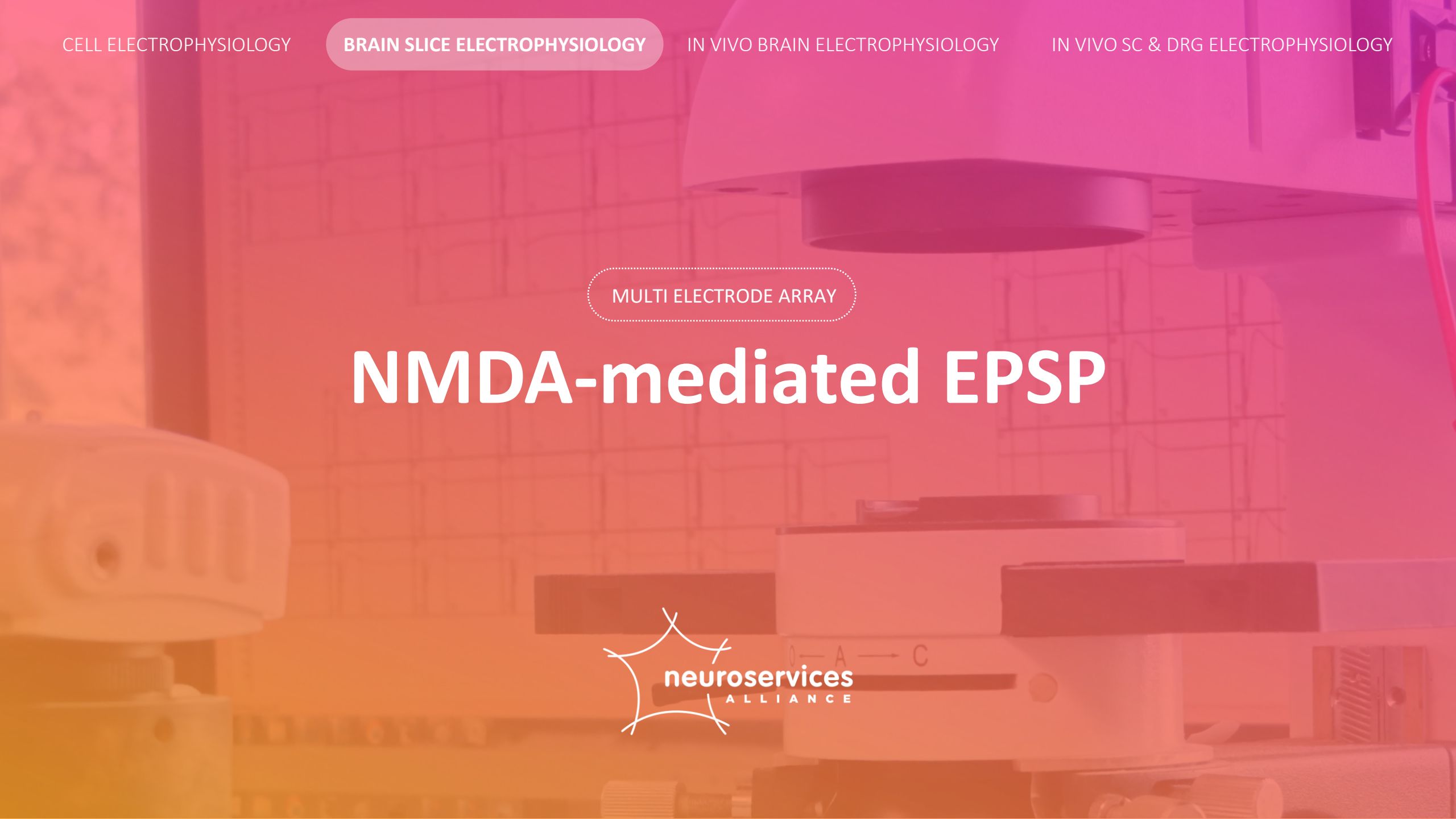
MULTI ELECTRODE ARRAY

NMDA-mediated EPSP



SUMMARY

NMDA-mediated EPSP

- Materials & Methods

Reference data

- AMPA antagonists – Perampanel
- NMDA antagonists – Ketamine, memantine, MK-801, amantadine
- NMDA agonists – D-serine
- NR2A/B subunits – MPX-004, MPX-006, MPX-007, RO-256981
- NR2A subunit – MPX-007

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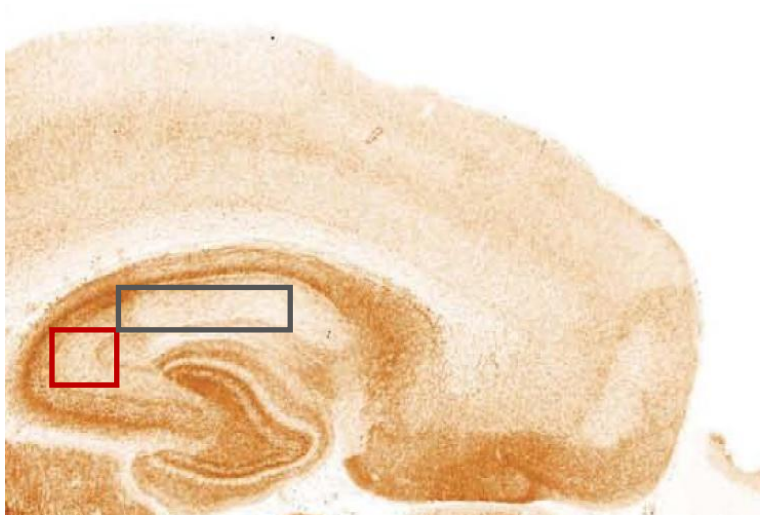
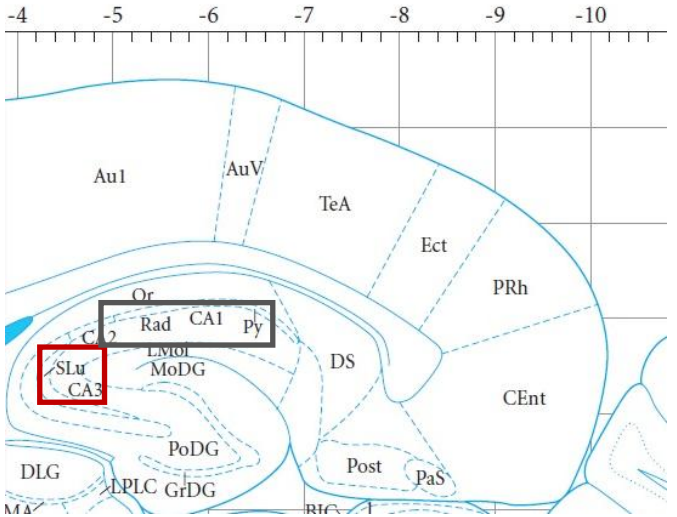
MATERIALS & METHODS

Area of recording – transverse hippocampal slices

summary





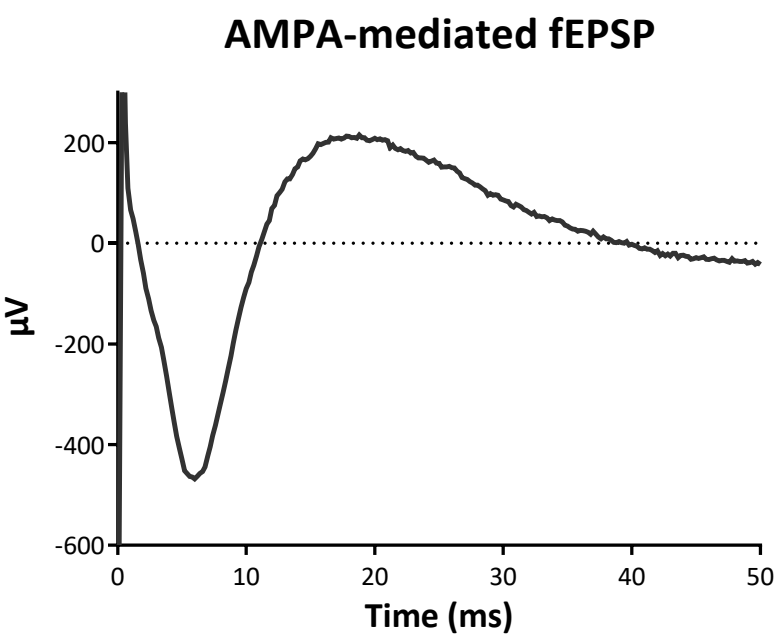
Stimulation area

Recording area

MATERIALS & METHODS

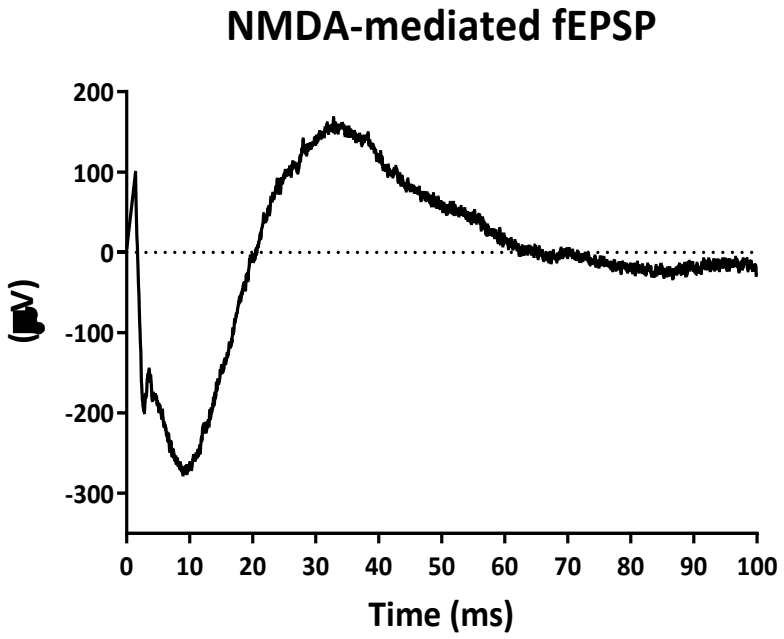
NMDA-mediated EPSPs recording

summary



Release NMDA receptor
(aCSF 0.1 mM $MgCl_2$)

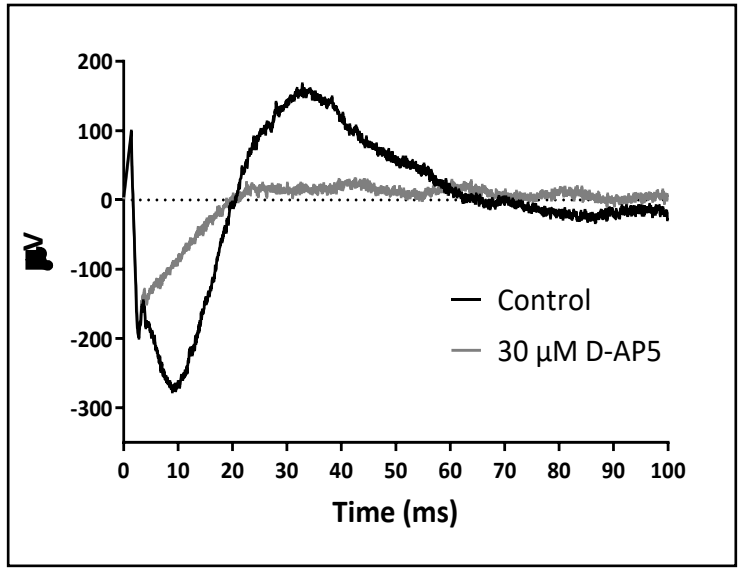
Block AMPA-mediated EPSP
(10 µM NBQX)



MATERIALS & METHODS

Data analysis

summary



Extracellular recording of Excitatory Post-Synaptic Potential (EPSP)

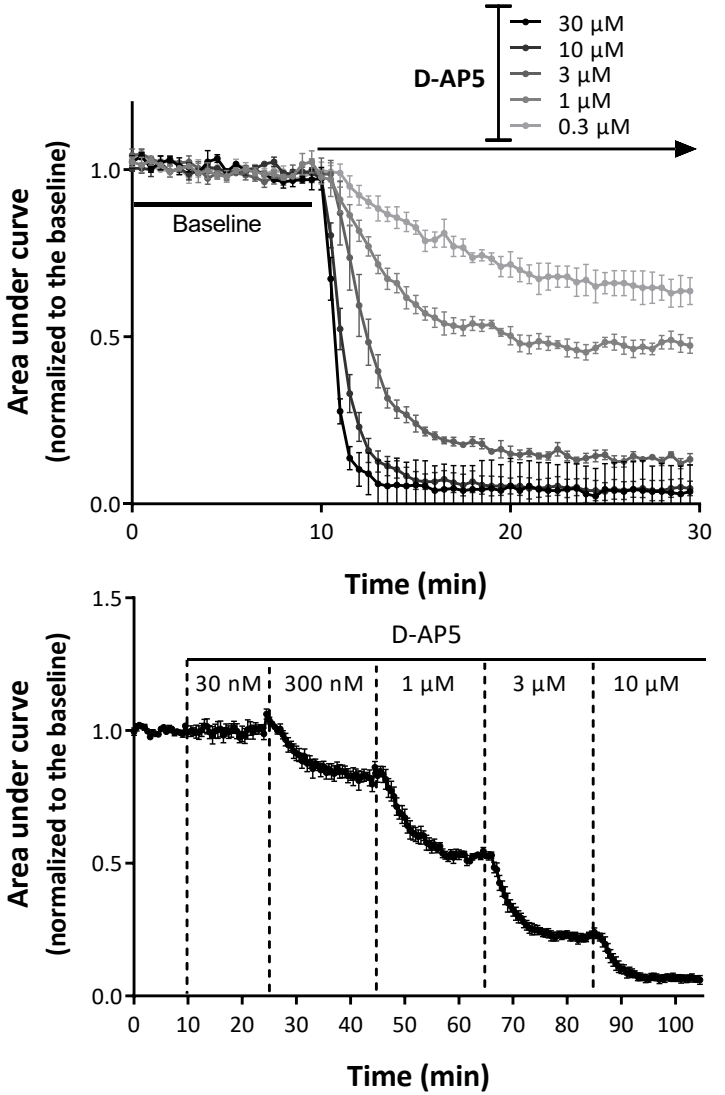
After 30 µM D-AP5 application, only the afferent volley and the system background noise remain (grey trace).

Area Under Curve
(NMDA-mediated EPSP AUC from 0 to 100 msec range)

Sampling
(1 minute bin)

Normalization
(over the baseline period)

Background noise And afferent volley suppression
(subtraction of AUC during the D-AP5 period)



Example of data with D-AP5, a NMDA antagonist

RESULTS

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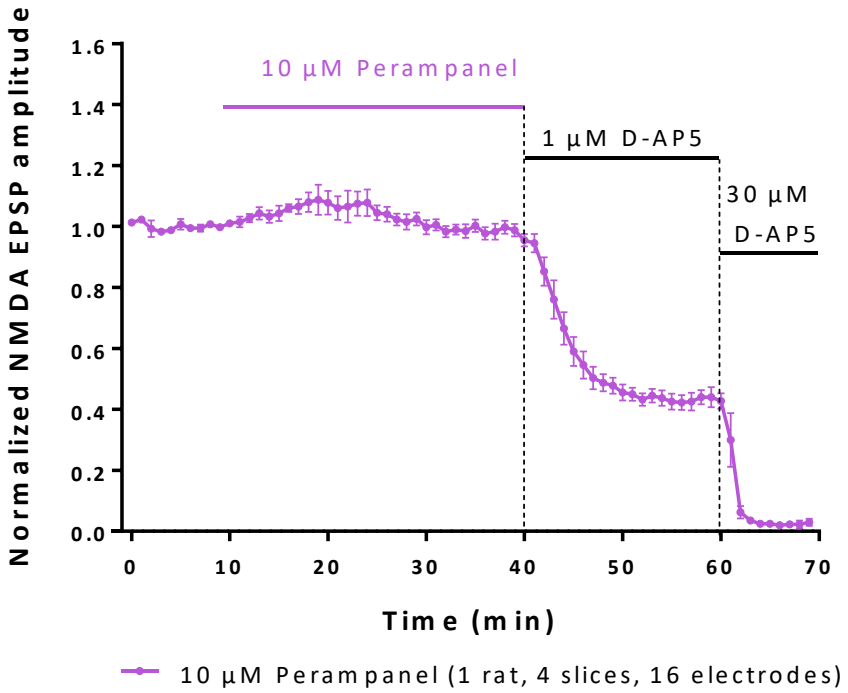
RESULTS

NR2A/B subunits

summary

Perampanel, D-AP5



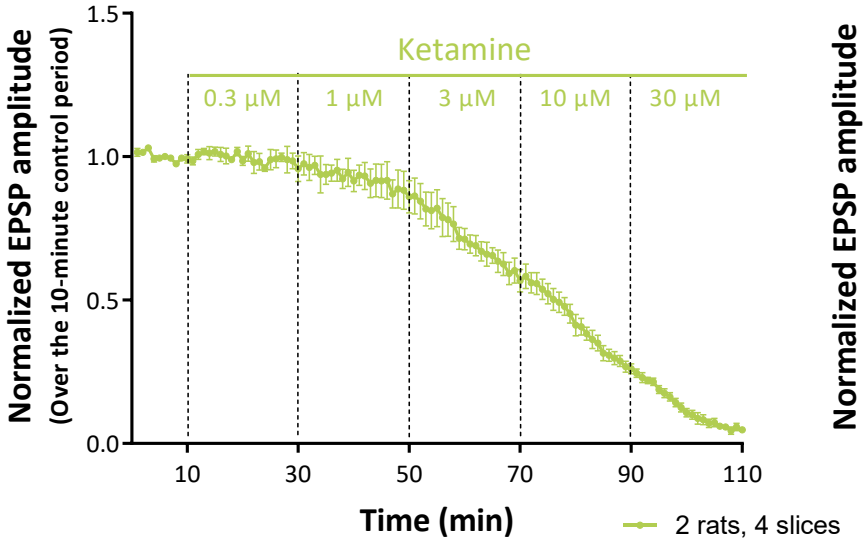
- 10 µM Perampanel – AMPA receptor antagonist – did not modify the amplitude of NMDA-mediated EPSP in rat hippocampal slices, over a 30-minute period.
- 1 µM D-AP5 decreased the amplitude of NMDA-mediated EPSP by about 55% after a 20-minute period, whereas a full inhibition of NMDA EPSP was observed after a few minutes of exposure to 30 µM D-AP5.

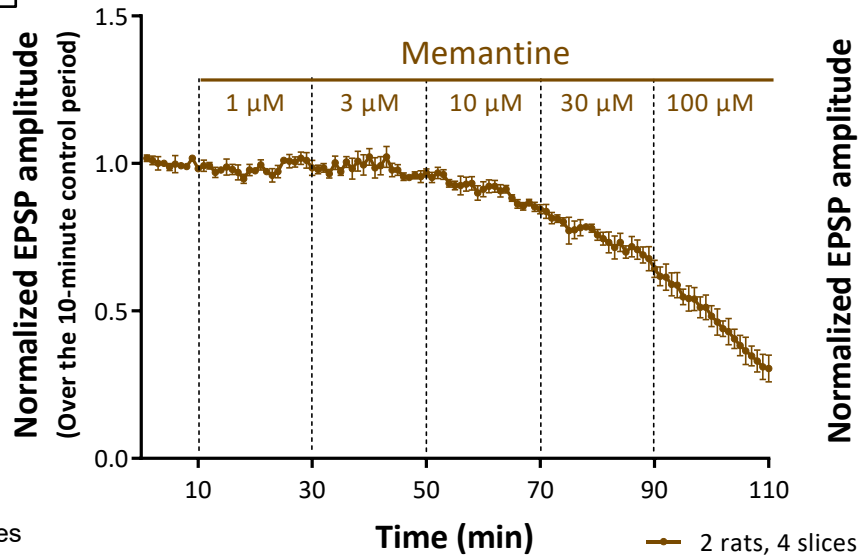
RESULTS

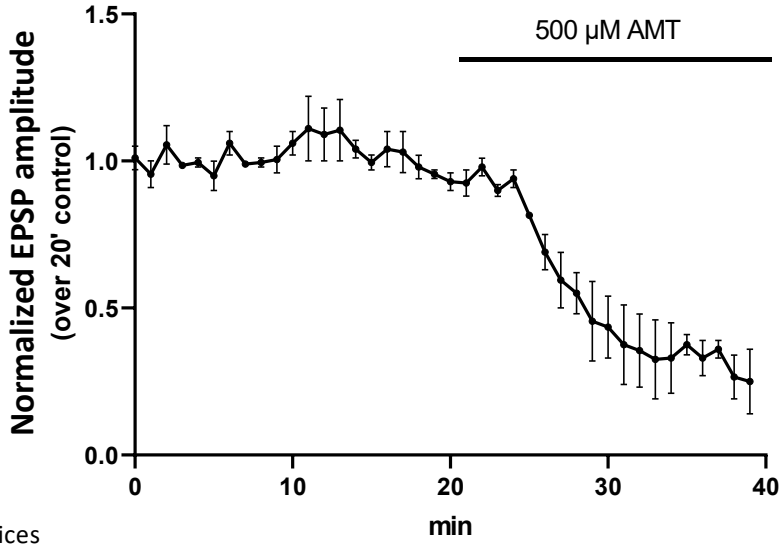
NMDA antagonists

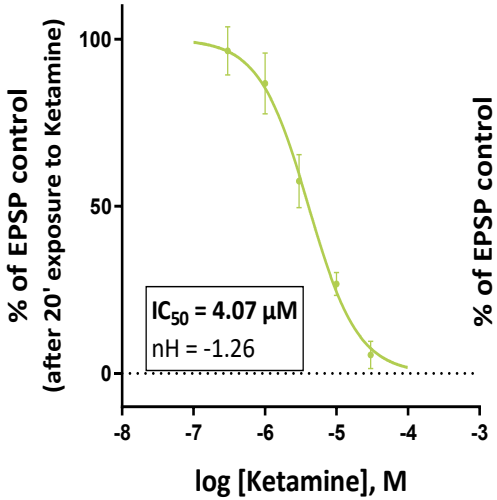
Ketamine, memantine, amantadine, MK-801

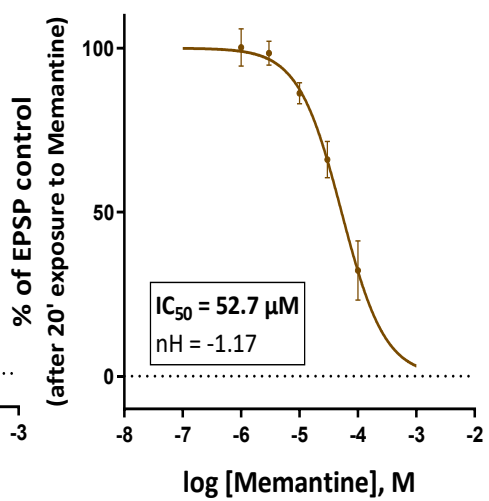
summary

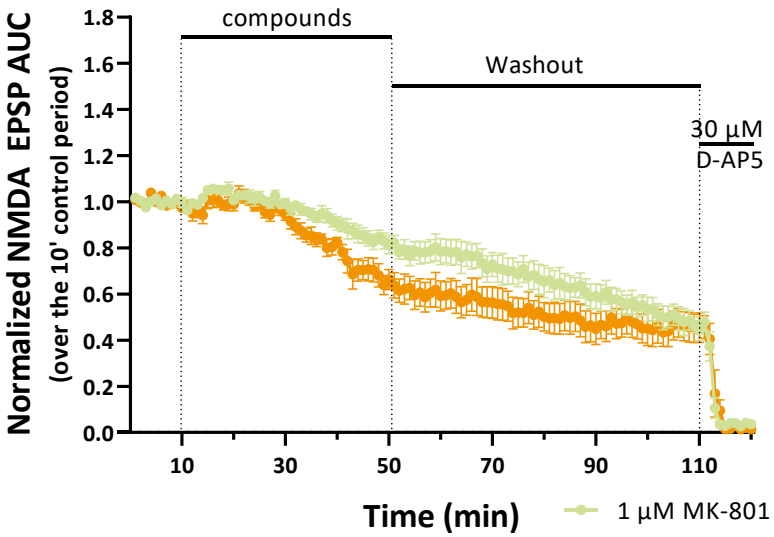












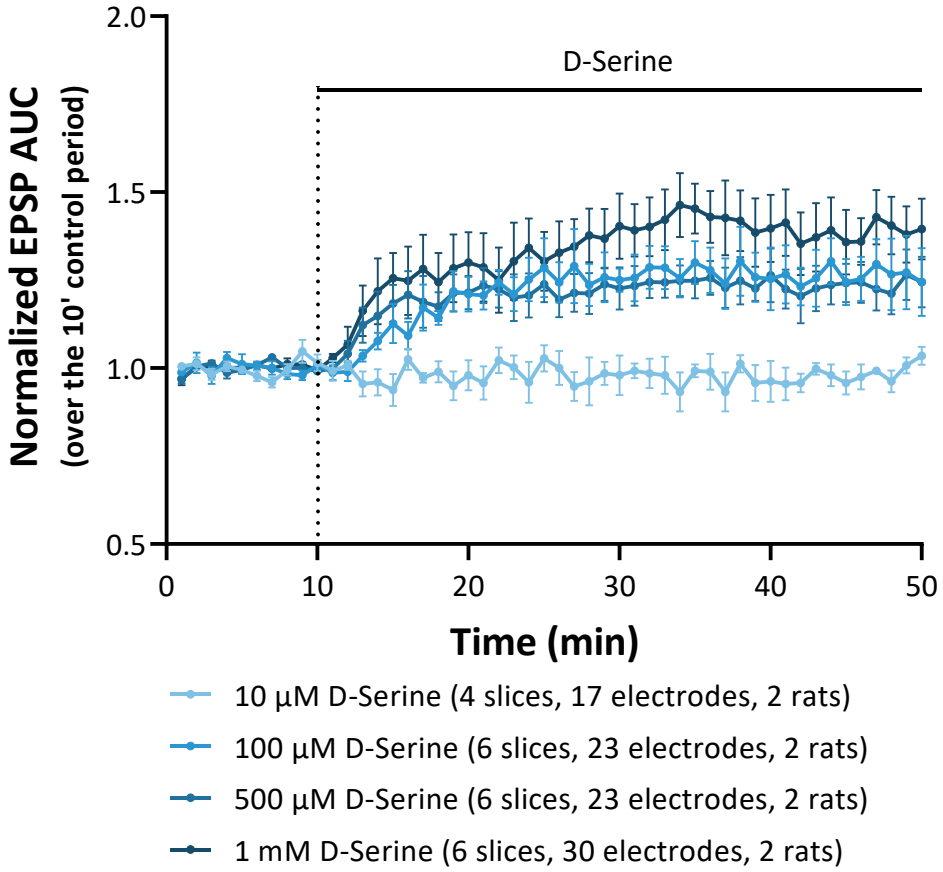
- The NMDA receptor antagonists ketamine and memantine dose-dependently inhibited NMDA-mediated EPSP with an IC_{50} close to 4 µM and 50 µM, respectively. The effect of memantine and MK-801 did not reverse at washout.
- Amantadine applied at 500 µM largely inhibited NMDA-mediated EPSPs.

RESULTS

NMDA agonists

summary

D-serine



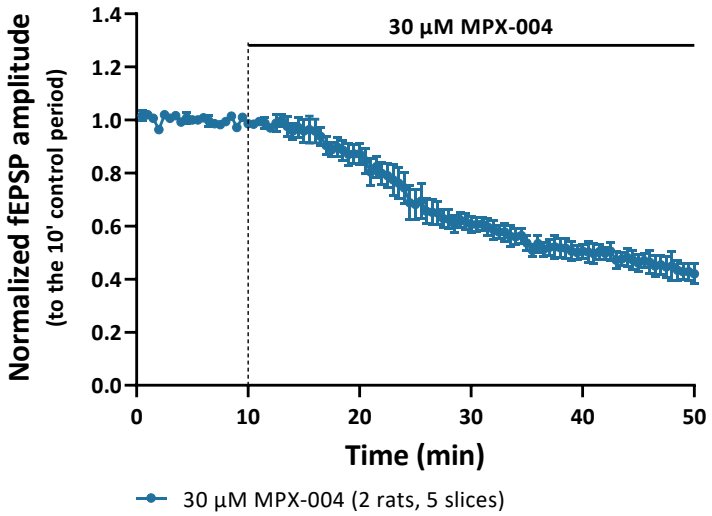
- D-Serine dose-dependently increased the NMDA-mediated EPSP amplitude over a 40-minute application period, in the CA1 region of rat hippocampal slices.

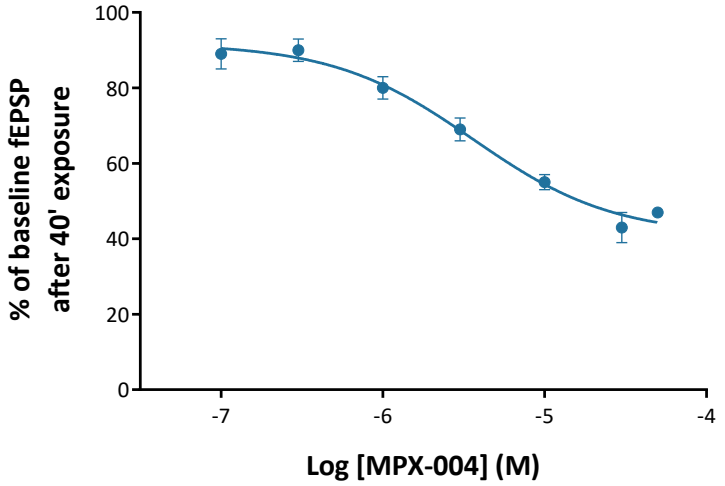
RESULTS

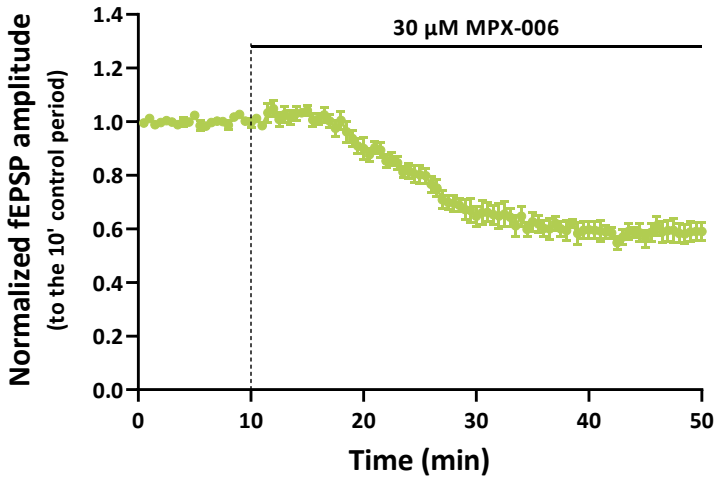
NR2A/B subunits

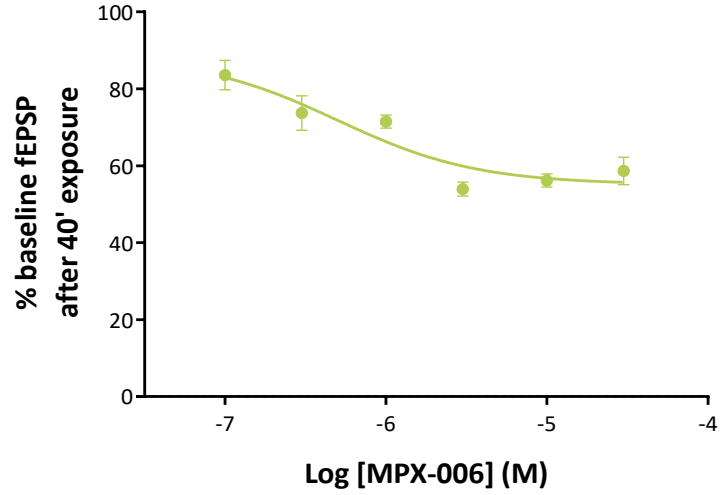
summary

MPX-004, MPX-006









Experiments performed from 3 week-old rats

- The NR2A Negative Allosteric Modulator (NAM) MPX-004 dose-dependently decreased the NMDA EPSP amplitude. MPX-004 IC_{50} is 3.6 µM, and the top of the concentration-response curve is reached with 30-50 µM MPX-004, causing a decrease of about 60 % of the evoked-responses.
- The NR2B Negative Allosteric Modulator (NAM) MPX-006 dose-dependently decreased 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, causing a decrease of about 40 % of the evoked-responses.

Such results are consistent with literature data, indicating that in CA1 pyramidal neurons, 40 % of NMDA currents are mediated by NR2B-containing NMDA receptors and 60 % mediated by NR2A-containing NMDA receptors, in 3 week-old rats.

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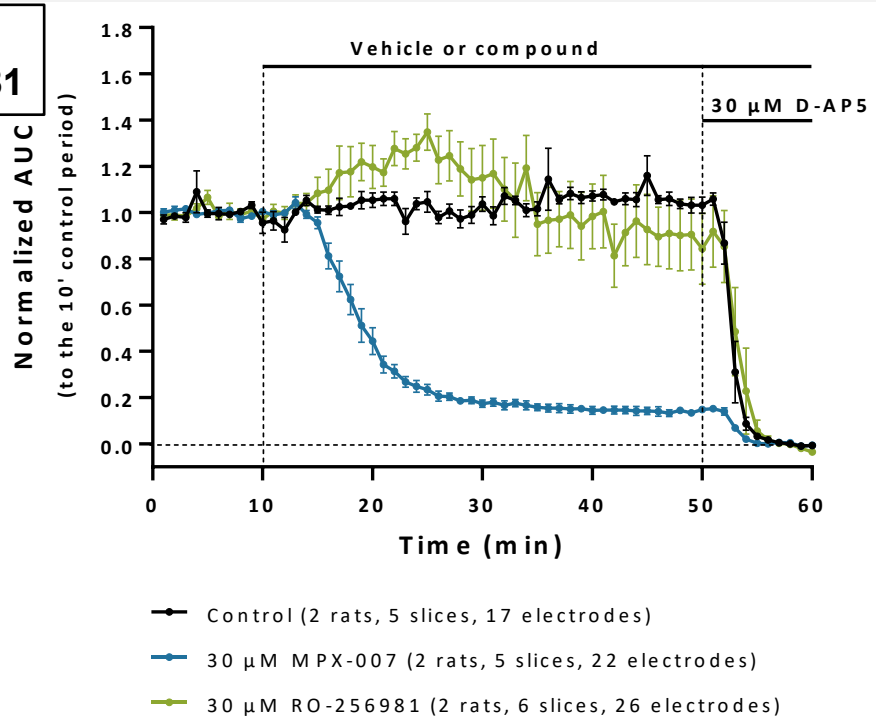
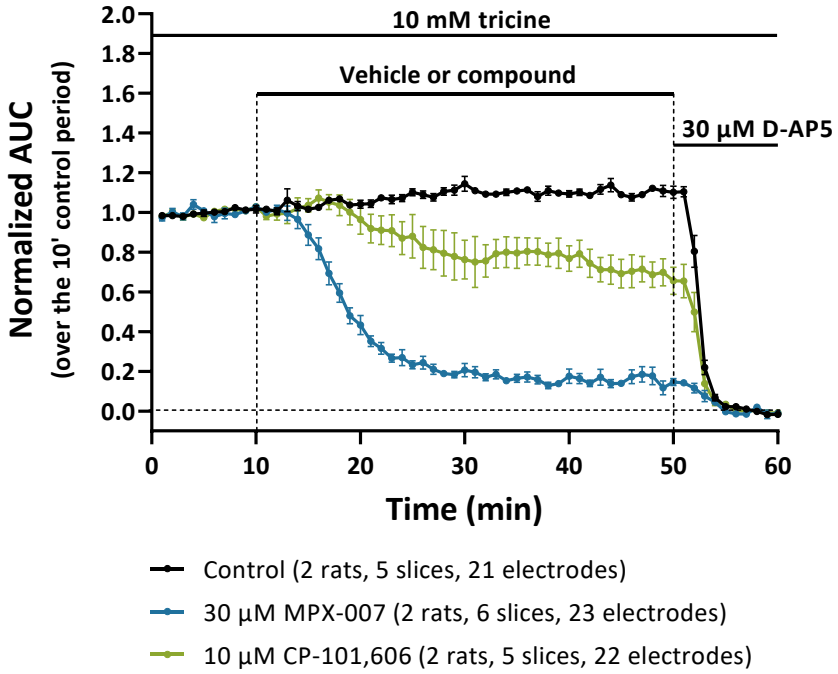
RESULTS

NR2A/B subunits

summary

MPX-007
RO-256981





Experiments performed from 6 week-old rats

- In the presence or absence of tricine, exposure to 30 µM MPX-007 (a NAM selective for NMDA receptors containing the NR2A sub-unit) drastically inhibited NMDA-mediated EPSP by about 85 %.
- 30 µM RO-256981 (a NAM selective for NMDA receptors containing the NR2B sub-unit) slightly decreased NMDA EPSP by about 15 % at end point.

- In the presence of tricine – used to chelate ambient zinc - 10 µM CP-101,606 (a NAM selective for NMDA receptors containing the NR2B sub-unit) decreased the NMDA EPSP AUC by about 35 %.
- For all experimental conditions, 30 µM D-AP5 (a selective NMDA antagonist) fully inhibited NMDA-mediated EPSP.

Consistently with the switch of NR2B to NR2A sub-units expression occurring along animals development, in 6 week-old Sprague-Dawley rats, the NR2A/NR2B components of NMDA-mediated EPSP were around 85% and 15%, respectively

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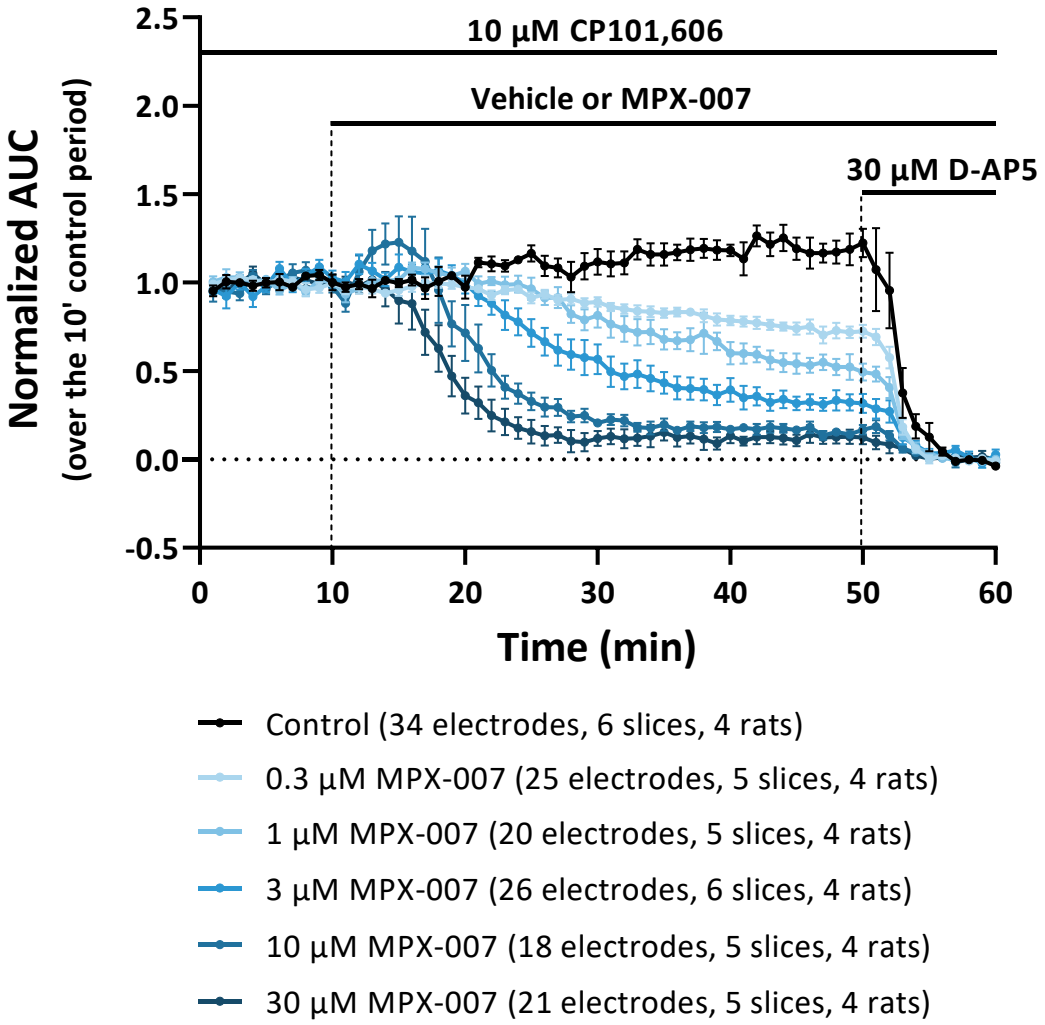
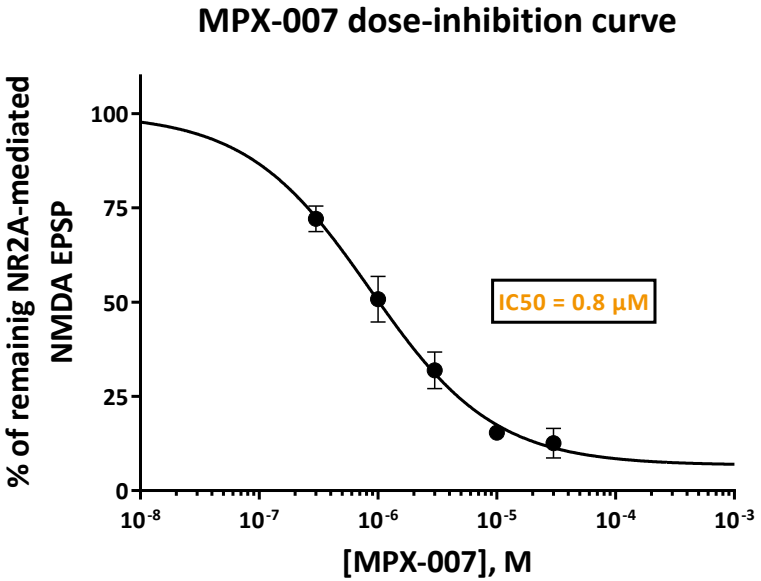
RESULTS

NR2A/B subunits

summary

MPX-007





- In the presence of tricine and CP101,606 (a NAM selective for NMDA receptors containing the NR2B sub-unit) MPX-007 - a NAM selective for NMDA receptors containing the NR2A subunit – dose-dependently inhibited NR2A-mediated NMDA EPSP.

- MPX-007 inhibited the NR2A-mediated NMDA EPSP in a concentration-dependent manner with an IC_{50} close to 0.8 µM.

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