

Introduction

Automated patch clamp platforms have become indispensable tools in increasing throughput for compound validation in drug screening.

Qube is a 384-format automated electrophysiology system with a state-of-the-art microfluidic channel system in the QChip.

The NMDA gated ion channel is activated by glutamate which is the primary excitatory neurotransmitter in the central nervous system (CNS), and in combination with the co-agonist glycine the NMDA channel is activated. Functional impairment of the NMDA receptor causes a variety of CNS-related diseases thereby making it a prime therapeutic target for drug discovery. Upon glutamate binding, the agonist-bound NMDA receptor opens an ion channel that is nonselective to cations. A property of the NMDA receptor is its voltage-dependent activation, a result of ion channel block by extracellular Mg^{2+} . We characterized the properties of NMDA ion channels in the presence and absence of the blocker ifenprodil and compared data generated on the Qube to literature values and found a good correlation.

In conclusion, the microfluidic design of Qube consumable; the QChip has proven to be effective for rapid and complete liquid exchange for fast ligand-gated ion channels.

Materials and methods

All experiments were done on NMDA NR1/NR2B expressed in HEK293 kindly provided by ChanTest. The cells were grown and induced according to SOP by adding 1 μ M/ml tetracyclin 24 hours before use. 200 μ M ketamine was added to the culture flask after induction. The cells were harvested using detachin kept on the Qube system in the cell hotel prior to the automatic preparation.

Solutions

Extracellular ringer

145 NaCl, 4 KCl, 1.8 $CaCl_2$, 10 HEPES, 10 glucose (pH 7.45), 315 mOsm.

Intracellular ringer

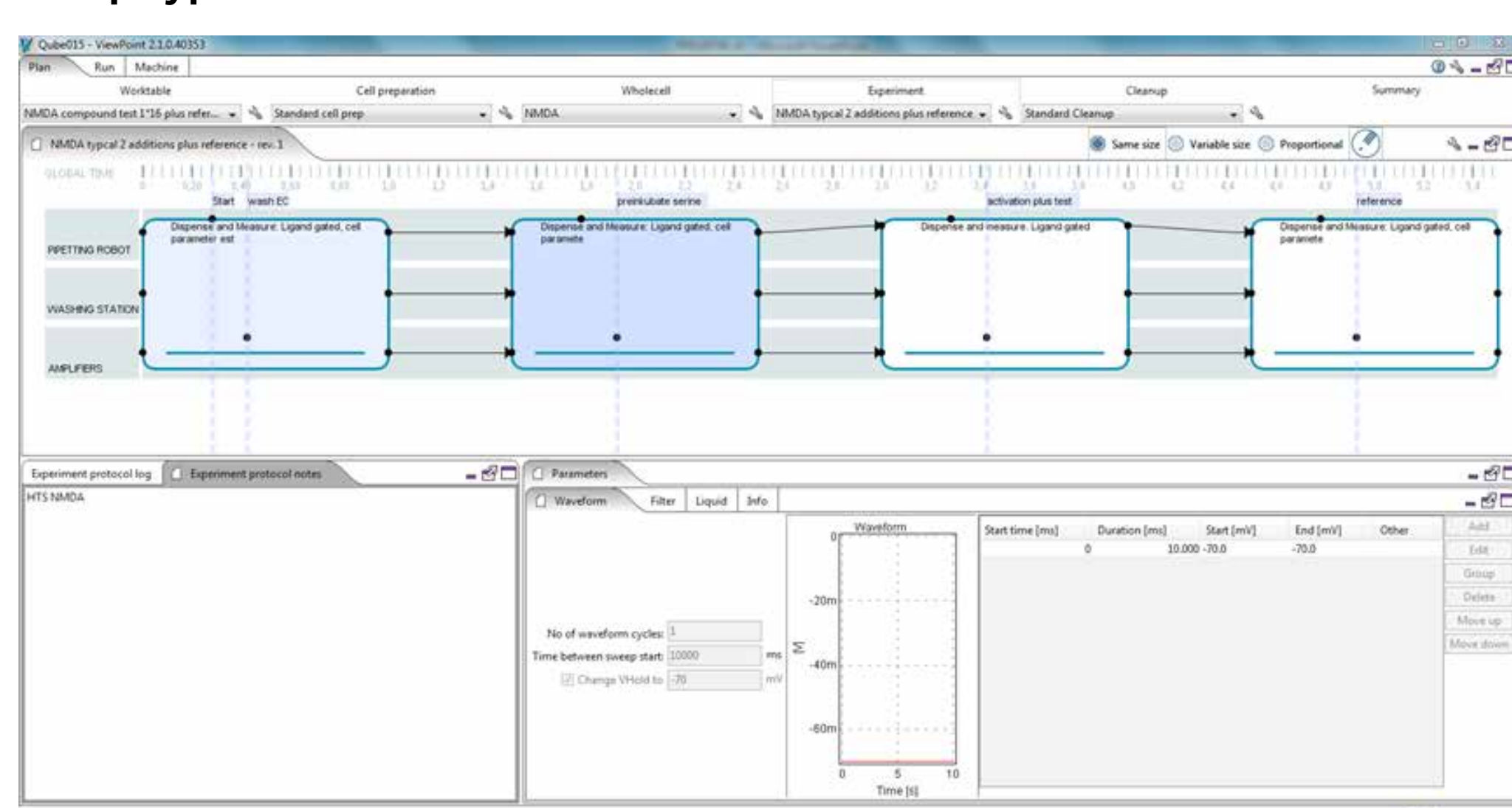
20 KCl, 130 CsF, 1 EGTA, 10 HEPES (pH 7.2), 295 mOsm.

The NMDA channel was typically activated with a combination of either 10 mM glycine/100 mM glutamate or 20 mM D-serine/20 mM glutamate made freshly every day of experiment. The compounds were also prepared freshly from DMSO stocks every day, and compound plates were made using a Zephyr liquid handling robot.

Patching and whole cell formation

Patching is done using a conventional standard WC protocol where the cells are positioned and patched with a -50 mbar pressure and whole cells are formed with 2 pressure pulses of -250 mbar – the holding potential is set to -60 mV throughout the experiment after break-in.

Setup typical 2 source test with reference



[Fig. 1]: Experiments on Qube consists of 5 parts

- "Worktable" defines where the compound plates to be tested are placed and also if a stacker of a reference is in use.
- "Cell preparation" defines the parameters for the on board spin down and resuspension of the cells.
- "Wholecell" is where the suction parameters for patching and whole cell formation are defined.
- "Experiment" is where the actual experiment protocol is defined. It consists of building blocks containing information on what liquid to be added and the applied voltage protocol for each liquid period.
- "Automatic clean up protocol" is the parameters for cleaning up between plates during unattended operation.

The experiment shown is the typical setup used for NMDA when testing modulators. It consists of a wash step, a preincubation step, an activation and compound test step followed by a reference step. The waveform is a basic constant holding potential of -70 mV.

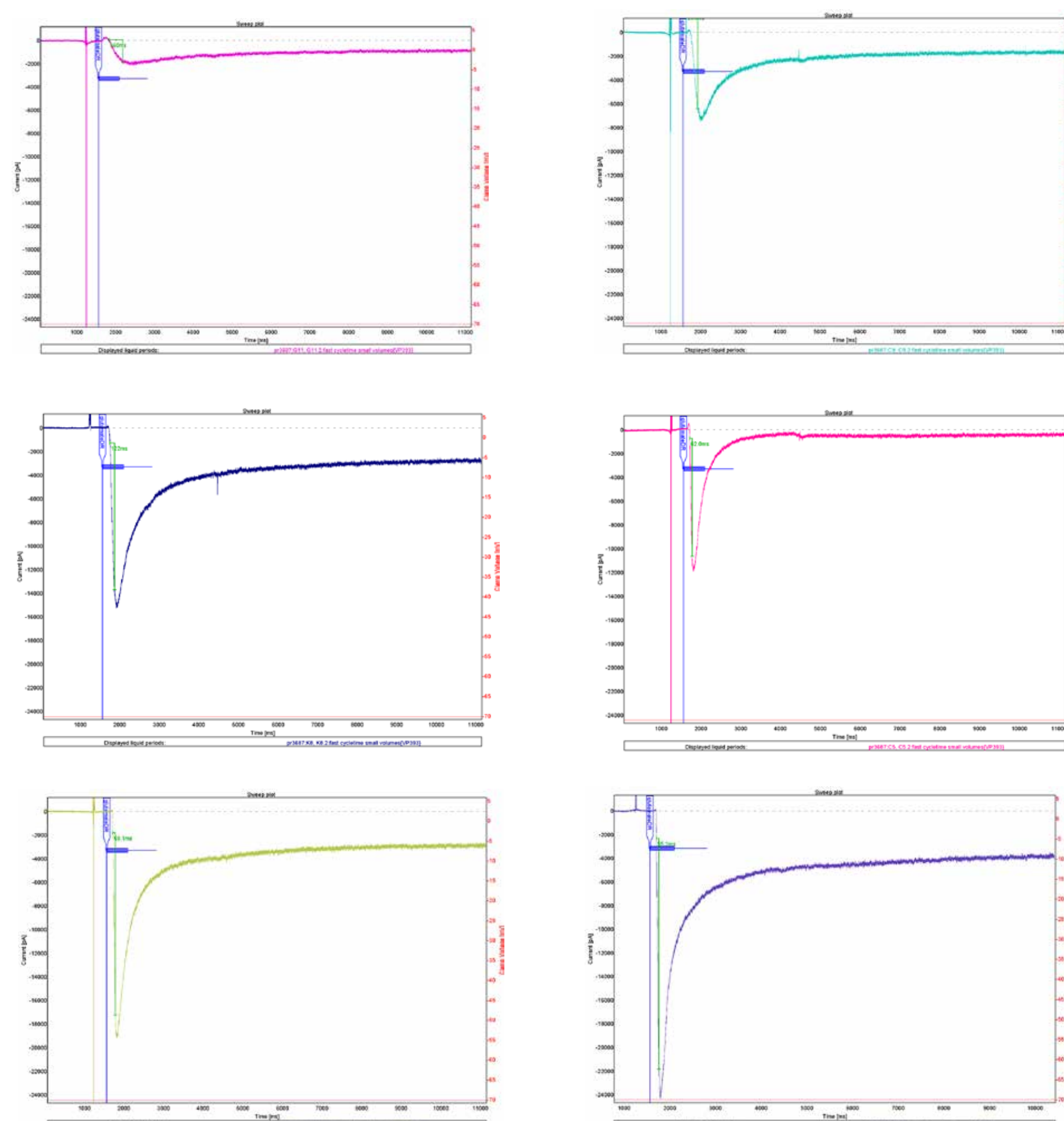
Conclusion

In this study we have investigated ligand gated NMDA channels in a 384 format on Qube. The data presented here demonstrates that Qube is capable of running NMDA assays in a robust and reproducible manner. The Z' factor presented here shows that the assay has a sufficient assay window for HTS use and is stable both within and between days.

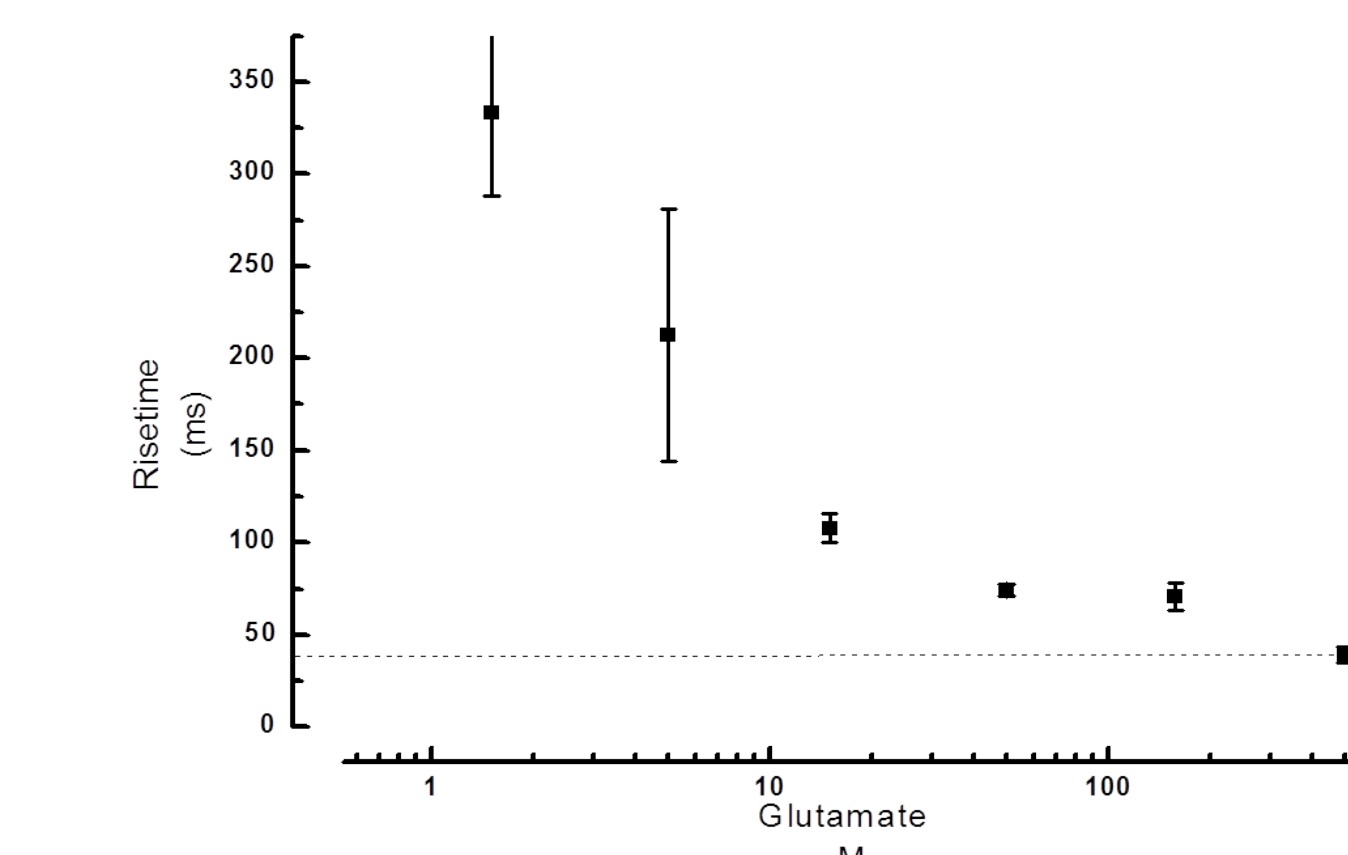
The IC_{50} values for ifenprodil and MK801 are as expected demonstrating that the Qube can also be used for profiling of NMDA blockers. The NMDA rise time on Qube is similar to the rise time measured on QPatch.

Combined these data demonstrates that Qube is capable of generating ligand data of a high quality in and high throughput manner.

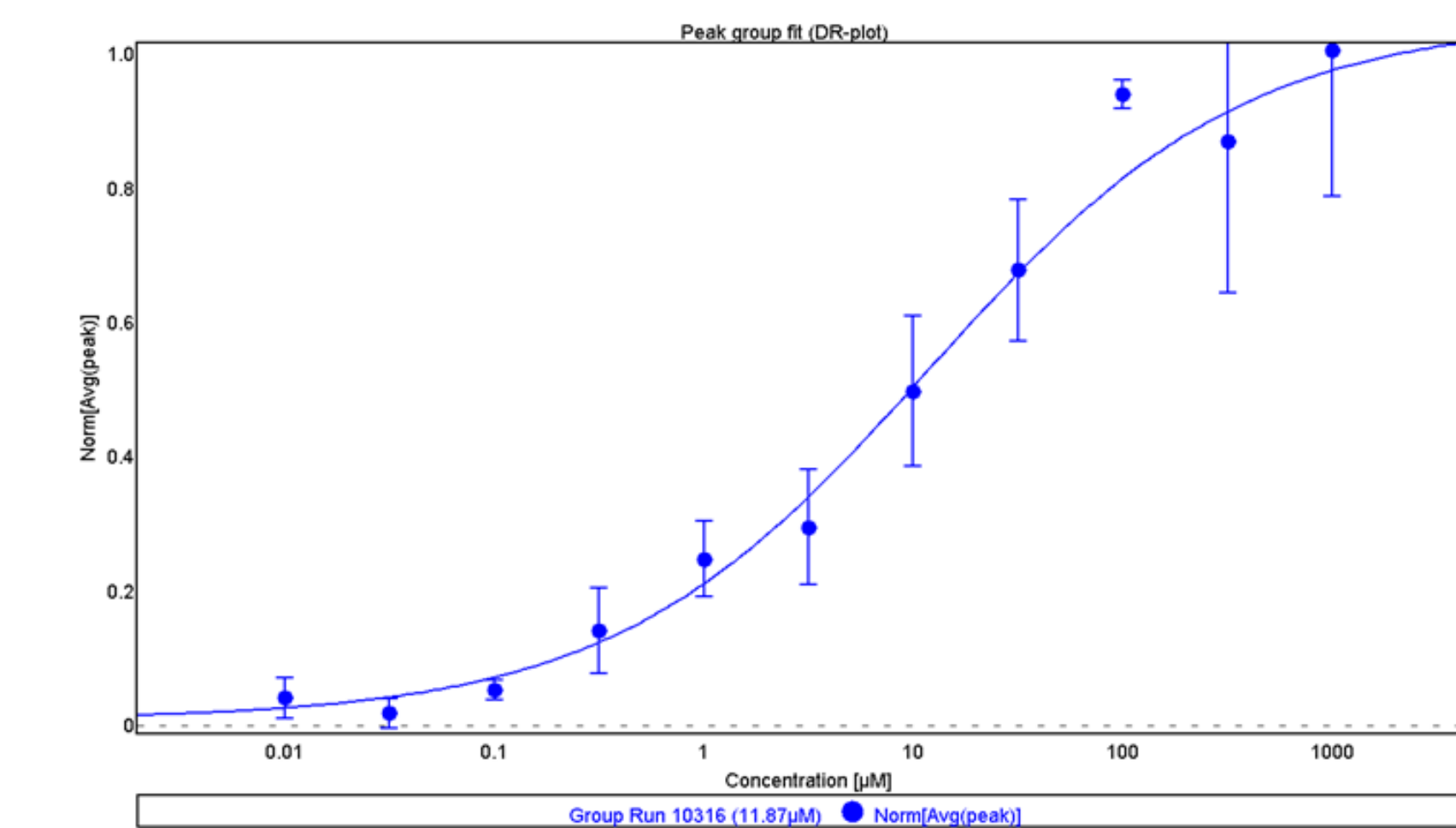
Glutamate dose response and rise times



[Fig. 2A]: Raw data sweeps from a glutamate dose response experiment
The channel was stimulated with different glutamate concentrations across a QChip384, 1, 3, 10, 30, 100 and 300 μ M glutamate was added together with a constant concentration of 10 μ M glycine. The sweeps represent typical responses from the various concentrations in that order.



[Fig. 2B]: Raw data sweeps from a glutamate dose response experiment
The 10-90 % risetime was derived from each sweep using Sophion Analyzer and is shown as a function of glutamate concentration. The rise time is 45 ms at 300 μ M. Error bars represent \pm SD.



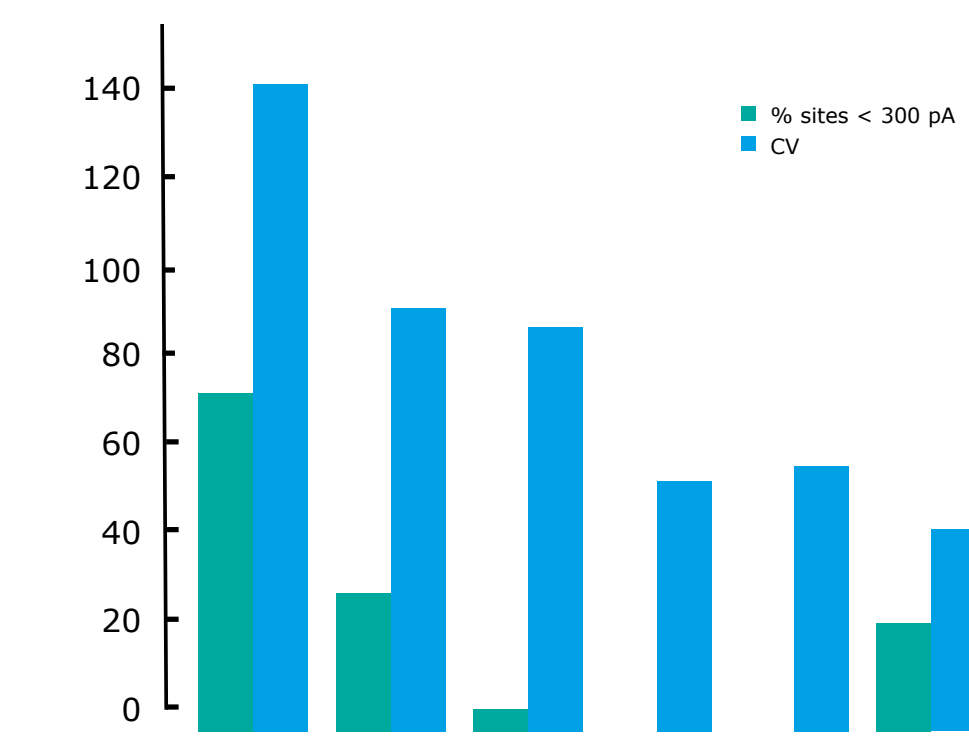
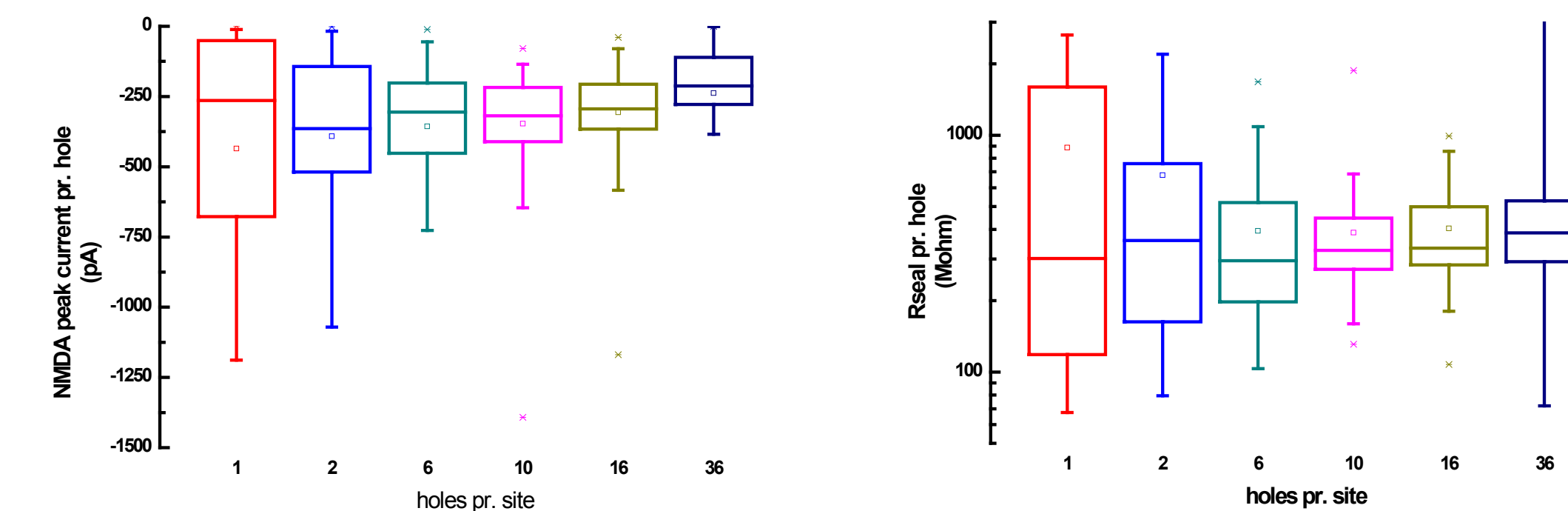
[Fig. 2C]: State glutamate dose response curve

The experiment was done using 2 baseline recordings (20 μ M D-serine/20 μ M glutamate), the test response (20 μ M D-serine/ X μ M glutamate), followed by a reference response (20 μ M D-serine/1000 μ M glutamate). Currents from state were derived from each sweep. The response were normalised to the baseline and the resulting result was as a function of glutamate concentration. EC_{50} is estimated to be 0.52 μ M. Error bars represent \pm SD.

References

Zhang JH, Chung TDY, Oldenburg KR (1999). "A simple statistical parameter for use in evaluation and validation of high throughput screening assays". Journal of Biomolecular Screening 4: 67-73.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KM, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010), Pharmacological Reviews 62, 405-496.

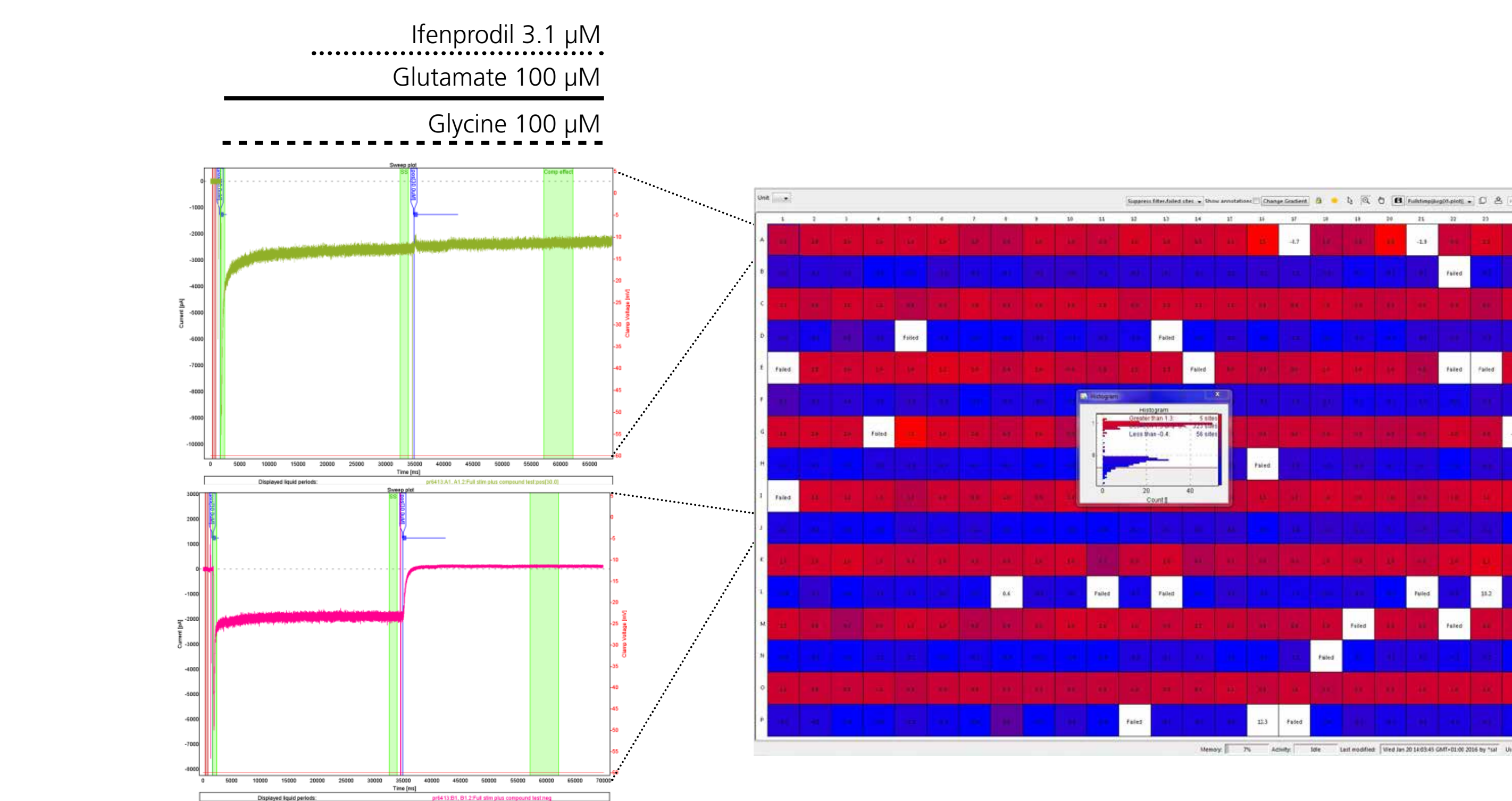


[Fig. 3B]: Basic properties of NMDA on Assay Development QChip.

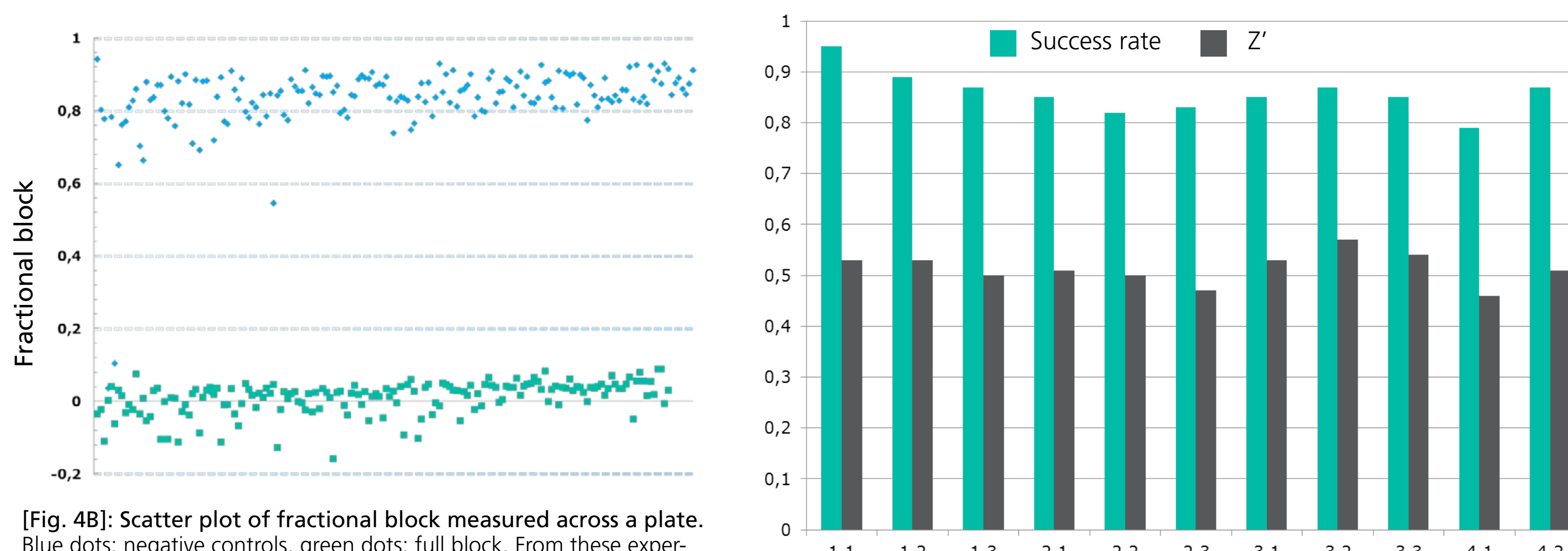
[Fig. 3A]: NMDA tested on Assay Development QChip.

Biolin provides a QChip that has a variety of holes pr. site. These plates can be used for an experimental determination of the optimal number of holes. It can be seen the variation in current amplitude decreases as a function of hole numbers pr. site. Also it was found that the number of sites with less than 300 pA current decreased and no sites with less than 300 pA are found using QChips with 10 holes. The remaining of the experiments with NMDA were run with QChip 384X with 10 holes.

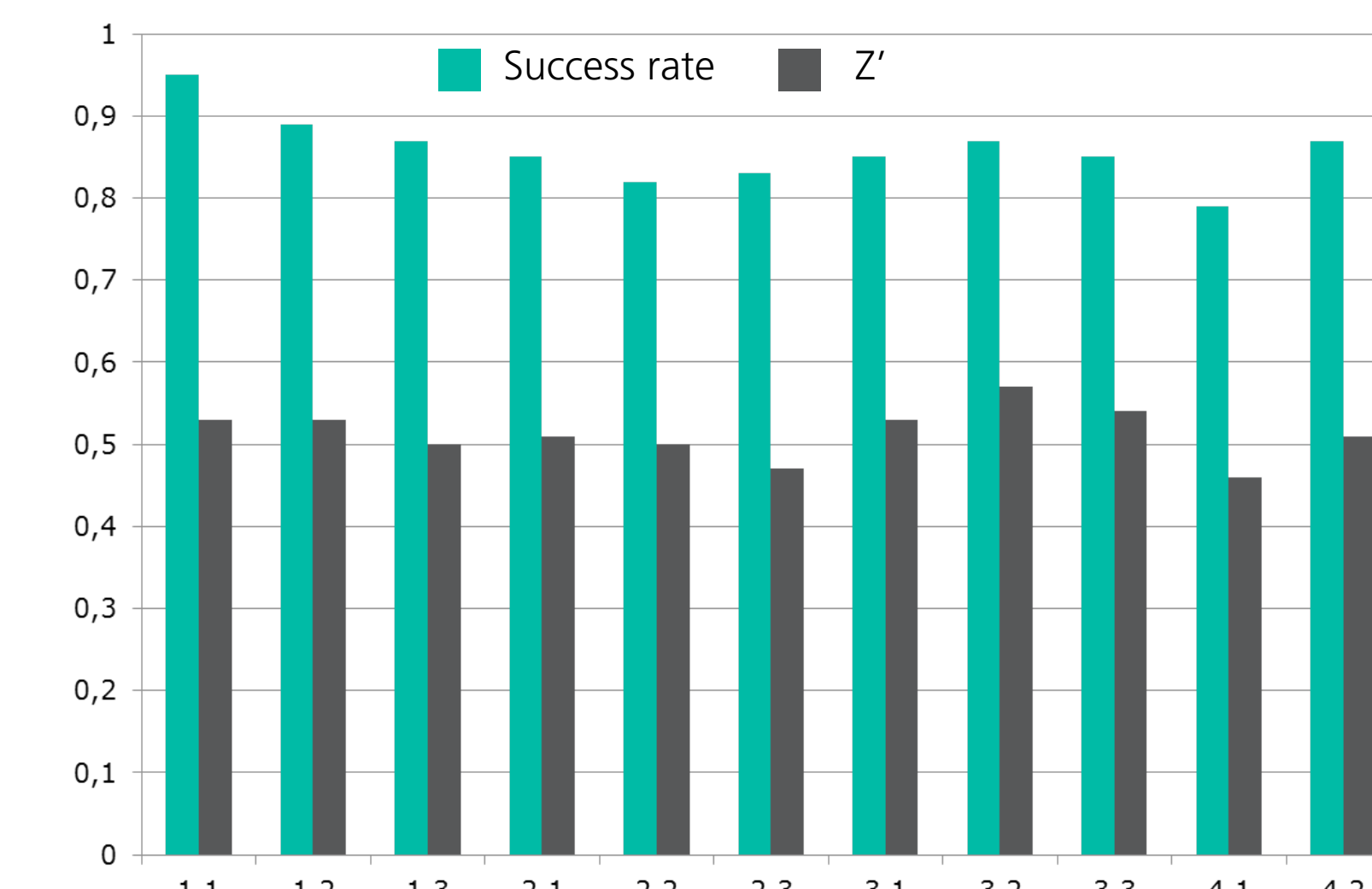
Success rates and stability



[Fig. 4A]: Plate view of fractional block from a typical Z' factor experiment.
A compound plate with positive (30 μ M Ifenprodil) and negative (0.1 % DMSO) controls was run 3 times over three days to evaluate the NMDA assay stability using the setup presented above. "Full block" is presented as blue, "no block" is red. A sweep from each situation is also shown. Z' factor was calculated as described in Zhang.

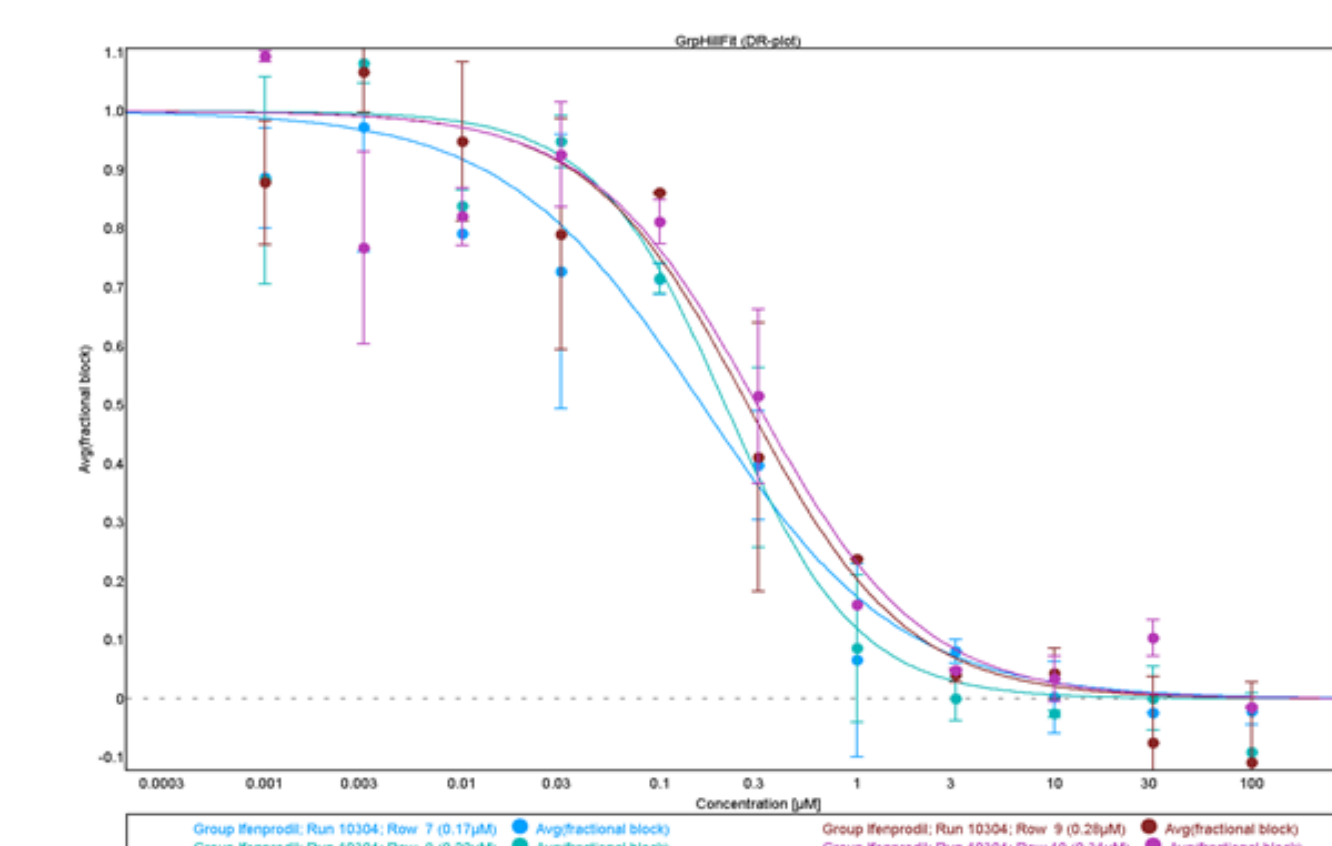


[Fig. 4B]: Scatter plot of fractional block measured across a plate.
Blue dots: negative controls, green dots: full block. From these experiments it was possible to derive a number of filters that gave the best success rate with the best Z' factor. These were steady state baseline current > 600 pA combined with standard deviation cursors detecting for noise again detecting for cell seal instability. For this run the success rate was 87% and the Z' factor 0.57.



[Fig. 4C]: Histogram of success rate and Z' factor from 3 days of experiments.
The Z' factor experiment was repeated on three different days in order to determine the stability of the assay. It was found that the average success rate was 86% with an average Z' factor of 0.51

Ifenprodil dose response



[Fig. 5A]: Ifenprodil. The resulting IC_{50} curves are shown to the left. The derived IC_{50} 's using 2 sites pr. point are summarised in the histogram. Error bars represent \pm SD.

