Evaluation of compounds on NMDA-mediated EPSP in rat hippocampal slices

May, 2019

MATERIALS & METHODS

Preparation of acute hippocampal slices

Experiments were carried out with 3 to 6 week-old male Sprague Dawley rats or 8 to 12-week old C57Black/6 mice provided by Elevage JANVIER (France). Septal hippocampal slices were cut (400 or 350 µm thick) with a McILWAIN tissue chopper. The slices were incubated at room temperature for at least 1 h in artificial Cerebro-Spinal Fluid (aCSF) of the following composition: NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11 (in mM).

Slices perfusion and temperature control

During experiments, slices were continuously perfused with oxygenated ACSF described hereinafter: NaCl 127, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 0.1, $CaCl_2$ 2, $NaHCO_3$ 25, glucose 11, pyruvate 0.8 (in mM) and 10 µM NBQX, at the rate of 3 mL/min with a peristaltic pump (MEA chamber volume: ~1 mL). Complete solution exchange in the MEA chamber was achieved 20 s after the switch of solutions.

The perfusion liquid was continuously pre-heated at 37°C just before reaching the MEA chamber with a heated-perfusion cannula (PH01, MultiChannel Systems, Reutlingen, Germany). The temperature of the MEA chamber was maintained at 37 °C with a heating element located in the MEA amplifier headstage.

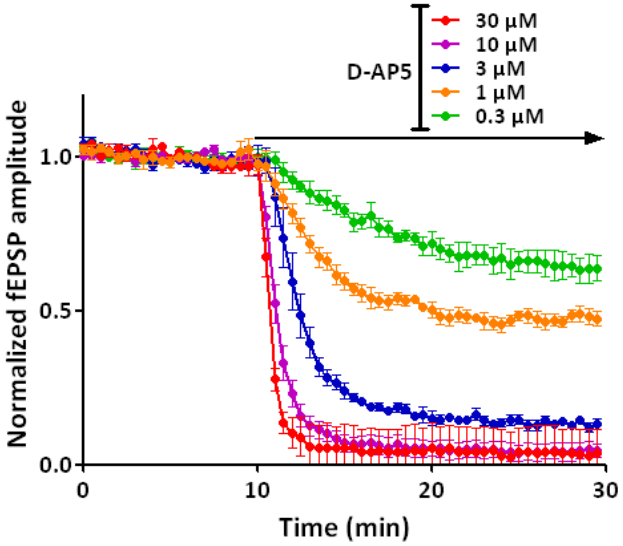
Stimulation/recording protocols

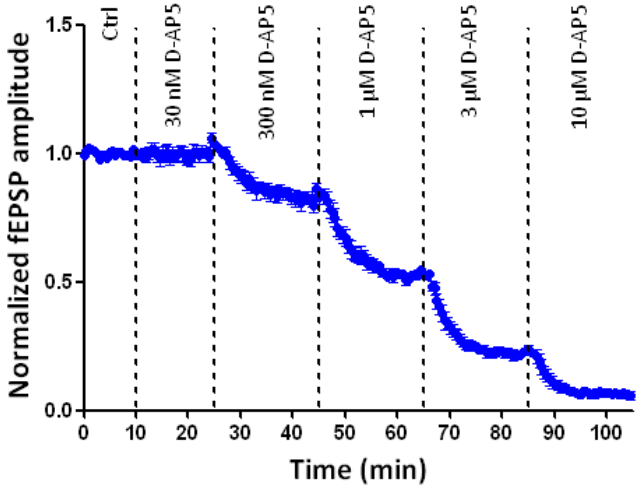
One electrode was chosen to stimulate SC at the CA1 border. The stimulus was consisting in a monopolar biphasic current pulse (−300 µA for 60 µs followed by +300 µA for 60 µs) applied every minute.

Since EPSP result from NMDA-mediated synaptic transmission consecutive to afferent pathway stimulation, and the complete inhibition of the AMPA/Kainate component due to NBQX, 30 µM D-AP5 was perfused on the slice at the end of each experiment, to validate the exclusive NMDA-mediated nature of synaptic transmission as well as to subtract background noise at individual electrode level.

RESULTS

Evaluation of NMDA antagonist or NAM on NMDA-mediated EPSP

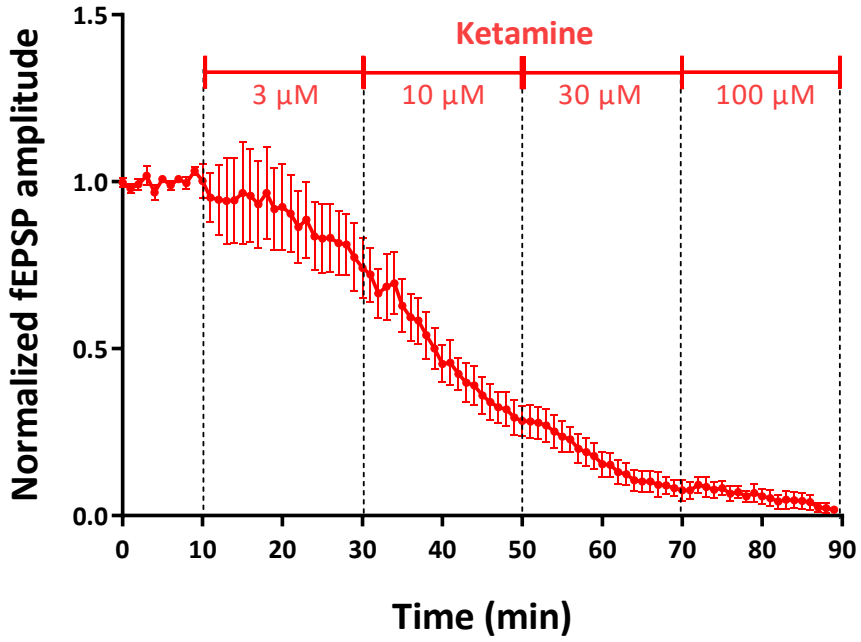


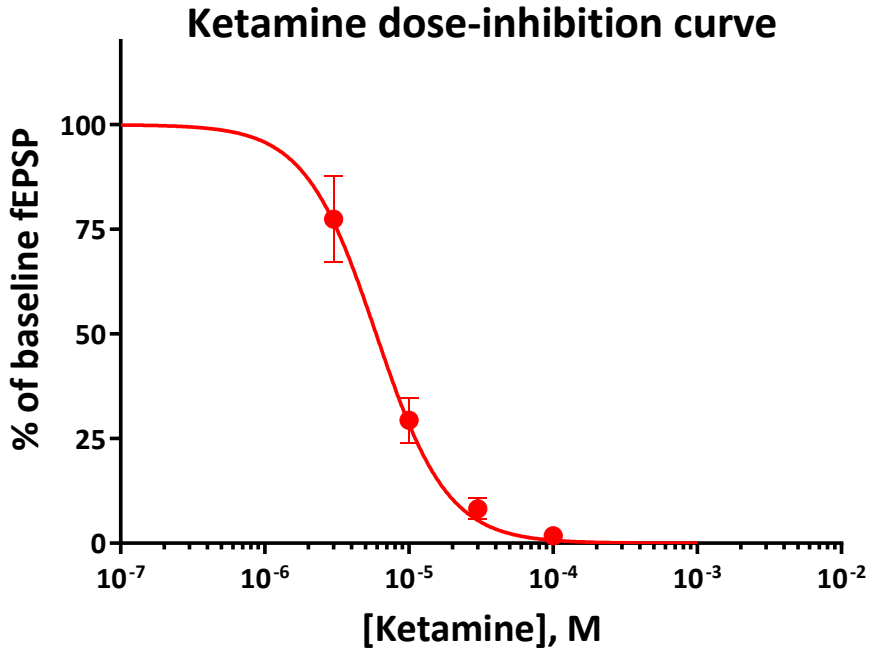


- Under certain conditions (low magnesium + NBQX) pure NMDA-mediated EPSP could be recorded in the CA1 region of hippocampal slices.
- D-AP5 dose-dependently decreased the NMDA-mediated EPSP amplitude in the CA1 region of rat and mouse hippocampal slices.
- Compound concentrations to be evaluated could be tested on independent slices (as in top graph, data from rat hippocampal slices) or sequentially on the same hippocampal slice (as in bottom graph, data from mouse hippocampal slices).

RESULTS

Evaluation of NMDA antagonist or NAM on NMDA-mediated EPSP



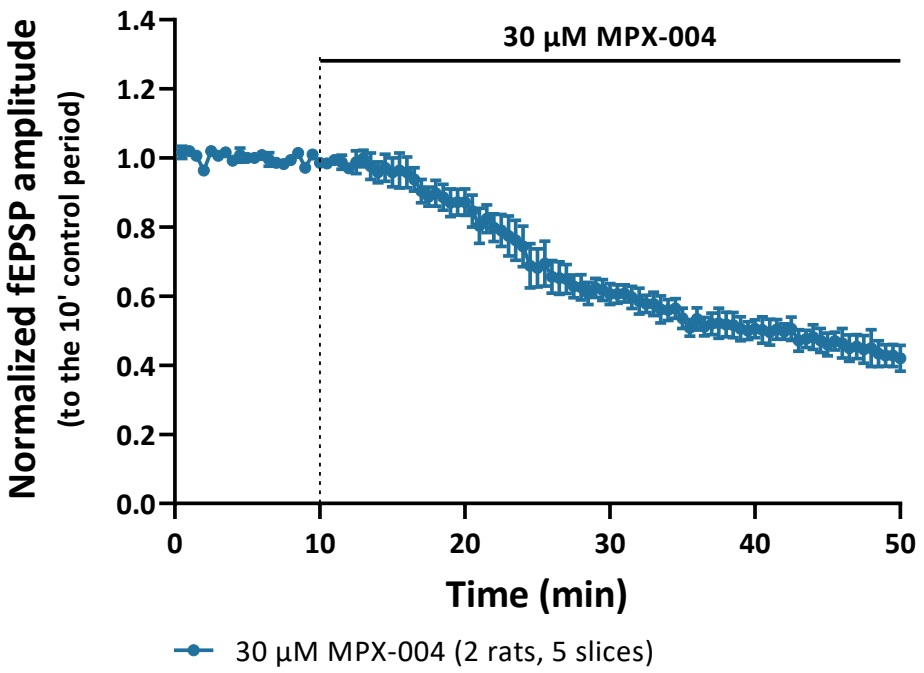


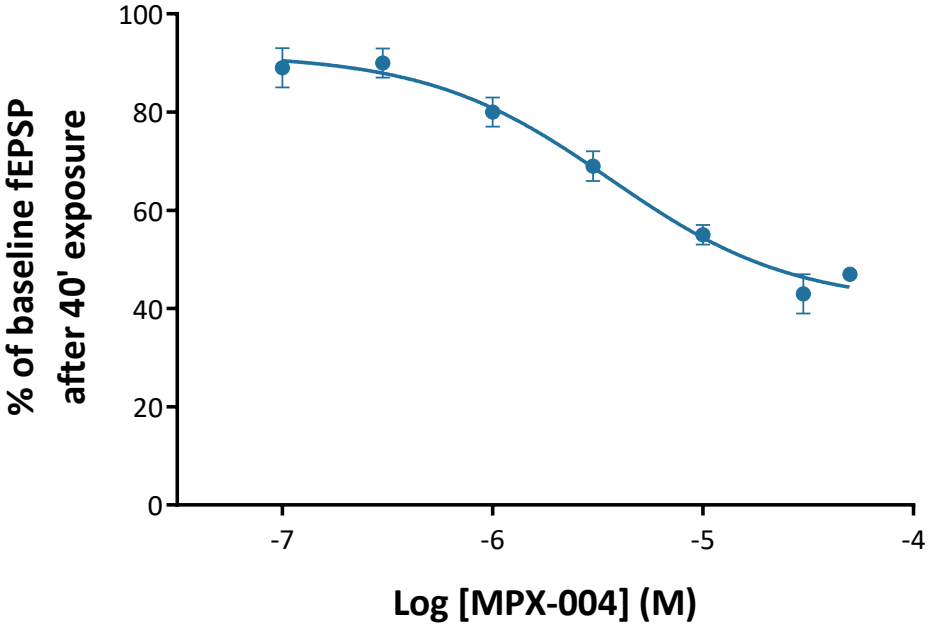
Ketamine dose-response (2 rats, 5 slices, 17 electrodes)

- The NMDA receptor antagonist ketamine dose-dependently inhibited NMDA-mediated EPSP amplitude in the CA1 region of rat hippocampal slices with an IC_{50} close to 4.8 µM.
- However, it is of value to note that stabilization of the NMDA-mediated responses were not stabilized at the end of 20-minute periods of ketamine application. Evaluating the effect of a dose dose-range of ketamine from distinct hippocampal slices would have provide d more accurate results.

RESULTS

Evaluation of sub-unit selective NMDA NAM

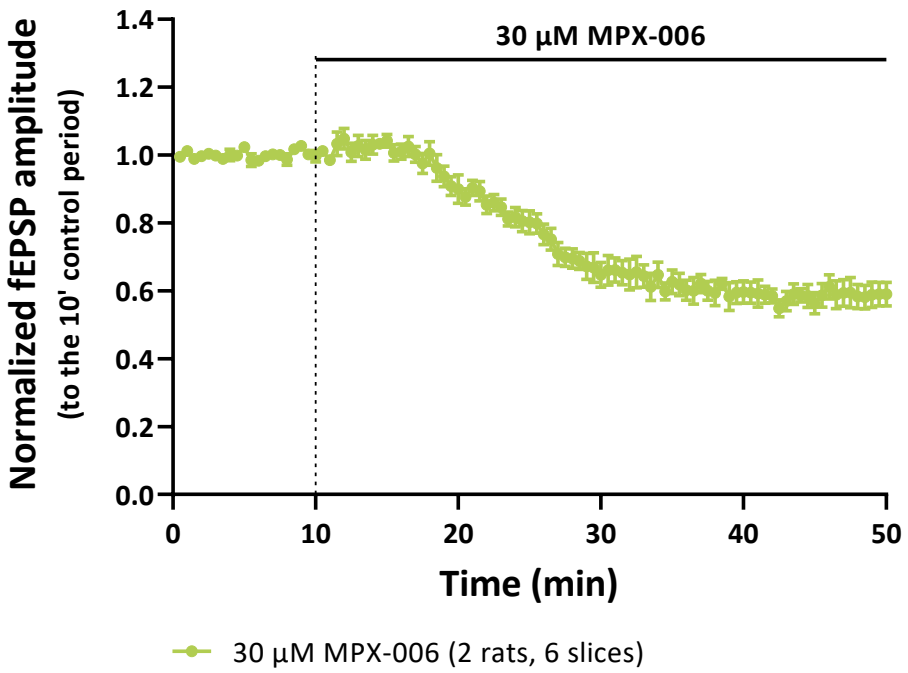


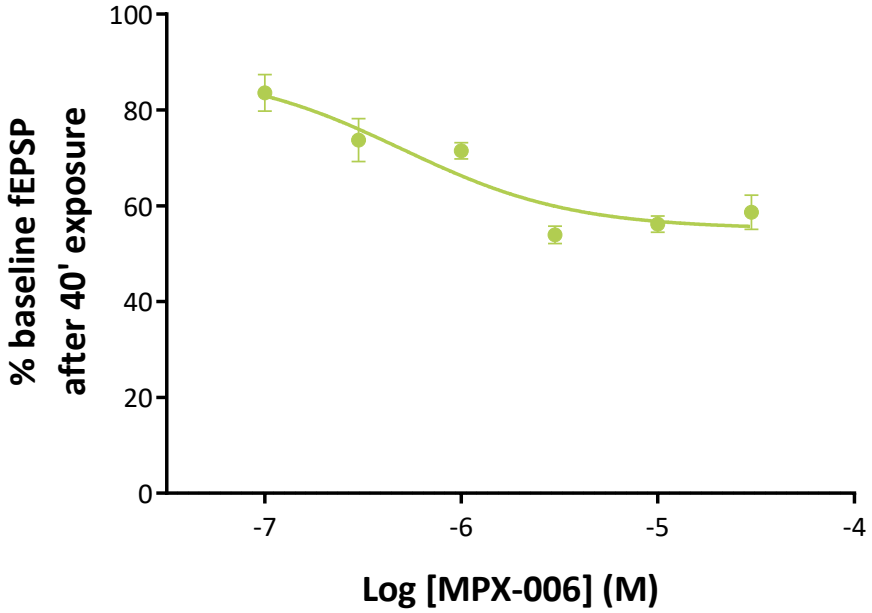


- The NR2A Negative Allosteric Modulator (NAM) MPX-004 dose-dependently decreases the NMDA EPSP amplitude.
- MPX-004 IC_{50} is of 3.6 µM, and the top of the concentration-response curve is reached with 30-50 µM MPX-004. The maximal inhibition of NMDA-mediated EPSP observed in the presence of MPX-004 is 60%. From these experiments, in consistence with literature data, it seems that NMDA currents in CA1 pyramidal neurons are 60 % mediated by NR2A-containing NMDA receptors in 3 week-old rats.

RESULTS

Evaluation of sub-unit selective NMDA NAM

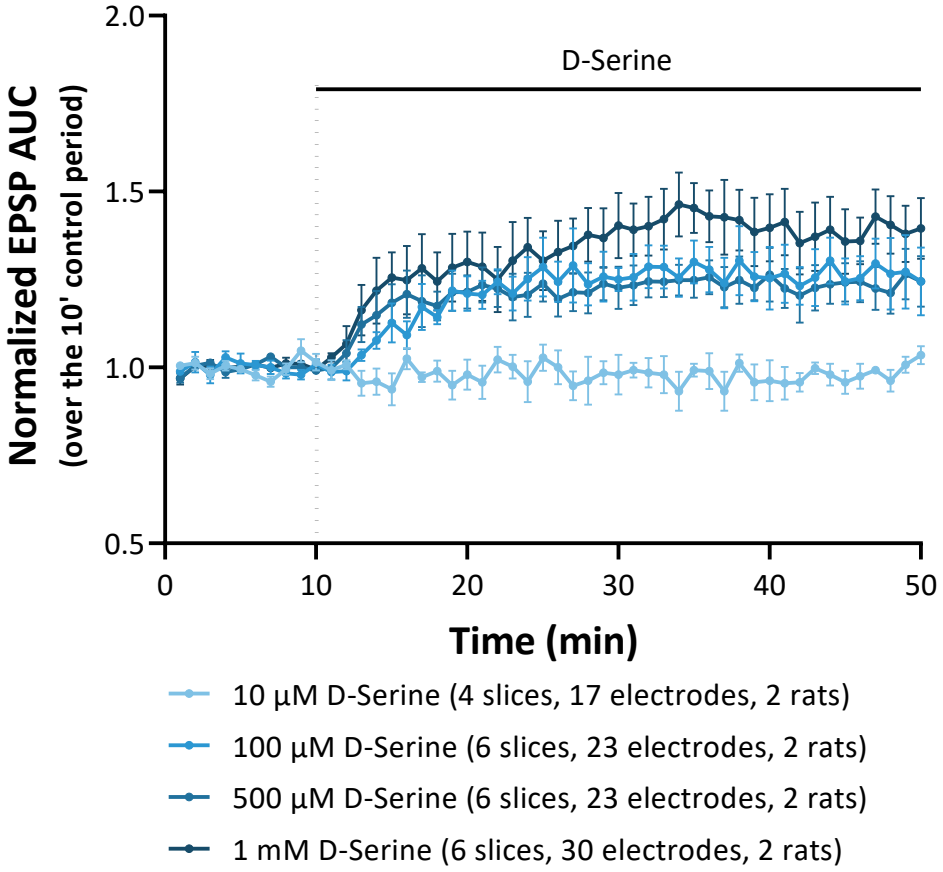




- The NR2B Negative Allosteric Modulator (NAM) MPX-006 dose-dependently decreases the NMDA EPSP amplitude with an IC_{50} of 0.5 µM. The maximal effect of MPX-006 seems reached from 3 µM MPX-006.
- From these experiments, in consistence with literature data, it seems that NMDA currents in CA1 pyramidal neurons are 40 % mediated by NR2B-containing NMDA receptors and 60 % mediated by NR2A-containing NMDA receptors, in 3 week-old rats.

RESULTS

Evaluation of NMDA co-agonist or PAM on NMDA-mediated EPSP



- D-Serine dose-dependently increased the NMDA-mediated EPSP amplitude over a 40-minute application period, in the CA1 region of rat hippocampal slices.
- The NMDA-mediated EPSP represents a rapid and sensitive model to screen compounds which act on NMDA receptors.

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