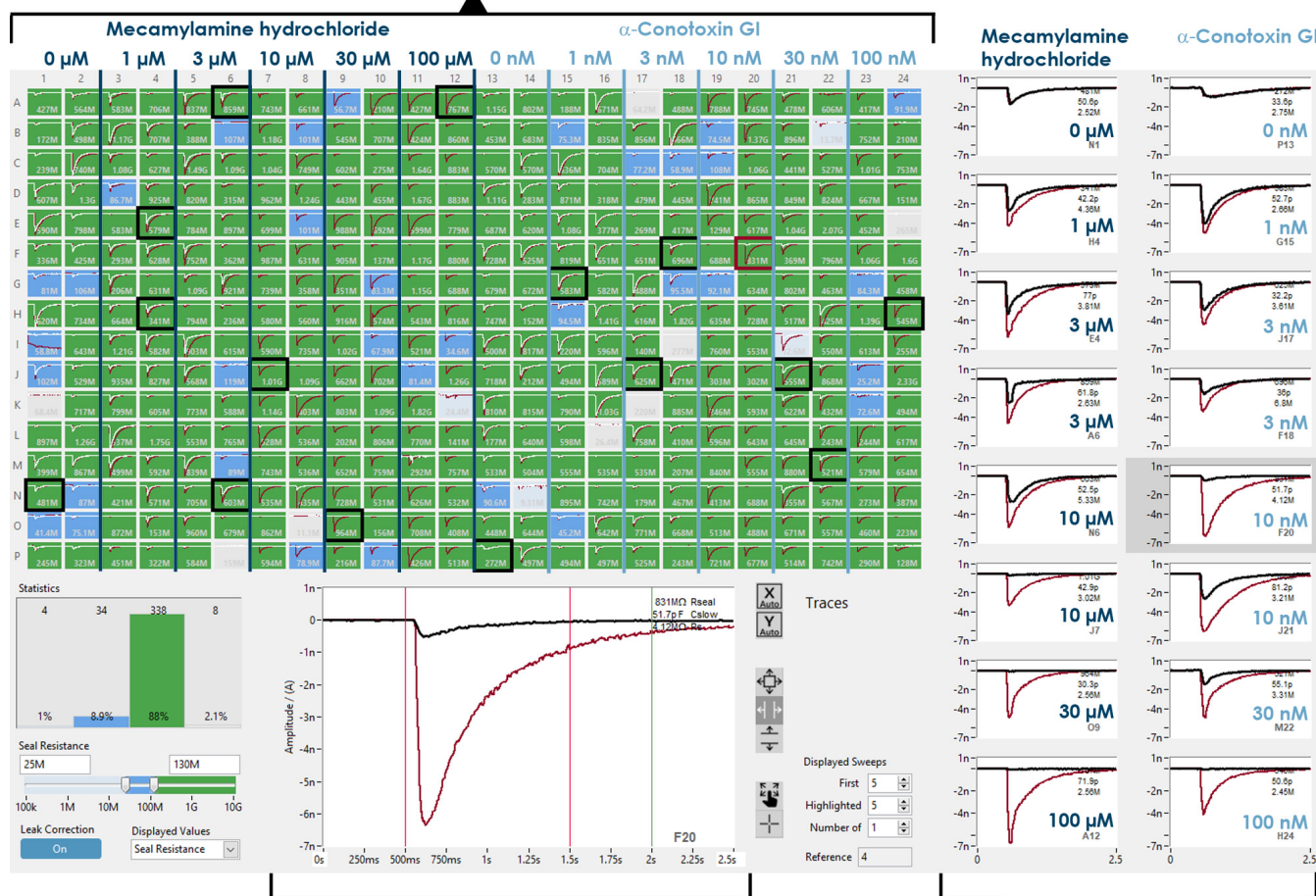


Figure 4: Average concentration response curve for α -conotoxin GI. A single concentration of α -conotoxin GI was co-applied with 3 μ M ACh and the concentration response curve for an average of 171 wells is shown. Average traces in different concentrations of α -conotoxin GI are also shown in the inset. The IC₅₀ for α -conotoxin GI was 6.8 ± 2.9 nM (n = 171).

Application Note

384 color coded depictions of data traces eases judgement of success rate



Highlighted raw current traces of one cell showing block of peak current by 10 nM α -conotoxin GI

Highlighted raw traces of 16 cells showing block of nAChR α 1 by 2 compounds in concentration dependent manner

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of raw current traces of nAChA1 β 1 γ 6 endogenously expressed in TE671 cells as recorded on one NPC-384 patch clamp chip (4x holes per well). Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Wells used for analysis are shown in green. Grey wells indicate failed wells based on the quality control criteria. One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current traces of nAChA1 β 1 γ 6 after activation with 3 μ M acetylcholine (red trace) and co-application with mecamylamine hydrochloride or α -conotoxin GI at the given concentrations (black trace).

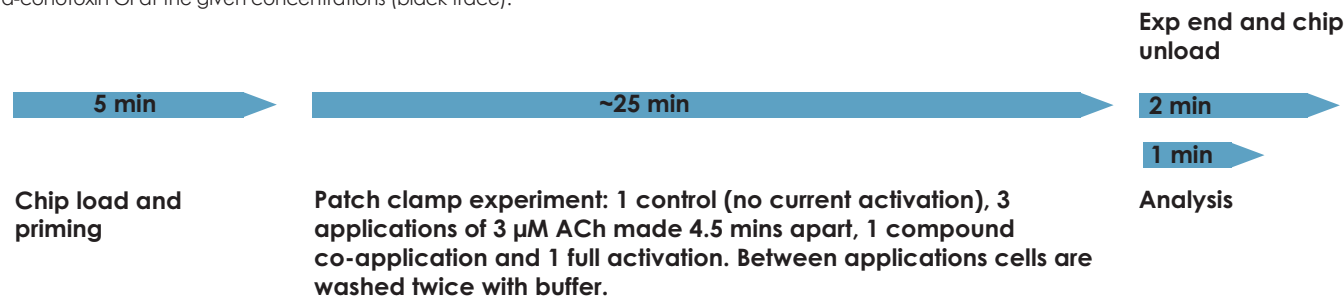


Figure 6: The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point concentration response curve plus repeated activation of nAChA1 β 1 γ 6 with acetylcholine took approximately 33 min.

Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. In this case, raw current traces are shown, red traces indicate control (3 μ M ACh) and black traces indicate the presence of either mecamylamine hydrochloride or α -conotoxin GI. An individual well can be highlighted to monitor the progression of the experiment.

In conclusion, nAChA1 β 1 γ 6 endogenously expressed in TE671 cells was recorded on the SyncroPatch 384 with a good success rate for completed experiments (>89%). The timeline of each experiment was about 33 minutes (start–end) and included activation with ACh (repeated three times), two washes between ligand application for complete ligand washout, application of a

single compound concentration to each well and a full activation with 100 μ M ACh.

Using the LigandPuff application procedure of the SyncroPatch 384, nAChA1 β 1 γ 6 could be repetitively activated. As expected, nAChA1 β 1 γ 6 could be activated by ACh and inhibited by mecamylamine hydrochloride and α -conotoxin GI in a concentration-dependent manner with IC₅₀ values similar to those reported in the literature^{9,10,12,13}.

The SyncroPatch 384 is a high throughput and highly reliable automated patch clamp device for recording nAChA1 β 1 γ 6 currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable, high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

nAChRa1 β 1 γ 6 endogenously expressing TE671 cells were obtained from CLS.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol for gentle cell handling .

Electrophysiology

Whole-cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384. A constant holding potential of -80 mV was used and a small volume of ligand (2 μ l) was applied to activate nAChRa1 β 1 γ 6. Multihole chips were used with 4 holes per well. For pharmacology experiments the inhibitor was co-applied with activator after 3 control applications of ligand and data normalized to peak amplitude in the absence of the blocker. A single concentration of blocker was applied to each well and the concentration response curve calculated across multiple wells.