Application Note

Channel: $hNa_V1.5$
Cells: CHO
Tools: SyncroPatch 384PE
SyncroPatch 384i

Increase throughput by recording in unattended mode on the SyncroPatch 384

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Cells were kindly provided by Charles River.

charles river

Summary

High throughput screening (HTS) is used in the pharmaceutical industry to aid drug discovery. Large numbers of chemical compounds can be tested for biological activity using a range of techniques. The patch clamp technique remains the gold standard to test activity of compounds on ion channels and automated patch clamp (APC) is increasingly adopted in HTS labs as an alternative to conventional patch clamp given its increased ease-of-use and higher throughput. APC is employed in all aspects of drug discovery from hit finding and lead optimization through to target validation and safety testing. This is only possible due to the increase in throughput toward HTS capabilities, the compatibility with HTS workflows, and a lower cost per data point which can compete with other techniques such as fluorescence imaging (using, for example, the FLIPR™ instrument) and calcium imaging with the added benefit of real-time kinetics of drug effects. Indeed, all the major contract research organizations worldwide use APC for ion channel screening and cardiac safety testing. Increased automation, including unattended operation, is also an important factor for increasing throughput, and instruments can reliably work beyond an 8-h day provided they are serviced with enough cells, solutions, and compounds[1]. For this to work effectively, data must be reliable with high success rates, low false positive and negative rates along with reproducible IC_{50} values.

Here, we demonstrate recordings of $hNa_V1.5$ expressed in CHO cells on the SyncroPatch 384 in unattended mode. In a 6 hour period, 10 compound plates were prepared, 10 NPC-384 chips were recorded and success rates, IC_{50}s and Z' values were calculated using 7 different compounds in duplicate on each plate, along with positive and negative controls.

Results

For efficient experiments, especially in unattended mode, success rates must be high. On the SyncroPatch 384, cells, solutions, compounds, and 10 x NPC-384 chips were loaded into position on the robot and the experiments were run unattended for 6 hours. First of all, compound plates were prepared for all the experiments, following this, the gripper arm moved a new NPC-384 chip and compound plate automatically into position when the previous experiment was finished. Alternatively, compound plates can be prepared between runs or pre-prepared compound plates can be stacked in the robot and the on-board barcode scanner can be used to document the compound plate used. Sufficient cells and solutions were loaded into position for the unattended period, including the use of four refillable reservoirs for internal, external and wash solutions. Success rates for completed experiments were 94-97% with little change in the success rate for the last (10th) plate (94%) at the end of the unattended period compared with the first plate (97%) at the start. The success rates for completed experiments for all 10 chips is shown in Figure 1.

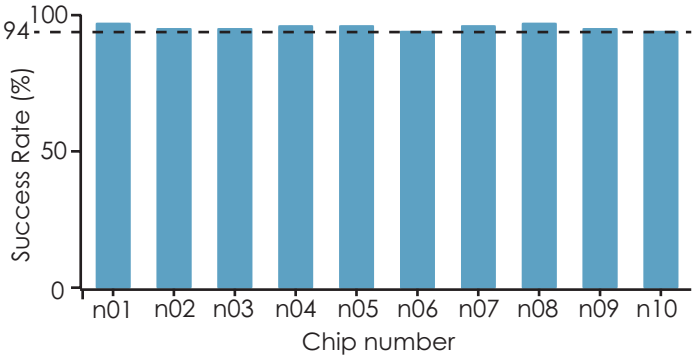

Figure 1: Success rate for completed experiments for 10 NPC-384 chips. Success rate (% of 384 wells used for analysis) of 10 NPC-384 chips is shown for recordings over a 6 hour period in unattended mode.

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Along with high success rates, data must also be reliable with low false positive and negative rates and a Z' value close to 1. The Z' value is a statistical parameter used in HTS (and elsewhere) to evaluate the overall quality of an assay without intervention of test compounds[2,3], it is calculated using positive and negative control data. Using the double step protocol shown in Figure 2A, Z' values were calculated for the 1st and 2nd peak for negative control (0.3% DMSO) and positive control (50 µM tetracaine) for the 10 chips (Shown in Table 1). Rundown was low which is shown in the scatter plot in Figure 2 for Peak 1 and Peak 2 and in Table 1. In 380 wells over the 10 plates which were used for negative control, just 3 wells for Peak 1 and 8 wells for Peak 2 showed a decrease in current >20% compared with reference giving a low false positive rate of 0.8% for Peak 1 and 2.1% for Peak 2. There were no wells which showed an increase in current >20% compared with reference giving a false negative rate of 0%.

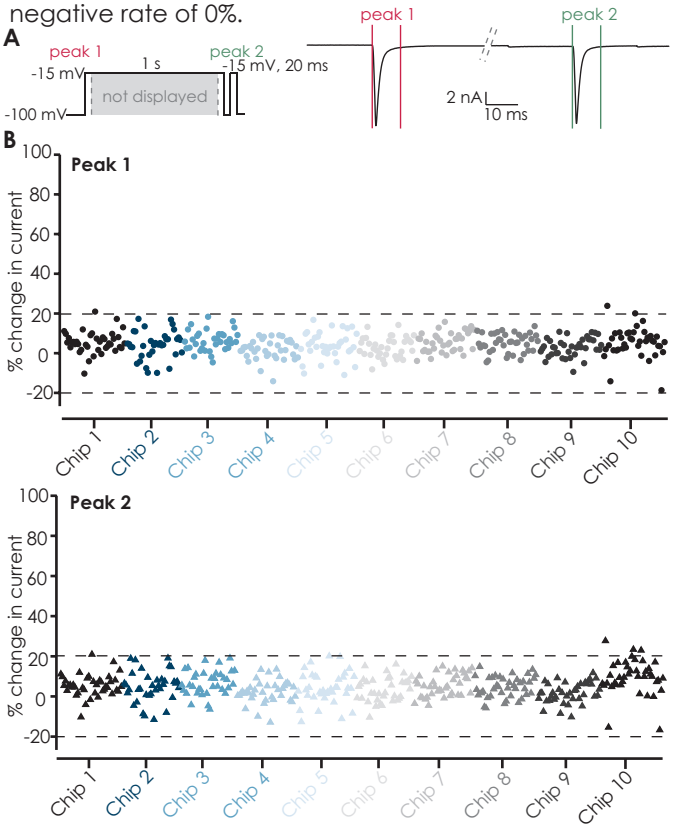

Figure 2: Low rundown of current responses. A. The voltage protocol used for the experiments is shown (left) along with an example of the current traces from a cell (right) showing also the cursors used for analysis. **B** Change in current (%) in negative control (0.3% DMSO) solution is shown for Peak 1 (top) and Peak 2 (bottom) for all chips. % change is shown normalized to reference at the beginning of the recording on each chip. Each point represents 1 well. Dotted lines indicate change of ±20%

	% change		Z' value	
Chip #	**Peak 1**	**Peak 2**	**Peak 1**	**Peak 2**
Chip 1 (40)	5.5 ± 0.9 (1)	10.9 ± 1.1 (4)	0.91	0.89
Chip 2 (36)	4.1 ± 1.2 (0)	4.9 ± 1.3 (0)	0.90	0.89
Chip 3 (35)	6.3 ± 0.9 (0)	6.8 ± 1.0 (0)	0.91	0.88
Chip 4 (38)	1.8 ± 0.8 (0)	2.4 ± 0.9 (0)	0.91	0.90
Chip 5 (37)	3.8 ± 1.1 (0)	4.3 ± 1.3 (1)	0.89	0.88
Chip 6 (36)	3.1 ± 0.9 (0)	3.6 ± 1.0 (0)	0.92	0.91
Chip 7 (38)	5.7 ± 0.8 (0)	5.4 ± 0.9 (0)	0.91	0.91
Chip 8 (40)	5.2 ± 0.7 (0)	4.7 ± 0.8 (0)	0.91	0.90
Chip 9 (40)	3.0 ± 0.7(0)	1.8 ± 0.8 (0)	0.93	0.93
Chip 10 (40)	5.3 ± 1.2 (2)	9.7 ± 1.4 (3)	0.88	0.87
Mean	**4.4 ± 0.3**	**5.4 ± 0.9**	**0.908 ± 0.004**	**0.893 ± 0.006**

Table 1: Negative control and Z' values for 10 NPC-384 chips for Peak 1 and Peak 2. Chip number is shown along with number of wells used for negative control in brackets. Mean ± S.E.M of % change in current for Peak 1 and Peak 2 and the number of wells which displayed >20% change in current given in brackets. Z' values were calculated from the negative and positive control data for each plate, mean ± S.E.M is also shown for Peak 1 and Peak 2. Z' are between 0.5 and 1 indicating an excellent assay[2,3].

During the unattended run, 7 compounds were applied in duplicate to each chip (see Methods). In addition to this, positive (8 wells; 50 µM tetracaine) and negative controls (40 wells; 0.3% DMSO) were performed on each plate. Figure 3 shows 16 concentration response curves for Peak 2 for an example compound (clozapine) overlaid. The concentration response curves were in excellent agreement across the plates, and could be almost exactly overlaid.

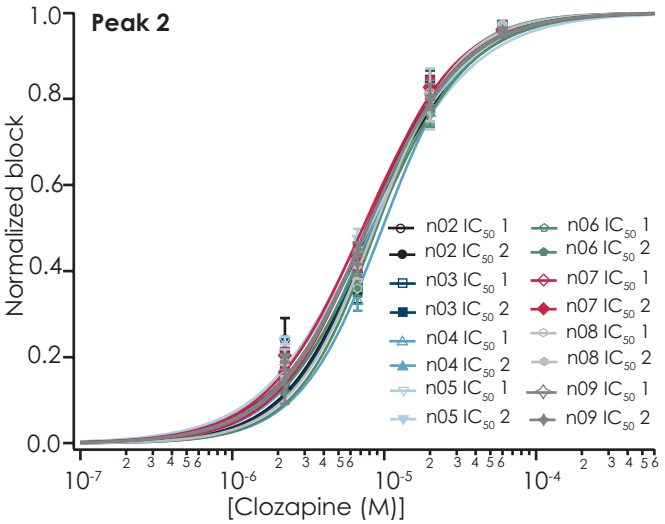

Figure 3: Concentration response curves for Peak 2 from 8 plates for clozapine overlaid. Concentration response curves are very similar across the plates.

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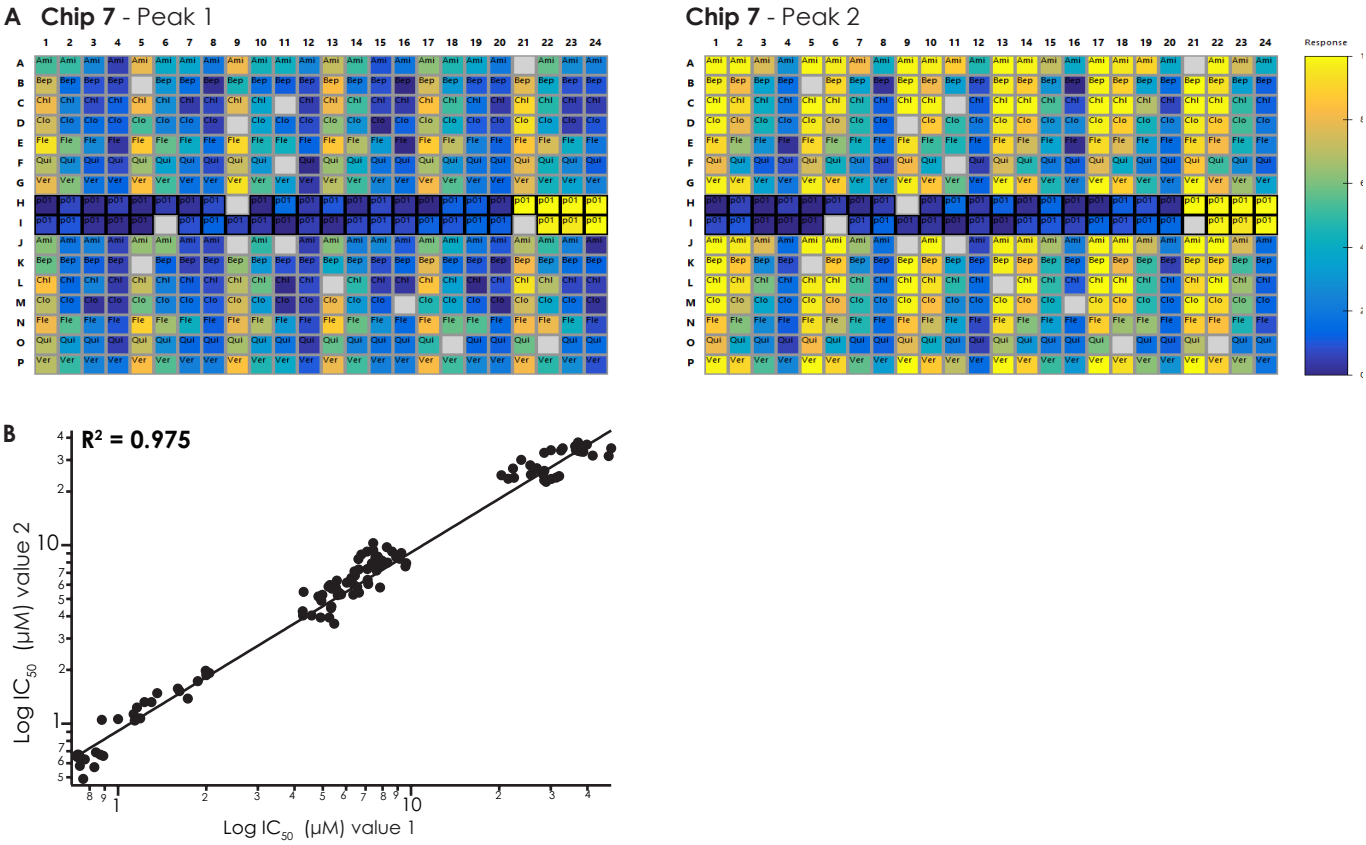

Figure 4: Heat maps of compound effects and IC_{50} correlation on the SyncroPatch 384. A Top left: Heat map of Chip 7 Peak 1 - effect of compounds, negative control (wells H1-20 and I1-20) and positive control (wells H21-24 and I21-24). Top right: Heat map of Chip 7 Peak 2 - effect of compounds, negative control (wells H1-20 and I1-20) and positive control (wells H21-24 and I21-24). Color code is shown on the top right. **B** Correlation plot of IC_{50} values. On the compound plates, the concentration response curves for each compound were run in duplicate on each plate. The two IC_{50} values for each chip for Peak 1 and Peak 2 are plotted against each other and fit with the Pearson's correlation coefficient. $R^2 = 0.975$ indicating excellent correlation.

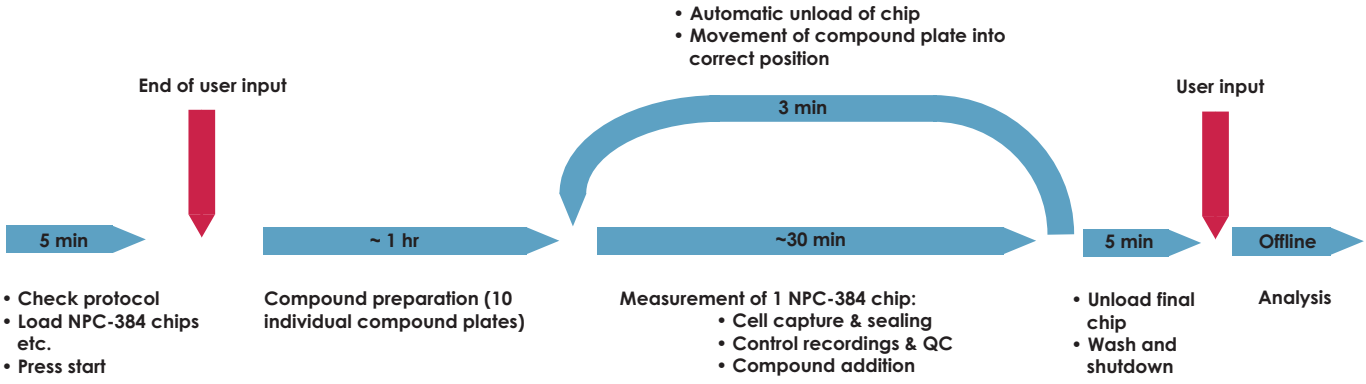

Figure 5: Timeline of unattended operation on the SyncroPatch 384 for the $hNa_v1.5$ assay. Chips, solutions and compound stock solutions were pre-loaded into the robot. The robot first prepared all the compound plates for the 10 experiments which took approx. 1 hour. Following this, one experiment, from chip load and priming, through cell capture & sealing, IV and QC, control recording and a single addition of compound to each well, took approximately 30 mins. At this point, the chip was unloaded and a new compound plate was added to the correct position, a new chip loaded and the experiment restarted. In a 5 hour measurement period, 10 x NPC-384 chips were recorded. After loading NPC-384 chips, compounds and solutions, no further user input was required until after the 6 hour unattended period. Data analysis was performed offline after completion of the experiments. Note that experiment time for other assays/targets will be different depending on whether single or cumulative addition of compound concentrations is performed, or where extended incubation times are required (e.g. for hERG experiments), and compound plates can be prepared prior to each NPC-384 chip recording rather than all at the start of the experiment, depending on user preference.

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Compound	IC_{50} (µM)		IC_{50} (µM) Literature	
	Peak 1	Peak 2	Ref. 4 Kramer (Peak 1)	Ref. 5 Crumb (Peak 1)
Amitriptyline	5.4 ± 0.3	0.67 ± 0.03	-	5.8
Bepridil	7.6 ± 0.3	1.73 ± 0.06	2.3 ± 0.3	2.9
Chlorpromazine	4.7 ± 0.1	1.14 ± 0.03	3.0 ± 0.4	4.5
Clozapine	35.4 ± 0.6	8.1 ± 0.2	15.1 ± 2.7	-
Flecainide	6.7 ± 0.2	5.5 ± 0.1	6.2 ± 0.6	6.7
Quinidine	36.3 ± 1.4	25.6 ± 0.8	14.6 ± 1.0	>5.4
Verapamil	26.1 ± 0.7	8.4 ± 0.3	32.5 ± 4.2	>1.0

Table 2: Average IC_{50} values for 7 compounds on $Na_V1.5$ Peak 1 and Peak 2 recorded in unattended mode on the SyncroPatch 384. IC_{50} values are mean ± S.E.M of 16 experiments. Values are also compared with the literature from Refs. 4 & 5, equivalent of Peak 1, where possible, or highest concentration is given (quinidine and verapamil, ref. 5).

Figure 4 shows the heat map of an example chip. For some compounds, Peak 2 is blocked with higher potency and this can be observed easily on the heat map (colour code is shown in the right of Figure 4). Figure 4B shows the correlation plot of all 7 compounds where the IC_{50} from each chip is plotted against its duplicate counterpart for Peak 1 and Peak 2 and Table 2 shows the average IC_{50} values for all compounds tested with comparison to the literature. All IC_{50}s recorded agreed well with the literature[4,5].

In conclusion, the SyncroPatch 384 instruments (SyncroPatch 384PE and SyncroPatch 384i) can reliably run in unattended mode as described in this Application Note. We chose $hNa_V1.5$ and a double voltage step protocol to investigate potency of compounds on the resting (Peak 1) and inactivated state (Peak 2) of the receptor. In a 6 hour unattended period, compound plates were prepared by the robot followed by recording of 10 NPC-384 chips. Seven compounds were used on each plate, along with positive and negative controls. A single concentration of the compound was added to the wells, with 6 replicates for each concentration. The concentration response curves were constructed across multiple wells (4 point). This was performed in duplicate on each plate on both Peak 1 and Peak 2 giving rise to 224 (4 point) concentration response curves. Data was reliable with high success rates (>94%) for completed experiments, low rundown or run-up, therefore low false positive and negligible false negative rates, excellent Z' values and concentration response curves with IC_{50} values in good agreement within plates, across plates and with the literature[4,5]. Therefore, users of the SyncroPatch 384 instruments can confidently extend their workday and increase productivity by using the instruments in unattended mode.

References

1. Obergrussberger, A., *et al*. 2020. EODD. doi: 10.1080/17460441.2020.1791079
2. Zhang, J-H., *et al*. 1999. JBS. 4 (2): 67-73
3. Iversen, P.W., *et al*. 2006. JBS. 11 (3): 247-252
4. Kramer, J., *et al.* 2013. Sci. Rep. 2013. 3: 2100
5. Crumb, W.J. Jr., *et al*. 2016. JPTM. 81:251-62

Methods

Cells

CHO cells expressing $hNa_V1.5$ were kindly provided by Charles River.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Perforated patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384 instruments using multihole chips (4x holes per well). Cells were held at -100 mV. A voltage step to -15 mV for 1 s for Peak 1 was followed by a brief step back to -100 mV for 40 ms and then a step to -15 mV for 20 ms for Peak 2. This double step protocol was repeated every 10 s. Single concentration addition was performed and concentration response curves calculated across multiple wells using DataControl 384. The same concentration of compound was added to 6 wells (i.e. 6 replicates) and 4 concentrations for each compound were used. Compounds were added in duplicate to chips 2-8, chips 1 (start of experiment) & 10 (end of experiment) received only negative control (0.3% DMSO) to check for rundown, with 8 wells receiving positive control (50 µM tetracaine). Analysis was performed offline using DataControl 384, Excel and IgorPro (Wavemetrics).

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