

Technical Report

Determination of IonFlux Fluid Exchange Speed

Introduction

The ability to apply compounds to the trapped cell is an important requirement for ligand-gated ion channels, which activate and desensitize rapidly (<100 ms). To characterize solution exchange speed on the IonFlux, channel blocker can be perfused while ion channels are kept open at a constant activation voltage. The speed with which solutions may be exchanged and can be characterized by measuring the corresponding ion channel activity.

Materials and Methods

1. Recording Plates. *FluxPlates* (design version I01, 96-well) were used for recording. In this design, a 96-well plate contained 6 separate recording regions, each with 8 cell trapping sites and 6 compound concentrations. The dimensions of cell trapping channels were approximately 2µm wide x 2µm high. The SBS-standard well plate format allows cells and solutions to be added to the plate using either manual pipettes or automated liquid handlers.

2. Cells. For ion channel recording experiments, freshly suspended CHO – Kv2.1 cells were used (Ionescu-Zanetti et al., 2005). Cells were maintained at 37°C with 5% CO₂, in DMEM with 10% fetal bovine serum. Tetracycline (Sigma) was added to the culture medium to a final concentration of 4 µg/ml, 24 hours prior to recording to induce Kv2.1 expression. Before the start of experiments, adherent cells were trypsinized, spun down at 1000 rpm for 0.5-1 minute, and resuspended in extracellular solution at a concentration 5x10⁶ cells/ml. The spin-down procedure was repeated to ensure a clean cell suspension.

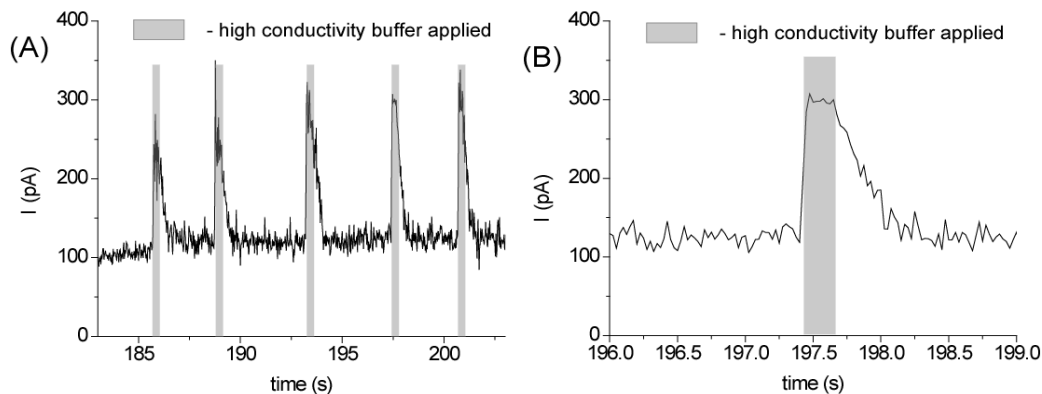


Figure 1. Timescales for microfluidic compound exchange. The compound application speed using the IonFlux microfluidic compound exchange system is measured by applying a series of pulses from a high conductivity buffer solution to the extracellular space of a trapped cell (A). High conductivity buffer results in an increase in current at a constant voltage. A zoom into one of the pulse profiles shows an 'ON' time of 25 ms, and removal times 500 ms (B).

3. Protocol. Wells of a 96 well plate were loaded with the appropriate fluid; either cell suspension, electrolyte, or compound and the microfluidic channels were primed by applying positive pneumatic pressure (3 inHg). Both the external and internal electrolyte solutions contained (mM): 140 KCl, 2 CaCl₂, 2 MgCl₂, 20 HEPES, 10 Glucose. pH was adjusted to 7.3 with KOH. Compound solutions consisted of a range of TEA concentrations substituted for external KCl (0.1 to 100 mM, Sigma). The main channel was filled last, establishing electrical connectivity and allowing for the measurement of access resistance. Cells were trapped sequentially by applying suction to the trapping channel. Whole cell sealing resistances above 100 Mohm were considered adequate for measurement. Perfusion on-time was characterized by introducing a higher conductivity extracellular buffer (150 mM KCl) as “compound”. Because of the increase in conductivity, a corresponding current spike is recorded.

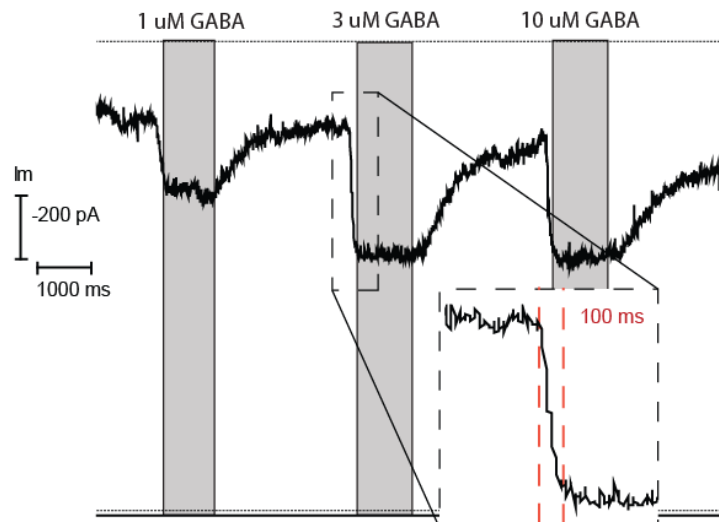


Figure 2. Compound exchange timescale – GABA A for a 20x cell ensemble. The compound application time is increased to 80ms for an ensemble of 20 cells (10 -90% ON time). Shown are averaged responses to agonist applications in increasing concentration from 1μM to 10μM. 1μM is the expected EC50 value.

Results and Discussion

Fig. 1 shows that the IonFlux Uni ‘ON’ time for a single cell under fluidic exchange is about 25ms. This fluid exchange time can be optimized by changing the geometry of the injection ports as well as increasing the flow rate in the main channel, but is a best case scenario in the sense that only one cell is being recorded from.

Fig. 2 shows exchange speeds in response to an agonist (GABA) for IonFlux Multi and an ensemble of cells arranged in a row along the side of the main flow channel. The ON time is increased due to the larger sample volume that needs to be replaced. Proof of concept data shows sub 100ms ON times.