

Pharmacological Characterization of the NMDA A-B-C by Automated Patch Clamp

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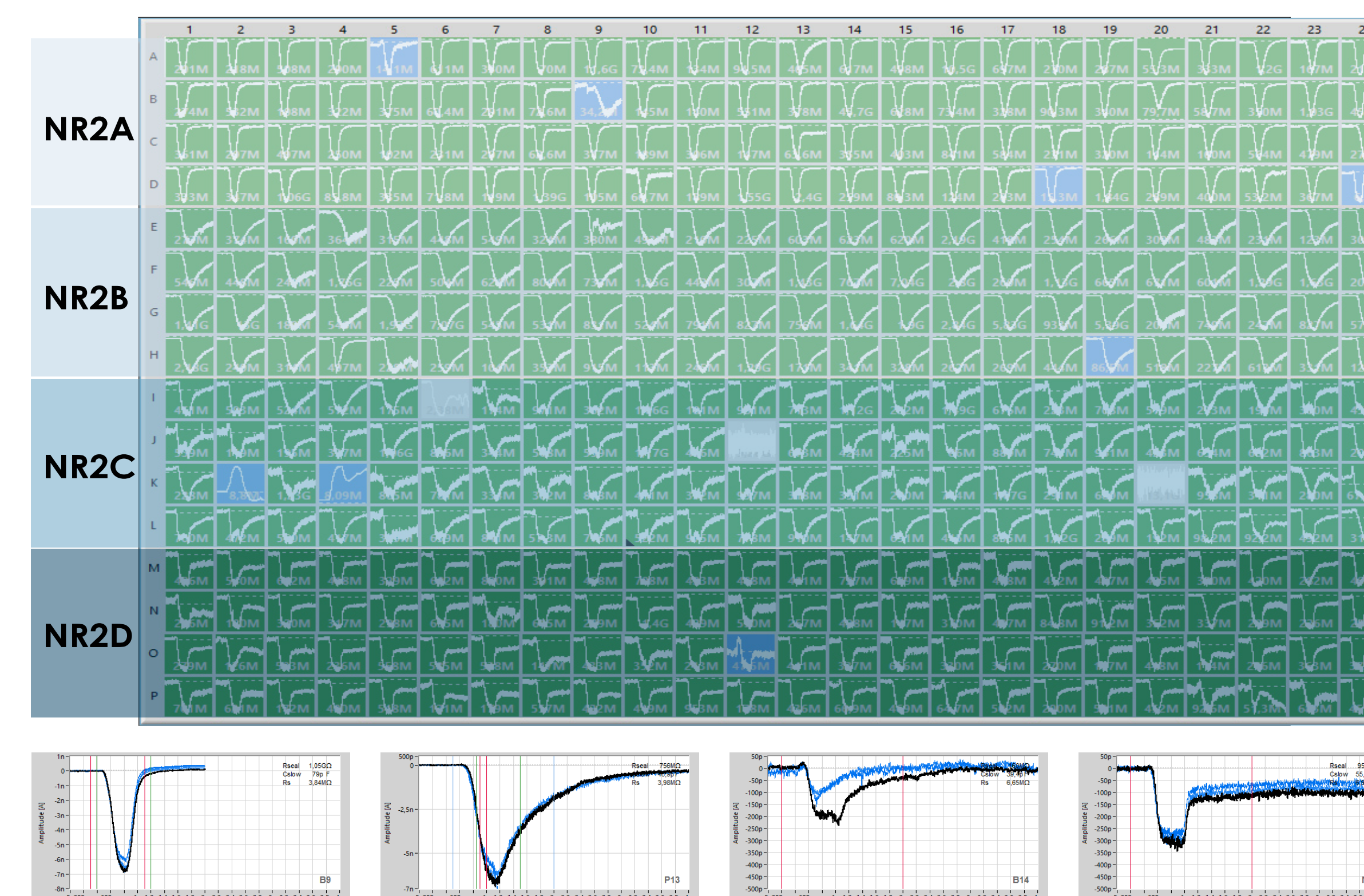
Introduction

N-Methyl-D-Aspartate receptors (NMDAR) are one of the key players in basic and complex excitatory neurotransmission. Seven subunits of NMDAR have been identified: GluN1, GluN2A-D and GluN3A-B1. Assembled as a tetramer¹, they consist of two NR1 subunits and either two NR2 subunits or a combination of NR2 and NR3 subunits².

Unique properties of NMDAR are the activation of the channel by simultaneous binding of glutamate and glycine, a voltage-dependent block by magnesium ions, a relatively slow current kinetic and a predominantly calcium carried conductance. The exceptional calcium entry through open NMDARs triggers on the one hand a number of regulatory processes important in development and synaptic plasticity processes of learning and memory³. On the other hand NMDARs are proposed to play a pathophysiological role in a number of neurological disorders such as epilepsy and Alzheimer's³. Given the importance in the treatment of such neurological diseases, it is of great interest developing clinically relevant NMDAR antagonists that would block excitotoxic NMDAR activation, without interfering with NMDAR function needed for normal synaptic transmission and plasticity.

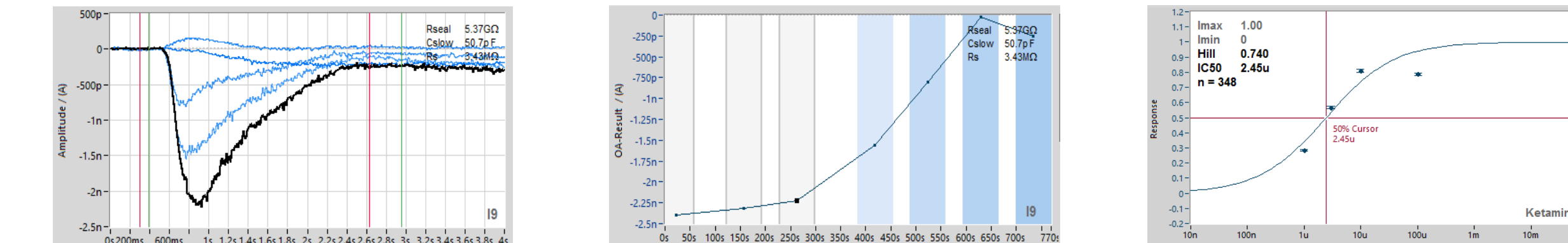
This study focuses on the basic biophysical properties of different NMDAR subtypes and the pharmacological relevance of modulation of NMDARs. Among others, we show activation kinetics of GluN2A, GluN2B, GluN2C and GluN2D subunit containing NMDARs. Moreover, we present data from the target screen of the positive allosteric modulator CIQ and blockers which were recorded with high throughput. All experiments were performed on the SyncroPatch384PE, the only APC device, which is able to stack the solutions inside a pipette and rapidly apply it to the cell, allowing for brief and accurate solution exchange (<10ms) and exposure times (<200ms). NMDAR expressing cell lines were kindly provided by Chantest.

NMDAR Kinetics on the SyncroPatch 384PE

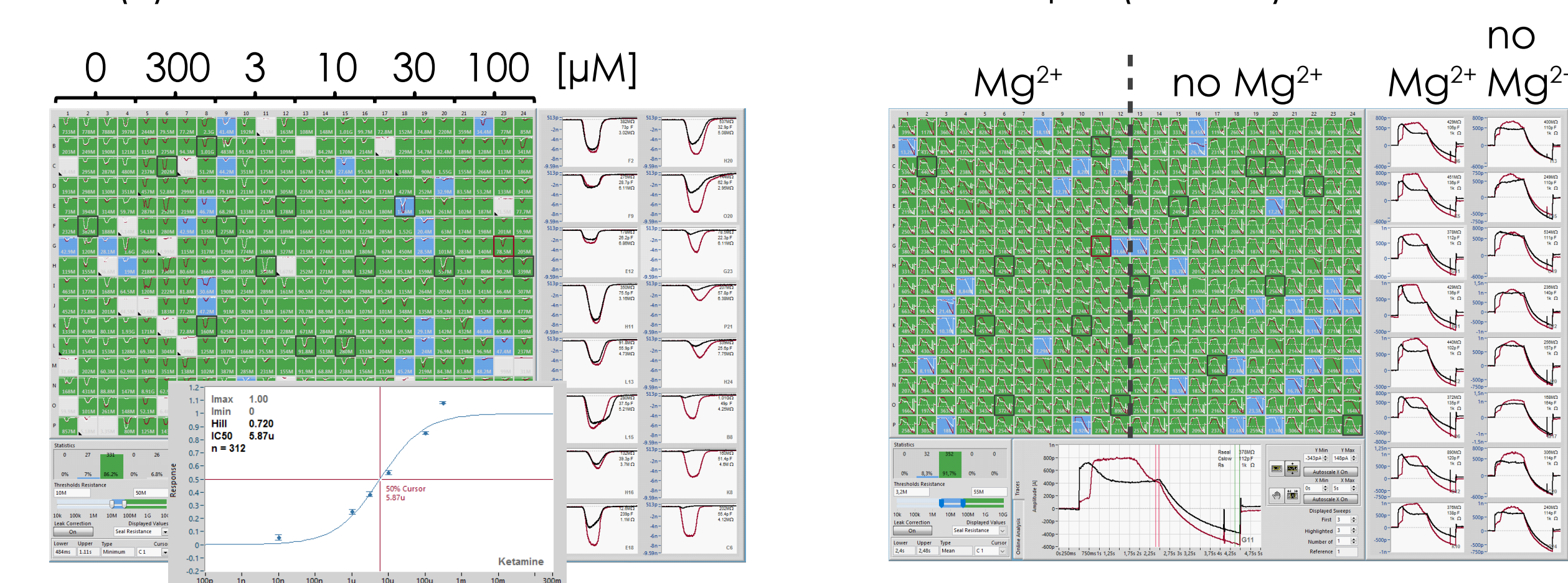


GluN1/GluN2A, GluN2B, GluN2C and GluN2D expressed in HEK cells simultaneously recorded on the SyncroPatch® 384PE. The experiment shows current activation and decay kinetics in response to glutamate/glycine repeated 3 times (with wash between applications) to show reproducibility of the signal. Holding potential was -80 mV. Note that multi-hole chips (4 holes per well) were used for this experiment.

Flexible Experimental Design



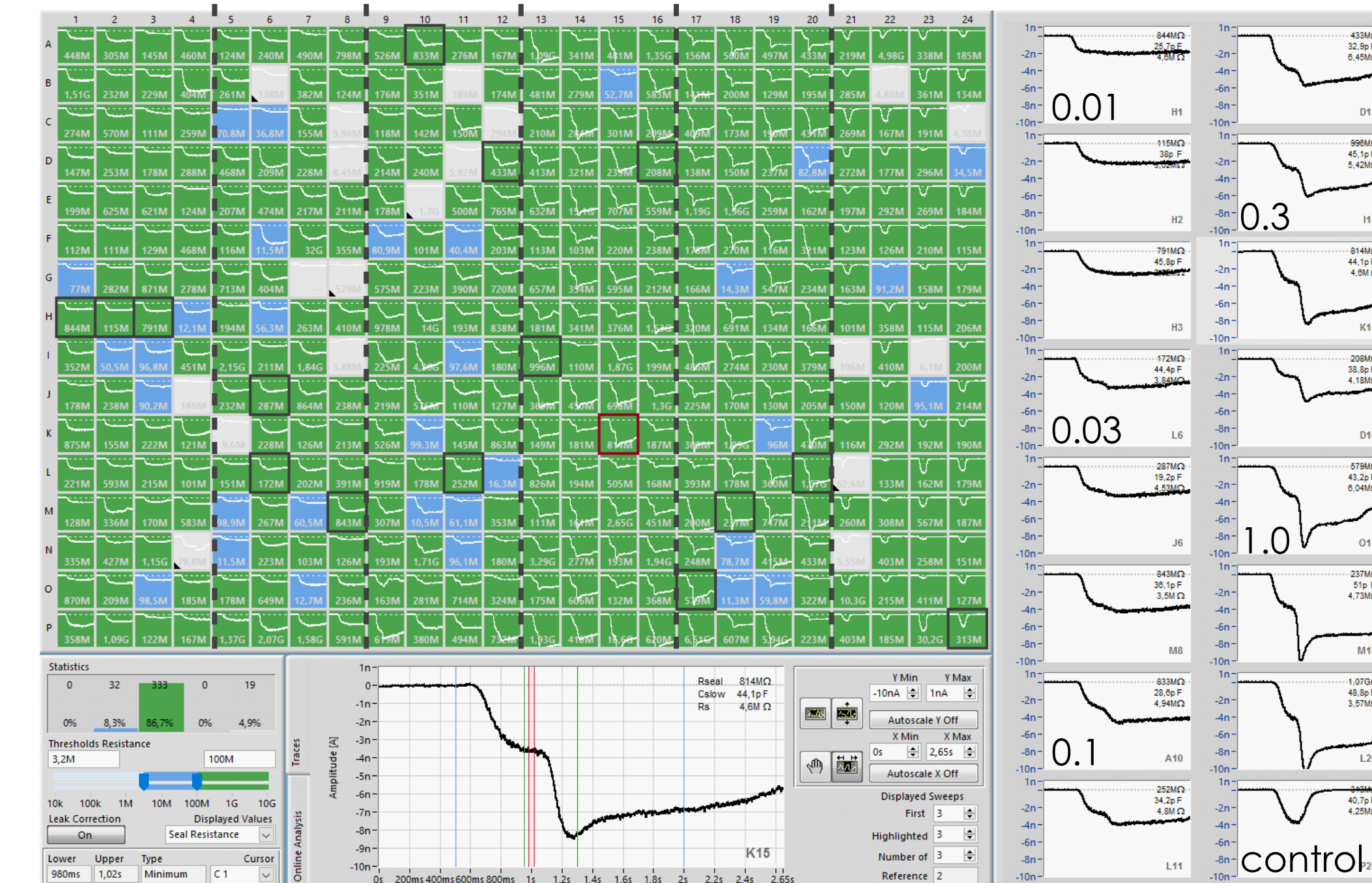
Cumulative dose response inhibition by the anesthesia ketamine. Data were normalized to maximum block and fitted with a standard Hill-equation. The IC₅₀ for (±)-ketamine block of NR1/NR2A was 2.45 ± 0.38 μM (n = 348).



Single point and coincident activation experiment. Left: Recordings were performed in solution without Mg²⁺. The IC₅₀ is within the range found in cumulative dose response experiments. Right: Recordings under physiological Mg²⁺ block conditions. Current response generated by activation with glutamate/glycine triggered to a depolarizing pulse protocol to overcome the natural Mg²⁺ block and to potentiate the open probability of the receptors. Currents were subsequently blocked by Ketamine.

Positive Allosteric Modulation of NMDA A-B-C-D

Spermine (mM)
0.01 | 0.03 | 0.1 | 0.3 | 1.0 | control



NMDAR modulation by spermine (GluN2B), pregnenolone sulfate (GluN2A) and CIQ (GluN2C and GluN2D). Left graph shows raw data traces of GluN2B 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. Currents in the presence of the individual PAM were normalized to the response generated by glutamate/glycine. Right graphs show dose response curves calculated across the plate. Each curve was fitted with a Hill equation and EC₅₀s were calculated to be for pregnenolone sulfate 39.6 μM (n = 284), spermine 260 μM (n = 303), CIQ modulating GluN2C 4.33 μM (n = 169) and CIQ modulating GluN2D 5.48 μM (n = 149).

Summary

The data collected on the SyncroPatch 384 PE reliably show the differences in current kinetics of NMDA receptors containing different subunit compositions. GluN1/GluN2A containing receptors show a higher open probability and faster glutamate deactivation kinetics than GluN1/GluN2B or GluN1/GluN2C and GluN1/GluN2D receptors, two characteristics which have been previously described in literature^{1,6}.

Current responses could be shown to be highly reproducible when recorded on the SyncroPatch 384PE providing the basis for accurate pharmacology. IC₅₀ of ketamine and EC₅₀s of the modulator experiments on all four GluN2 subunit containing receptors were in agreement with the literature^{2,4,5}.

Brief ligand application mimicking synaptic transmission and the ability to precisely time the ligand addition to a voltage pulse mimicking membrane depolarisation make the SyncroPatch 384PE predestinated to record NMDA receptors under physiological nuptical conditions.

In conclusion, the SyncroPatch 384 PE is highly suitable to study NMDA receptors and their role in synaptic plasticity, which includes mechanisms underlying learning and memory.

References

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