

Application Report:

Nicotinic $\alpha 7$

From Galantos



This study presents data of the validation and optimization study of CHO cells expressing the homomeric nicotinic ligand gated $\alpha 7$ ion channel cloned and provided by Galantos.

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Introduction

Nicotinic $\alpha 7$ currents are challenging on automated platforms due to the fast desensitizing properties of the channel. This property calls for a cell line with exceedingly good expression of the channel.

Sophion has previously successfully tested a rat cell line GH4-C1 expressing $\alpha 7$ however this cell line expresses the isoform of the rat and the cell line is not commercially available (Friis et al, 2009).

Galantos has cloned a human isoform into a CHO background and this report describes the data obtained with this cell line.

Nicotinic $\alpha 7$ is of interest since it has been found to be involved in many normal and pathological conditions. It is important in memory formation, arousal, and attention, nicotine addiction, and nicotine-induced reversal of age-related memory deficits. $\alpha 7$ activation leads to neuroprotection against amyloid- β -induced neurotoxicity, which suggests that the receptor may play an important role in Alzheimer's disease. Also their role in hippocampal auditory gating has linked the receptor to schizophrenia.

Materials & Methods

Cells were detached from the flask with trypsin and kept in culture medium according to Sophion standard procedures.

The formation of gigaseals and whole cell formation on QPatch was set up using the QPatch assay software and was done by application of negative pressure pulses.

Addition of agonists and test compounds was also set up using the QPatch assay software. During recording, the membrane potential was held at -90 mV.

The ringers used:

Extracellular (mM): 145 NaCl, 4 KCl, 10 HEPES, 2 CaCl₂, 1 MgCl₂

Intracellular (mM): 120 KF, 20 KCl, 0.1 EGTA, 10 HEPES, 2 MgCl₂

The cell line was tested on both single hole and multi hole patch plates.

Results

Success rates

The sealing rate with CHO $\alpha 7$ is very good and the formation of whole cells does not pose a problem. The whole cell rate is about 80% in single hole mode with this cell line. Below is a snap shot from the QPatch assay software.

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [M Ω]	R seal [M Ω]	R whole-cell [M Ω]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	2.11	1315.6	1272.7	1377	1
B1	✓	✓	✓	✓	2.13	2606.6	1153.7	1378	1
C1	✓	✓	✓	✓	2.08	0.0	0.0	0	0
D1	✓	✓	✓	✓	2.19	2100.6	1325.3	1425	1
E1	✓	✓	✓	✓	2.10	2133.0	0.0	0	0
F1	✓	✓	✓	✓	2.12	2234.9	1736.9	1423	1
G1	✓	✓	✓	✓	2.19	2461.3	1093.6	1472	1
H1	✓	✓	✓	✓	2.26	2631.8	1861.1	1484	1
A2	✓	✓	✓	✓	2.20	2212.2	1876.1	1389	1
B2	✓	✓	✓	✓	2.12	1147.2	907.7	1389	1
C2	✓	✓	✓	✓	2.18	1276.0	1136.9	1406	1
D2	✓	✓	✓	✓	2.19	108.3	188.1	422	0
E2	✓	✓	✓	✓	2.17	1784.1	1457.1	1460	1
F2	✓	✓	✓	✓	2.07	1340.3	590.1	1461	1
G2	✓	✓	✓	✓	2.18	1956.6	1555.0	1497	1
H2	✓	✓	✓	✓	2.19	1299.6	1024.2	1496	1
Total	16	15	14	14					13
Success rate	100 %	94 %	88 %	88 %					

Figure 1: Typical overview of chip, seal (on cell) and whole cell resistances obtained on a single hole QPlate.

The same was true for multi hole plates here the resistances are app. ten fold lower due to the fact that there are ten holes patched in parallel at the same time pr. site.

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [M Ω]	R seal [M Ω]	R whole-cell [M Ω]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	0.25	49.9	46.7	1495	1
B1	✓	✓	✓	✓	0.25	53.3	55.8	1495	1
C1	✓	✓	✓	✓	0.23	12.6	10.3	1528	1
D1	✓	✓	✓	✓	0.24	96.1	77.5	1529	1
E1	✓	✓	✓	✓	0.23	101.4	87.0	1582	1
F1	✓	✓	✓	✓	0.24	39.3	48.9	1582	1
G1	✓	✓	✓	✓	0.26	263.5	171.7	1619	1
H1	✓	✓	✓	✓	0.24	77.4	102.5	1619	1
A2	✓	✓	✓	✓	0.23	29.3	55.6	1508	1
B2	✓	✓	✓	✓	0.24	100.0	67.0	1508	1
C2	✓	✓	✓	✓	0.24	319.2	255.4	1539	1
D2	✓	✓	✓	✓	0.26	18.5	14.3	1537	1
E2	✓	✓	✓	✓	0.25	3.8	12.5	1597	1
F2	✓	✓	✓	✓	0.25	93.5	121.7	1597	1
G2	✓	✓	✓	✓	0.26	149.7	272.1	1632	1
H2	✓	✓	✓	✓	0.28	30000.0	30000.0	1632	1
Total	16	16	16	16					16
Success rate	100 %	100 %	100 %	100 %					

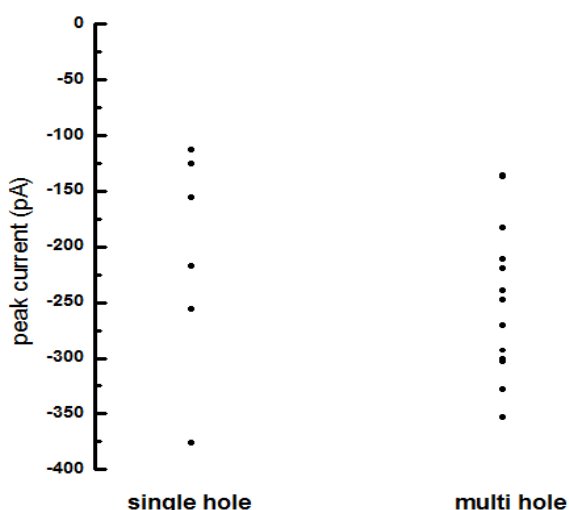
Figure 2: Typical overview of chip, seal (on cell) and whole cell resistances obtained on a multi hole QPlate.

The number of cells expressing enough current to be useful, >50 pA when stimulated with 0.3 mM acetyl choline (ACh), was optimized by testing some of the different growth arrest methods that previously has been shown to boost channel expression: prolonged 30 degree incubation and nocodazole. It was found that incubation at 30 degree for 5 days boosted the expression level while still maintaining the cells viable enough to form good patches. Data from such an experiment is shown in table 1 below.

Incubation, plate type	Avg peak @ 0.3 mM ACh (pA)	Success rate (%)
2 days, single hole	65 +/- 10	28
5 days, single hole	124 +/- 12	37
5 days, multi hole	511 +/- 23	90

Table 1: Effects of prolonged 30 degree incubation on $\alpha 7$ amplified and success rates >50 pA current.

Data from a similar experiment on a different day using 1 mM ACh is shown below. This method was found to be extremely robust and good patchable well expressing cells were generated routinely.



Figur 3: $\alpha 7$ responses recorded from the same batch of cells on either single or multi hole QPlates. These measurements were done on a QPatch 16 i.e. 16 possible recordings pr. plate.

Signal stability

To ensure the best stability of the signal we added acetyl choline esterase to the washing solution – a method which has previously been described and found to stabilize responses in these assays.

It was found that the stability of the current was sufficient for all the typical types of assays that are run on QPatch. Sweep responses demonstrating the stability is shown below.

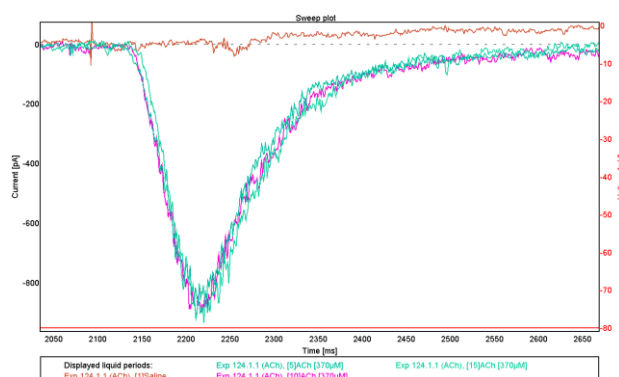


Figure 4: raw data sweeps from a multi hole experiment demonstrating the stability of the responses. The cells are stimulated 3 times every 180 seconds with 370 μM acetyl choline.

Agonist dose response

Full agonism – acetyl choline

$\alpha 7$ can be stimulated with a variety of different agonists - we have made assays to test both full and partial agonists on the QPatch.

Acetyl choline is considered to be a full agonist for this receptor. The dose response relationship for acetyl choline was tested in a set of 6 point dose response experiments. The raw traces from one of these experiments in multi hole mode is shown below in Figure 5.

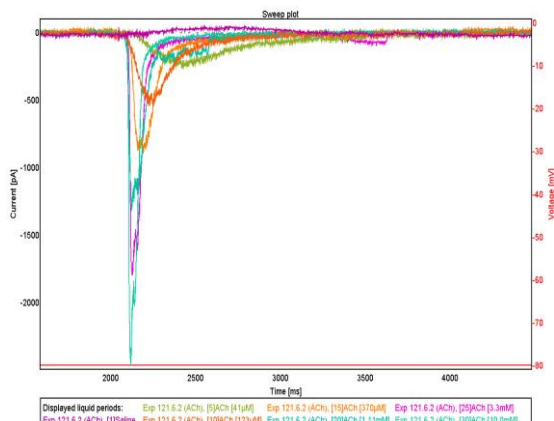


Figure 5: raw data sweeps from a multi hole mode ACh 6 point dose response experiment. The cells are stimulated ACh at increasing concentrations ranging from 0.041 – 10 mM. The time between stimulation is 180 sec.

The peak response data never reach saturation – therefore we have fixed the full response to be the 10 mM response i.e. the EC₅₀ is not estimated very accurately see figure 6 below.

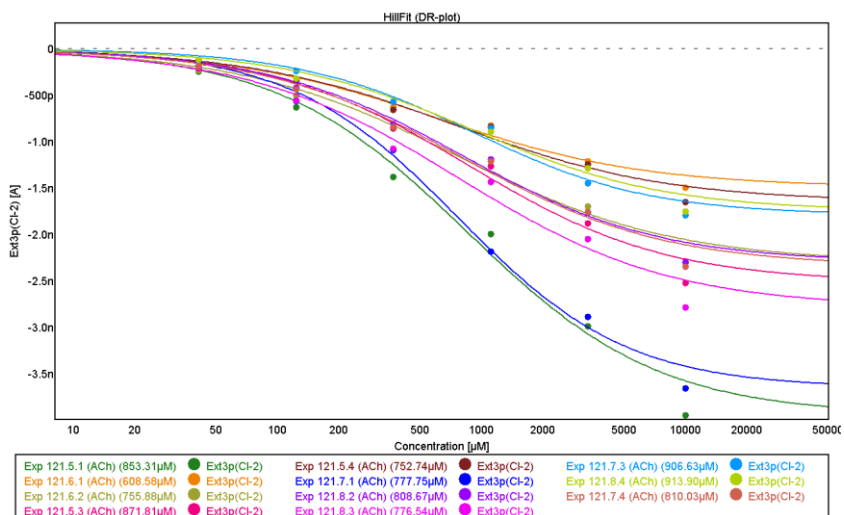
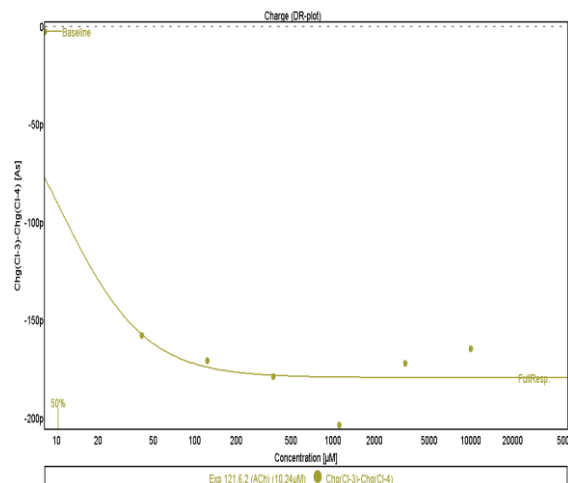


Figure 6: Hill fit curves derived from the peak response from increasing concentrations of ACh.

In order to get a saturating response it has been shown that the use of area under curve (AUC) is a tool to normalize differences between setups with different flow systems (Papke & Papke, 2002). Estimation of EC_{50} with the various methods and on different plate types is summarized in table 2.



Figur 7: Hill fit derived from AUC from increasing concentrations of ACh.

Acetyl Choline EC_{50}		
Plate type	Acetyl Choline	Acetyl Choline
	Peak EC_{50} (mM)	AUC EC_{50} (μ M)
Single hole	5.0 +/- 0.1	56 +/- 1.7
Multi hole	2.9 +/- 0.5	35 +/- 0.7

Table 2: summary of acetyl choline data collected on $\alpha 7$ from Galantos.

Patial Agonism - Nicotine

Nicotine was used to demonstrate a partial agonist assay. Two additions of 300 μM acetyl choline were used as controls before increasing concentrations of one of the partial agonists of interest was added. The sequence of a typical experiment is shown below in an It-plot.

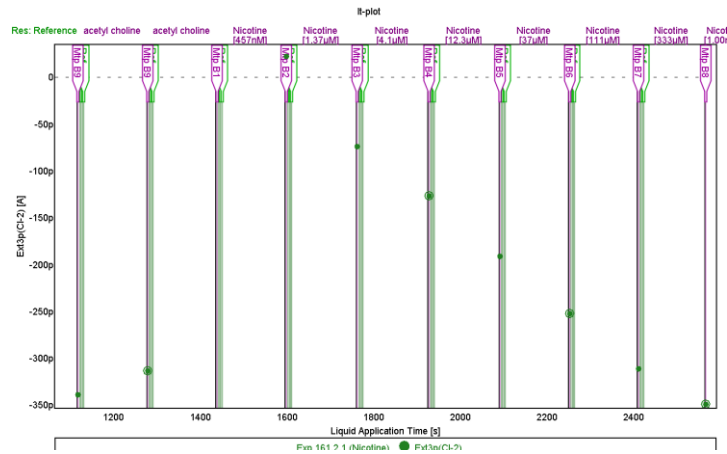


Figure 8: Typical time course of a partial agonist experiment. The first responses are baseline responses followed by increasing concentrations of nicotine.

It was found that nicotine acts as a partial agonist with a higher affinity ($EC_{50}=25 \mu\text{M}$) and with a lower efficacy towards $\alpha 7$ which is perfectly in line with previous observations made on the QPatch.

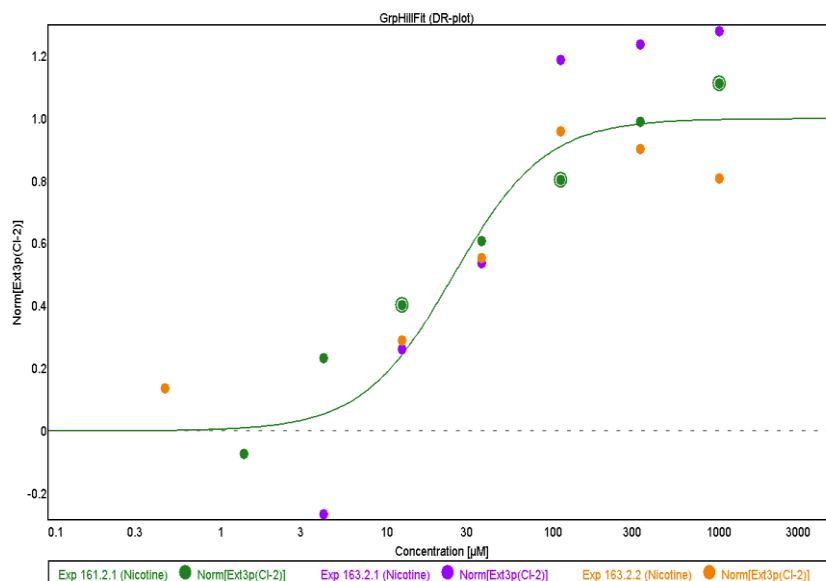


Figure 9: Peak responses normalized to the baseline ACh response.

Antagonist assay

Assays for examining the effect of antagonists (and also modulators) is set up in a very similar manner as the partial agonist assay: A sequence of 3 controls with 300 μM acetyl choline is made before the test compound is added to the cell. The test compound is added alone as a part of the wash from the previous cycle and the cell is thus preincubated for a specified period of time (typically 180 seconds) and then the cell is stimulated with acetyl choline plus the test compound.

The effect of the two known blockers - methyllycaconitine ac (MLA) and mecamylamine (MECA) were tested to validate this type of assay. Typical rawdata sweeps, time course and IC_{50} estimation of such experiments are shown below.

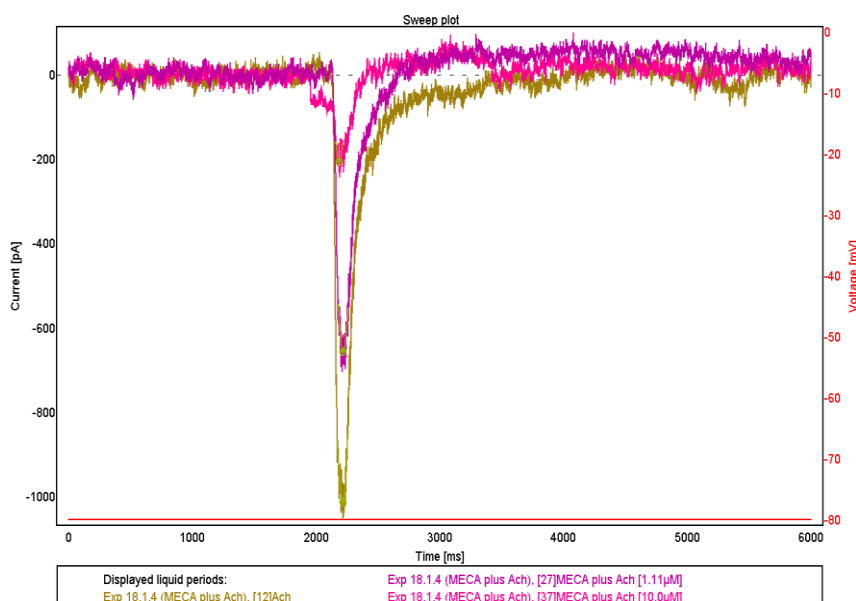


Figure 10: Raw data traces from a multihole experiment of responses to increasing concentrations of MECA. Note that the shape of the response does not change - only the size is decreased.

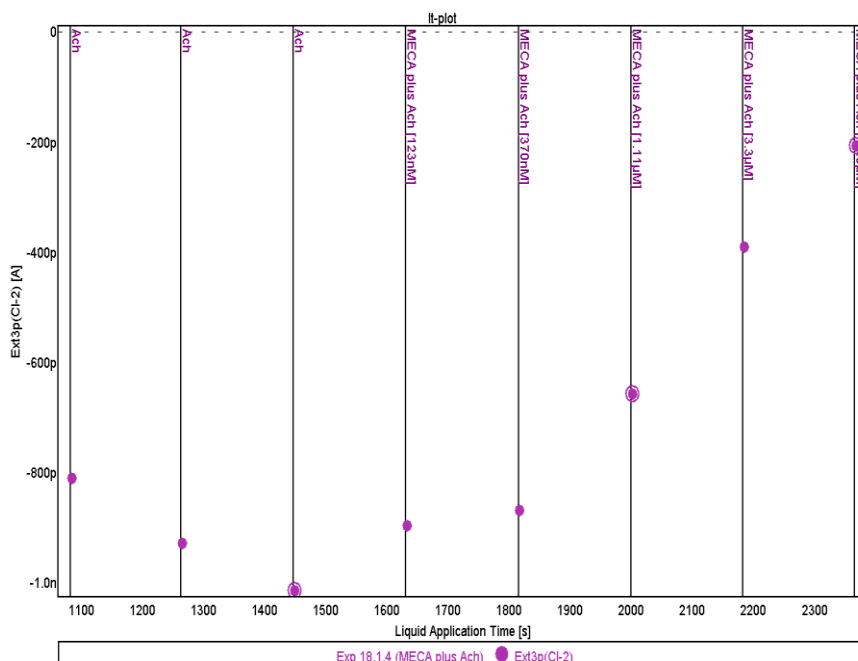


Figure 11: IT-plot of typical antagonist experiment. The response shown is the peak response measured. Three additions are used as controls/baseline estimation before increasing concentrations of test compound is added. 16 experiments of this kind in parallel takes app. 25 minutes total to complete.

The IC_{50} 's obtained with MLA and MECA is summarized in the table below:

	MECA	MLA
	Peak IC_{50}	peak IC_{50}
	(μM)	(nM)
Single hole	4.4 +/- 2.0	3.3 +/- 1.8
Multi hole	2.7 +/- 0.3	1.8 +/- 0.4

Table 3: IC_{50} values of reference blockers obtained on $\alpha 7$ from Galantos.

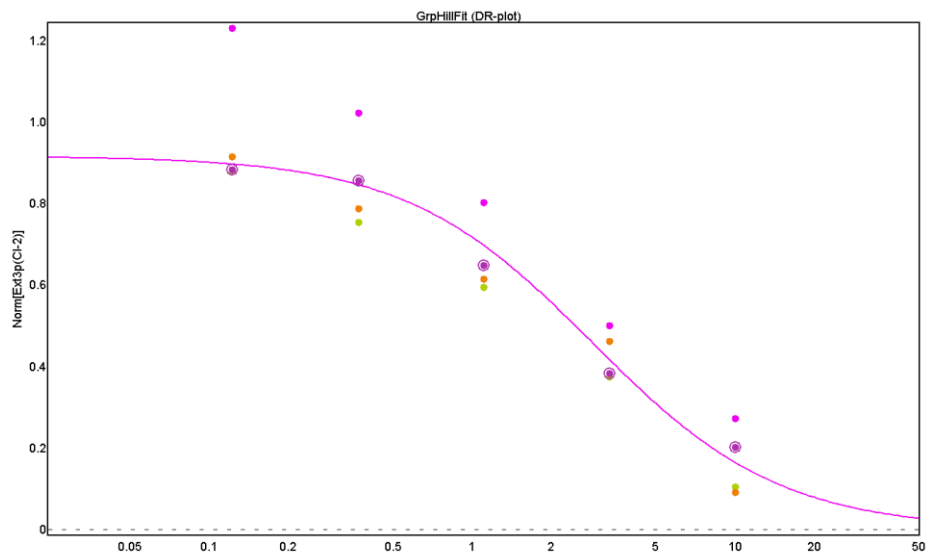


Figure 12: Normalized peak current responses from a multi hole experiment as a function of increasing concentration of MECA. The data is grouped together and averaged thus generating one single curve is fitted to the average of the points to estimate IC_{50} .

References

Friis S, Mathes C, Sunesen M, Bowlby MR, Dunlop J. Characterization of compounds on nicotinic acetylcholine receptor $\alpha 7$ channels using higher throughput electrophysiology. *Journal of neuroscience methods*. 2009;177(1):142-8.

Papke RL, Porter Papke JK. Comparative pharmacology of rat and human $\alpha 7$ nAChR conducted with net charge analysis. *British journal of pharmacology*. 2002;137(1):49-61

Conclusion

CHO $\alpha 7$ from Galantos were evaluated on QPatch and was found to be a very well suited cell line for automated patch clamp platforms since it expresses $\alpha 7$ at a sufficiently high rate. The success rate i.e. number of sites with $\alpha 7$ current could be increased app. two fold by using multi hole plates rather than single hole plates.

The cell line was validated with both full and partial agonists and antagonists and all data generated are in agreement with previously obtained data on QPatch.