Investigating pain pathways by inhibition of voltage-gated sodium channels

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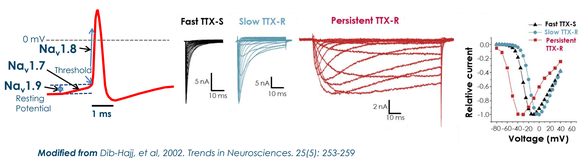
Abstract

Voltage-gated sodium channels (Na_V) are attractive targets for investigation of chronic and neuropathic pain, due to their physiological role in action potential generation and propagation and, thus, neuronal excitability. The $Na_V1.7$ is found primarily in the peripheral nervous system and is thought to play a role in nociception and pain sensing. The TTX-resistant $Na_V1.8$ is selectively expressed in dorsal root ganglion (DRG) neurons. We present recordings of $Na_V1.7$ and $Na_V1.8$ channels on a high throughput screening patch clamp platform.

$Na_V1.7$ was expressed in CHO cells and the current voltage relationship recorded was consistent with $Na_V1.7$ obtained using other methods. Vhalf of activation was -24 mV (n=275). Using a double-step voltage protocol we were able to investigate whether compounds, such as tetracaine, exhibit state dependence. We show that tetracaine exhibited a lower IC on the second pulse, i.e. the inactivated state of the receptor, compared with the resting state. $Na_V1.8$ expressed in CHO cells started to activate at approximately -40 mV, peaking at between 10 mV and 20 mV with a Vhalf of activation of -2.7 mV (n=380). In order to study Na_V channels involved in pain pathways in a more physiological environment, we used stem cell-derived neurons, more specifically with an overexpression of $Na_V1.8$. In these cells, endogenous Na_V-mediated currents were recorded with activation parameters consistent with NaV1.7.

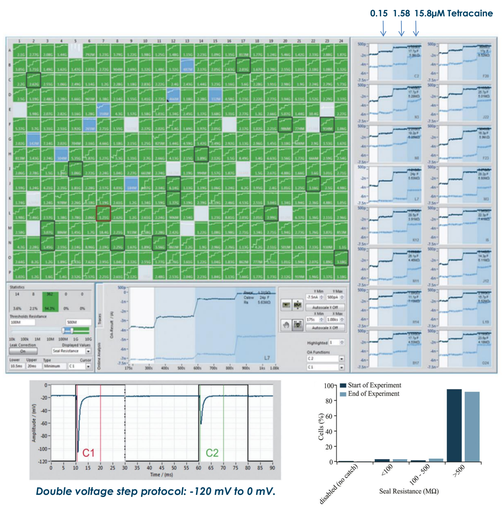
Our results demonstrate that Na_V channels can be successfully studied on high throughput electrophysiological systems, facilitating the discovery of novel pain therapeutics.



***Modified from** Dib-Hajj, et al, 2002. Trends in Neurosciences. 25(5): 253-259*

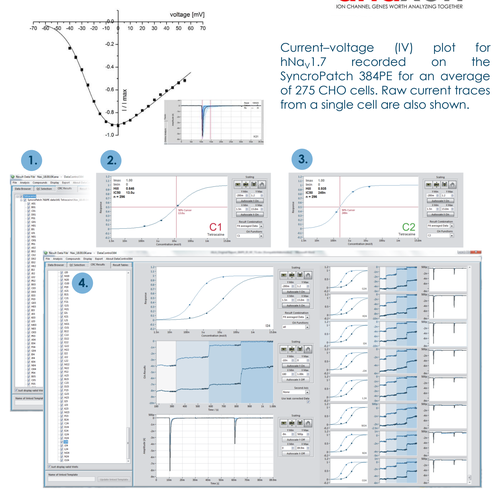
State-Dependence of Tetracaine on $hNa_V1.7$ Channels

anaxon® ION CHANNEL GENES WORTH ANALYZING TOGETHER



Double voltage step protocol: -120 mV to 0 mV.

Screenshot of the PatchControl 384PE software showing an experiment using CHO cells expressing $hNa_V1.7$. Peak current amplitude of the first peak (light blue) and the second peak (dark blue) is plotted against time. First, control solution without tetracaine was added (white), and then increasing concentrations of tetracaine in different shades of blue (cumulative concentration response). Bar graph showing seal resistance at the start (dark blue) and end (light blue) of the experiment. Here, 95% of cells had a seal resistance of >500 MΩ at the beginning, and 91% at the end of the experiment (after 20 min).



Current–voltage (IV) plot for $hNa_V1.7$ recorded on the SyncroPatch 384PE for an average of 275 CHO cells. Raw current traces from a single cell are also shown.

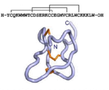
Analysis of tetracaine block of $hNa_V1.7$ expressed in CHO cells using DataControl 384. Results file is loaded into DataControl 384 and sorted by compound name (**1**). Average concentration–response curve (CRC) for tetracaine block of the first peak (**2**) and the second peak is shown (**3**). DataControl 384 software shows the raw data traces, online analysis, and concentration–response curves for each individual cell. The concentration–response curves for the first and second peaks are displayed overlaid (**4**).

***Data from** Obergrussberger, et al, 2016. JALA. 21(6) 779–793*

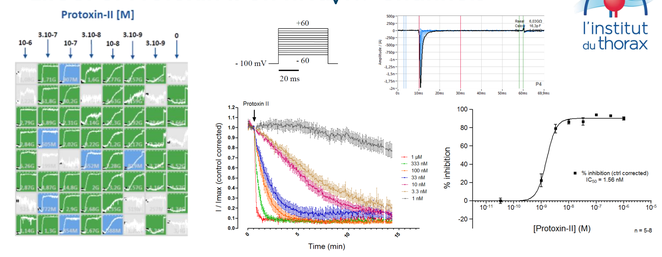
Effect of Protoxin II on $hNa_V1.7$ channels

Smartox Biotechnology

Thrixopelma pruriens
Peruvian green velvet tarantula



Amino acid sequences and structures of spider-derived $Na_V1.7$ blocker protoxin-II (Pennington et al. 2017)



Protoxin-II inhibits both tetrodotoxin-sensitive and -resistant voltage-gated sodium channels, efficiently blocking the human $Na_V1.7$ channel. Here, selective inhibition of $Na_V1.7$ channel IC_{50} by Protoxin-II was measured in SyncroPatch 384PE. Currents were evoked by depolarizing pulses from a holding potential of -100 mV to step potential varying from -60 to 60 mV. The IC_{50} value for current inhibition by Protoxin-II was 1.56 nM.

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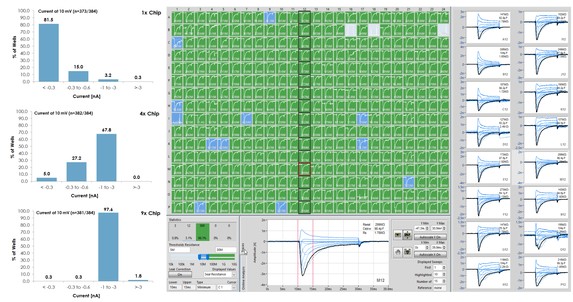
$hNa_V1.8$ Current-Voltage (IV) Relationship Recorded with Multihole Substrate

discovery from charles river

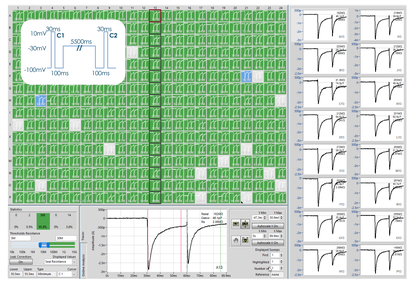


	V_{half} (mV)	V_{hold} (mV)	Cell type
4x Chip	-8.6 (n=382)	-100	CHO
9x Chips	-12.8 (n=381)	-100	CHO
Manual	-15.7	-100	DRG

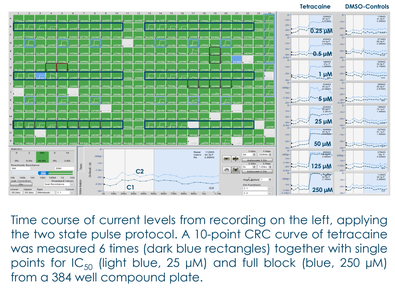
These values agree well with the literature *(Cummins and Waxmann. 1997. J.Neurosci., 17(10):3503-14).*

Bar graphs (left): comparison of current levels obtained from single- and multihole (4x, 9x) chips from similar recordings. Screenshot depicts $Na_V1.8$ IV currents recorded with 9x multihole chip.

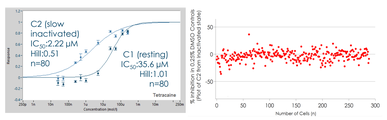
Compound Affinity and Assay Stability for the Slow Inactivated State of $hNa_V1.8$ Channels



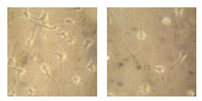
Screenshot of a representative recording of $hNa_V1.8$ channels using the voltage protocol shown on the left to asses the resting (C1) and slow inactivated state (C2). Currents are leak subtracted, holding potential was -100 mV and pulse was applied once every 60s. Average CRC for tetracaine (n=80/82) fitted with a standard Hill-equation highlighting a ~16 fold shift for the IC_{50}s obtained from the slow inactivated compared to the resting state. Bottom right graph shows a distribution of data points obtained from DMSO controls wells (n=291/302) which shows little rundown and highly consistent results.



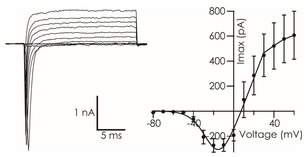
Time course of current levels from recording on the left, applying the two state pulse protocol. A 10-point CRC curve of tetracaine was measured 6 times (dark blue rectangles) together with single points for IC_{50} (light blue, 25 µM) and full block (blue, 250 µM) from a 384 well compound plate.



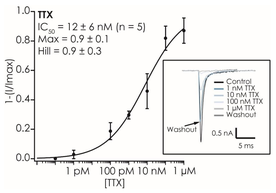
Stem Cell-Derived Neurons Recorded on the Patchliner



Adherent iCell Neurons (left) and in suspension for use on the Patchliner (right).



Current responses to a voltage step protocol of iCell Neurons (left) and the corresponding IV plot for an average of 54 cells (right). V_{half} was -20 mV when calculated using a Boltzmann equation.



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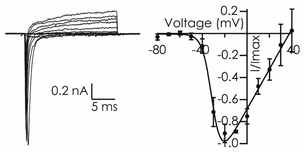
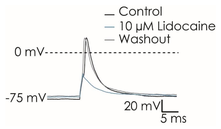
Na_V-mediated currents recorded from iCell Neurons were blocked by TTX with an IC_{50} = 12 ± 6 nM (n = 5) revealing a TTX-sensitive current in these cells.

***Data from** Haythornthwaite, et al, 2012. JBS. 17(9) 1264-1272*



Current responses to a voltage step protocol of Dopa.4U Neurons (left) and the corresponding IV plot for an average of 5 cells (right). V_{half} was -31 mV when calculated using a Boltzmann equation.



Action potential elicited using an 1 ms current pulse of 80 pA. Action potential was abolished by 10 µM lidocaine and returned upon washout.

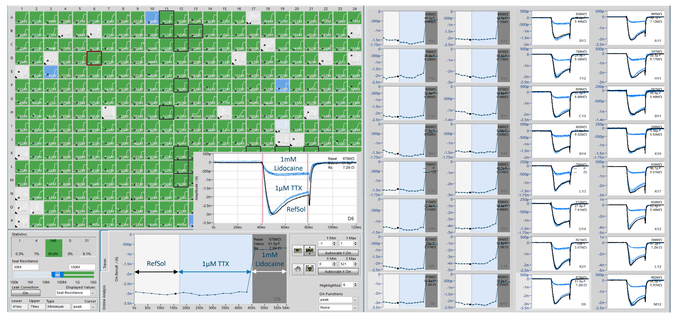
Ncardia Stem cell experts

High Throughput Automated Patch Clamp of $hNa_V1.9$

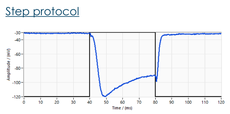
ICAGEN

$hNa_V1.9$ is TTX-Insensitive

90% success rate
QCs: R >100MΩ, I > 0.2nA (4x)



Step protocol

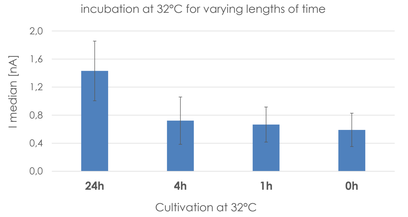


Sodium channels are classified according to their sensitivity to the neurotoxin TTX. They are separated into two classes, TTX-sensitive channels (e.g. $Na_V1.7$) and TTX-resistant channels (e.g. $Na_V1.8$ and $Na_V1.9$).

The figure (left) shows a screenshot of the data acquisition displaying OA results and the overlaying current amplitudes in the absence and presence of 1 µM TTX, followed by a block of 1mM Lidocaine (full block)

As expected, $hNa_V1.9$ currents are TTX-resistant.

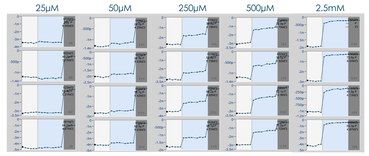
Incubation at 32°C Increases Channel Expression



After 2 days of cell incubation at 37°C cell were kept at **32°C** for varying length of time -> **24h, 4h, 1h, 0h.**
All cells were subsequently measured side by side on one chip (4x).

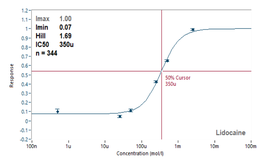
-> Current amplitudes are significantly higher (~2.4-fold) with overnight incubation (24 hrs) at 32°C.

Concentration-Dependent Block of $hNa_V1.9$ by Lidocaine



A five point CRC for Lidocaine block of $hNa_V1.9$ was constructed across the plate.

The IC_{50} matches the literature value of 356µM[8] exactly.



90% Success Rate
QCs: R >100MΩ, I > 0.2nA (4x)

Summary

- ✓ $hNa_V1.7$, 1.8 and 1.9 were recorded on the SyncroPatch 384PE with high success rates for Rseal, current levels and completed experiments.
- ✓ Current-voltage relationships and values for V_{half} of activation for $hNa_V1.7$ and 1.8 calculated using automated patch clamp agree well with the literature.
- ✓ IC_{50} values for tetracaine and lidocaine were as expected. Tetracaine exhibited state dependence on $Na_V1.7$ and 1.8.
- ✓ Stem cell-derived neurons were used on the automated patch clamp device, the Patchliner, where TTX-sensitive Na_V-mediated currents were recorded. In current clamp mode, action potentials were abolished by lidocaine.

✓ Acknowledgements: special thank you to Matthew Fuller at Icagen for contributing the $Na_V1.9$ data, and to Sébastien Nicolas and Michel de Waard at L'insitut du thorax and Rémy Béroud from Smartox for contributing the Protoxin-II data.