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, these results showed that there is fast compound application (<100ms). The time course of compound application was confirmed using fluorescent indicators and biological reporters, such as GABA-A expressing cells. These data also validated our ability to record from fast desensitizing ligand gated ion channels without appreciable desensitization. We compared the time course of solution exchange with and without a protective layer technique and additionally characterized application of multiple compounds to the same ensemble of cells. In conclusion, the novel microfluidic approach allows for the fast exchange of compounds and facilitates the recording of fast activating voltage and ligand-gated channels.

Materials and Methods

Instrument: The IonFlux system includes the following novel features:

•Well-plate integration: Unlike other automated electrophysiology systems, the IonFlux instrument is similar to plate readers in both footprint and workflow. 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 without the requirement for liquid addition or removal during the recording protocol. Plates can be filled by standard liquid handlers.

• Fast compound addition: Integrated flow channels enable the rapid application of multiple compounds and wash sequences.

• "Single Head" integration: Electrodes are directly integrated with a pneumatic flow control 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 64 multiplexed amplifiers to facilitate throughput. Groups of cells can be addressed by up to 8 different compounds or concentrations so that each trapping/recording protocol generates as many as 8 data points.

• Microfluidics: IonFlux plates incorporate a parallel array, trapping channels have dimensions of 1.5um wide x 2um high. Interfaces provide pressure and electrical connectivity to run experiments automatically.

Cells. For ion channel recording experiments, freshly suspended mammalian cells expressing human voltage and ligand gated ion channels were used. Cells were maintained at 37ºC in DMEM with 10% fetal bovine serum. Before the start of experiments, adherent cells were detached, spun down at 1000 rpm, and resuspended in extracellular electrolyte solution at a concentration $5x10^6$ cells/ml.

Procedure. Devices were first primed using positive pressure to fill all of the channels. Trapping well/channels were filled with electrolyte and compound introduction channels with the compound of interest. The main channel was filled last, establishing electrical connectivity and allowing for the measurement of access resistance.

System Description

Figure 1. 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 preloaded with cells,compounds, and reagents using conventional liquid handlers. Once filled, plates are fed automatically into the IonFlux system. All fluidic control necessary to trap cells and deliver compounds is handled within the instrument, and integrated electrodes record currents.

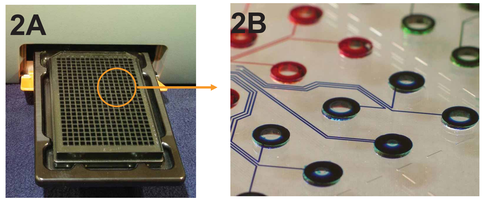

Figure 2. The IonFlux plates are based on the 384 well SBS-standard format (2A). Figure 2B shows the microfluidic network attached to the bottom of a well plate. Trapping and whole cell protocols are configured and controlled via software control.

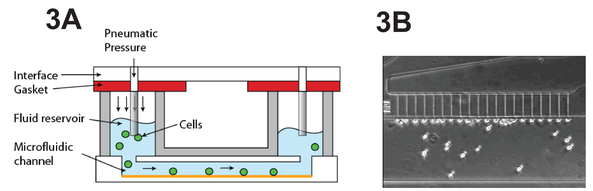

Figure 3. Pneumatic pressure controls rapid and precise fluid delivery (3A). A gasketed interface seals to an entire 384 well plate after it loads in the instrument. The interface includes pneumatic connections to each well, with positive and negative pressure regulation from computer-controlled electro-pneumatic regulators. Ag/AgCl electrodes are integrated in the interface. The IonFlux system includes up to 64 multiplexed amplifiers. Each 384-well plate includes 32 experimental zones, each containing 8 compound wells and 2 multi-cell trapping regions. Throughputs in excess of 1,000 data points per hour can be achieved. Figure 3B shows a magnification of the parallel trapping region. Each cell trapping junction is approximately 1.5X 2 um.

Rapid Compound Application

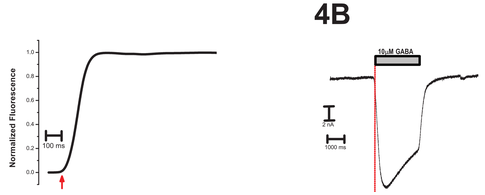

Figure 4. Rapid Compound Application. Figure 4A shows the rapid time course of compound application measured with the fluorescent dye calcein. The red arrow indicates the start of compound application. There was rapid exchange of solution from 0 to 90% in 100 ms. Figure 4B shows an inward Cl- current during exposure of 10 μM GABA to an ensemble of HEK cells expressing hGABA A receptor/channels. Maximum current was recorded within 400 ms in agreement with known $GABA_A$ current rise-time.

Ionflux Allows for Rapid Temperature Control

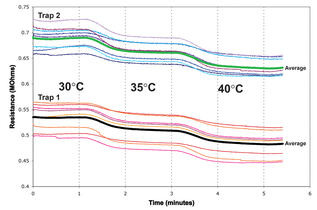

Figure 5 Ionflux allows for Rapid Temperature Control. The temperature can be adjusted from ambient to 40 °C within minutes. Ionflux data showing Resistance (Ropen) versus time with varying temperatures. The conductivity changes with increasing temperature.

Plate-to-plate Consistency in Current Intensity

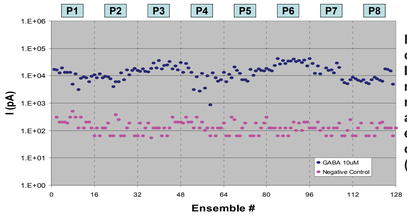

Figure 6. Plate-to plate consistency in current intensity: IonFlux data showing the magnitude of the stimulus response (10μM GABA application) for 128 cell ensembles across 8 plates, and comparison to a negative control (ECS buffer application).

Average current and Z'-values per plate

Figure 7. Key assay metrics: Z'-values were obtained by comparing the distribution of 10μM GABA applications with control application of ECS buffer (left axis, scatter plot). The overall Z'-value for the run was 0.58±0.15. Also plotted are average current per ensemble and standard deviation of the same (bar chart, right axis).

Kv2.1 Current Distribution

The Kv2.1 channel is a slowly inactivating voltage-gated potassium channel. In these experiments, current recordings as a function of voltage were generated. A comparison to "gold standard" manual patch clamp recordings shows good agreement between the two methods.

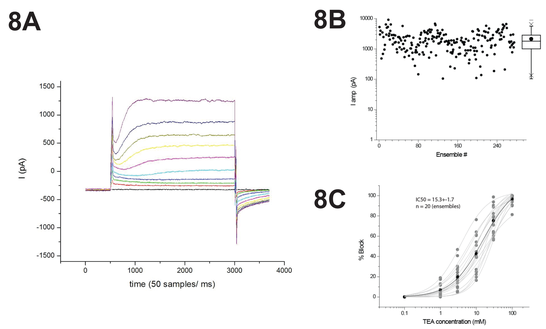

Figure 8. (8A) Current as a function of voltage for the Kv2.1 channel using a 20-cell ensemble. (8B) Representative Imax values. (8C) Individual TEA dose-response curves superimposed from n=20 ensemble recordings.

Recording from Fast Desensitizing Channels

GABA EC50

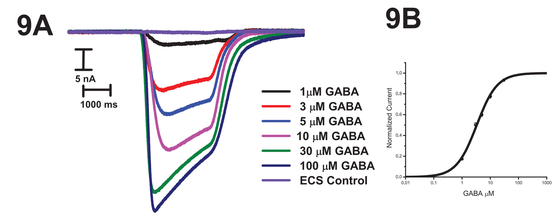

Figure 9. Representative sweeps (9A) showing the response of the same cell ensesmble exposed to increasing GABA concentrations. Figure 9B shows average response to GABA (n = 30 EC50 experiments), standard error, and a fit function (EC50 = 3.4 μM, R2=0.995) as compared to a literature value of 3.9 (Hollands et al., J Biomol Screen (2009) 7:769-80).

Bicuculine IC50

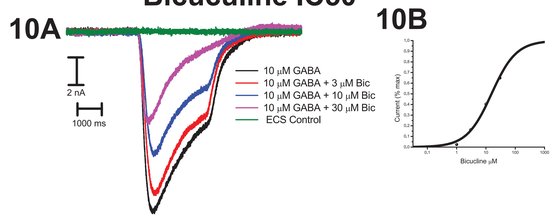

Figure 10. Response of a cell ensemble exposed to 10μM GABA co-applied with increasing concentrations of bicuculline (10A). Each blocker concentration is pre-incubated for 3 min before the GABA co-application. Plot showing inhibition of mean GABA-sensitive currents (n=8) during exposure to increasing blocker concentrations. A hill fit (10B) provides an IC50 value of 15 μM, as compared to a literature value of 2μM (Hollands et al., 2009).

Multiple Compound Application to same Ensemble

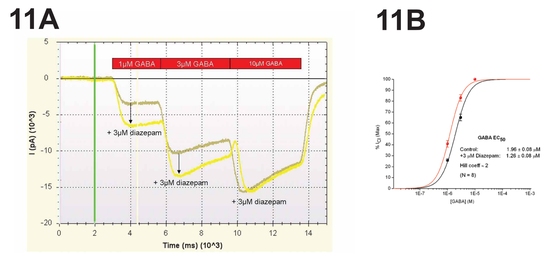

Figure 11. Cumulative dose response curves (1uM, 3uM and 10uM GABA) were obtained in the presence of the benzodiazepine, diazepam (11A), reproducing the known shift in GABA potency attributable to positive allosteric modulators (11B; Hollands et al., 2009).

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.**

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