MULTI ELECTRODE ARRAY

Spinal cord – Dorsal horn

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MAIN SUMMARY

Materials & Methods

- Spinal cord – Lumbar part
- Laminar organization
- 4-AP-induced Epileptiform Discharge (ED)
- Spontaneous firing activity (SF)
- Evoked-responses (EPSPs paired-pulse)
- Synaptic plasticity – Long-Term Potentiation

Rats data

- Epileptiform discharge activity in CCI model neuropathic pain
- Spontaneous firing activity (SF)
- Capsaicin-induced firing activity as a pain model
- Evoked-responses (EPSPs paired-pulse)
- NMDA-mediated EPSP

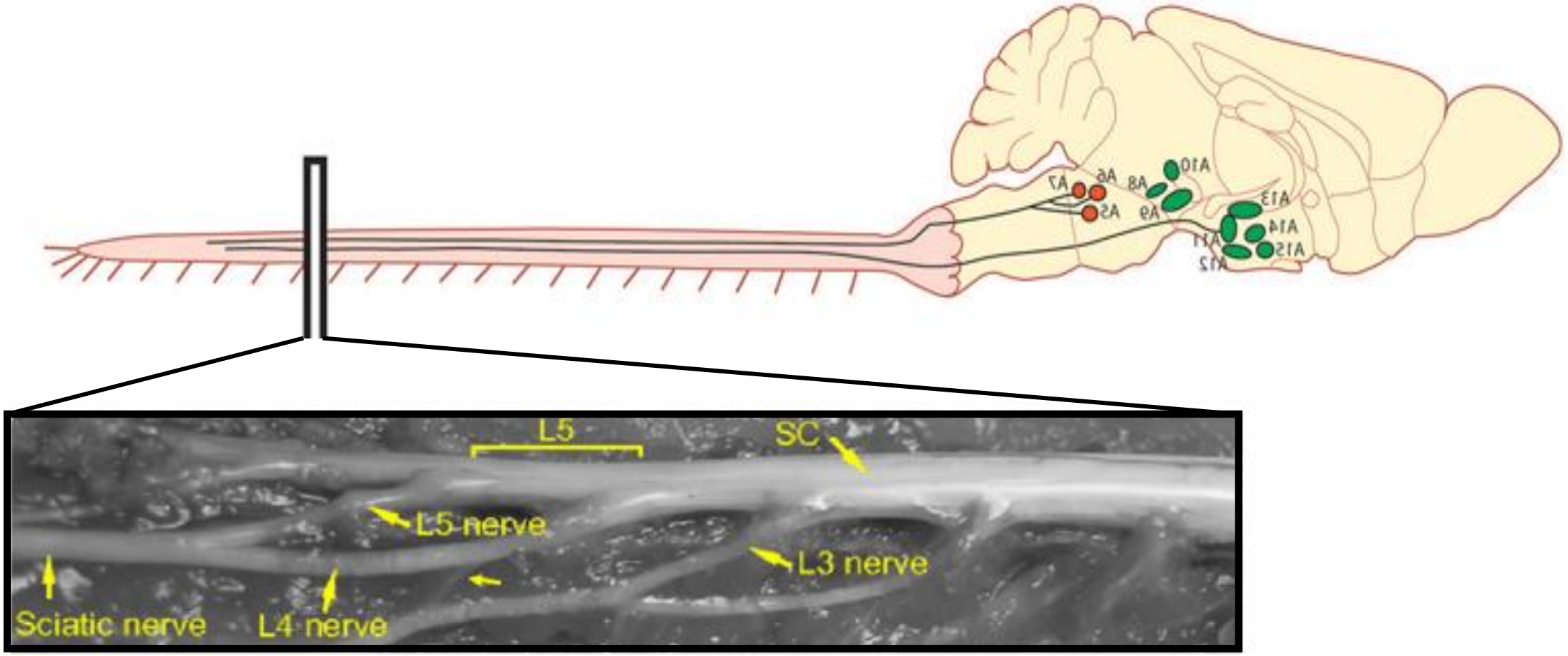
Mice data

- Spontaneous firing activity (SF)

MATERIALS & METHODS

Spinal cord – Lumbar part

Main summary

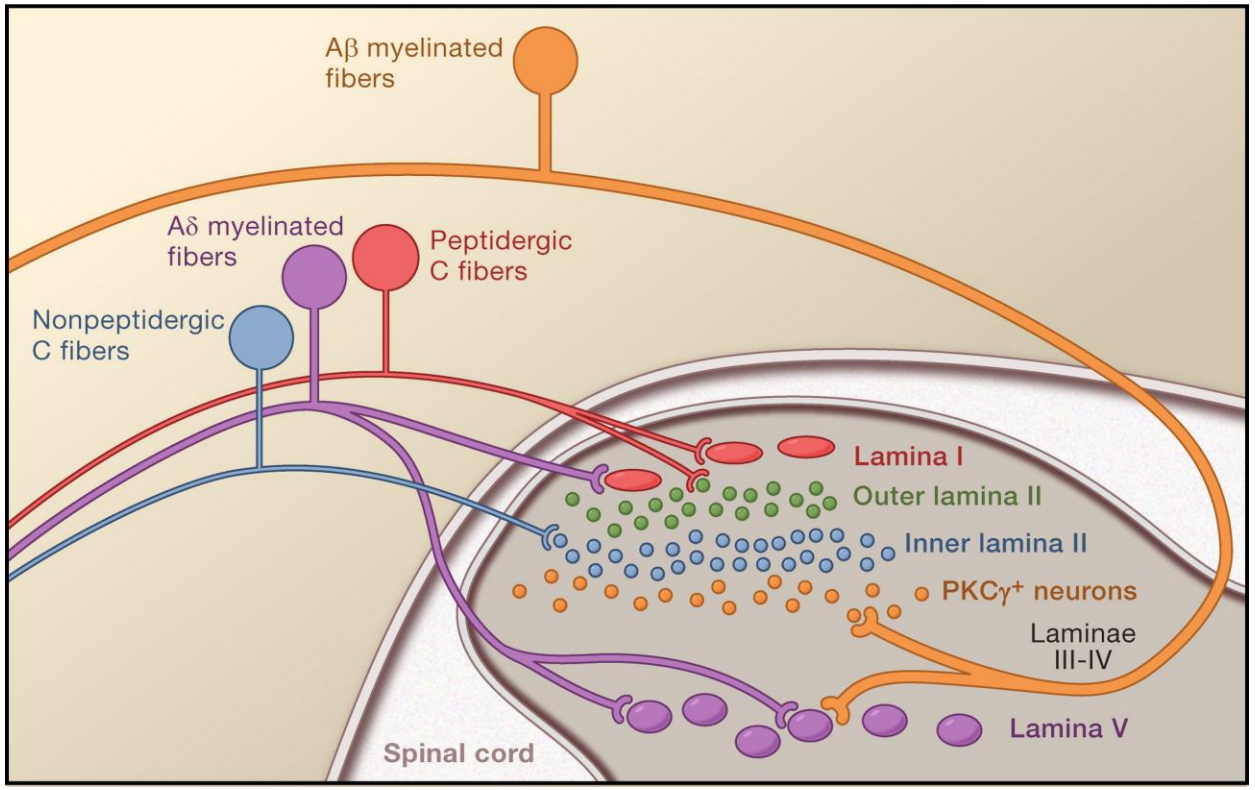


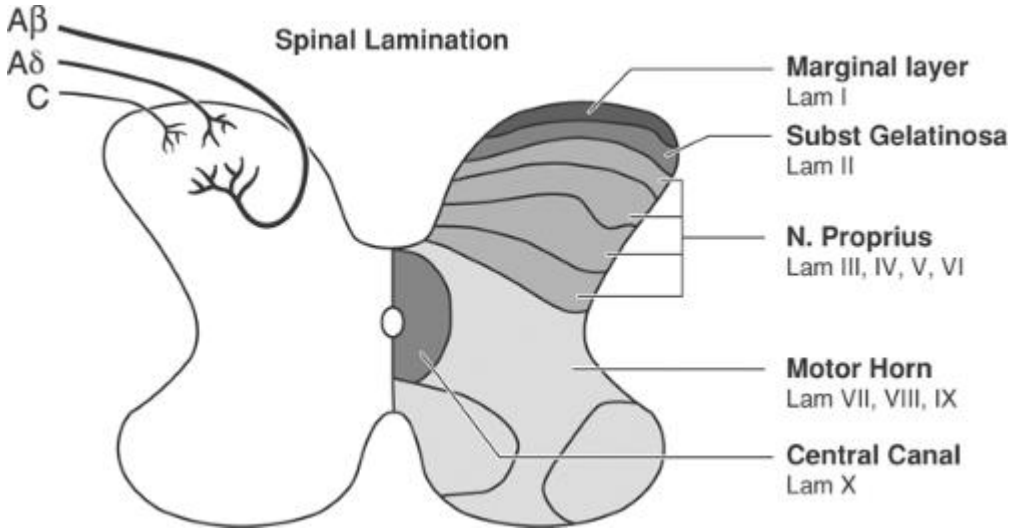
- After a dorsal laminectomy, the lumbar part of the spinal cord is isolated and cut in 300 µm thick coronal slices.

MATERIALS & METHODS

Laminar organization

Main summary



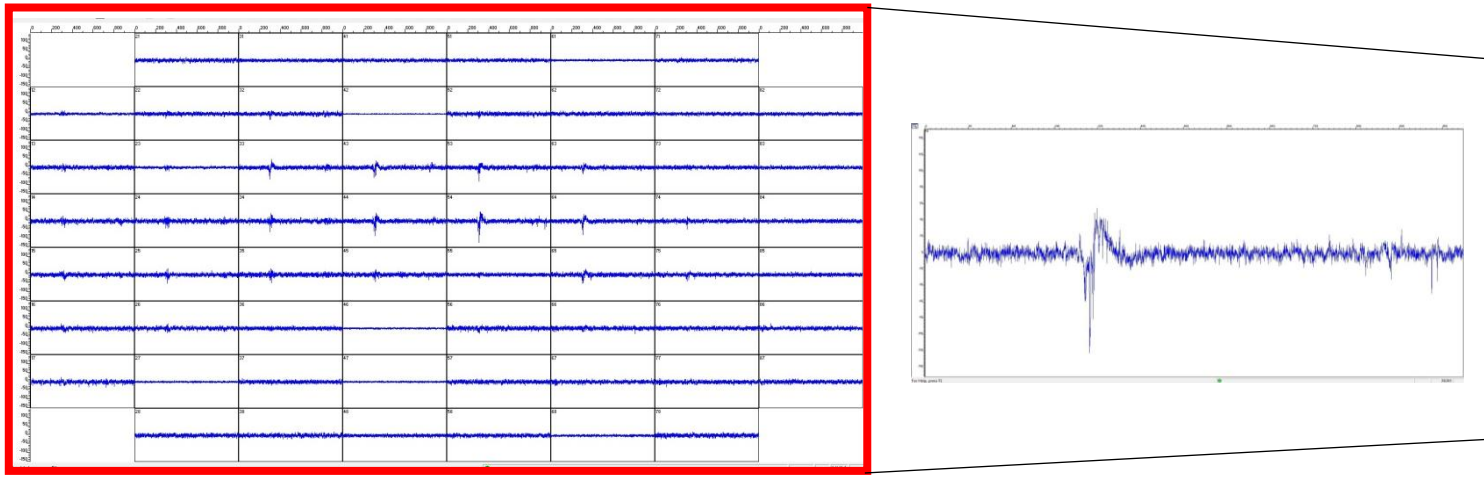


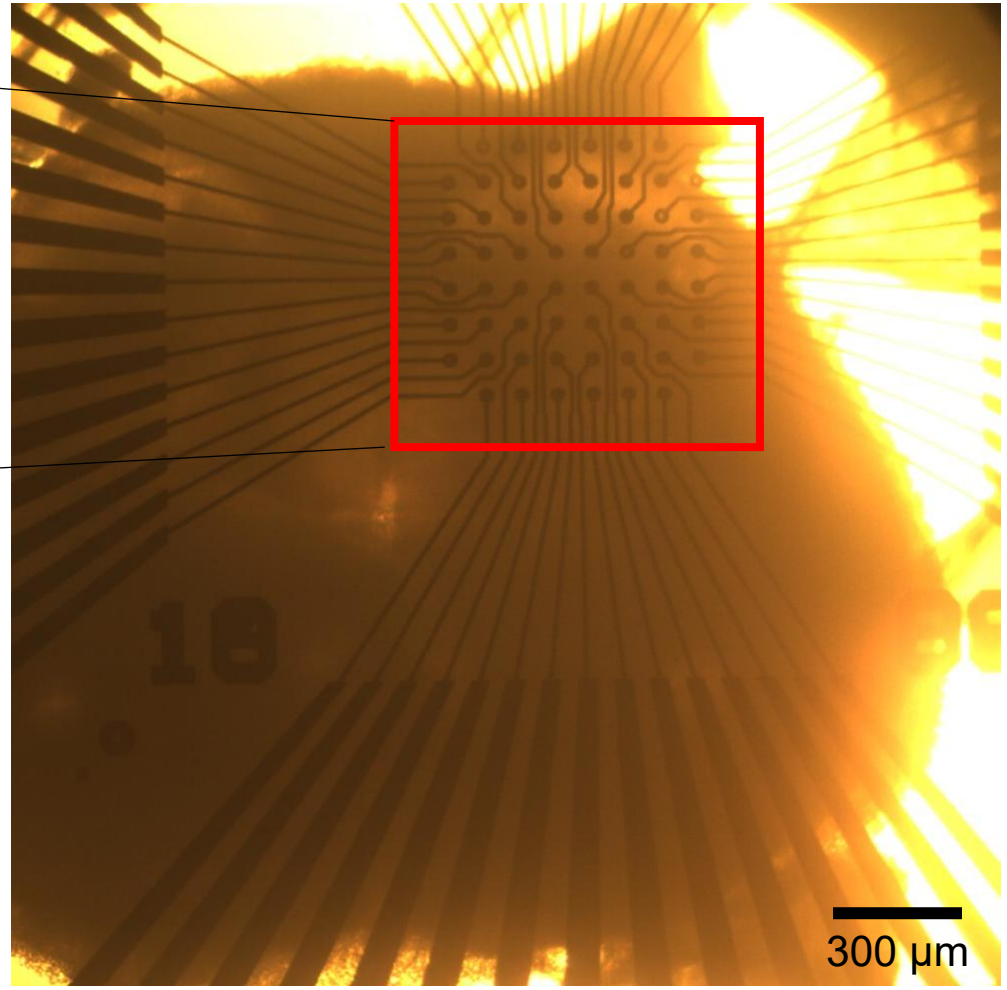
- The dorsal horn of the spinal cord displays a laminar organization; subsets of primary afferent fibers target spinal neurons within discrete laminae. The unmyelinated, peptidergic C (red) and myelinated Aδ nociceptors (purple) terminate most superficially, synapsing upon large projection neurons (red) located in lamina I and interneurons (green) located in outer lamina II. The unmyelinated, nonpeptidergic nociceptors (blue) target interneurons (blue) in the inner part of lamina II. By contrast, innocuous input carried by myelinated Aβ fibers (orange) terminates on PKCγ expressing interneurons in the ventral half of the inner lamina II. A second set of projection neurons within lamina V (purple) receive convergent input from Aδ and Aβ fibers *(Basbaum et al. - 2009 - Cellular and Molecular Mechanisms of Pain)*.

MATERIALS & METHODS

4-AP-induced Epileptiform Discharge(ED)

Main summary





- Paroxysmal forms of neuropathic pain have common features with epileptic seizures. These observations have led to the successful attempt to relieve neuropathic pain by anticonvulsant drugs (Rusheweyh, 2003).

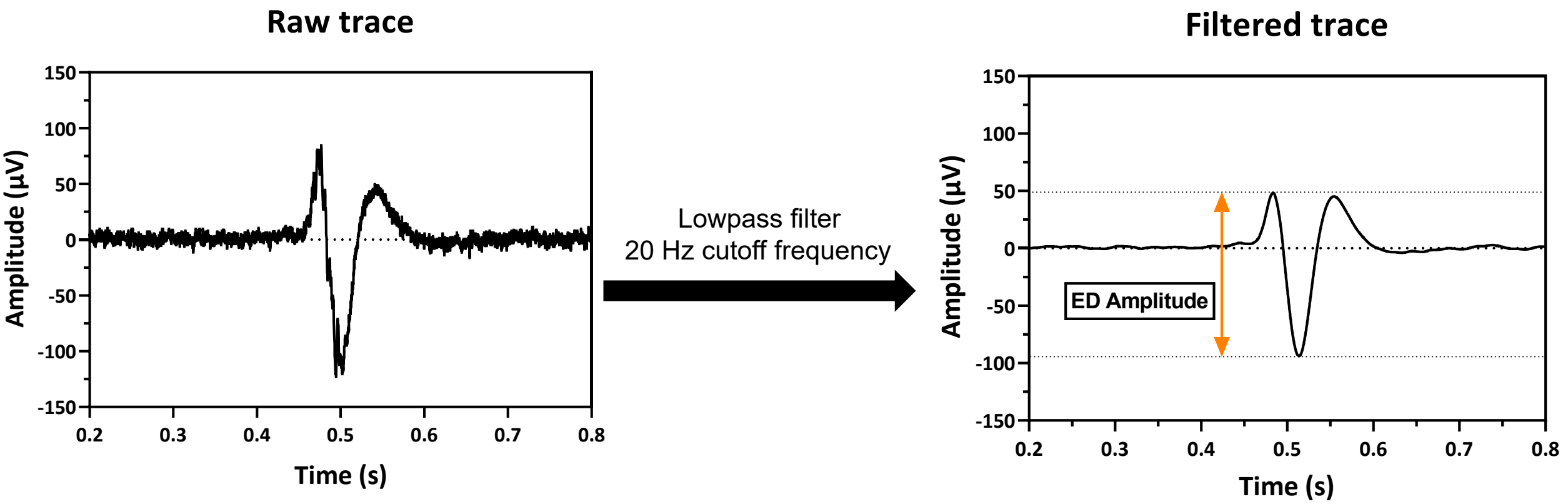
- A single epileptiform discharge consist of multiple superimposed inward postsynaptic currents.

- The MEA technique allows to monitor the effect of different drugs on Epileptiform Discharges in the superficial laminae (I-II-III) of the dorsal horn spinal cord slices.

MATERIALS & METHODS

4-AP-induced Epileptiform Discharge(ED)

Main summary



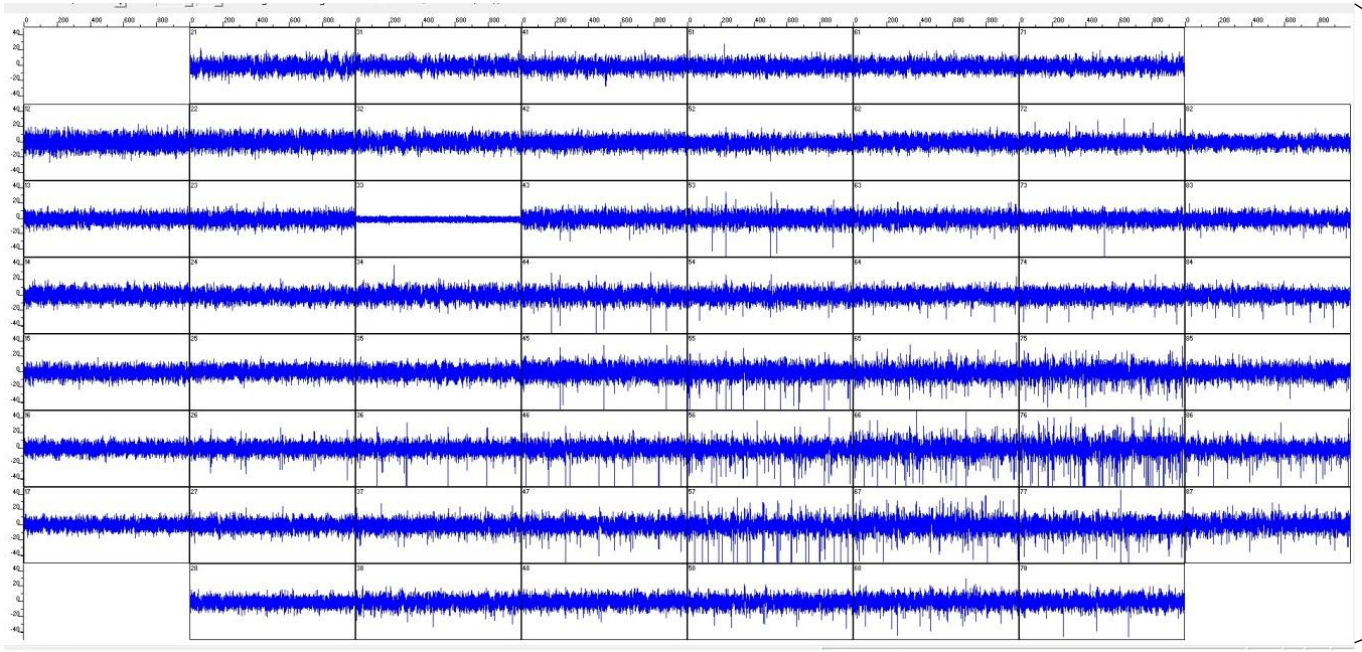
Validation criteria

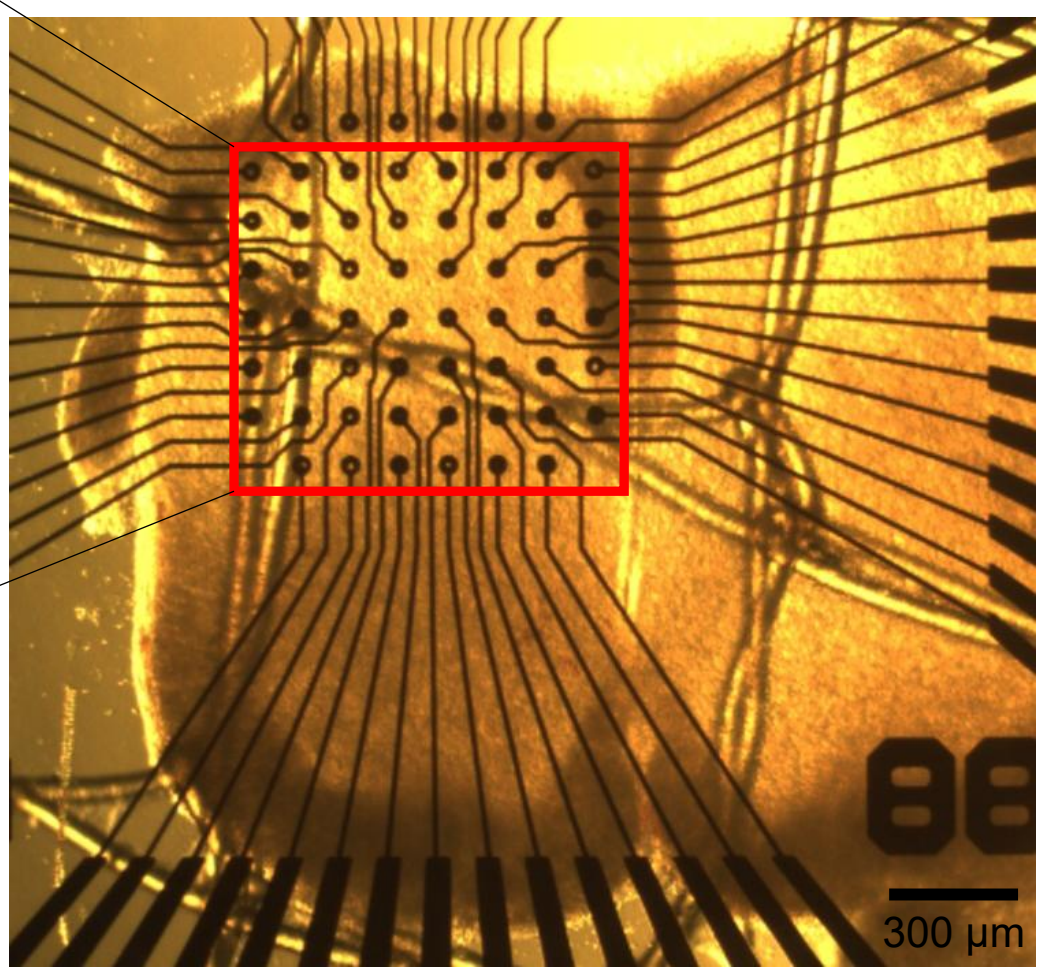
- Epileptiform Discharges (EDs) have to be higher than 15 µV to be counted.

MATERIALS & METHODS

Spontaneous firing activity(SF)

Main summary





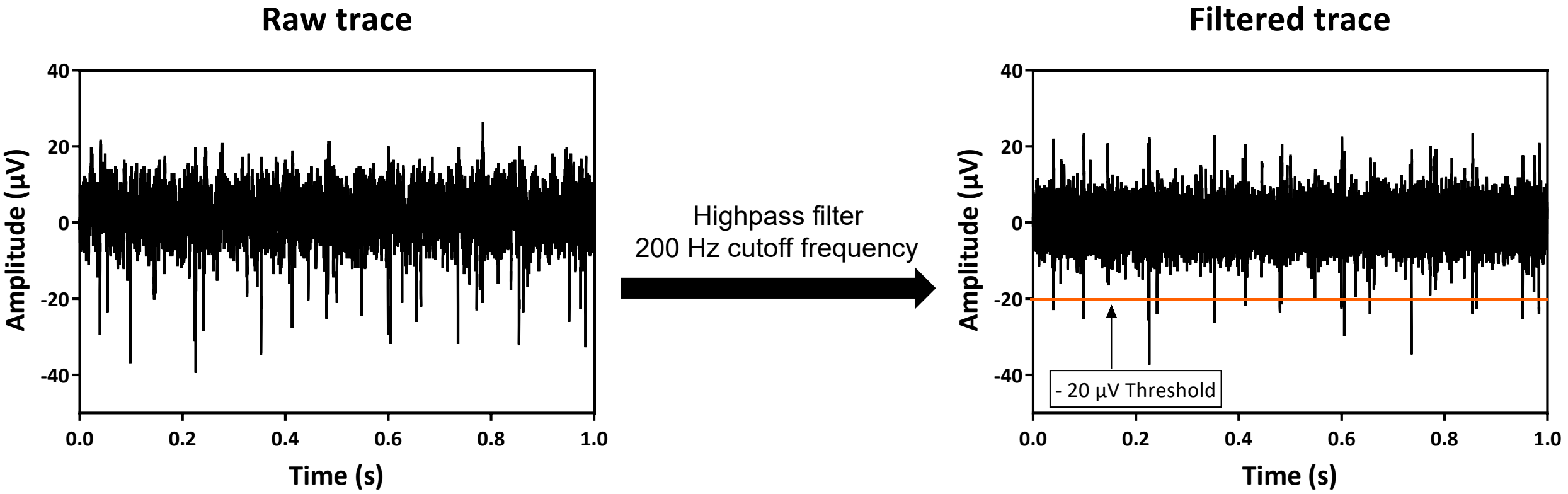
- Neurons of the different layers of the dorsal horn are active in physiological conditions.

- The MEA technique allows to monitor the effect of different drugs on firing activity in the whole dorsal horn of spinal cord slices from both rats and mice.

MATERIALS & METHODS

Spontaneous firing activity(SF)

Main summary



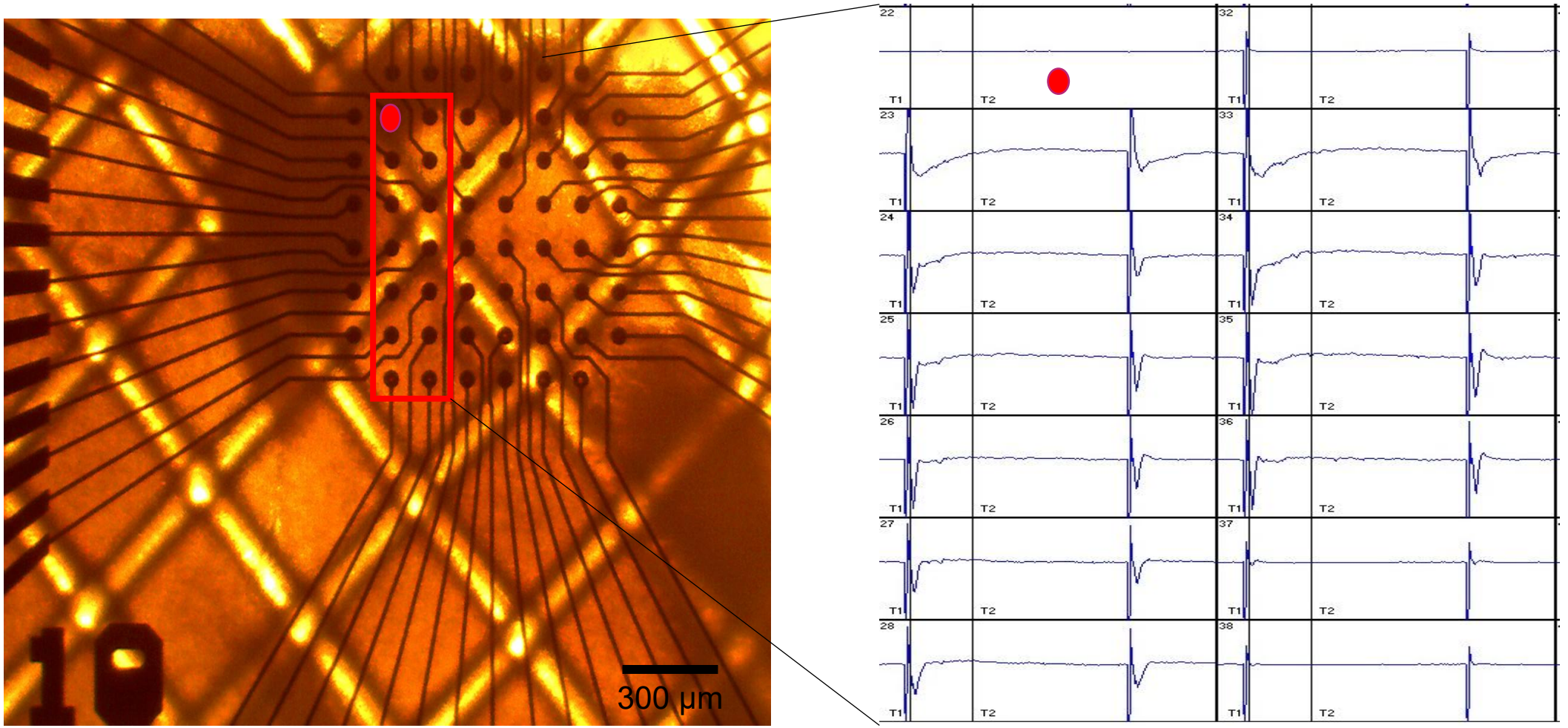
Validation criteria

- Action potentials (APs) have to be higher than 20 µV to be counted.
- After a 10-minute period of 1 µM TTX application, firing activity has to be abolished.

MATERIALS & METHODS

Evoked-responses (paired-pulse stimulation)

Main summary

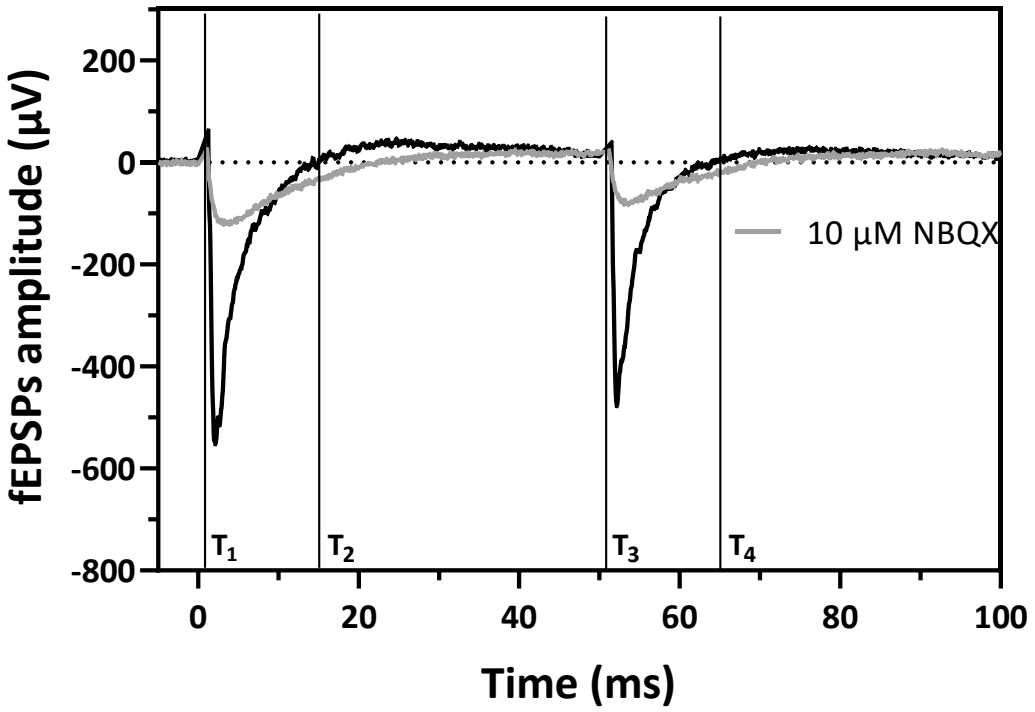


- Stimulation of afferent pathways allows to record evoked-responses in all the laminae of the dorsal horn of spinal cord slices. The stimulus consisted in a monopolar biphasic current paired-pulse (−200 µA for 60 µs followed by +200 µA for 60 µs repeated twice with a 50 ms interval) applied at 60 s intervals. The electrode used to stimulate Aβ/C fibers is surrounded in red in the example above. The electrodes in the red frame (Laminae I-VI) that display evoked-responses are recorded.

MATERIALS & METHODS

Evoked-responses (EPSPs paired-pulse)

Main summary



- The region of interests to determine the EPSPs amplitude was set between T1 = 1 ms and T2 = 15 ms (for EPSP1) and between T3 = 51 ms and T4 = 65 ms (for EPSP2).
- EPSP2 / EPSP1 amplitudes = paired-pulse ratio (PPR).

- Paired-pulse depression is observed in all laminae when Aβ/C fibers are stimulated (PPR < 1).
- Paired-stimulation allows to document pre- and post-synaptic effects of compounds in the dorsal horn.
- Raw PPR and normalized EPSP1 and EPSP2 amplitude averaged from all laminae are presented as a function of time.

Validation criteria

- fEPSPs must be abolished after a 10-minute period of exposure to 10 µM NBQX.

RATS DATA SUMMARY

Main summary

Epileptiform Discharge in CCI model of neuropathic pain

- Non-selective voltage-dependent K^+-channel blocker– **4-Aminopyridine**

Spontaneous firing activity

- SST2,3,5 receptors agonist – **octreotide**
- SST2,3,5 receptors agonist – **octreotide** + SST2 receptors antagonist – **CYN154806**
- TRPV1 receptor agonist – **capsaicin**

Capsaicin-induced firing activity as a pain model

- μ-opioid receptor agonist – **morphine**
- sodium channel blocker – **lidocaine**
- $GABA_B$ receptor agonist – **baclofen**
- SST2,3,5 receptor agonist – **octreotide**

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RATS DATA SUMMARY

Main summary

Evoked responses (EPSPs paired-pulse)

- $GABA_A$ receptor antagonist - **Bicuculline**
- $GABA_B$ receptor agonist - **Baclofen**
- Non-competitive NMDA receptors antagonist - **Ketamine**
- Binding α2δ subunit of voltage-sensitive calcium channels - **Pregabaline**

NMDA-mediated EPSP

- Non-competitive NMDA receptors antagonist – **ketamine**

CCI MODEL RATS DATA
4-AP-induced Epileptiform Discharges

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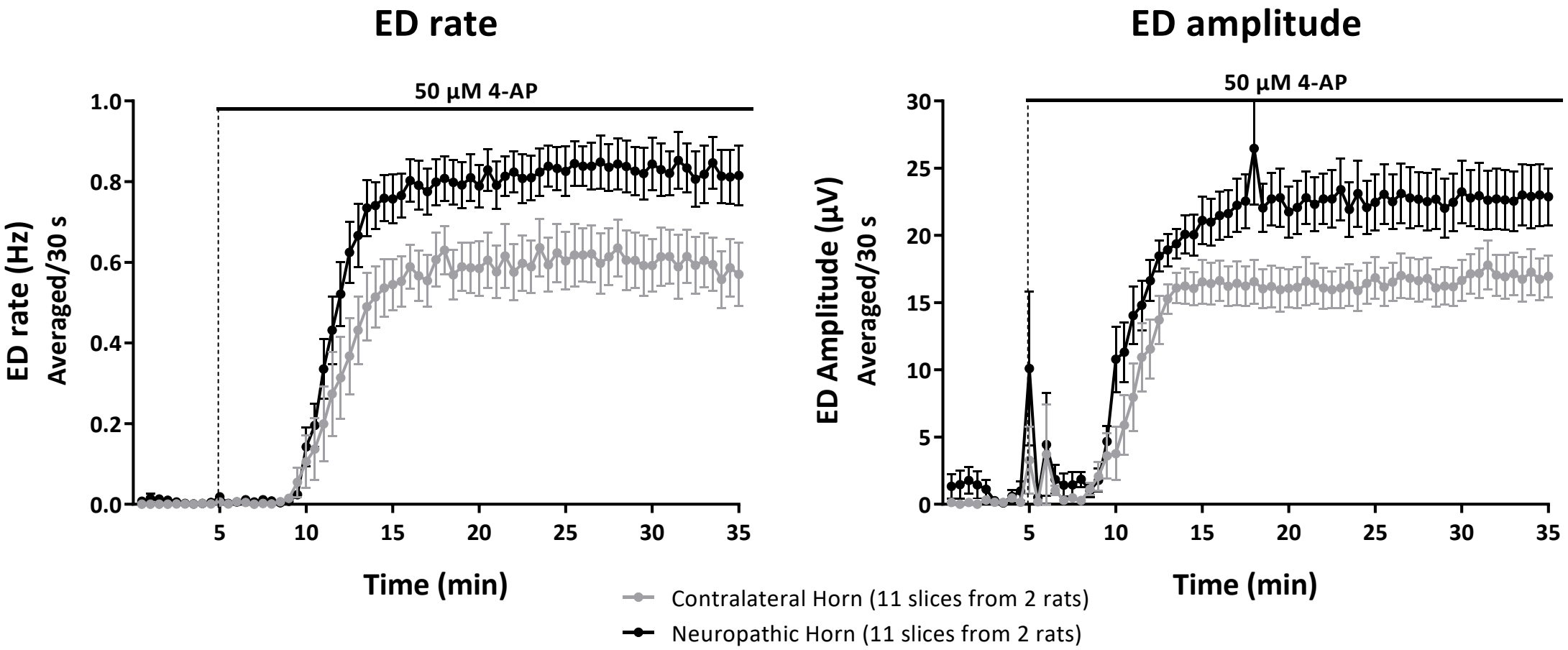
REFERENCE DATA

Main summary

Rats data summary

Non-selective voltage-dependent K+-channel blocker– 4-Aminopyridin



- In CCI rat model of neuropathic pain, the non-selective votage dependent K^+ channel blocker 4-aminopyridine (4-AP) induced larger EDs at a higher rate in neuropathic horn than in the contralateral dorsal horn and trended to onset faster.

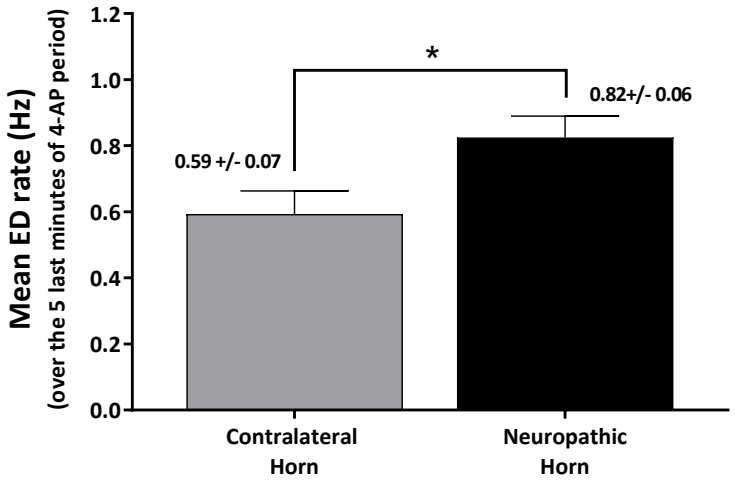
REFERENCE DATA

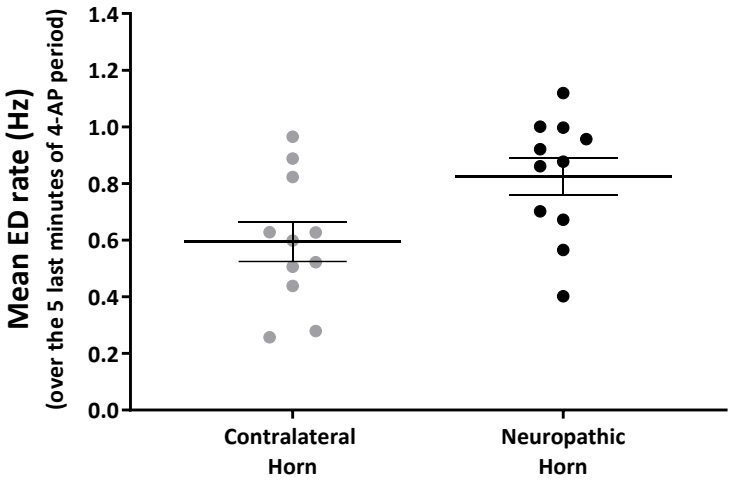
Main summary

Rats data summary

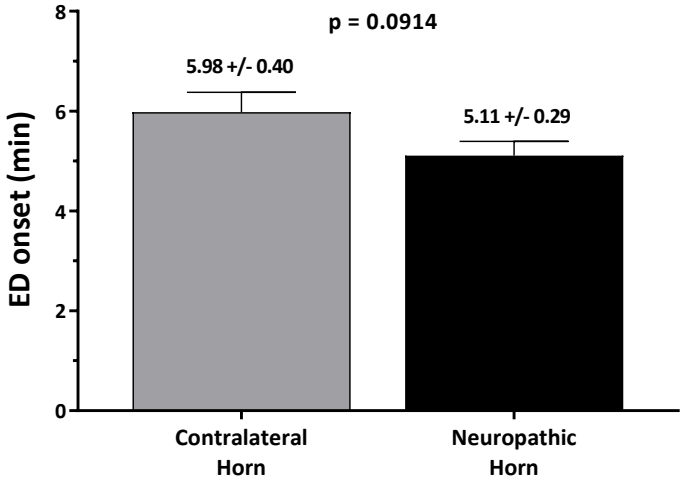
Non-selective voltage-dependent K+-channel blocker– 4-Aminopyridin

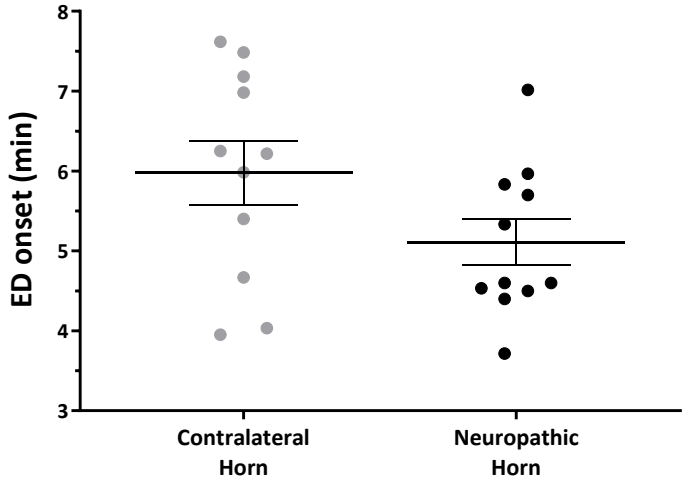
EDs Mean rate





EDs onset





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P7-11 RATS DATA
Spontaneous firing activity (SF)

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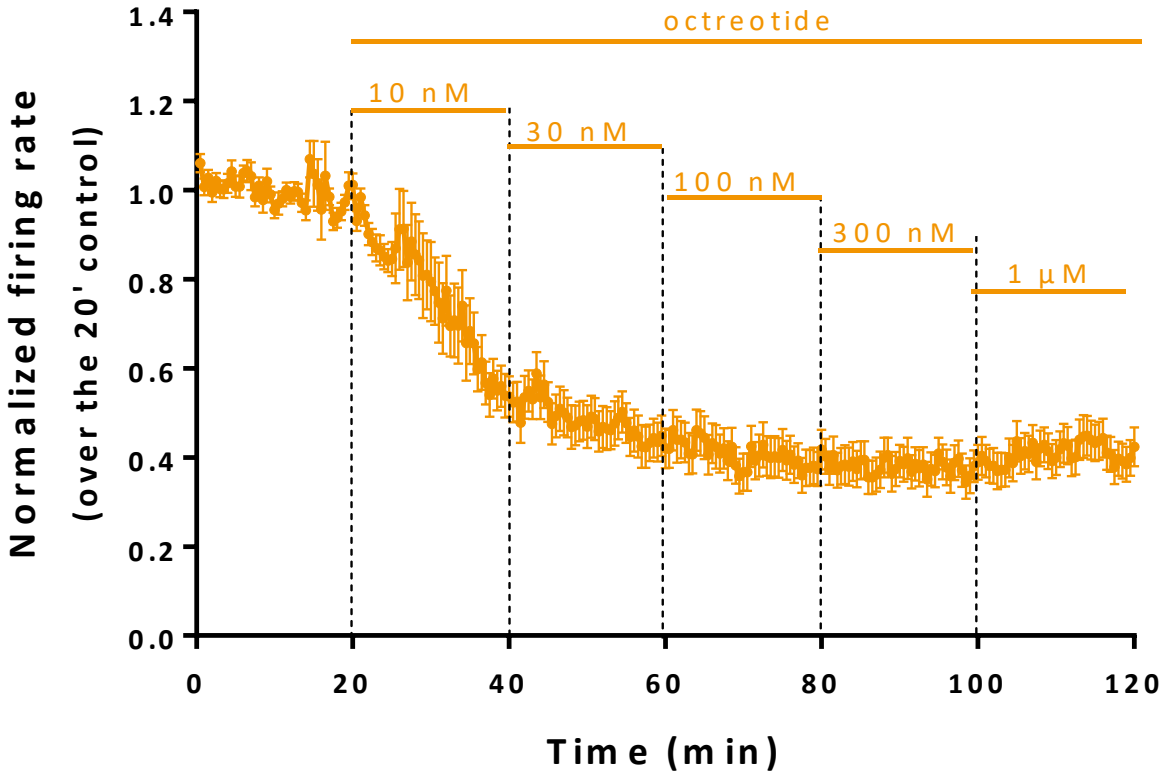
REFERENCE DATA

SST2,3,5 receptors agonist – octreotide

Main summary

Rats data summary



Concentration-range of octreotide (58 electrodes, 8 slices from 2 animals)

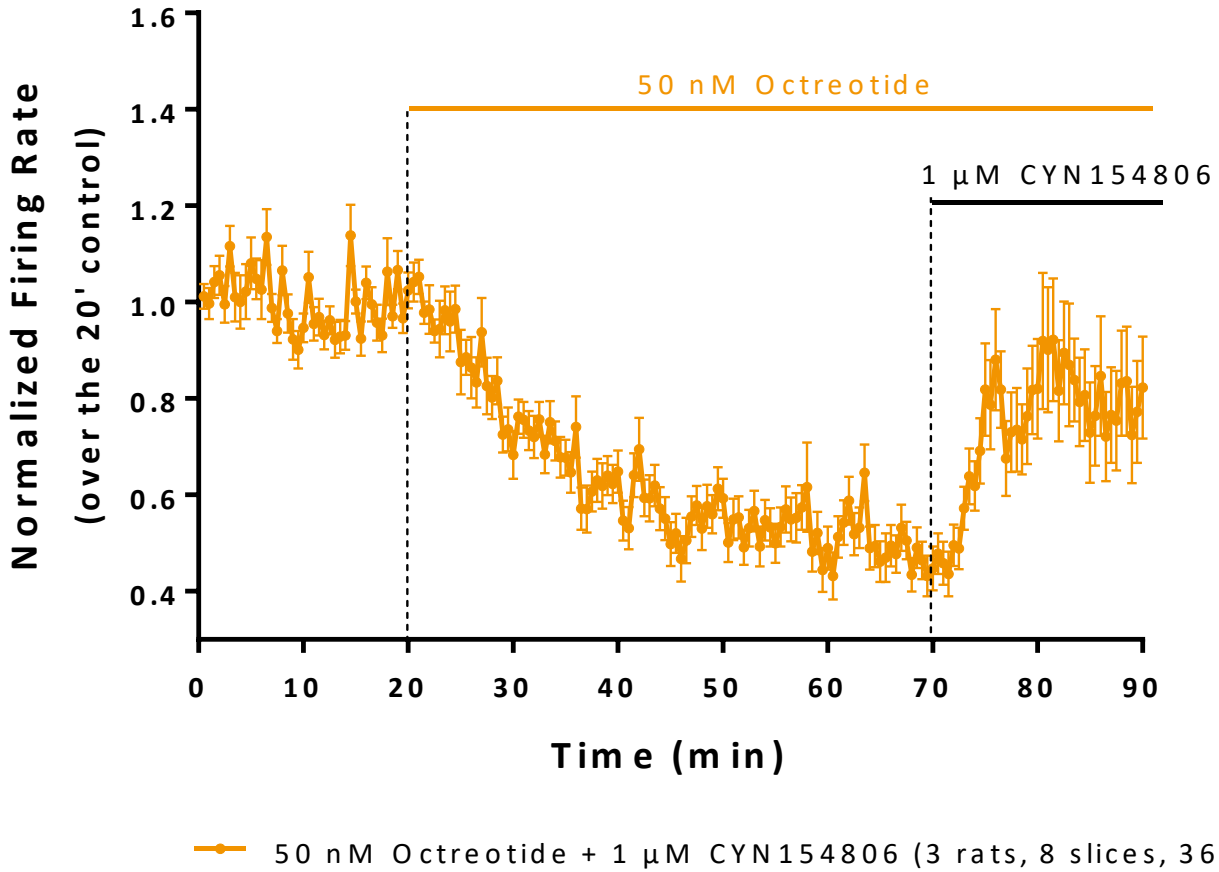
- The SST2,3,5 receptors agonist octreotide largely decreased the dorsal horn neurons firing activity in rat spinal cord slices from 10 nM concentration.

REFERENCE DATA

Main summary

Rats data summary

SST2,3,5Rs agonist – octreotide + SST2Rs antagonist – CYN154806



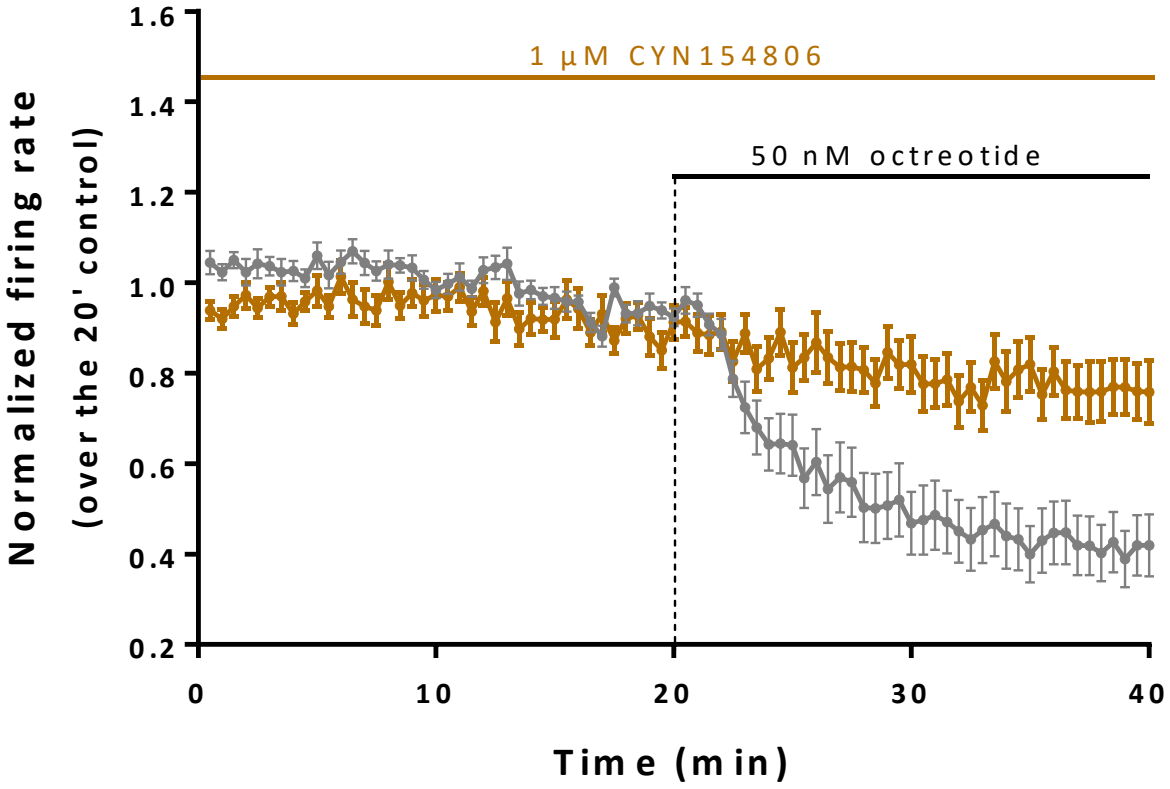
- 1 µM CYN154806 (a SST2R antagonist) partially reversed the octreotide effect on firing of dorsal horn neurons, thereby confirming that octretotide effect is largely mediated by SST2 receptors activation.

REFERENCE DATA

Main summary

Rats data summary

SST2,3,5Rs agonist – octreotide + SST2Rs antagonist – CYN154806



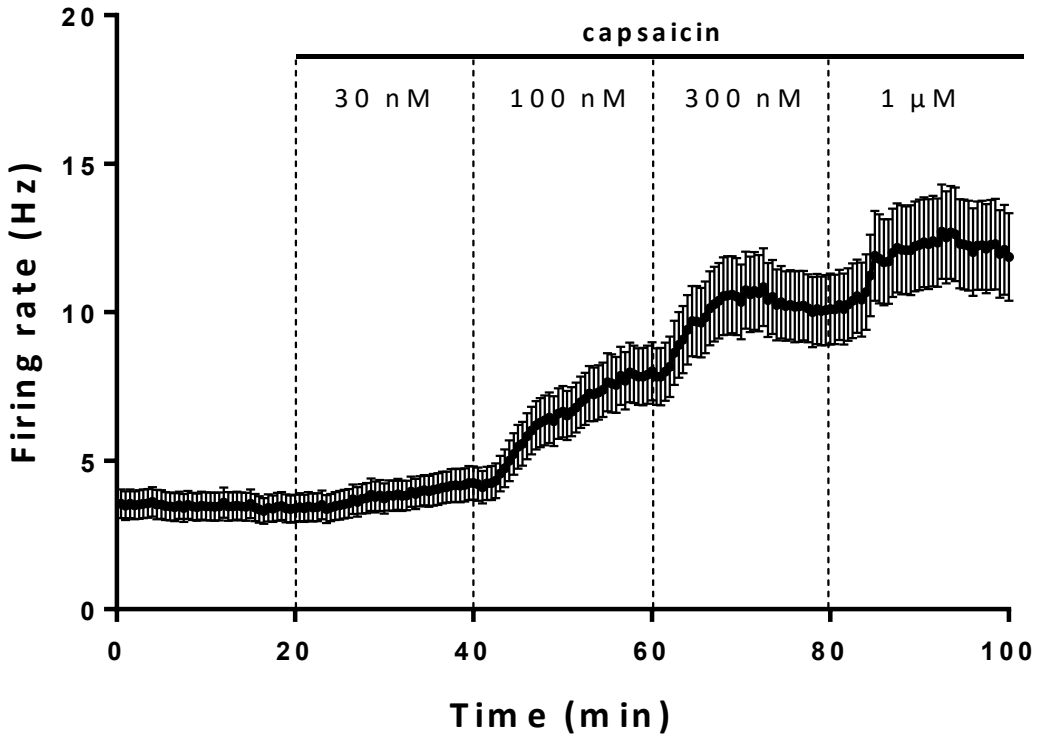
- SSTR2 are mainly involved in the inhibition of dorsal horn firing activity as demonstrated by the use of the selective SST2 receptors antagonist CYN154806. While pre-applied CYN 154806 strongly inhibited octreotide effect.

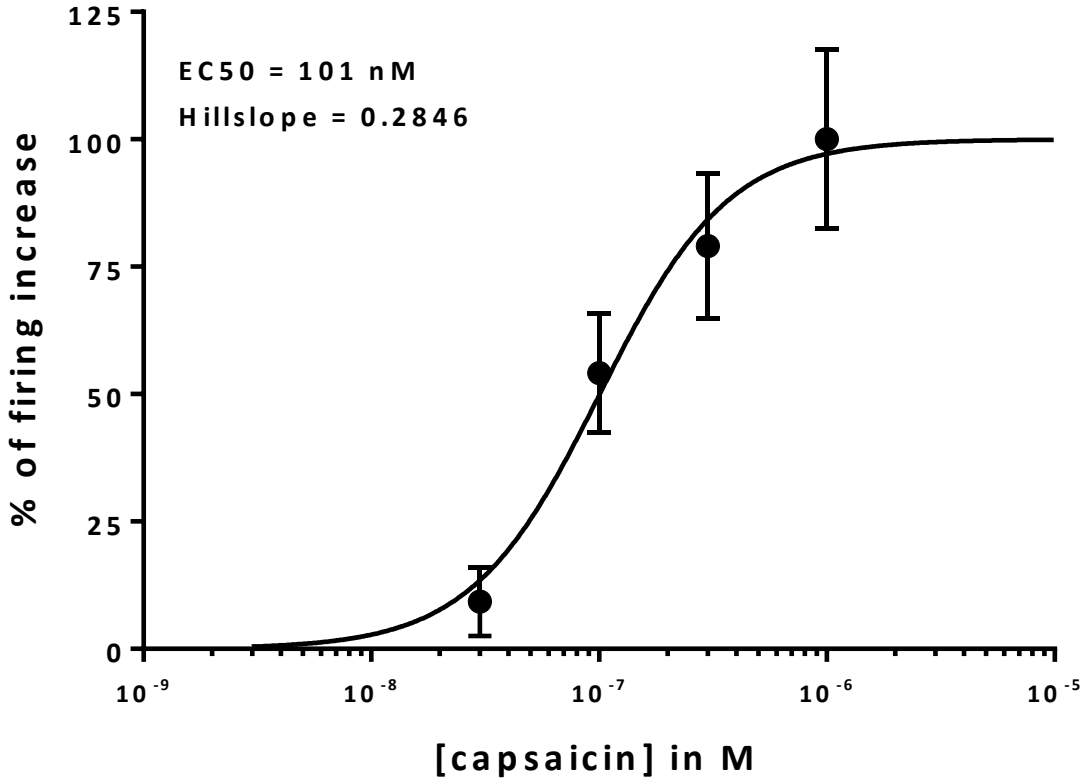
REFERENCE DATA

TRPV1 receptor agonist – capsaicin

Main summary

Rats data summary





Concentration-response of capsaicin (2 rats, 10 slices, 82 electrodes)

- The TRPV1 agonist receptor capsaicin dose-dependently increased the dorsal horn neurons firing activity in rat spinal cord slices with an apparent EC_{50} of 101 nM.
- TRPV1 receptors are nonselective cation channels that are sensitized from noxious stimuli, leading to inflammatory conditions and pain.

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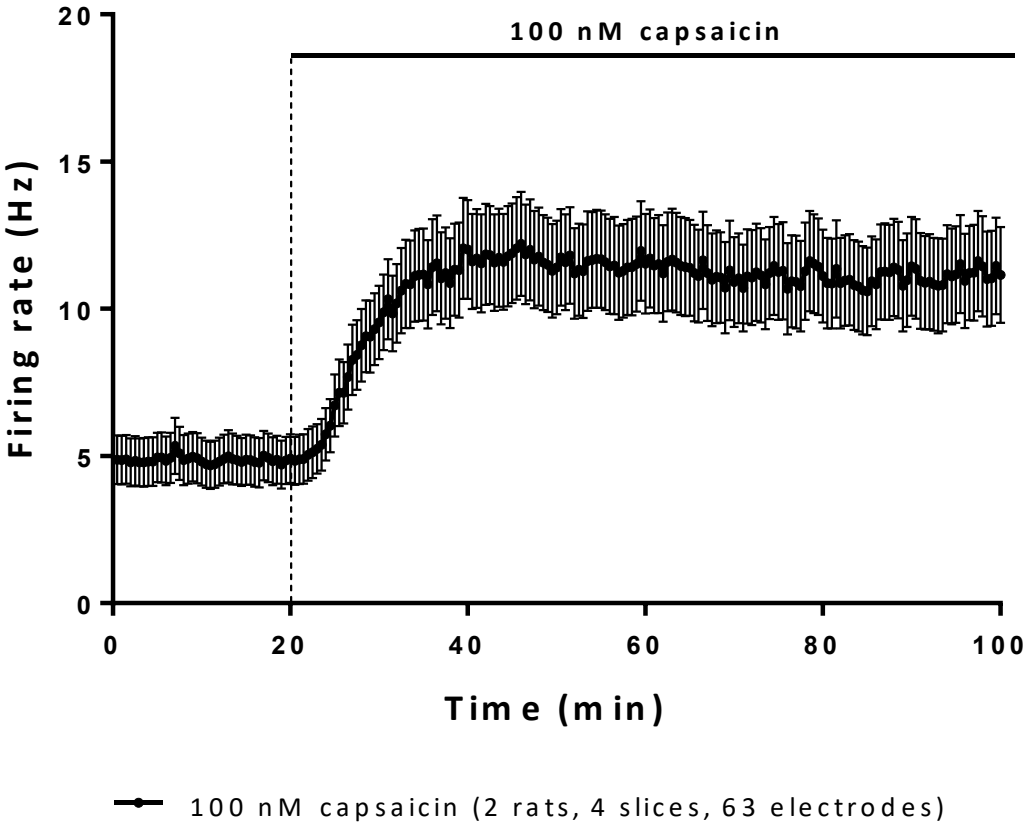
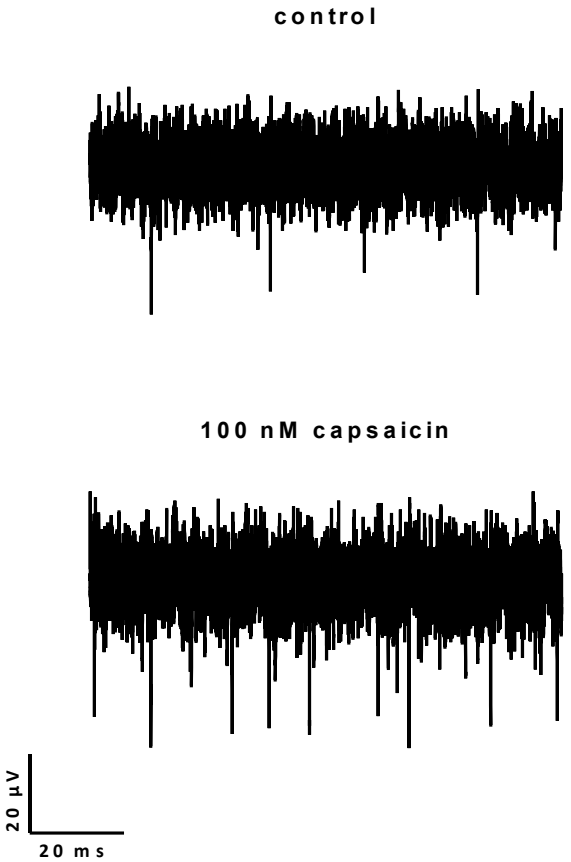
REFERENCE DATA

TRPV1 receptor agonist – capsaicin

Main summary

Rats data summary





- 100 nM capsaicin increased the firing activity over 20 minutes and next the firing remained stable for 60 minutes.

P7-11 RATS DATA
Capsaicin-induced firing activity

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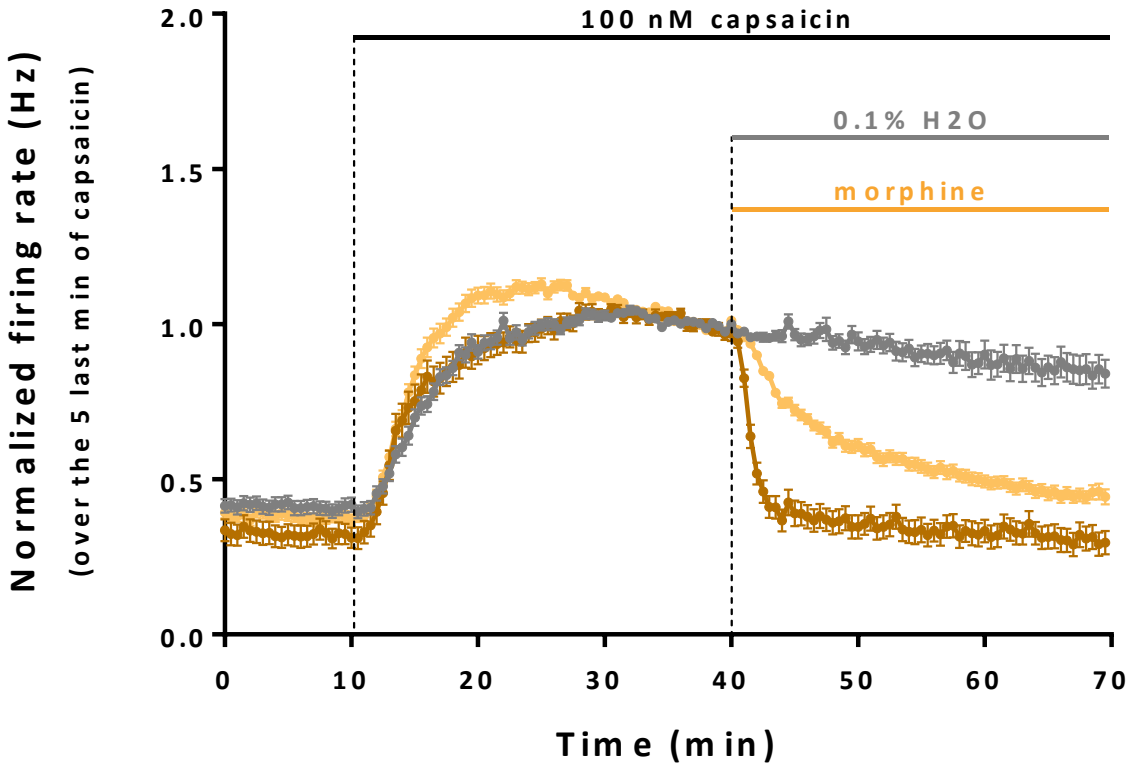
REFERENCE DATA

µ-opioid receptor agonist – morphine

Main summary

Rats data summary



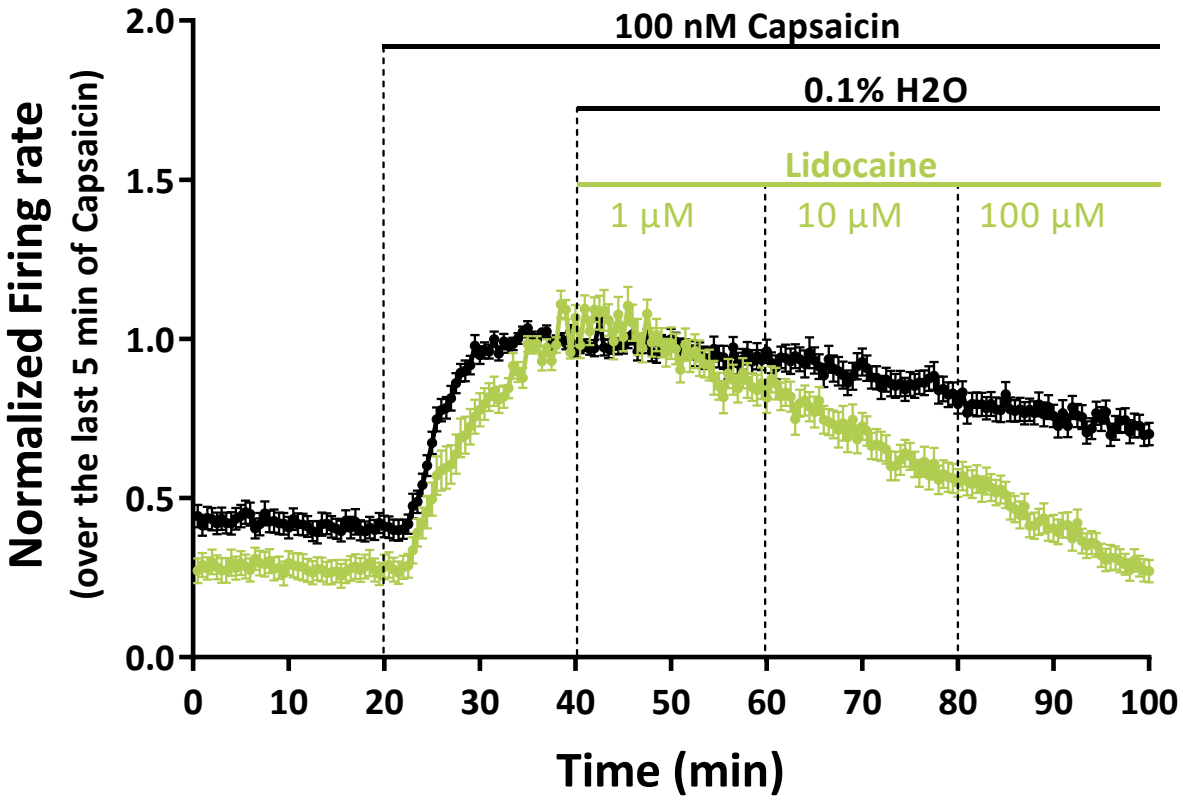
- The µ-opioid receptor agonist morphine dose-dependently inhibited the capsaicin-induced firing. Indeed, addition of 100 nM morphine substantially inhibited the capsaicin-induced firing over a 30-minute period, whereas a full inhibition was observed within 10 minutes with 10 µM morphine.

REFERENCE DATA

Sodium channel blocker – Lidocaine

Main summary

Rats data summary



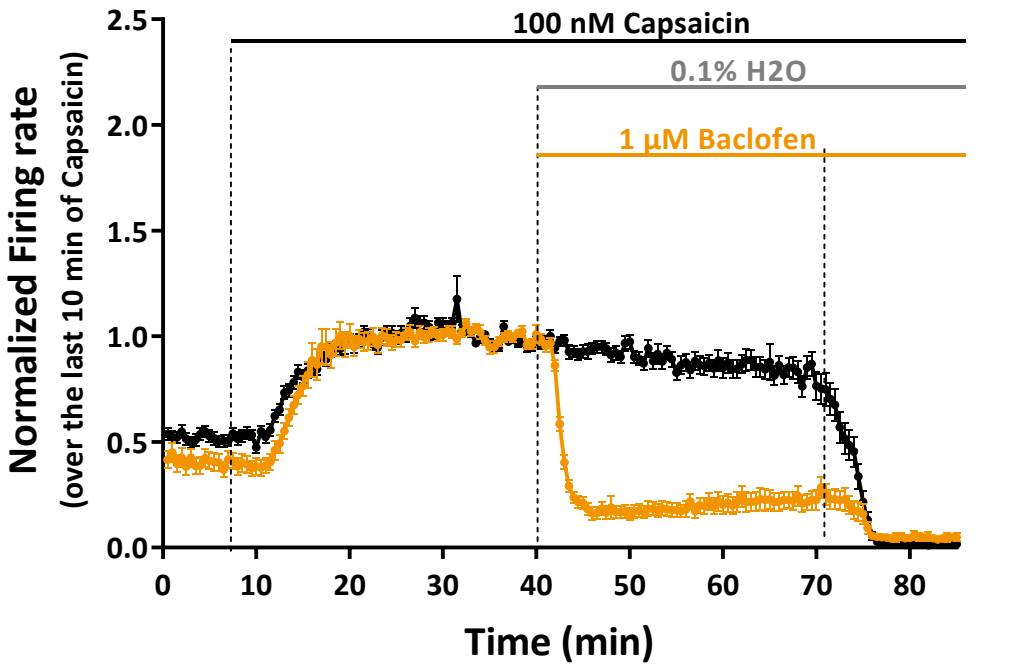
- The sodium channel blocker lidocaine efficiently decreased the capsaicin-induced firing. Indeed, addition of increasing concentrations of lidocaine for 20 minutes each concentrations (cumulative application) led to a progressive and complete inhibition of the capsaicin-induced firing.

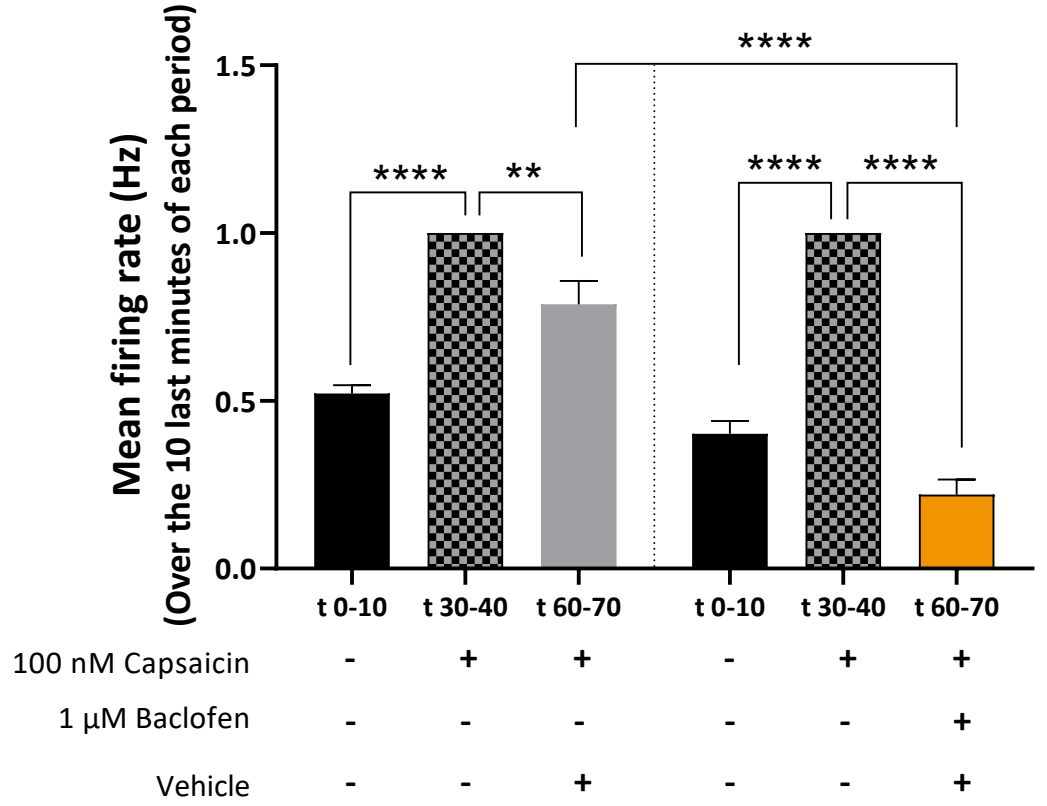
REFERENCE DATA

$GABA_B$ receptor agonist – baclofen

Main summary

Rats data summary



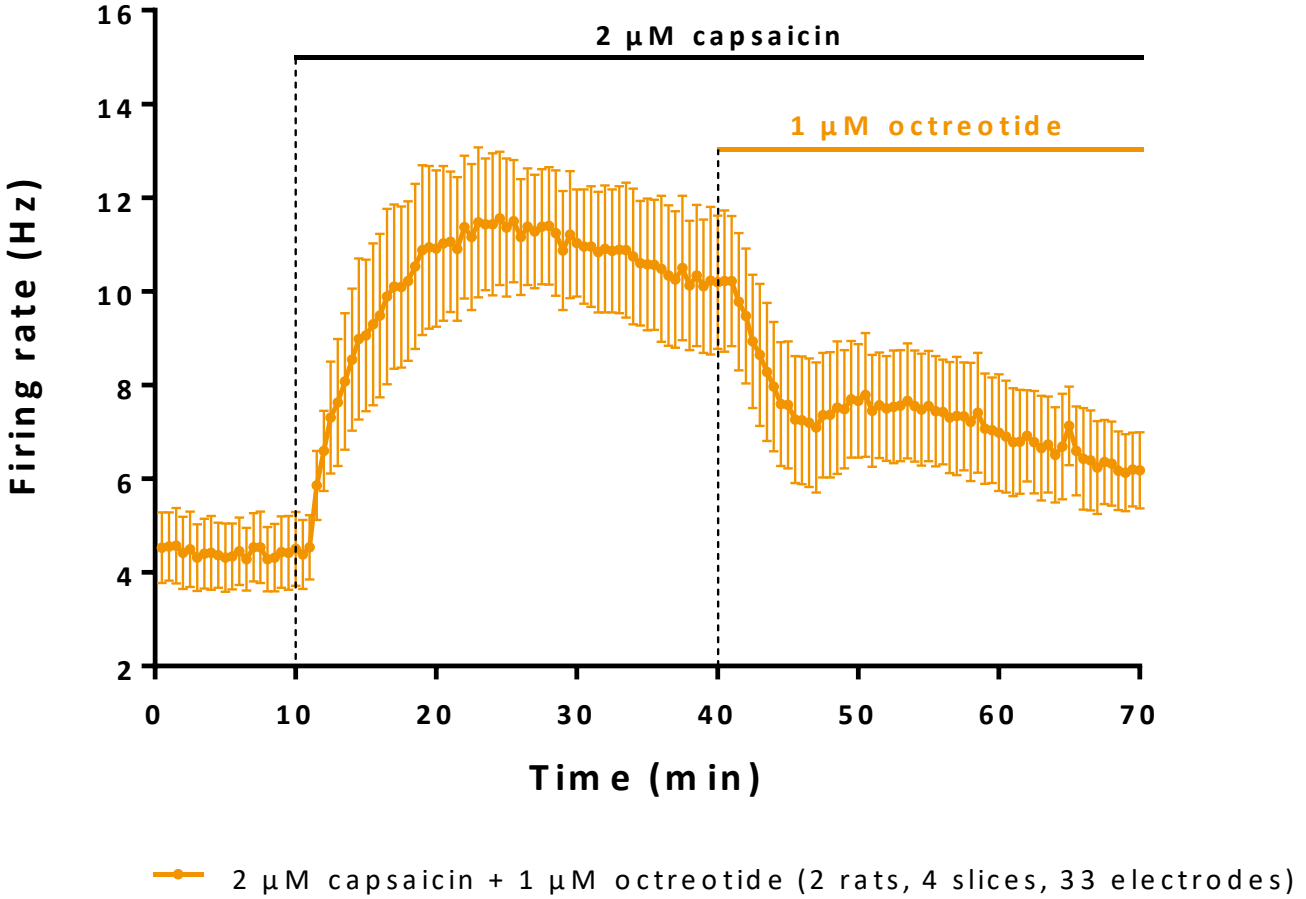


REFERENCE DATA

SST2,3,5 receptors agonist – octreotide

Main summary

Rats data summary



- The SST2,3,5 receptors agonist octreotide, a pain-killer used as an alternative to opioid compounds, substantially inhibited the capsaicin-induced firing.

P7-11 RATS DATA
Evoked-responses
(EPSPs paired-pulse)

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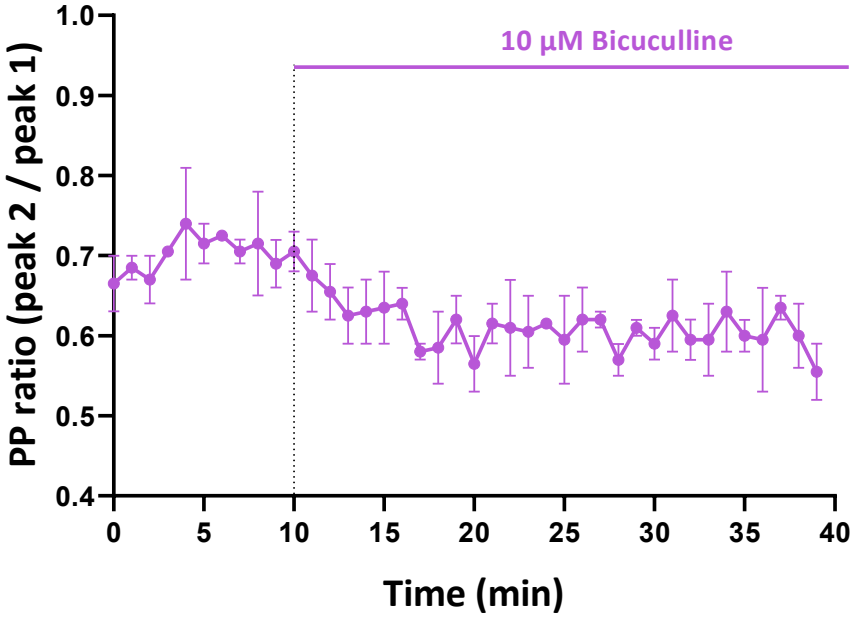
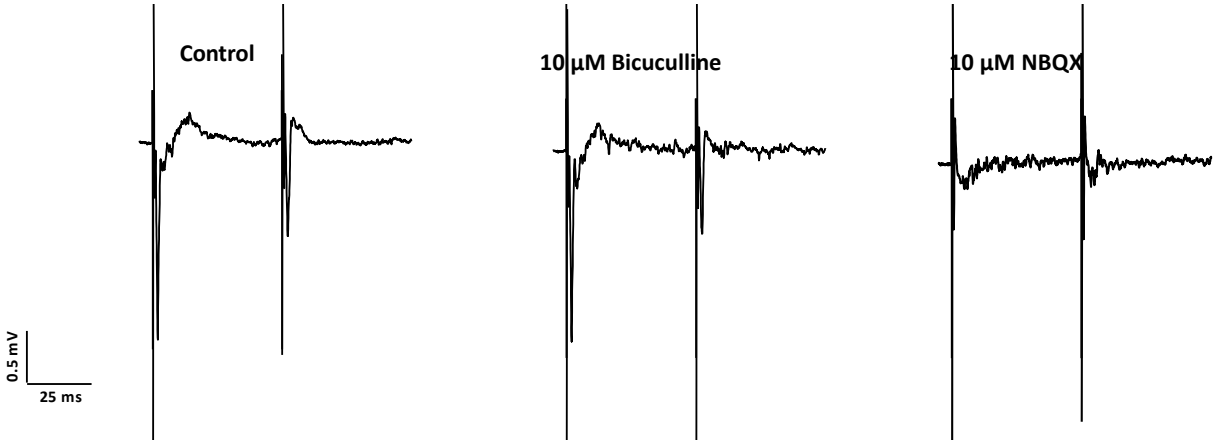
REFERENCE DATA

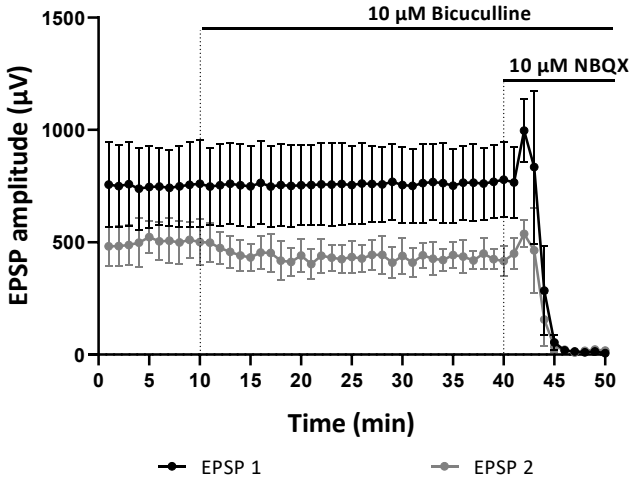
$GABA_A$ receptor antagonist - bicuculline

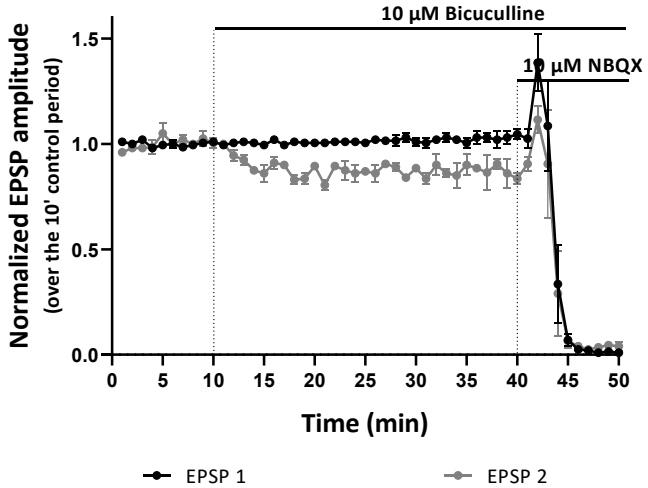
Main summary

Rats data summary









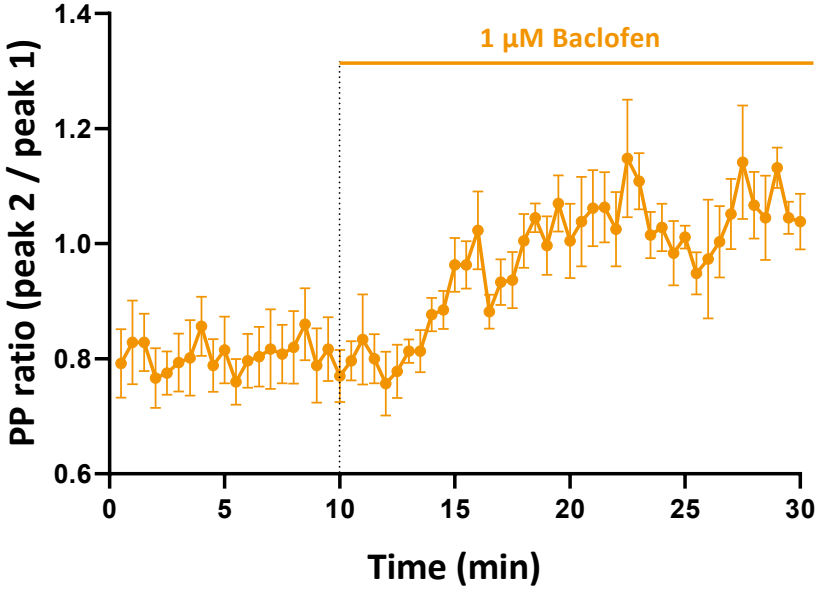
- The $GABA_A$ antagonist Bicuculline at 10 µM decreased the PPR from 0.7 to 0.6.
- Only EPSP2 was decreased by about 15%.
- Consistent with *Malan et al. – 2002.*

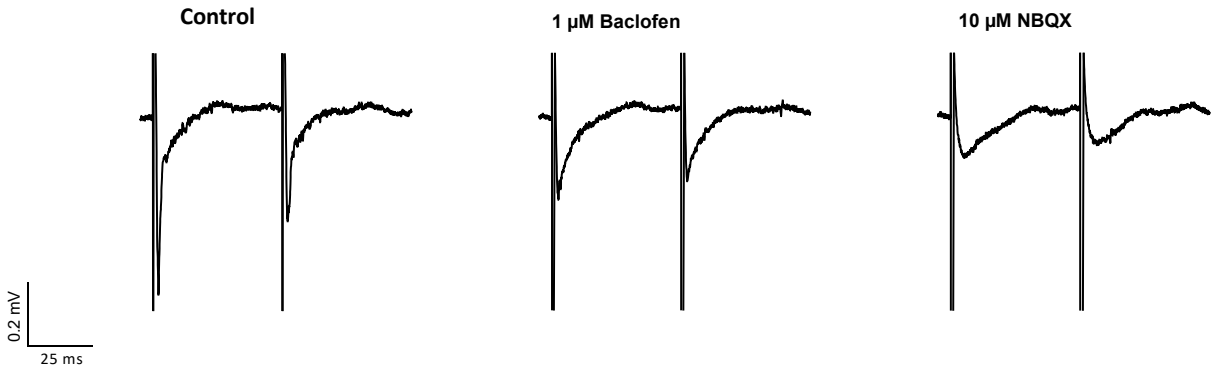
REFERENCE DATA

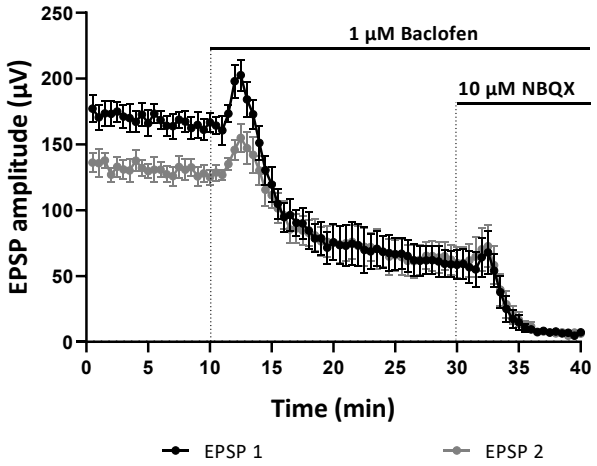
$GABA_B$ receptor agonist - baclofen

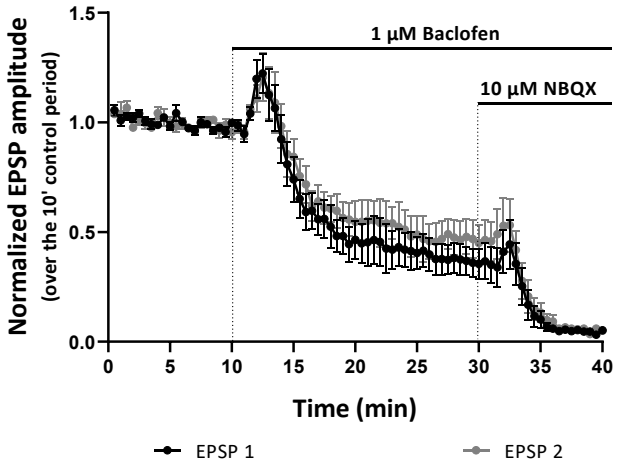
Main summary

Rats data summary









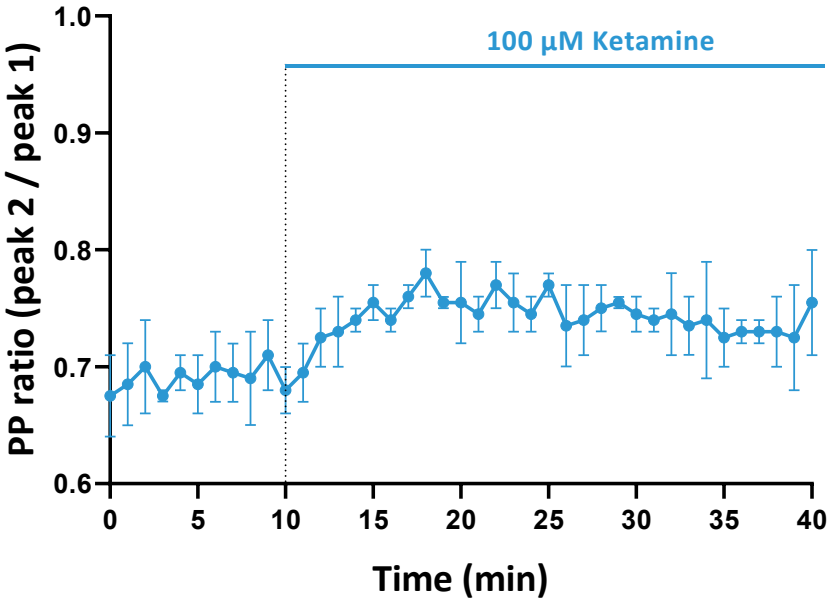
- The $GABA_B$ agonist Baclofen at 1 µM increased the PPR from 0.8 to 1.
- Both EPSP1 and EPSP2 were decreased
- Consistent with *Salio et al. - 2017*

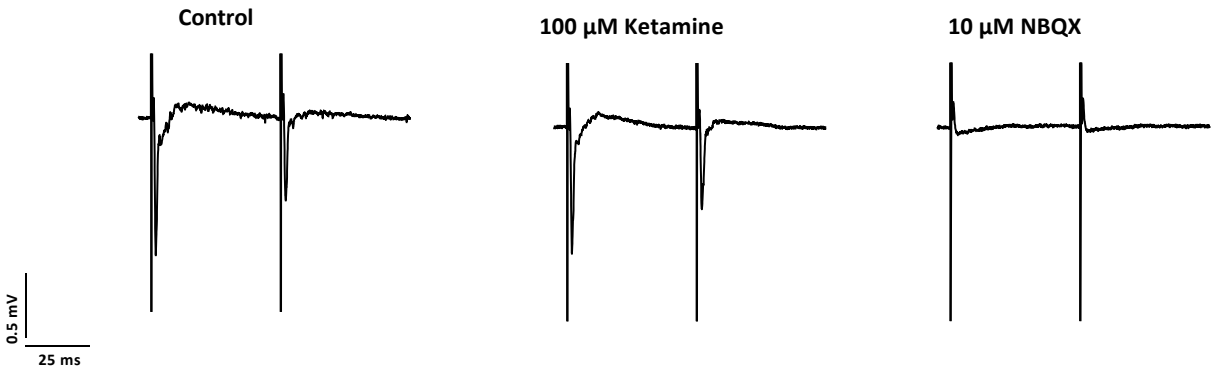
REFERENCE DATA

Main summary

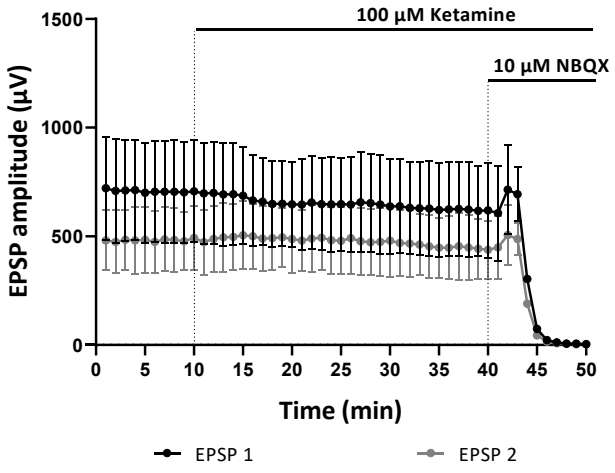
Rats data summary

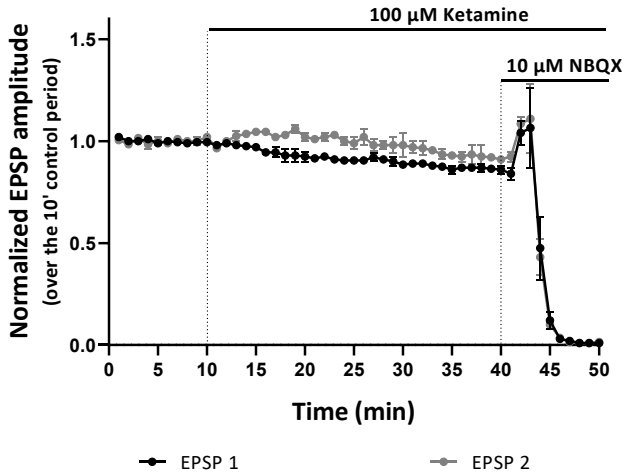
Non competitive NMDA receptor antagonist – ketamine





47 electrodes, 2 slices from 1 rat





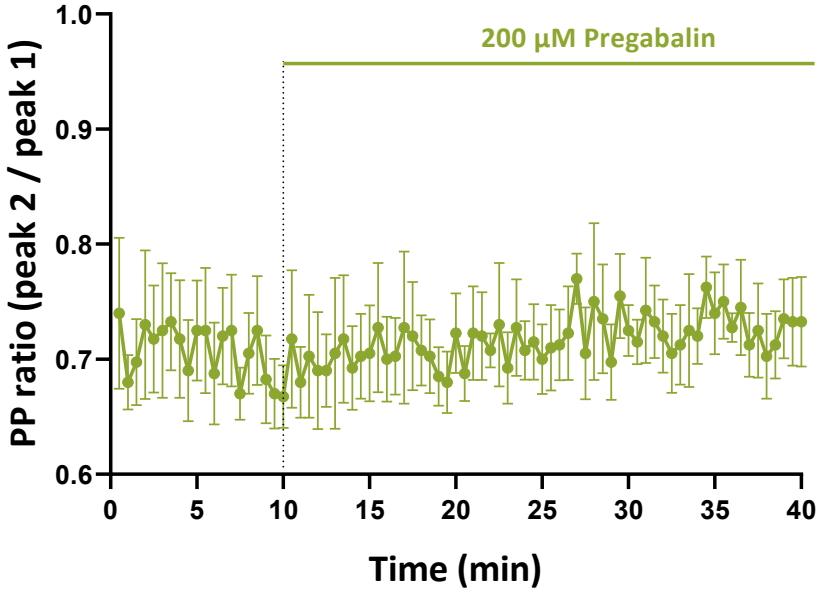
- The non competitive NMDA antagonist ketamine at 100 µM slightly increased the PPR from 0.68 to 0.75.
- EPSP1 was decreased whereas EPSP2 was increased.

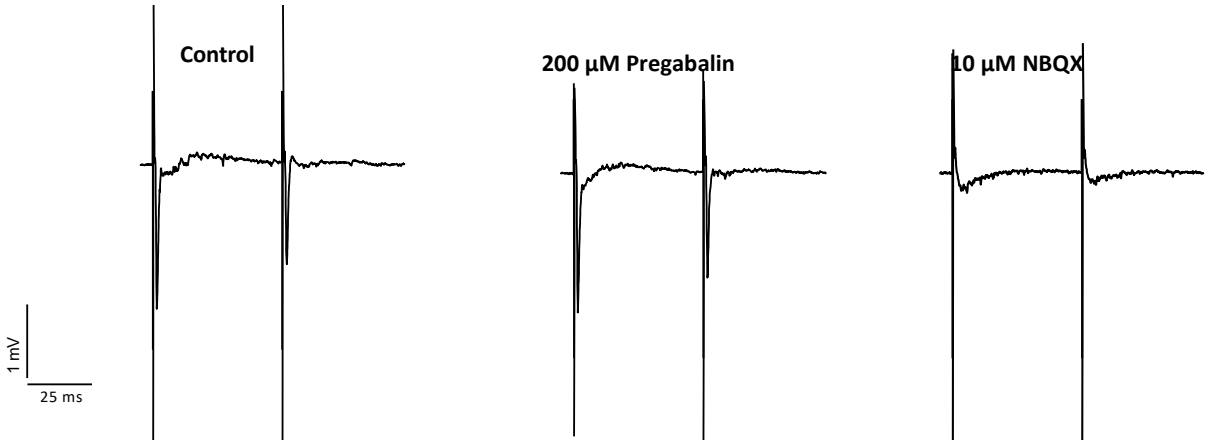
REFERENCE DATA

Main summary

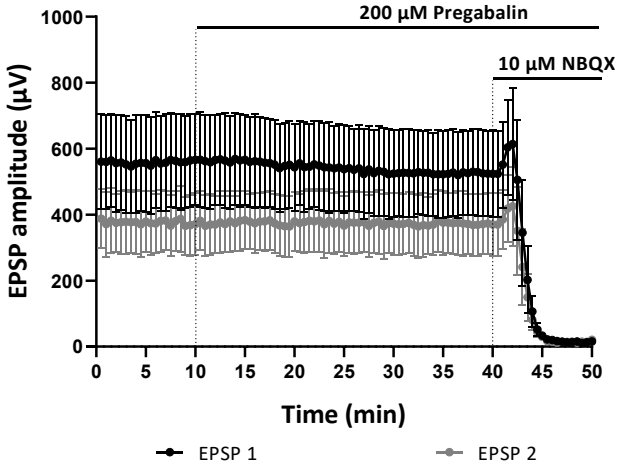
Rats data summary

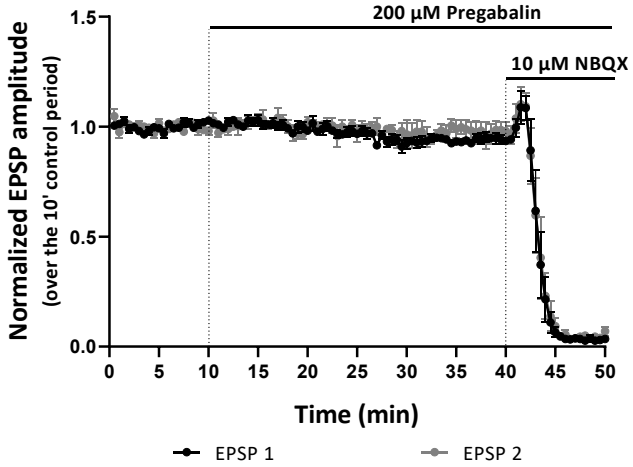
Binding α2δ subunit of voltage-sensitive calcium channels - Pregabalin





76 electrodes, 4 slices from 2 rats





- The binding α2δ subunit of voltage-sensitive calcium channels at 200 µM did not modify the PPR.
- Neither EPSP1 nor EPSP2 were modified.

P7-11 RATS DATA
NMDA-mediated EPSP

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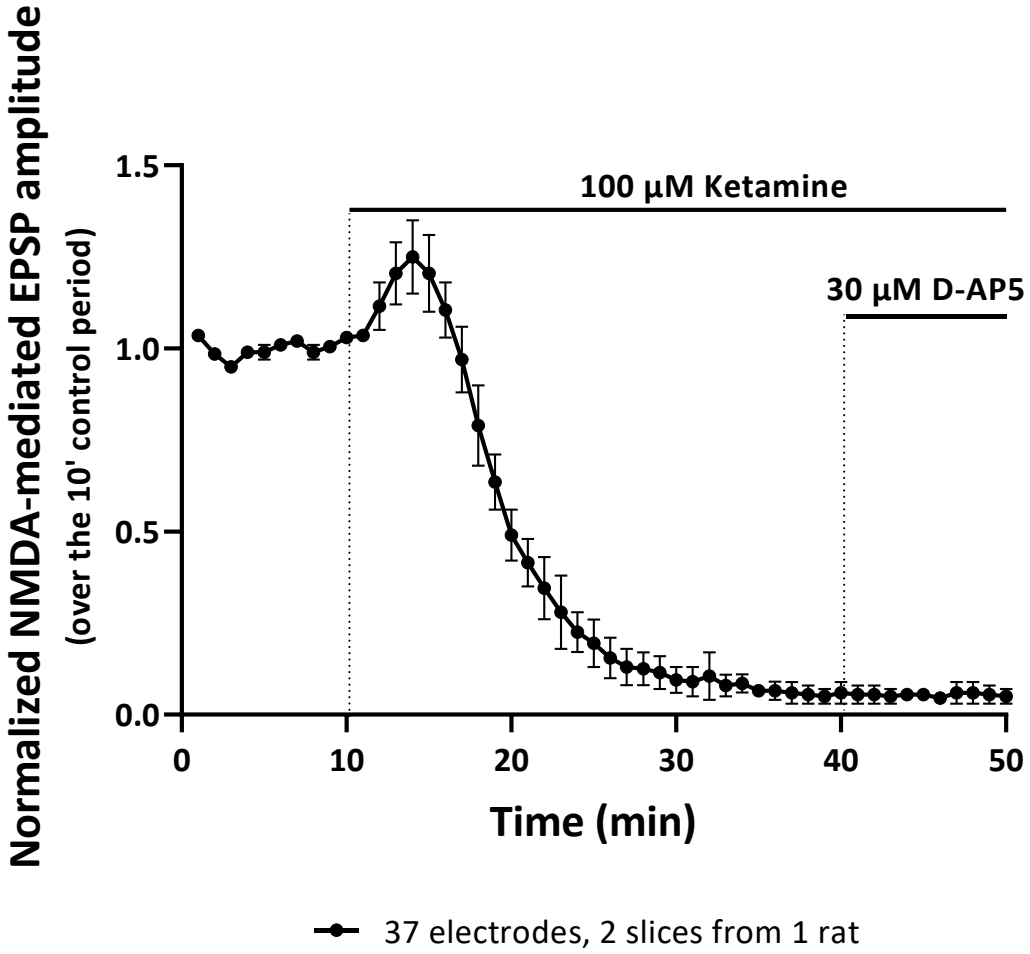
ALLIANCE

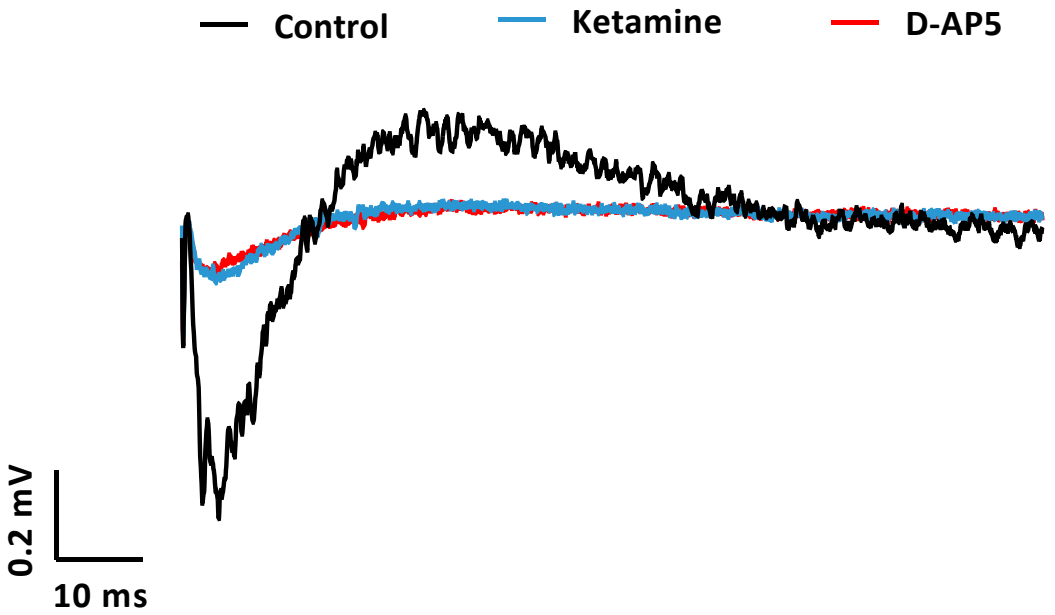
REFERENCE DATA

Main summary

Rats data summary

Non competitive NMDA receptor antagonist – ketamine





- The non competitive NMDA antagonist ketamine fully blocked the NMDA-mediated EPSP when applied at 100 µM.

MICE DATA SUMMARY

Main summary

Spontaneous firing activity (SF)

- TRPV1 receptor agonist – capsaicin

MICE DATA
Spontaneous firing activity (SF)

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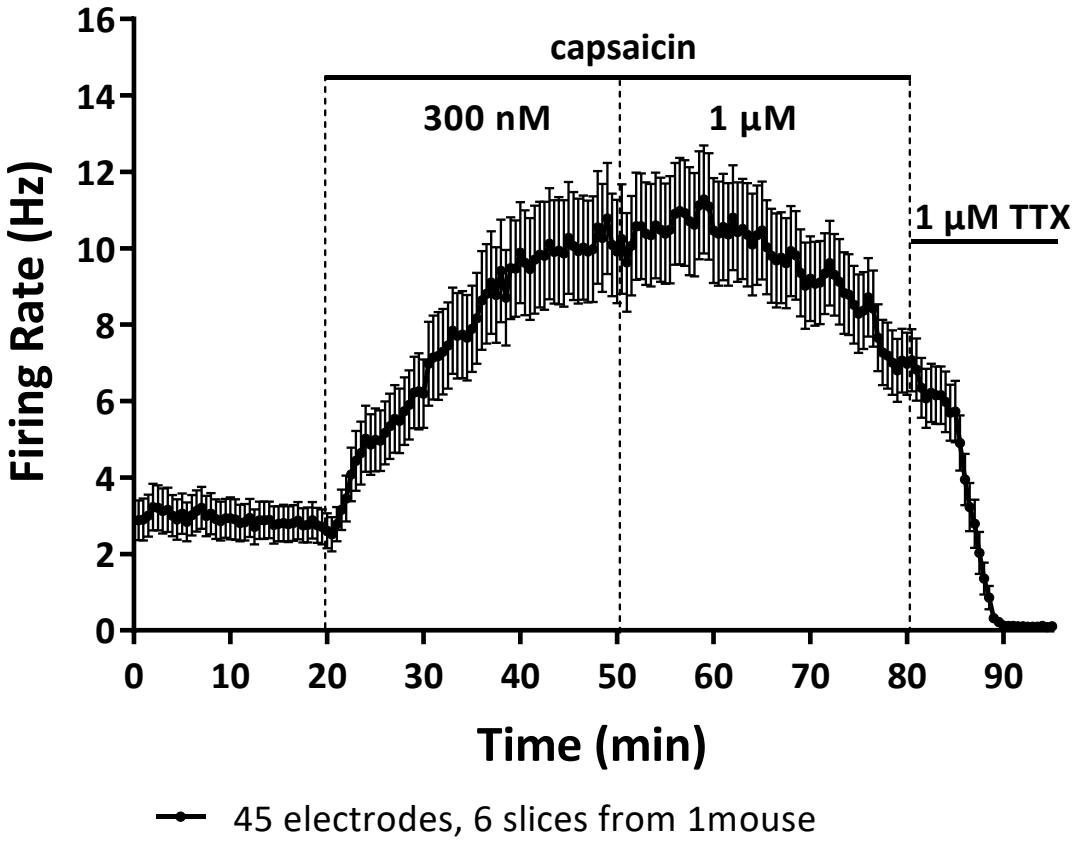
ALLIANCE

REFERENCE DATA

TRPV1 receptor agonist – capsaicin

Main summary

Mice data summary



- The TRPV1 receptor agonist capsaicin increased the firing activity at 300 nM and whereas desensitization was obsevered at 1 µM after about 10 min, in 6 month-old C57Bl6 mice.