Multi-parameter ion channel screening: mechanism-of-action data directly from HTS

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1 ABSTRACT

High-throughput automated patch clamp (APC) is quickly emerging as the new gold standard technology for screening compounds against ion channel targets. The latest APC platforms, such as the Qube, enable true giga-seal quality data generation, comparable to conventional patch clamp.

Traditional plate-based screening technologies rarely allowed for analysis of multiple parameters to detect potential differences in the mechanism of action of novel compounds. Using multiple data analysis cursors in the Sophion Analyzer software, it's possible to gain valuable insight into compound activities on a particular ion channel target, which may translate into different results in further in vivo experiments. This also increases the value of APC data, as multiple parameters can be extracted from each well for both data quality control as well as hit detection.

Here we present Qube data for a 10,000 compound pilot screen for a sodium channel activator project, with analysis for multiple hit parameters, demonstrating the ability of the assay to detect novel compounds with different mechanisms of action.

2 MATERIALS AND METHODS

Cell Culture: HEK-Nav1.1 cells were produced at Charles River Laboratories and are commercially available. All cells were grown according to their respective SOPs as developed by Charles River. Cells were kept in a serum-free medium in the cell hotel for up to 6 hours during experiment.

Solutions: Extracellular Saline (EC0.0.0) and intracellular saline (IC5.0.0) prepared according to Sophion Bioscience SOP. For compound additions, 0.05% Pluronic acid F-127 was used to prevent adsorption to the plastic.

Qube experiments: All experiments were carried out using the Qube platform which performs 384 parallel and independent patch-clamp recordings on a disposable, multi-hole QChip. Experiments were run unattended using the automated stackers.

Analysis: Data analysis was performed using Qube Analyzer and Dotmatics Vortex software.

3 RESULTS

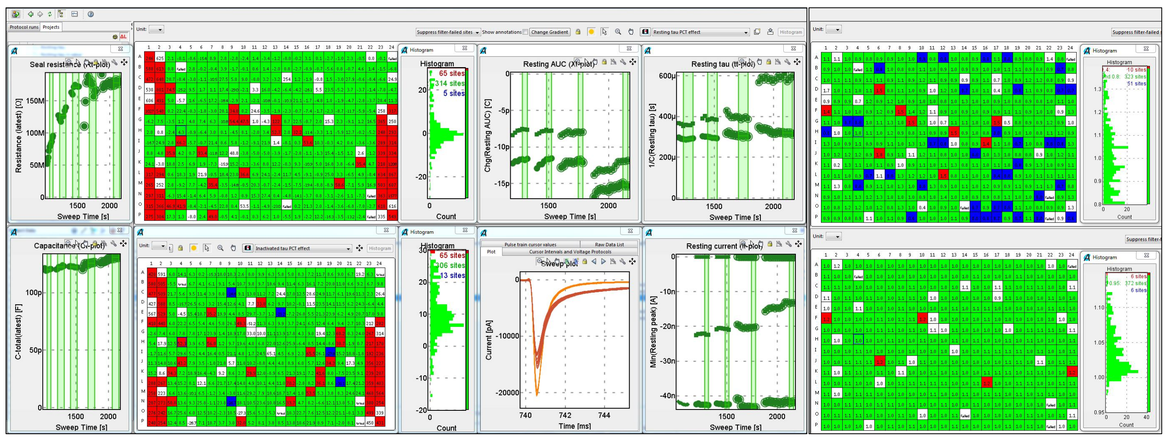

Figure 1. Multi-parameter analysis of sodium channel activator data. Results are plotted using four hit parameters (area-under-curve (AUC) and tau for resting and inactivated states) and three quality control parameters (peak current for resting and inactivated states, seal resistance and capacitance). Cut-off limits were defined individually from 3*SD of the DMSO control wells for each parameter, and each plate is coloured using these limits, shown in the histograms. Control wells are in columns 1,2, 23 and 24. Well H4 is highlighted, showing a compound with an effect on AUC in both states, but only on the inactivated state tau and peak current.

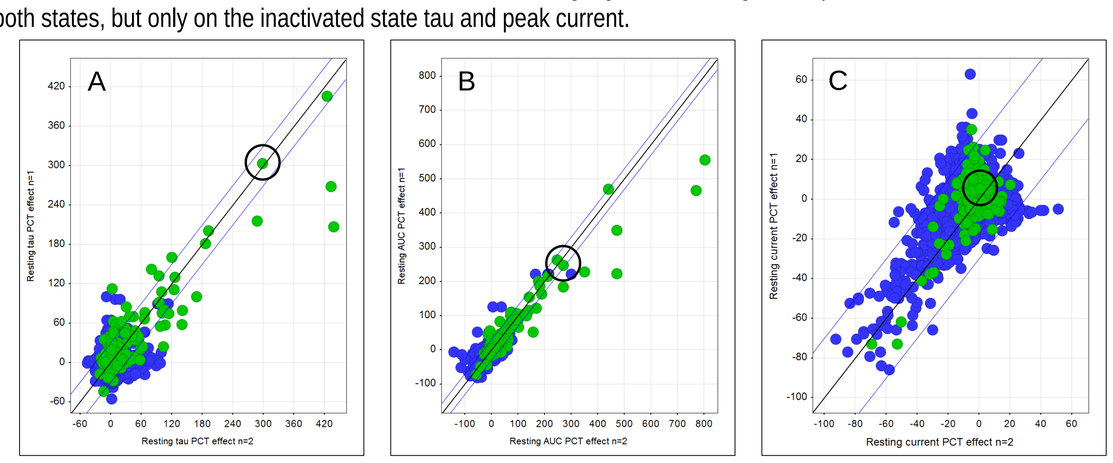

Figure 2. Correlation data for each hit parameter n=1 versus n=2 from the pilot screen. Compound data correlated well for each hit parameter. The highlighted compound had a significant effect on the resting state tau (A), average effect on resting state AUC (B) and no effect on the resting state peak current (C). Compounds from a plate containing known activators are highlighted in green.

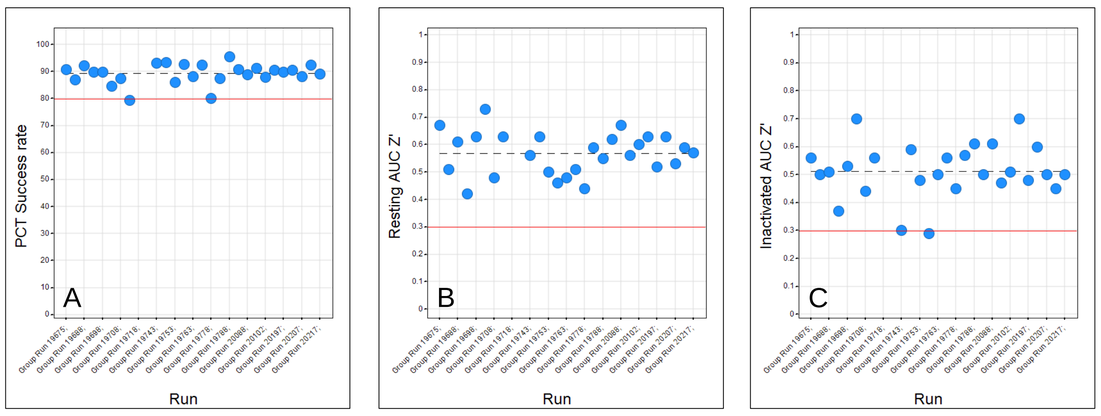

Figure 3. Assay quality parameters from the pilot screen. Success rates were acceptable, at above 80%, with average success rate at ~89% (A). The Z' values for the primary plate QC parameters, resting and inactivated state AUC, were above 0.3 (B and C, respectively), which was considered acceptable due to the variability of the activator compound effect.

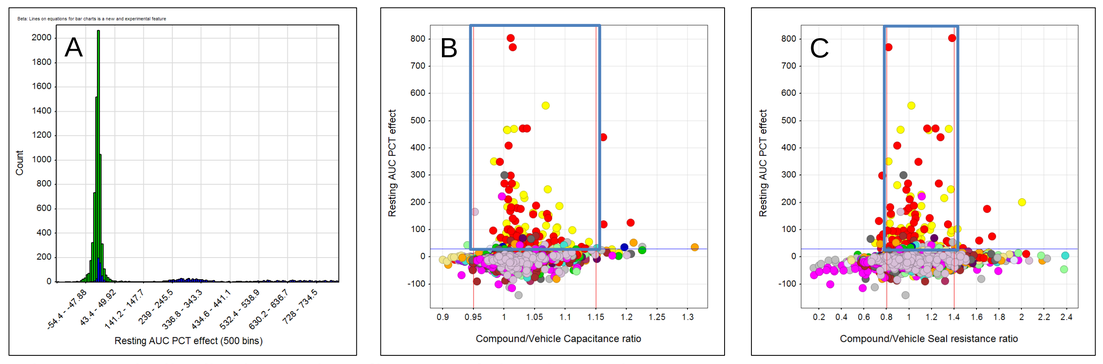

Figure 4. Hit distribution and quality control data for resting state AUC. Control wells are shown in blue, and compound wells in green in A. The peak of the hit distribution histogram was centred on ~0% effect, with 3*SD of the DMSO control wells ±30%. Using these values as the hit cut-off, data were verified using the change in seal resistance and capacitance, with acceptable limits denoted by the red lines in B and C. Verified hits are highlighted in the blue box in B and C. Hits outside the limits for seal resistance and capacitance change were labelled artefacts.

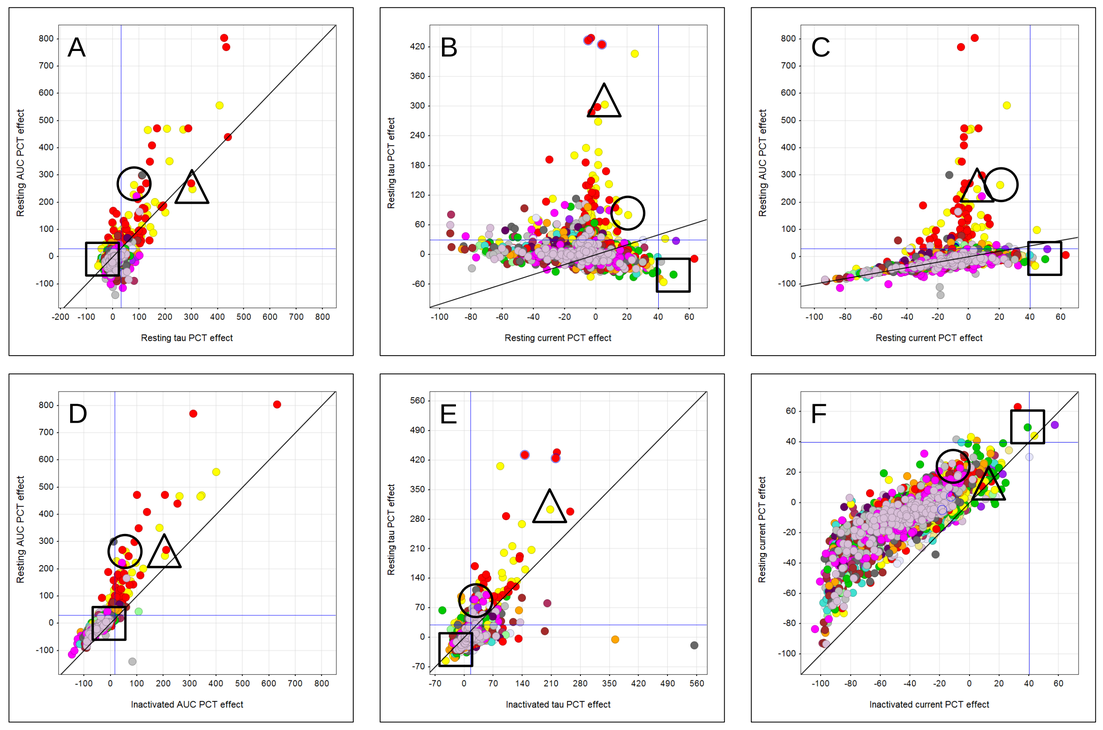

Figure 5. Comparison of hit parameters to identify compounds with different mechanisms of action. By analysing all hit parameters in parallel enabled the rapid detection of compounds with different effects on each parameter, including state-dependency, in order to optimise the compound profile for further *in vivo* validation of the mechanisms. For example, the desirable compound profile might be an increase in the resting state peak current amplitude and AUC, with minimal effect on tau or inactivated state peak current amplitude, such as the compound highlighted in the black circle. Alternatively, the preference might be for a compound with no effect on the peak current amplitude, which slows down the current inactivation and therefore increases the tau and AUC in parallel in both states, such as the compound highlighted in the black triangle. Furthermore, it may be possible to develop compounds that have effects on multiple parameters, such as the compound highlighted in the black square, with an increase in the peak current amplitude in both states, decrease in tau in both states but minimal effects on AUC.

4 SUMMARY

Here we present Qube screening data from a sodium channel activator pilot screen for approximately 7,500 compounds. Data quality was good, with Z' values greater than 0.3 for the critical plate QC parameters and success rates greater than 80% for the majority of the plates. The hit rate was approximately 1.5% with artefact wells removed, depending on the hit parameter and cut-off for hit selection. Known activator and inhibitor compounds were detected by the assay, with high confidence in the hits due to the simultaneous verification of multiple hit and quality control parameters. As a result of the successful execution of the pilot screen, a full HTS campaign was started.

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