Modulators of ligand gated ion channel (LGIC) activity are being actively developed by a number of leading pharmaceutical companies. Electro-physiology assays remain the gold standard for determining functional compound effects on these targets, and pose unique challenges due to the need for accurate temporal control of agonist and compound application. In this study, we present results from complex assays enabled by a novel microfluidic automated patch clamp platform. The data includes case studies from NMDA receptors, nicotinic receptors and GABA receptors and trade-offs between the different available measurement modalities developed. For targets where cell byproducts activate the channel (such as NMDA) continuous perfusion is required for removing such products from the extracellular space to maintain a robust assay. Continuous perfusion is also essential for accurate measurements of channel recovery times which are important (nicotinic receptors).

IonFlux System Description

![Image: IonFlux System Description](image)

**Figure 1.** The IonFlux system is designed to operate much like a plate reader. The entire recording protocol, including cell loading, 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 transferred automatically into the IonFlux system. After the initial loading, each well is filled with the designated loading solution and treated within the instrument, and integrated electrode record currents continuously.

**Figure 2.** Each well contains 2 wells, 2 trap recordings, 1 inlet for cells, and 1 outlet for waste. The cells and compounds are loaded at the same time, which eliminates the liquid handling robotics in the instrument. The microfluidic approach enables continuous perfusion, and fast fluid exchange. Ensembles sum the signal over 20 pach cells per recording with high consistency. The single cell consumable records from one cell per recording with blazed noise.

**Figure 3.** IonFlux Principles of Operation. Extracellular solution flows in the main flow channel during experiment. Compound is programmed to flow into the main flow channel at a specific time, and compound addition time is calculated to ensure solution in main channel serves as compound versus channel depletion. Continuous perfusion perfusion and signal acquisition ensures the IonFlux was well suited for ligand gated ion channel recording.

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**Figure 4.** Cumulative concentration response (EC50) for Glu. Since the NMDA receptor shows little desensitization in presence of Glutamate, it is possible to do a fast, cumulative concentration-response experiment on the IonFlux platform. Shown are 16 ensemble recordings from 1 plate run, there are 64 recordings (2 plates, 8 Comp Channels, 1 inlet for cells, and 1 outlet for waste). The cells and compounds are loaded into the plate, delivery of compounds is programmed to inject compound wash between compound applications. The IonFlux system has been utilized for robust averaging (defined as 100%). Minimum response is 0%.

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**Figure 6.** One of the hallmarks of the NMDA receptor is that it requires both Glutamate and Glycine to open fully. This experiment can be challenged by having to use reporters from neighboring cells. Due to the superior perfusion scheme employed in the IonFlux microfluidic platforms, contamination from nearby cells is a problem. Continuous perfusion is not necessary to maintain high levels of magnesium in the extracellular solution to keep the channels blocked between agonist applications.

**Figure 7.** Characterization of the NMDA receptor. Panel A shows ensemble currents evoked by escalating concentrations of Glutamate in the presence of 30 µM Glycine. Panel B shows peak current responses over time for 10 µM compound exposed to the escalating compound concentrations. Panel C shows the Hill fit of the concentration-response response on an extra-log scale. The individual recordings were normalized to the largest peak response.

**Figure 8.** Recovery from desensitization. The NMDA receptor shows rapid desensitization in response to a short application of ACh. Panel A shows the response to a 1 second long application of ACh followed by a 1 second long wash and then a 2nd 1 second long application of ACh. Expanding the wash time is between the ACh, the response is visible to 15 seconds. In a heterogenous expression system, one expects a mixed population of these two forms. These two forms vary in their sensitivity, up to 100% sensitivity, to reversible desensitization. The upper panel are currents evoked by Glu and Gly from neighboring cells and Ca2+ toxicity once the channels have been opened.

**Figure 9.** The Neuronal nAChR tetramer consisting of two NR1 subunits and two NR2 subunits, they can assemble into different stoichiometries. In a heterologous expression system, they can assemble into different stoichiometries. The Neuronal nAChR, therefore, is important for investigating the role of nAChR in learning and memory. Amnesiacs typically have reduced levels of functional nAChRs.

**Figure 10.** Pharmacology of the nicotinic nAChR. Panel A plots EC50 (defined as 100%) and Hill coefficients (nH) for compounds. The EC50 is determined by a competition with 1 nM [3H]QNB(35). Binding of [3H]QNB is competed by increasing diamox (7A) and nicotine (7B) concentration. (C) EC50 is determined by competitive binding with the unlabeled nicotine (7B) and competitive binding with [3H]QNB (7A). The data were normalized to peak response (defined as 100%). Minimum response was obtained by subtracting 5% from the total peak values. The concentration-response curves are fitted a Hill equation (not shown) or to a double hill equation (B) using EC50. The data were normalized to peak response (defined as 100%). Minimum response was obtained by subtracting 5% from the total peak values. The concentration-response curves are fitted a Hill equation (not shown) or to a double hill equation (B) using EC50.