PARALLEL PATCH CLAMP OF α7 NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS

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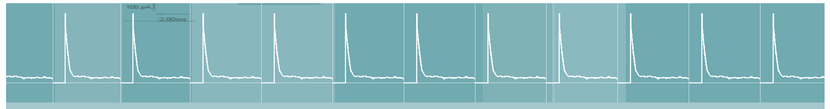


The human α7 nicotinic acetylcholine receptor (α7-nAChR) is a neuronal ligand-gated, fast desensitizing, non-selective cation channel. It is pentahomomeric, consisting of five 50 kD α7 subunits, each composed of 502 amino acids. The α7-nAChR is involved in memory and cognition, and it is widely distributed throughout the nervous system, especially in cholinergic neurons projecting to hippocampus and cortex. The α7-nAChR has also been found to be associated with pathophysiological states. Importantly, it is involved in widespread human neuro-degenerative and psychotic disorders, including Alzheimer's disease and schizophrenia. Therefore the therapeutic potential of α7-nAChR is substantial, and electrophysiological and pharmacological characterization of the receptor has become an increasingly important issue. The fast kinetics (milliseconds) makes precise patch clamp measurements a challenging task. The present study addresses the possibility of efficient and precise characterization of α7-nAChR function and pharmacology by means of the QPatch automated patch clamp system. Compared to conventional whole-cell patch clamp the system enables a highly increased throughput by simultaneous and asynchronous operation of 16 parallel patch clamp experiments.

MATERIALS AND METHODS

Cells: For the studies were used GH4-C1 cells expressing rat α7-nAChR. The cells were growth-arrested 48 hours prior to use with 0.5 mM Na-Butyrate, detached from the flask with trypsin and sub-sequently kept in culture medium according to Sophion standard procedures. The isolated cells in solution appeared spherical. For a large number of these experiments frozen vials of cells were thawed just before experiment execution and used directly on the QPatch. This has been found to be an ideal to way to maintain a stable supply of suitable cells for this and other cell lines.

Ringer's solutions: Extracellular (mM): 145 NaCl, 4 KCl, 10 HEPES, 2 $CaCl_2$, 1 $MgCl_2$. Intracellular (mM): 120 KF, 20 KCl, 0.1 EGTA, 10 HEPES, 2 $MgCl_2$.

Compound application: We used the QPlates XL that hold 250 µL in the waste. This allowed execution of advanced ligand- and compound application protocols including multiple applications. Acetyl choline (ACh) and nicotine were used as agonists. Agonist and test compound application protocols were defined in the QPatch assay software. Eight teflon coated pipettes applied the compounds via pipetting wells and the QPlate integrated glass flow channels. During recording, the membrane potential was held at -90 mV.

Sealing rates: The whole-cell success rate was about 80%. However, the final success rate with useful completed experiments was less due to lack of expression in all cells.

Figure 1. Current rise time
Addition of ligands, either ACh or nicotine, elicited typical α7-nAChR currents with rise times of 5-10 ms (5 - 95 %). Here the current is induce by 10 mM ACh. The rise time reflects the solution exchange rate rather than the actual opening rate of the α7-nAChR.

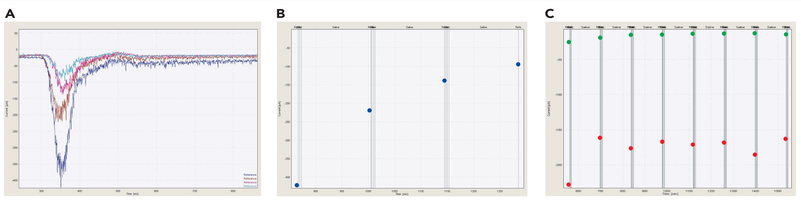


Figure 2. ACh esterase eliminates residual ACh
Multiple additions of the same ligand concentration (1 mM ACh) led to decreasing amplitudes of peak α7-nAChR currents, as seen from the 4 sequential raw current recordings (**A**) and the resulting I-t plot (**B**). Because the concentration-response graph for desensitization is left-shifted to that of activation, even minute agonist concentrations remaining after washes will partially desensitize α7-nAChR. Enzymatic removal of the ACh with ACh esterase reduced the problem, as seen from the I-t plot presenting 8 successive applications of 1 mM ACh (**C**). Green points represent leak currents.

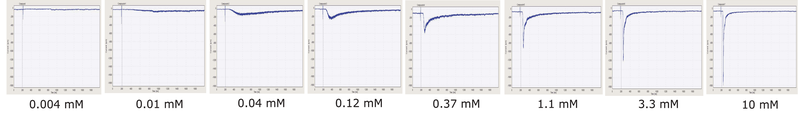


Figure 3. Effect of ACh - Full agonism
Sequence of original current recordings of α7-nAChR currents induced by increasing ACh consentrations (4 nM - 10 mM). Between each application is a 150 sec resting period.

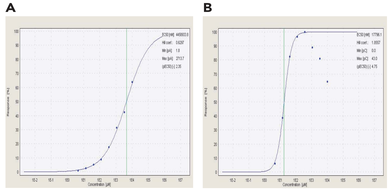
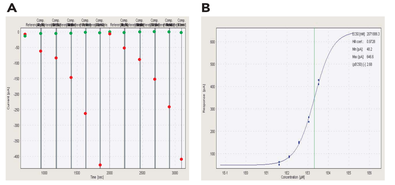


Figure 4. ACh: Peak vs. area under curve (AUC) analysis
A: Concentration-response relationship for the effect of ACh on α7-nAChR current amplitudes. The graph led to EC_{50} = 2.0±0.7 mM (n=8). Similar recordings with the ultra-fast solution exchange system Dynaflow led to IC_{50} = 0.38 mM (data kindly provided by Wyeth Research) indicating that the 5-fold increased QPatch IC_{50} value is a result of a slower solution exchange.
B: An alternative 'area-under-curve' (AUC, i.e. the integrated charge transfer) analysis led to IC_{50} = 26±7 µM (n=6) which compares well to the value obtained with Dynaflow, 29 µM.



[ACh]	(peak 1/peak 2)·100 (% ± SEM)
41 µM	101 ± 13
123 µM	105 ± 8
370 µM	107 ± 10
1.11 mM	101 ± 11
3.33 mM	87 ± 12

Figure 5. Assay reproducibility and stability
A: Two ACh concentration-response experiments were performed with a time interval of approximately 45 minutes. Green points represent leak currents. **B**: The two Hill fits were practically identical demonstrating a high degree of reproducibility.
Table: Comparison between mean peak α7-nAChR current amplitudes of 5 experiments similar to the one presented in the panel A. At no ACh concentration is the ratio between the two time-separated peak current amplitudes significantly different from 1.00.

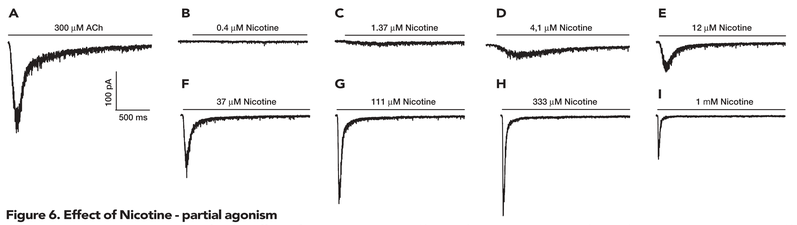


Figure 6. Effect of Nicotine - partial agonism
Concentration-response analysis for the effect of nicotine on α7-nAChR peak currents. Trace **A** shows the response to 300 µM ACh for comparison. Eight increasing concentrations (traces **B-I**) of nicotine were employed.

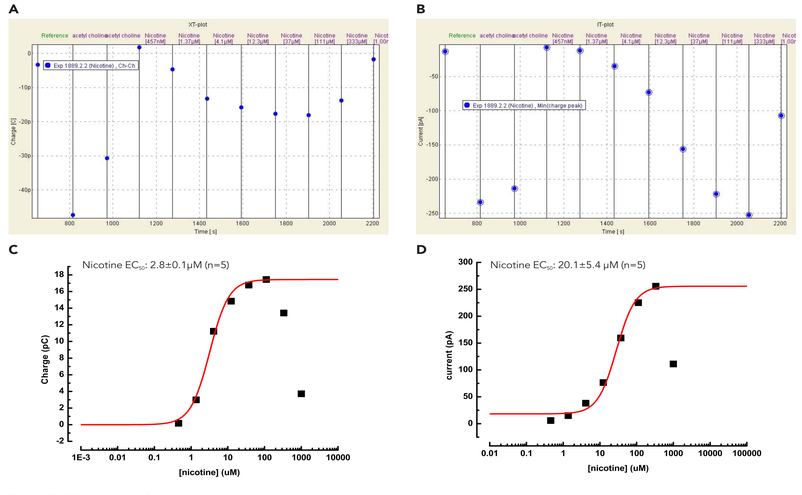


Figure 7. Nicotine: AUC vs. peak current analysis
A and **C**: AUC-t plot and Hill fit for the effect of increasing concentrations of nicotine on AUC. **B** and **D**: I-t plot and Hill fit for the effect of nicotine on peak α7-nAChR currents. EC_{50} value compare well to values obtained with Dynaflow (EC_{50}=9.9 µM).

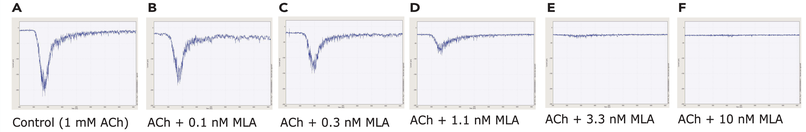


Figure 8. Effect of methylcaconitine (MLA)
Concentration-response analysis for the antagonist MLA. The agonist employed was ACh at a concentration of 1 mM.
A: current response to ACh only (control). **B-F**: progressive current inhibition in response to 5 increasing concentrations of MLA.

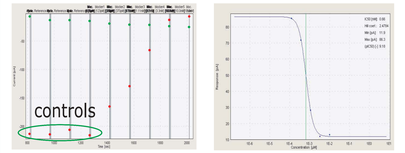


Figure 9. Effect of MLA
I-t plot (**A**) and Hill fit (**B**) for data presented in Figure 8. IC_{50} = 0.79±0.08 nM (n=9). Literature value: 0.64 nM (Biggs & McKenna, Neuropharmacology, **35**: 407-414, 1996).Note that the first four points in the I-t plot represents ACh only (no antagonist). Green points represent leak currents.

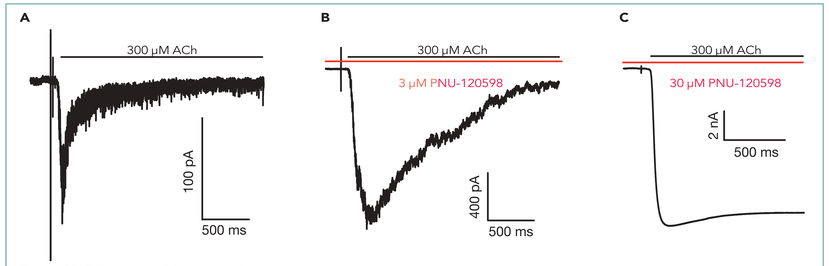


Figure 10. Effect of positive allosteric modulators
Effect of the desensitization inhibitor PNU-120598 on ACh-induced α7-nAChR currents. **A**: Response to 300 µM ACh, only (control). **B**: 3 µM PNU-120598 partly inhibits desensitization of ACh - induced currents. **C**: 30 µM PNU-120598 almost completely inhibits desensitization of ACh - induced currents.

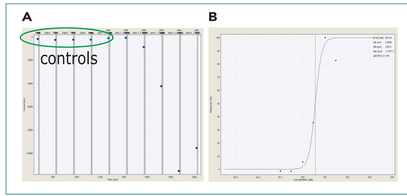


Figure 11. Effect of PNU-120598
Concentration-response analysis of the stimulatory effect of PNU-120598. **A**: I-t plot. The first 5 points are controls (no PNU-120598). **B**: Hill fit of the AUC data. EC_{50}=5.5±1.1 µM (n=8).

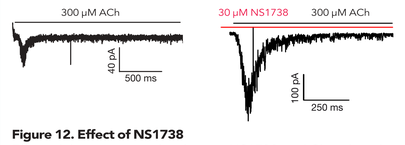


Figure 12. Effect of NS1738
Effect of the current peak enhancer NS1738 on ACh-induced α7-nAChR currents. **A**: Original current recording of a pure agonist (300 µM ACh) response (control). **B**: Original current recording of NS1738-stimulated α7-nAChR current. The NS1738 concentration was 30 µM.

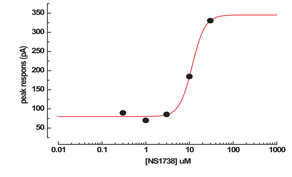


Figure 13. Effect of NS1738
Hill fit of NS1738-stimulated α7-nAChR currents. EC_{50}=13.0±1.5 µM (n=5).

CONCLUSION

Despite fast activation and subsequent desensitization which leaves a very brief time window for recordings, it was possible to efficiently study the effects of agonists (full or partial), antagonists and modulators on α7-nAChR expressed in cultured GH4-C1 cells. Current peaks with rise times of 5-10 ms were routinely recorded. The results demonstrate that EC_{50} and IC_{50} values for peak currents or charge (AUC) can be measured with accuracy in a highly reproducible manner. Optimization of experimental conditions and unmanned operation makes it possible to obtain higher throughput compared to manual patch clamp.