

Activation and Inhibition of human NMDA Channels on Nanion's SyncroPatch 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by Chantest, a Charles River Company.



Summary

N-Methyl-D-aspartate (NMDA) receptors are a member of the ionotropic glutamate receptor family, ligand-gated ion channels that mediate the majority of excitatory neurotransmission in the mammalian CNS. They are expressed primarily in the CNS but also in peripheral locations such as pancreatic islet cells, sensory nerve terminals in skin and cardiac ganglia¹. Seven subunits of the NMDA receptor have been identified, NR1, NR2A-D and NR3A-B², they assemble as a tetramer² consisting of two NR1 subunits and either two NR2 subunits or a combination of NR2 and NR3 subunits³. Activation of NMDA receptors requires the simultaneous binding of glutamate and glycine³.

Calcium entry through NMDA receptors plays an important role in development and synaptic plasticity and is proposed to underlie higher processes such as learning and memory¹. It is also proposed to play a role in a number of neurological diseases such as epilepsy and Alzheimer's¹. Indeed, memantine is an NMDA antagonist which has been approved for the treatment of moderate to severe Alzheimer's². NMDA antagonists may also be targets for the treatment of neuropathic pain, major depression and Parkinson's disease².

Here we present high quality data at a high throughput collected on the SyncroPatch 384PE showing activation and inhibition of NMDA NR1/NR2B expressed in HEK cells. Stable recordings of NMDA receptor were achieved and modulation of the response by spermine and ketamine is shown.

Results

For the evaluation of the performance of HEK-NR1/NR2B cells, seal resistance was determined from one experiment (Fig. 1) using single-hole chips. At the start of the experiment, 66% of cells had a seal resistance (RSeal) > 1 GΩ, with a further 26% with a RSeal > 500 MΩ giving a success rate of 92% for cells with RSeal > 500 MΩ. This was only marginally reduced by the end of the experiment (89% of cells had RSeal > 500 MΩ, experiment time ~ 20 min).

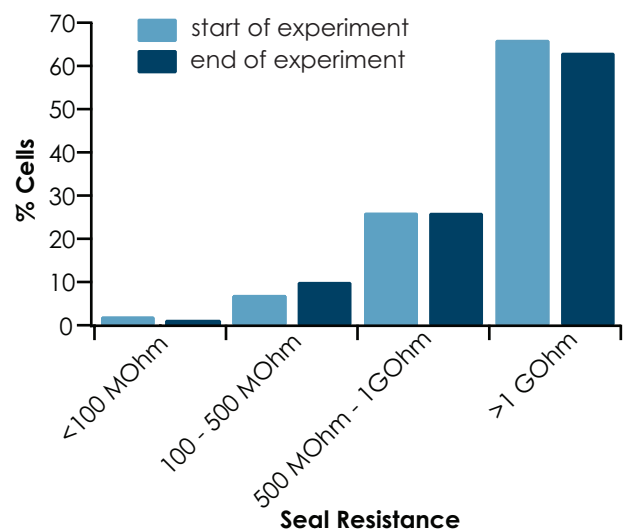


Figure 1: Statistics of NR1/NR2B expressing HEK293 cells recorded on one NPC-384 chip on the SyncroPatch 384PE. Success rate (seal resistance) of individual HEK cells on the SyncroPatch 384PE. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue).

Application Note

Currents mediated by NR1/NR2B could be reliably recorded on the SyncroPatch 384PE with a high success rate. Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch 384PE during an experiment recording the current response of NR1/NR2B to 3 applications of 3 μM glutamate plus 10 μM glycine. In this experiment, multi-hole chips containing 4 holes per well were used.

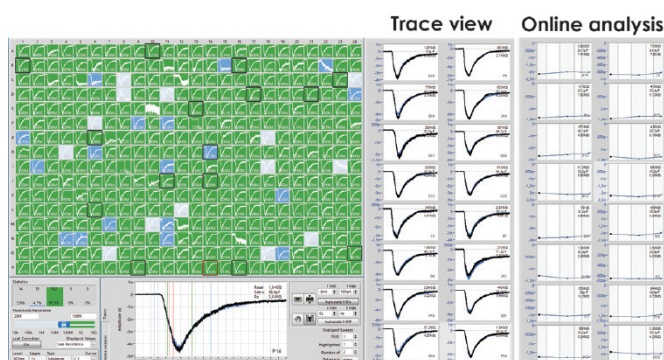


Figure 2: Typical recording from NR1/NR2B expressed in HEK cells on the SyncroPatch 384PE. The screenshot shows the data acquisition and analysis software used on the SyncroPatch 384PE. Three hundred and eighty-four small color-coded pictures shown in the upper left part from A1 to P24 display 384 recordings. Wells are color-coded based on seal resistance (green: $R_{\text{memb}} > 100 \text{ M}\Omega$, blue: $R_{\text{memb}} = 25 - 100 \text{ M}\Omega$, light blue or grey: $R_{\text{memb}} < 25 \text{ M}\Omega$ or disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. On the far right, the online analysis view showing peak current amplitude is provided. Vertical lines and grey shaded areas show application of solution containing 3 μM glutamate plus 10 μM glycine and white shows incubation in wash solution. The experiment shows current activation in response to glutamate/glycine repeated 3 times (with wash between applications) to show reproducibility of the signal. Holding potential was -80 mV. Note that multi-hole chips (4 holes per well) were used for this experiment.

NR1/NR2B-mediated responses could be reliably and reproducibly recorded on the SyncroPatch 384PE as shown in Figure 2. The current responses to multiple applications of 3 μM glutamate plus 10 μM glycine could be exactly overlaid.

Figure 3 shows the response curve to increasing concentrations of spermine. Spermine has been shown to potentiate NMDA responses^{3,4,5} but does not activate NMDA currents alone³. Cells were activated by application of 10 μM glutamate/10 μM glycine followed by co-application with different concentrations of spermine in a double-stacked application approach. The concentration response curve was calculated across the whole plate and revealed an $\text{EC}_{50} = 134 \text{ }\mu\text{M}$ ($n = 290$). Multi-hole chips (9 holes per well) were used for the experiments.

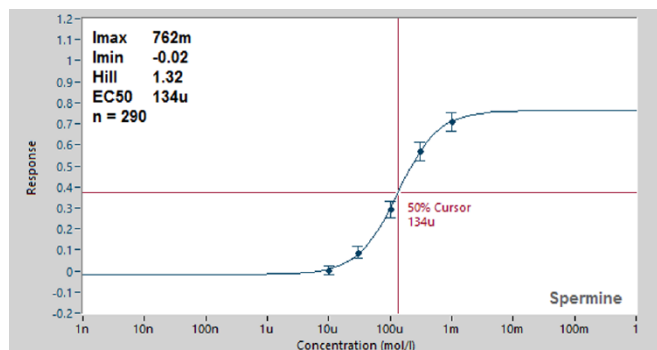


Figure 3: Positive allosteric modulation of NR1/NR2B by spermine. Currents in the presence of the PAM were normalized to the response generated with glutamate/glycine and the concentration response curve for spermine calculated across the whole plate as shown in Figure 5. The curve was fitted with a Hill equation and the EC_{50} calculated to be 134 μM ($n = 290$).

Ketamine is an NMDA receptor blocker used clinically as a pediatric and veterinary anesthetic. It is also an important tool in schizophrenia research as it is used to generate animal models of the disease. ($\pm$)-Ketamine was co-applied with glutamate and glycine and cumulative concentration response curves were performed, i.e. multiple concentrations applied to each cell. Figure 4 shows the concentration response curve for ($\pm$)-ketamine for an average of 321 cells. Multi-hole chips were used (4 holes per well). The IC_{50} was $2.26 \pm 0.38 \text{ }\mu\text{M}$ ($n = 321$) which is within the range found in the literature^{6,7} although a direct comparison with the literature is difficult due to experimental variables (e.g. Mg^{2+} concentration, glycine concentration, racemic ketamine) which affect the IC_{50} .

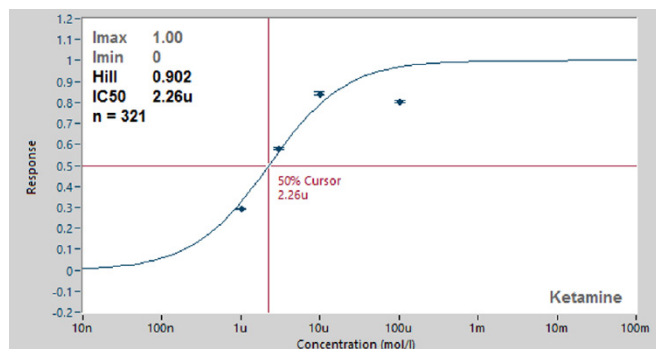
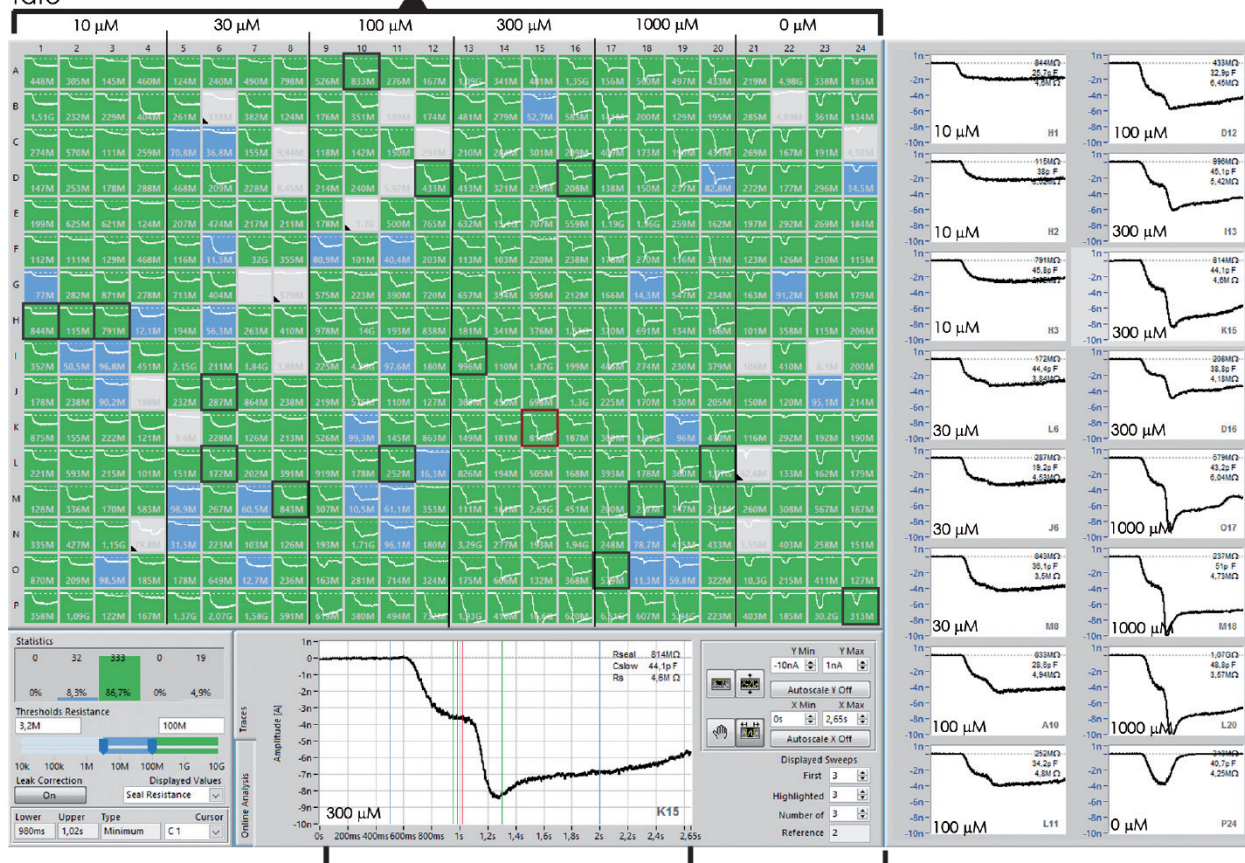


Figure 4: Average concentration response curve for ($\pm$)-ketamine block of NR1/NR2B. Cumulative concentration response curves were performed on each cell. The SyncroPatch 384PE analysis software (DataControl 384) was used to calculate the average concentration response curve, normalized to maximum block and fitted with a standard Hill-equation. The IC_{50} for ($\pm$)-ketamine block of NR1/NR2B was $2.26 \pm 0.38 \text{ }\mu\text{M}$ ($n = 321$), within the range found in the literature^{6,7}.

Application Note

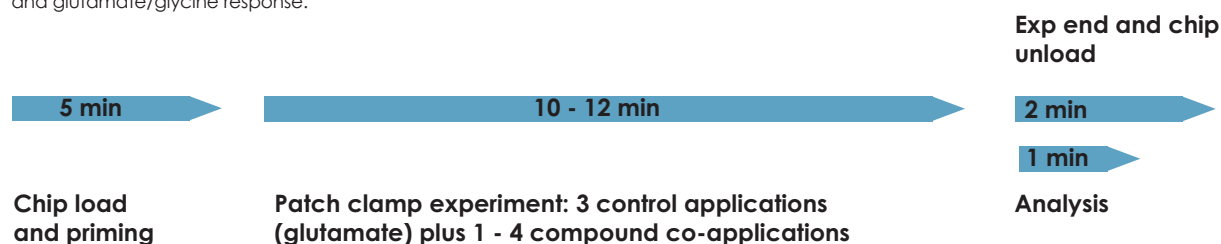
384 color coded depictions of data traces eases judgement of success rate



Highlighted raw traces of 1 cell showing positive allosteric modulation of NR2B by spermine co-applied with glutamate

Highlighted raw traces of 16 cells showing positive allosteric modulation of NR2B by spermine

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot of depiction of raw data traces of NR1/NR2B-expressing HEK cells as recorded on one NPC-384 patch clamp chip. A single compound (in this case spermine) was co-applied with glutamate and glycine to individual wells at varying concentrations (including control wells) across the plate. Multi-hole chips were used where 4 holes were present per well. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green ($R_{\text{memb}} > 100 \text{ M}\Omega$), blue ($R_{\text{memb}} = 3.2 - 100 \text{ M}\Omega$), light blue or grey ($R_{\text{memb}} < 3.2 \text{ M}\Omega$ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show raw data traces of NR1/NR2B channels following activation first by glutamate and glycine (red cursor), followed by co-application with spermine (green cursor) at the concentrations indicated. Spermine potentiates and glutamate/glycine response.



Chip load and priming

Patch clamp experiment: 3 control applications (glutamate) plus 1 - 4 compound co-applications

Exp end and chip unload

Analysis

Figure 6: Timeline of an experiment on the SyncroPatch 384PE. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a 1 - 4 point concentration response curve on NMDA-mediated currents took approximately 17-19 min.

Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, raw traces are chosen and the graphs show control responses to glutamate plus glycine followed by potentiation of the response by co-application of spermine. An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, NMDA receptors with the subunit combination NR1/NR2B expressed in HEK cells can be recorded on the SyncroPatch 384PE with high success rates for completed experiments (typically >75%). The timeline of each experiment was about 17-19 minutes (start – end) and included 3 control applications with glutamate plus glycine followed by co-application with 1 - 4 concentrations of a modulator. The NR1/NR2B-

mediated response could be potentiated by spermine in a concentration-dependent manner as expected^{3,4,5} and blocked by ketamine with an IC_{50} within the range found in the literature^{6,7}.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording NMDA receptor-mediated currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

HEK293 cells expressing NR1/NR2B kindly provided by ChanTest (a Charles River company).

Cell culture

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

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384PE using single- or multi-hole (4 or 9 holes per well) chips. Cells were held at -80 mV for the duration of the experiment. For potentiator concentration response curves, a double-stacked solutions approach was used for the pipette. In this way, the cells were first activated by glutamate/glycine and then spermine was co-applied. The peak amplitude in the presence of the PAM was normalized to the current amplitude in glutamate/glycine alone. Single point concentration response curves were performed and the concentration response curves calculated across the whole plate. For inhibitor experiments, ($\pm$)-ketamine was co-applied with glutamate/glycine after 3 control applications without inhibitor. Data was normalized to the maximum peak amplitude without inhibitor. Cumulative concentration response curves were performed. For all experiments, Mg^{2+} -free external solution was used.