Comparing Ensemble versus Single Cell Recordings of Voltage-Gated Channels with a Microfluidics-Based Automated Patch Clamp

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Abstract
Although automated patch clamp systems are now established systems in the drug screening pipeline, manual patch clamp is still considered the gold standard in studying voltage-gated channels. Most of the reasoning stems from the need of the highest quality in recordings matched with the lowest cost per data point. Although automated systems in recent years have achieved an improved balance between quality of channel recordings and cost, it is mostly to the detriment of effective solution exchange. In this study, we use a unique automated patch clamp system with precise in-place solution exchange and continuous flow to compare results between ensemble (optimized for quantity) and single cell (optimized for quality) recordings. The IonFlux plates are the first microfluidic patch clamp devices to achieve GΩm seals for high quality recording. We present data from potassium and sodium voltage-gated channels and compare results relative to membrane resistance and recorded current density.

Materials and Methods

CHO-HERG
CHO (Chinese Hamster Ovary) human ERG (a荨er-a-go-go related gene) cells were cultured following internal guidelines. Cell culture was done in 37°C / 5% CO2 conditions using HRS Culture Media. The cells were then transferred to a 30°C incubator for 1-5 days before running experiments to boost channel expression. On the day of the experiment, cells were lifted using DetachX (100077-508 VWR) for 15 minutes at 37°C. Verapamil was purchased from Sigma. Verapamil was first dissolved in DMSO as a high concentration stock solution (10 mM), then diluted in DMSO and 1% FBS (Fluxion Biosciences) before being diluted into the final concentrations in HRS Extracellular Solution. A negative control of DMSO solution (0.1%) was always applied before compound applications and was not found to reduce a change in current amplitudes recording 10%.

Voltage steps:
- An initial step to +50 mV for 10 ms from -80 mV holding potential, followed by an activating step to +20 mV for 2.5 seconds then a step-down to -50 mV (step 2) for 2.5 seconds. Tail recordings were taken at tail-to-tail minus current start at step 1.

hERG Culture media:
F-12 (hMEM) with GlutaMAX (1% HEPES- Intronig) | 10% FBS (E2422 - Sigma) | 1% Penicillin/Streptomycin (15444-vetgood) | Selection Antibiotics
hERG Extracellular Solution (mM):
2 CaCl2 | 1 MgCl2 | 10 D-glucose | 74 KCl | 14 NaCl | 10 Glucose

hERG Intracellular Solution (mM):
5.374 KCl | 1.75 NaCl | 130 HEPES | 10 HEPES | 120 KCl | 4 Na+6P

HEK Nav 1.7 & HEK Nav 1.8
HEK-293 cells expressing the channels of interest were utilized. The cells were cultured according to internal guidelines. Cell culture was done in 37°C / 5% CO2 conditions using Nav Culture Media. On the day of the experiment, cells were lifted using DetachX (100077-508 VWR) for 10 minutes at 37°C. A stock solution containing 10 mM labodoxil in ECS was diluted in a three-fold dilution series for dose response experiments.

Voltage steps:
- Starting from a holding potential of -80 mV, an initial step to -20 mV for 50 mV to maximize channel availability, and then to -30 mV for 50 mV. All voltage protocols were applied at 1 Hz.

Nav Culture Media:
Gibco DME/HAM12 (10082-012-vetgood) | 10% FBS (H2422 - Sigma) | 1% Penicillin/Streptomycin (15444-vetgood) | Selection Antibiotics

Nav Extracellular Solution (mM):
137 NaCl | 5 KCl | 2 CaCl2 | 1 MgCl2 | 10 glucose | 100 mM NMDA

Nav Intracellular Solution (mM):
100 CsF | 65 CsCl | 50 HEPES | 5 Na2 | 6.67 Gluconate

IonFlux Mercury
The IonFlux Mercury fully integrated automated patch clamp system features a next-generation pneumatically driven in-plate perfusion, providing precise fluid control and continuous flow for IonFlux plates, based on standard configurations of 96 or 384 wells can be preloaded with cells, compounds, and reagents using conventional liquid handling or pipettes. Plates are loaded into the system like a simple plate reader. Each pattern in an IonFlux plate has 8 compound wells, 2 trap recordings filled with intracellular fluid, 1空for cells and extracellular solution, and 1 outlet for waste (right). Cells are captured from suspension and then patched in whole cell configuration. Plates come in two configurations: 20-cell ensemble and single cell. Both plate formats provide high quality recordings (with GigaOhm seals recorded in single-cell plates).

CHO-hERG Single Cell Recordings

- CHO-hERG Single cell recordings: Left: Superimposed single cell recordings under an increasing concentration of Terfenadine. Right: HEK 1F plot of Terfenadine blocking hERG, IC50, 0.6 µM (hERG).
- CHO-hERG Giga Seals & Current Recordings

- CHO-hERG giga seals and currents success rate: Left: percentage of cells within a plate showing membrane resistance of >200 GΩm and above. Right: Percentage of cells showing a measured tail current of 200 pA and above. Tail currents were measured at -50 mV with a preceding depolarizing step to +20 mV for 2 seconds. Measurements were taken at 5, 10, 15, and 20 minutes (N = 24).

Conclusion
IonFlux combines enhanced next generation fluidic control with in-plate continuous solution flow. It has long been used for facilitating ligand-gated assay development where complex fluidic exchanges are required. Although ensemble recordings have been most utilized due to their suitability for pharmacological studies of ion channel activity, single cell recordings with IonFlux provide a great cost-effective path to high quality data from an automated patch clamp device. This data set shows that GigaOhm seals with non-glass coated plates are achievable as well as sustainable.