



## Application Report

### Short compound exposure times for fast-desensitizing ion channels recorded on QPatch Compact

Short ligand exposure times (<1 s) enable stable recordings of fast-desensitizing ion channels, exemplified by the nicotinic acetylcholine receptor alpha1

#### Summary

The nicotinic acetylcholine receptor alpha1 (nAChR  $\alpha$ 1) is a fast-desensitizing channel that makes the development of a stable high throughput assay on automated patch clamp systems challenging.

- The solution stacking feature on QPatch Compact (QPC) reduces ligand-exposure times below 1 s. Using this feature, it is possible to evoke stable acetylcholine signals over several agonist stimulations.
- The QPlates microfluidic flow channel architecture ensures rapid and complete solution exchange of compound and agonist and complete washout, both of which are critical for obtaining stable recordings.

Box 1: Short compound exposure is relevant for several ion channel classes, including:

- » Nicotinic ACh Receptors
- » GABA<sub>A</sub> Receptors
- » AMPA Receptors
- » Kainate Receptors
- » P2X Receptors
- » Serotonin 5-HT Receptors

#### Introduction

##### Transient ligand delivery for fast-desensitizing ion channels

Fast-desensitizing ligand-gated ion channels require short agonist application times to accurately study their function. Applications with short compound exposure are relevant for several classes of ligand-gated ion channels, see box 1. These receptors activate within milliseconds but also rapidly enter desensitized, non-conducting states if exposed to agonists for too long. In pharmacological characterization and drug screening assays, brief agonist application prevents cumulative desensitization, improving reproducibility and sensitivity. Thus, rapid and transient ligand delivery is essential for studying these channels.

A programmable automatic pipette allows the user the stacking of two solutions on top of each other in the same pipette, sep-

arated by an air gap. A variety of pipettes offer the possibility of programming and liquid stacking, including Gilson Pipette P20M (PIPETMAN M Connected), which was used in this study. Using the stacked solution technique, it is possible to reduce the ligand exposure time below 1 s.

In this present study, we demonstrate this feature employing the relatively fast-desensitizing nAChR  $\alpha$ 1 using the solution stacking feature.



Fig. 3: Illustrating two liquids stacked in a pipette tip. Upon ejection, the cell will first be exposed to the compound (red), followed by washout with saline (blue). The compound exposure time can be set by adjusting the air gap volume and pipetting speed.

##### Desensitization: physiological relevance, but assay challenge

The nAChR  $\alpha$ 1 subunit is predominantly expressed in skeletal muscle cells and it mediates fast agonist transmissions of electrical signals from motor neurons. Following agonist stimulation, the channel becomes non-selectively permeable to cations. Consequently, the membrane potential depolarizes, potentially leading to an excitatory postsynaptic potential in the muscle cell (Kalamida *et al.*, 2007).

Prolonged or repeated agonist exposure to the channel leads to a decreased responsiveness over time, a process called desensitization (Quick & Lester, 2002). Whilst this process is of great physiological relevance, it poses a challenge when studying compound effects on the channel in an *in vitro* setting.

In an ideal assay, the exact same current response is evoked following each repeated agonist stimulation. In this way any possible change in current can be attributed to the compound tested.

## Results and discussion

First, the nAChR response was characterized with regard to ACh sensitivity and biophysical properties. At a holding potential of -90 mV, the application of 5  $\mu$ M ACh (~EC<sub>20</sub>) evoked an inward current with fast opening kinetics that slowly desensitized over the short exposure time (<1s). A following wash step with saline completely abolished this current. As illustrated in Figure 2, the agonist exposure time was below 1 s.

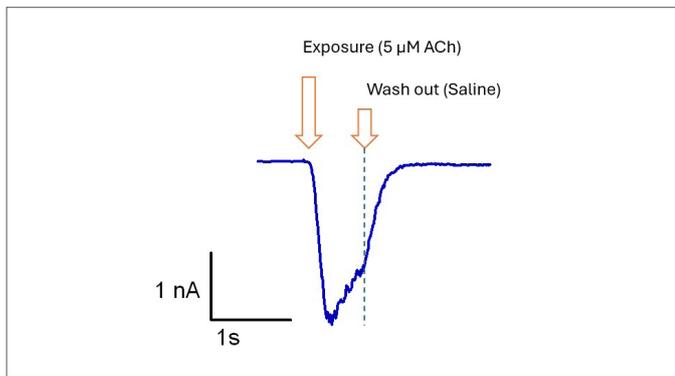


Fig. 2: Typical ACh (5  $\mu$ M) - evoked current at  $V = -90$  mV in RD (TE671) cells recorded using multi-hole QPlate. The wash solution (saline) was stacked in the pipette on top of the agonist solution; in this way, the agonist could immediately be washed out following application. The flow channel architecture of the QPlate allows for the removal of the agonist, a fact that further reduces desensitization of the channel.

Thereafter, the stability of the assay was tested: The ACh-concentration increased to 70  $\mu$ M (~EC<sub>80</sub>) and each cell was exposed to 3 stacked additions of ACh (Figure 3). Between the ACh exposures, there was an additional wash step with saline. The nAChR-mediated current was stable over time and showed no signs of cumulative desensitisation or signal run-down.

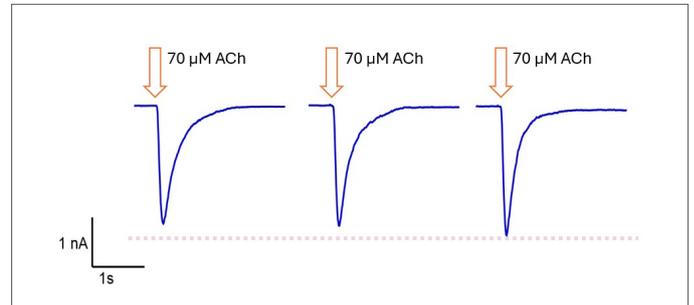


Fig. 3: Current stability over time: 3 stacked applications of 70  $\mu$ M ACh (~EC<sub>80</sub>), each followed by an additional wash step with saline. The traces are from 3 consecutive liquid periods with >1 minute in between. No accumulative desensitization or run-down was observed.

## Methods

### Cells

- RD (CCL-136, TE671) cells endogenously expressing the nicotinic ACh receptor alpha1 subunit (nAChR  $\alpha$ 1) were cultured at 37  $^{\circ}$ C and 5% CO<sub>2</sub> in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin and streptomycin, and 2 mM sodium-pyruvate. Cells were purchased from ATCC.
- On the day of the experiment, cells were harvested using detachin (Gelantis) and kept in serum-free media until further use. QPC's automated cell preparation unit was used to resuspend cells in saline just before the start of the experiment.

### Electrophysiology

- Whole-cell was gained using a single pressure pulse to -250 mbar for 4 s. Cells were afterwards clamped at  $V_{\text{hold}} = -90$  mV.
- All experiments were performed using multi-hole QPlate 8X (10 holes/well).
- The data were analyzed using Sophion Analyzer and Graph-Pad Prism 10 software.

## References

1. Kalamida, D., Poulas, K., Avramopoulou, V., Fostieri, E., Lagoumintzis, G., Lazaridis, K., ... Tzartos, S. J. (2007). Muscle and neuronal nicotinic ACh receptors: Structure, function and pathogenicity. *FEBS Journal*, 274(15), 3799–3845.
2. Quick, M. W., & Lester, R. A. J. (2002). Desensitization of neuronal nicotinic receptors. *Journal of Neurobiology*, 53(4), 457–478.

### Authors:

Kim Boddum, Senior Research Scientist  
Daniel Sauter, Science & Engineering Manager

## Sophion Bioscience A/S

info@sophion.com  
sophion.com