A HIGH THROUGHPUT MICROFLUIDIC APPROACH ENABLES FAST EXCHANGE OF SOLUTIONS AND LIGAND GATED ION CHANNEL RECORDING FROM CELL ENSEMBLE

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Abstract
Electrophysiology is the preferred technique for characterizing ion channel function and kinetics. It is the most functionally pertinent assay for screening in terms of information content. High throughput pharmaceutical screens often use a population patch approach, which eliminates cell-to-cell variability of single cell recordings. However, currently available population patch platforms have key shortcomings such as: a) the inability for fast exchange of solutions, b) the inability to apply multiple compounds to the same ensemble of cells, and c) the inability to record fast desensitizing channels.

Here we present novel data showing that by using a microfluidic network design along with population patch recording we are able to overcome these obstacles. We validated our system using cells expressing voltage-gated channels in ensembles of up to 30 cells under voltage clamp. Moreover, we are able to overcome these obstacles. We validated our population patch platforms have key shortcomings such as the inability for fast exchange of solutions and the inability to apply multiple compounds to the same ensemble of cells.

System Description

The IonFlux recording plate microfluidics integrate to SBS-standard well plates. The IonFlux system is designed to operate much like a plate reader. The entire recording protocol, including cell trapping, whole cell formation, compound perfusion, and ion channel recording is managed automatically via software. Plates are preseeded with cells, compounds, and reagents using conventional liquid handlers. Once filled, plates are fast automatically into the IonFlux system. All fluidic control is trapped to facilitate compound trapping and deliver compounds is hand within the instrument, and integrated electrodes record currents. It is the most function of single cell recordings. However, currently available population patch platforms have key shortcomings such as the inability for fast exchange of solutions and the inability to apply multiple compounds to the same ensemble of cells, and the inability to record fast desensitizing channels.

Materials and Methods

Instrument: The IonFlux system includes the following novel features:
-Whole plate integration: Unlike other automated patch-clamping systems, the IonFlux instrument is a single plate reader in both batch and window. The consumable is a microfluidic network bonded to a conventional well plate and after loading with solutions, IonFlux plates are "read" by the bench top system.
-"Single Head" Electrophoresis: Dishes are directly integrated with a pneumatic force clamping interface. There is no liquid handler.
-Continuous recording: Continuous recording is achieved by the use of multiple amplifier channels and systems are available with either 16 or 32 multiplexed amplifier (Infinitea instruments). GigaOhmohm can be addressed to 100 cells in 30 seconds. The IonFlux protocol allows for the fast trapping/mapping protocol generation as many as 60 plates.
-Microfluidics: IonFlux plates incorporate a parallel array, trapping channels have dimensions of 50 μm wide x 2 μm high. Interface provide pressure and electrical connectivity to run experiments automatically.

Cell Trapping: Ion channel recording experiments, high resistant membrane cells expressing human voltage and ligand gated ion channels were used. Cells were maintained at 37°C in ECS buffer with 10% fetal bovine serum. After the start of experiments, adherent cells were detached, spun down at 1000 rpm, and resuspended in extracellular solution on a solution warming 60°C capillary.

Procedure: Devices were first primed using positive pressure to fill all of the channels. Trapping wells were filled with electrode and compound introduction channels with the compound of interest. The main channel was filled first, establishing electrical connectivity and allowing for the measurement of ionic resistance.

Rapid Compound Application

Figure 4. Rapid Compound Application. Figure 4A shows the rapid time course of compound application measured with the fluorescent dye calcein. The red arrow indicates the start of compound application. The blue line shows the rapid exchange of solution from 0 to 90% in 100 ms. Figure 4B shows an inward Cl- current during exposure of 16 μM GABA to an ensemble of HEK cells expressing hGABA A receptor/channels. Maximum current was recorded within 400 ms in agreement with known hGABA A current rise-time.

Recording from Fast Desensitizing Channels

Figure 9. Representative averages (A) showing the response of the same cell ensemble exposed to increasing GABA concentrations (1–36 μM EC50 experiments), standard error, and a fit function (EC50 = 3.4 ± 0.8, ΔH2 = 0.66) as compared to a literature value of 15 µM (11B; Hollands et al., 2009). A 15 µM GABA co-applied with increasing concentrations of bicuculline (1A). Each blocker concentration is pre-incubated for 5 min before the GABA co-application. Plate showing inhibition of mean GABA-excitatory currents (1A) by increasing exposure to increasing bicuculline concentrations. A 15 µM (1B) provides a near complete inhibition of GABA mediated currents. A further increase in bicuculline concentration reduces the percent inhibition of the GABA mediated currents.

Multiple Compound Application to Same Ensemble

Figure 11. Cumulative dose response curves (A,B, D and H) GABA were obtained in the presence of the benzodiazepine, diazepam (1A), reproducing the known shift in GABA potency attributable to positive allosteric modulators (1B; Hollands et al., 2009).

Conclusions

- IonFlux microfluidic automated patch-clamp system provides the ability to record from an ensemble of up to 30 cells simultaneously with good agreement to manual patch recordings.
- IonFlux recording plate microfluidics integrate to SBS-standard well plates for compound and cell loading, facilitating plate prep by standard liquid handlers.
- IonFlux allows for fast exchange of solutions, application of multiple compounds to the same ensemble of cells, and the ability to record fast desensitizing channels.

Figure 8. Plate-to-plate Consistency in Current Intensity

Plate-to-plate Consistency in Current Intensity

Offset: 0.45
Frequency: 1
Figure 12. The IonFlux plates are based on the 384 well SBS-standard format (2A). Figure 2B shows 1 of 12 plates exposed to increasing ECS buffer application.

3A

2B

Figure 2. The IonFlux plates are based on the 384 well SBS-standard format (2A). Figure 2B shows 1 of 12 plates exposed to increasing ECS buffer application.