Application Note

Channel: nAChR α4β2
Cells: HEK
Tools: SyncroPatch 384PE

Pharmacology of human α4β2 nAChR recorded on the SyncroPatch 384PE

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Summary

Nicotinic Acetylcholine Receptors (nAChR) are cation-permeable ion channels, which mediate fast synaptic transmission when activated by the endogenous neurotransmitter acetylcholine (ACh) and the exogenous natural alkaloid, nicotine. Neuronal nAChR form pentameric channels which are composed of two α (α2 to α10) and three β subunits (β2 to β4)[1,2]. Mutations of nAChR are associated with some forms of epilepsy and many other neurological disorders such as Alzheimer's Disease, Parkinson's, Tourette's Syndrome, Schizophrenia and depression[3].

The most abundantly expressed nAChR in the mammalian brain are the α7 homomeric and α4β2 heteromeric receptors[4]. In contrast to the α7, α4β2 nAChR has a high affinity for nicotine[5]. This property, the up-regulation during chronic exposure to nicotine[6], and the receptor expression location in addiction sensitive regions of the brain like the ventral tegmental area[6], strongly indicate that the α4β2 nAChR is a potential target for addiction to nicotine[3].

Here we present data collected on the SyncroPatch 384PE showing activation and block of α4β2 nAChR currents expressed in HEK cells with rapid application of ligand ('Ligand Puff'). ACh activates α4β2 nAChR in a concentration-dependent manner with an EC_{50} value similar to those reported in the literature[3,7,8,9]. Reproducible currents were achieved when cells were preincubated with acetylcholinesterase (AChE). Finally, α4β2 nAChR were blocked by dihydro-b-erythroidine hydrobromide (DHßE), a well known competitive antagonist of the α4 subunit[3] with an IC_{50} in good agreement with the literature[7,9].

Results

Currents mediated by nAChR were activated by adding ACh using the Ligand Puff function in PatchControl 384. A single concentration of ACh was applied to each well, and cells were maximally activated with 200 µM ACh which was added to all wells. Currents were normalized to the maximal response of each well and concentration response curve was calculated across the whole plate. Data was fitted using a Hill equation revealing an EC_{50} = 3.4 µM (342 wells), in excellent agreement with the literature[3,7,8,9] (Figure 1). The success rate for completed experiments was 89%.

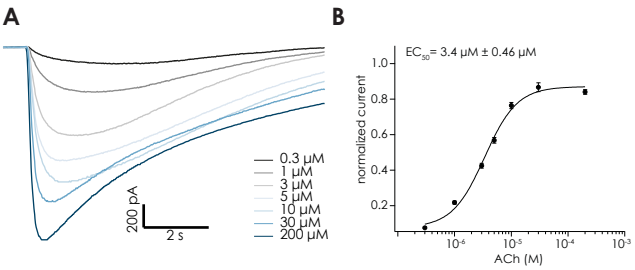

Figure 1: Activation of nAChR α4β2 expressed in HEK cells on the SyncroPatch 384PE by ACh. A. A single concentration of ACh was applied to each well and the average traces of all 342 cells are shown. The concentration response curve was calculated across the plate and is shown in Panel **B**. Currents from each well were normalized to the maximal response within the well, averaged and fitted with a Hill equation revealing an EC_{50} of 3.4 µM (n = 342) in excellent agreement with the literature[3,7,8,9].

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Repeated or extended exposure of ACh lead to desensitization of the nAChR and resulted in receptor inactivation. AChE, a natural enzyme located on the postsynaptic membrane, hydrolyzes ACh, and the liberated choline is then recycled into the presynaptic membrane. Consequently, the postsynaptic nAChRs quickly recover from desensitization and ACh is resynthesized in the presynapse which is able to reactivate nAChRs.

Figure 3 shows α4β2 nAChR desensitization after repetitive activation of the receptor with 2 µM ACh and recovery from desensitization when ACh is co-applied with AChE. When ACh is co-applied with AChE peak amplitudes varied with each application by no more than 5%.

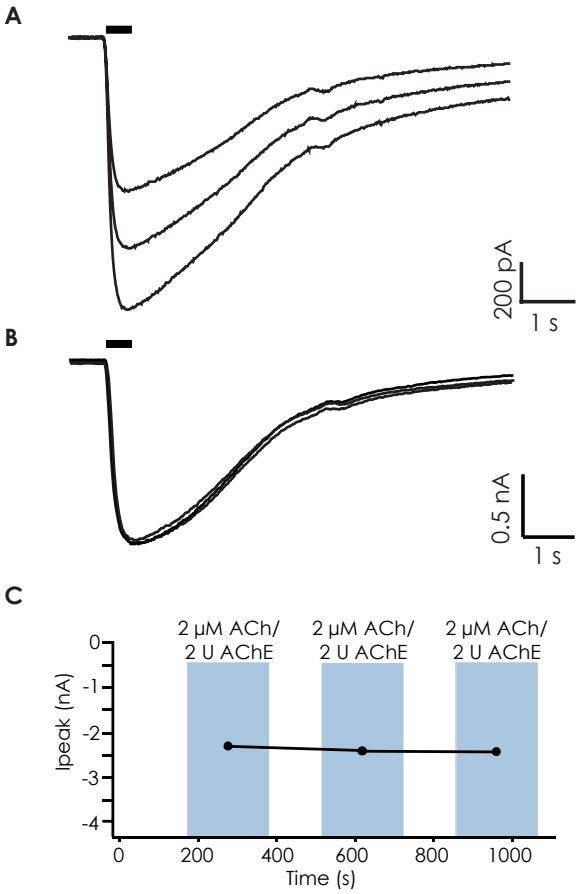

Figure 3: Desensitization and recovery of nAChR mediated currents recorded on the SyncroPatch 384PE. A. Averaged raw current traces from 64 wells showing current activation by repetitive application of 2 µM ACh. **B.** Averaged raw current traces from 64 wells showing current activation by application of 2 µM ACh in the presence of 2 units AChE repeated 3 times in the well. Peak amplitudes were very similar with each application of ACh/AChE, differing by no more than 5%. **C.** Online analysis plot showing peak amplitude versus time for one cell.

For pharmacological experiments, DHβE, a competitive nicotinic acetylcholine receptor antagonist, was co-applied with ACh. Single point concentration response curves were calculated by combining the results of the application of nine different concentrations distributed across the plate (Figure 4). This gave an IC_{50} = 48.2 ± 14.8 nM (n = 362), in excellent agreement with the literature[7,9]. The success rate for completed experiments was 94 %. For normalization 1 µM ACh was applied first, followed by co-application of DHβE and 1 µM ACh.

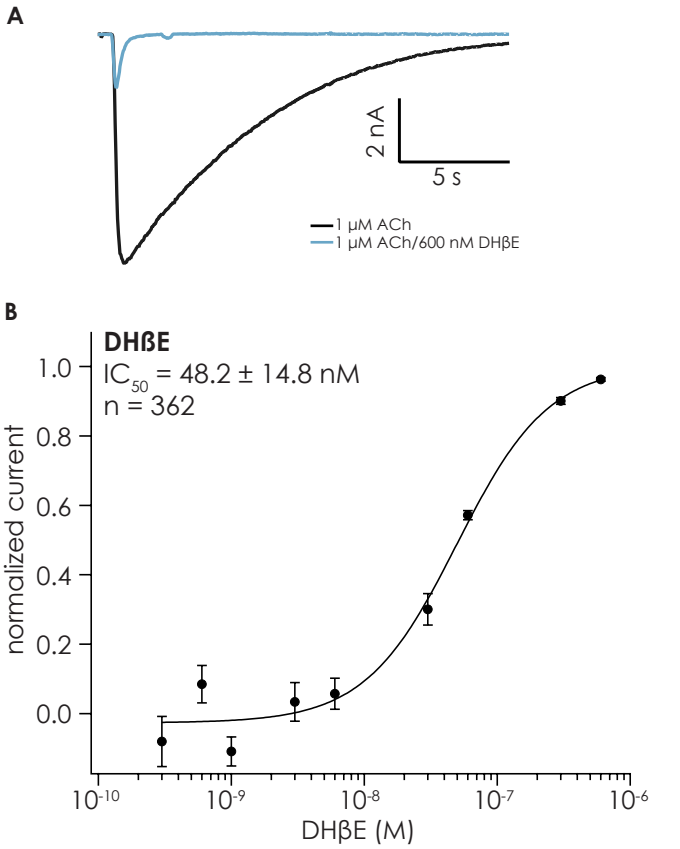

Figure 4: Block of nAChR α4β2 by DHβE. A. Exemplary averaged traces after addition of 1 µM ACh (black) and after co-application with 600 nM DHβE (blue). **B.** Currents in the presence of the antagonist were normalized to the control current activated by ACh only. The concentration response curve for DHβE was calculated across the plate. Data were fitted with standard Hill equation and IC_{50} calculated to be 48.2 ± 14.8 nM (362), in good agreement with values[7,9].

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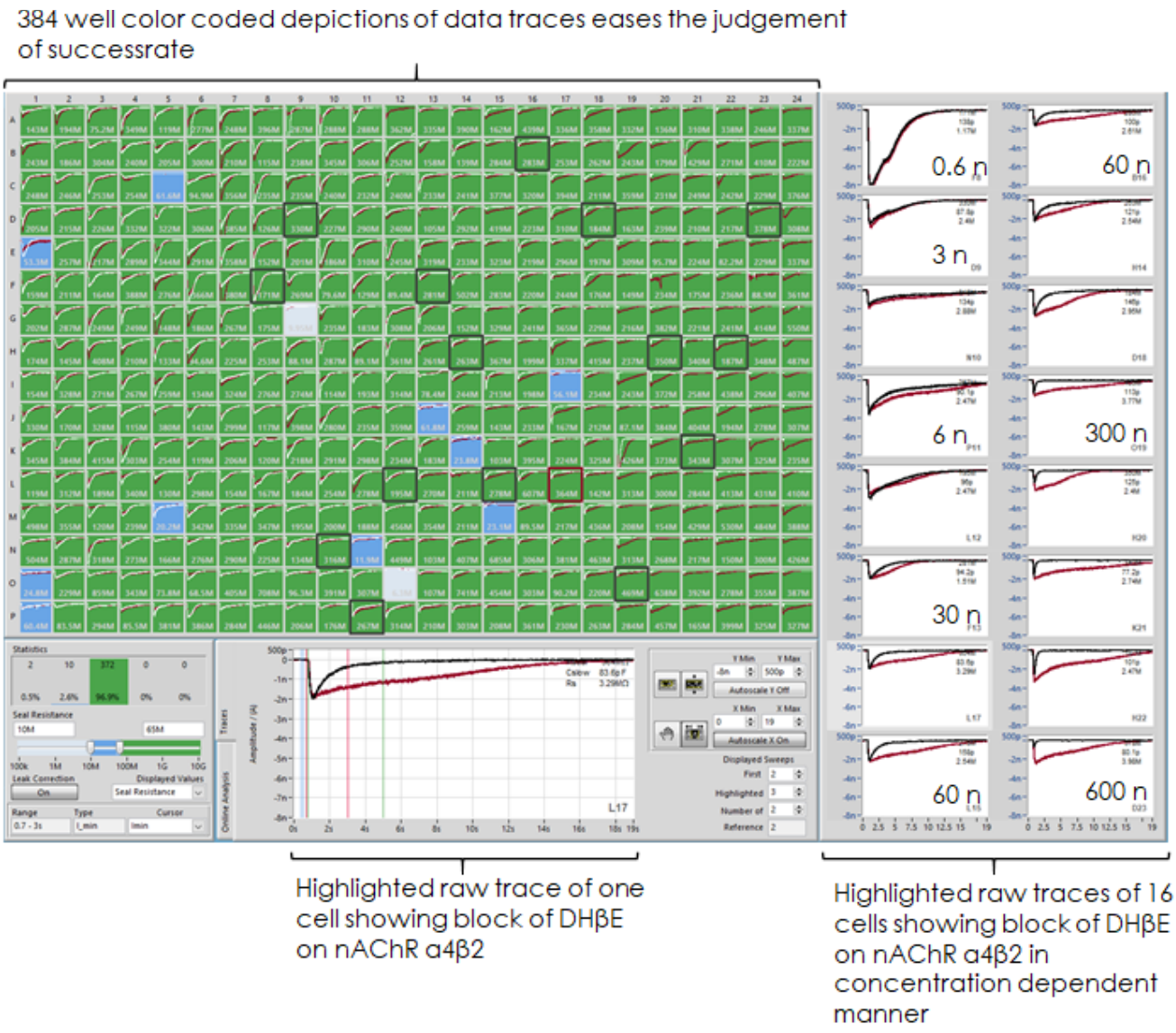

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot depicts raw data traces of nAChR a4β2 expressing HEK cells as recorded on one NPC-384 patch clamp chip. A single point concentration response curve was performed for DHβE on individual wells. One control application of 1 µM ACh was followed by one concentration of DHβE on each well and increasing concentrations across the plate. DHβE was co-applied with ACh. Multi-hole chips were used where 8 holes were present per well. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green (Rmemb > 70 MΩ), blue (Rmemb 10 - 70 MΩ) or light blue (Rmemb > 10 MΩ). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show raw data traces of nAChR a4β2 channels following application DHβE co-applied with ACh.

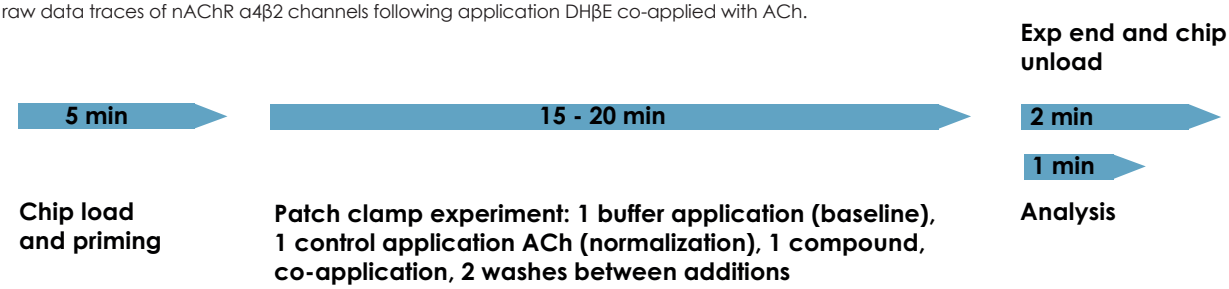

Figure 6: Timeline of an experiment on the SyncroPatch 384PE. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point concentration response curve on a4β2 nAChR-mediated currents took approximately 20-25 min.

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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 easily toggle between raw traces and online analysis. In the example shown, raw traces are chosen and the graphs show current responses to ACh followed by inhibition by co-application of DHßE. The combination of brief compound application and the performance of single point concentration response curves provides an easy and quick way to discover agonists and antagonists of fast desensitizing receptors such as nAChR. The cells were stable for about 30 minutes and compound could be rapidly applied to the cells to minimize desensitization. An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, α4β2 nAChR expressed in HEK cells can be reliably and reproducible recorded on the SyncroPatch 384PE. The timeline of each experiment was approximately 20 minutes (start – end) and included baseline recording after application of standard physiological solution, single control application of ACh followed by co-application with single concentration of antagonist. Finally, a maximum concentration of ACh (200 µM) was typically applied. The α4β2 nAChR-mediated response was inhibited by DHßE in a concentration-dependent manner as expected with an IC_{50} within the range round in the literature[7]. The EC_{50} values for ACh and the kinetics of desensitization were consistent with α4β2 nAChR heteromers[7].

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for studies on α4β2 nAChR-mediated currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each well 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

HEK293 cells expressing nAChR with the subunit configuration α4β2 are kindly provided by SB Drug Discovery

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol. Nicotinic ACh α4β2 Receptor expression was induced by incubation in 1 µg/ml tetracycline 18 - 24 hours prior to experiments.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384PE using multi-hole (8 holes per well) chips. Cells were held at -100 mV for the duration of the experiment, if not state different. For ACh concentration response curves, AChE (1U/µM ACh) was used to prevent desensitization. For pharmacology experiments, cells were pre-incubated in DHβE for 5 min and then compound was co-applied with 2 µM ACh. Receptors were activated using the 'Ligand Puff' command in PatchControl 384: Agonist volume: 5 µM, wash volume: 7 µM, application speed 40 µl/s. Cells were then washed 3 x with external solution before re-application of agonist. Integral and the minimum peak current amplitudes were analyzed to determine the EC_{50} or IC_{50}. Data were fitted by Hill equation either to normalized values or raw amplitudes.

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