

CELL ELECTROPHYSIOLOGY

**BRAIN SLICE ELECTROPHYSIOLOGY**

IN VIVO BRAIN ELECTROPHYSIOLOGY

IN VIVO SC & DRG ELECTROPHYSIOLOGY

MULTI ELECTRODE ARRAY

# CA1 neurons firing



# SUMMARY

## Materials & methods

- Advantages of multipoint recording
- Experimental conditions and data analysis

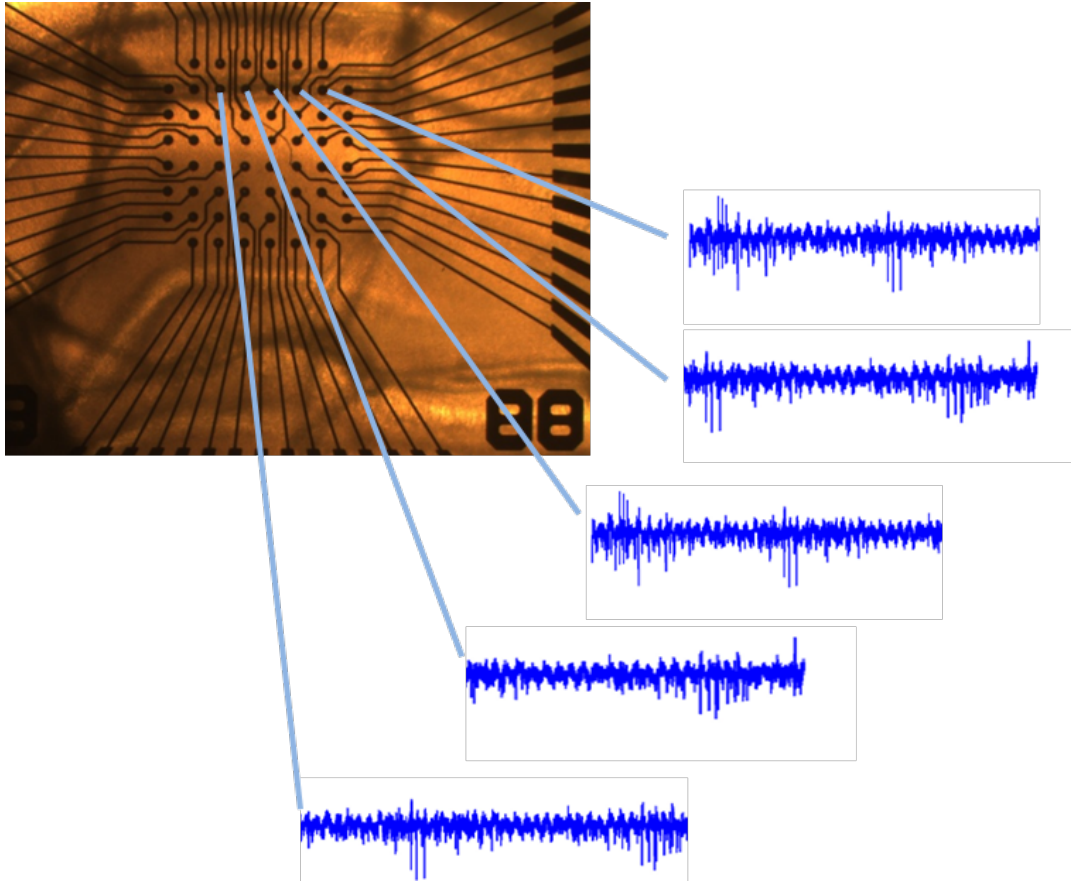
## Firing modulation

- GABA<sub>A</sub> receptor – [PTX, Diazepam](#)
- NMDA receptor – [NMDA, D-AP5, CIQ](#)
- Kainate receptor – [Kainate](#)
- Cholinergic receptors – [Carbachol, Pirenzepine](#)
- Voltage-activated K<sup>+</sup> channels – [Retigabine](#)
- Serotonergic receptors – [5-HT](#)
- Somatostatin receptors – [L-803,087](#)



# MATERIALS & METHODS

## Advantages of multipoint recording



Within each tested slice, the CA1 neurons spontaneous activity is recorded at 3 to 8 electrodes, each electrodes recording the activity of several neurons located in the vicinity. The results obtained are averaged from a large number of neurons and are then very robust. Several concentrations of a compound could also be evaluated on a single slice.

### Advantages

- Recording of a steady firing activity over a long period of time provide very accurate information about the compound evaluated.
- The firing activity is recorded from neurons located in a native network.
- The technique is non-invasive and the solution bathing the neurons is close to the cerebro-spinal fluid composition, allowing to precisely document the pharmacological profile of compounds, in conditions close to the in vivo situation.
- Multipoint recordings largely increase the number of neurons recorded within a single slice and reduce the cost associated with compounds evaluation.

### Limitations

- The MEA technique does not allow to investigate single neurons parameter such as rheobase or to apply depolarizing step to the recorded neuron.



# MATERIALS & METHODS

## Experimental conditions and data analysis

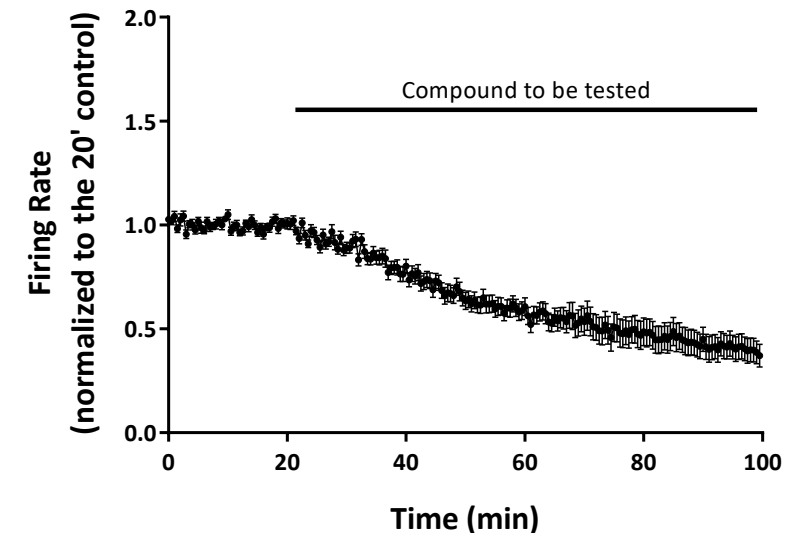
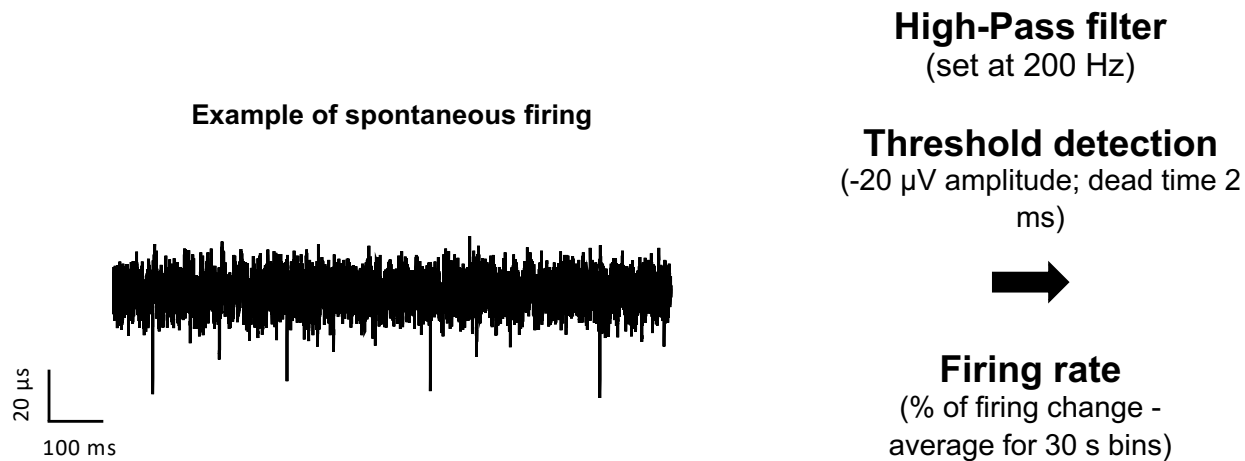
### Rodent hippocampal slices

- Experiment is conducted with Sprague Dawleys rats or C57Black/6 mice between 3 and 6 weeks of age.
- Hippocampal slices (350  $\mu\text{m}$  thickness) are cut with a vibratome

### Slices perfusion

- aCSF composition: glucose 11,  $\text{NaHCO}_3$  25,  $\text{NaCl}$  126,  $\text{KCl}$  3.5,  $\text{NaH}_2\text{PO}_4$  1.2,  $\text{MgCl}_2$  1.3,  $\text{CaCl}_2$  2 in mM
- The concentration of potassium can be adjusted to 5 or 7 mM to induce firing activity of CA1 neurons

### Analysis





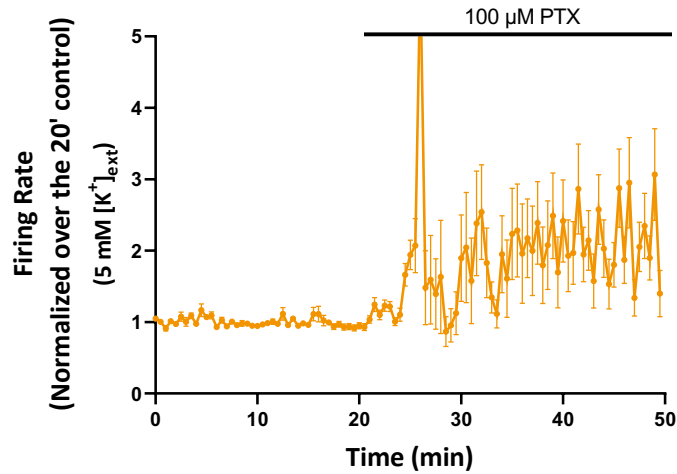
# RESULTS



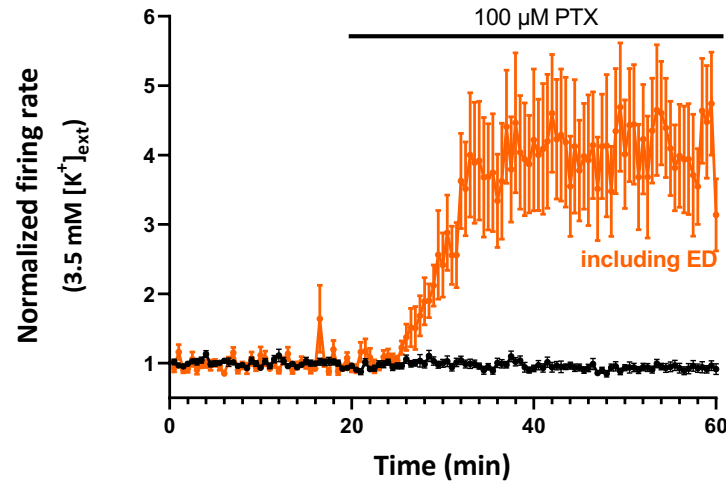
# RESULTS

## GABA<sub>A</sub> receptors

### Picrotoxin, diazepam

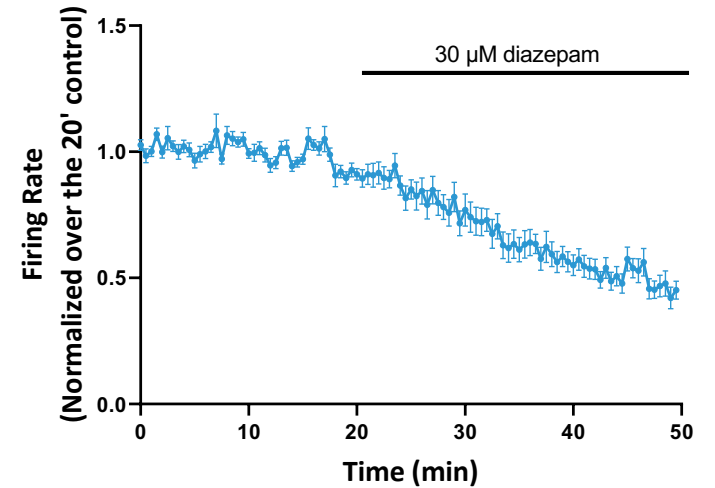


— 100 μM PTX (2 mice, 4 slices, 28 electrodes)



— Sucrose cutting method (39 electrodes, 6 slices, 2 mice)

— K-glu/choline/hepes holding cutting method (29 electrodes, 6 slices, 2 mice)



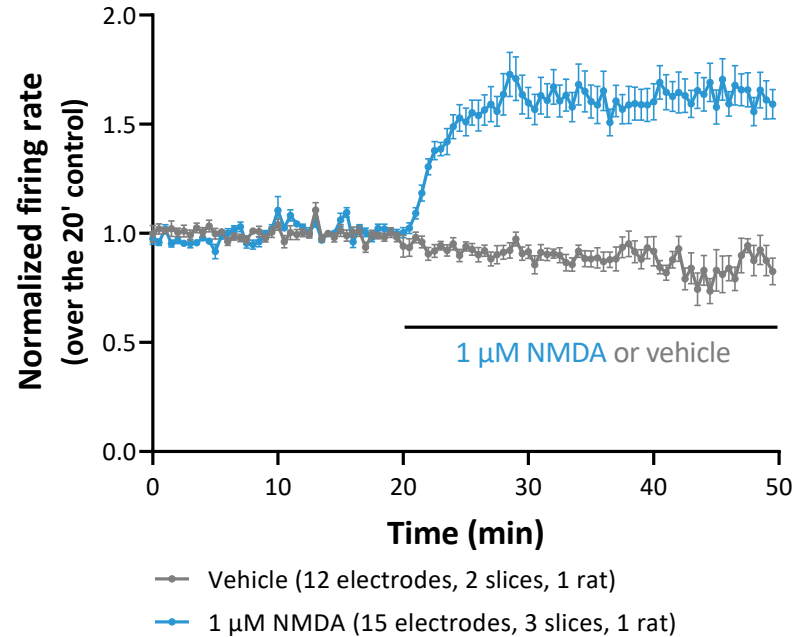
— 30 μM diazepam (2 mice, 6 slices, 22 electrodes)

- When cutting or recordings are performed in hyperpolarizing medium (5 mM K<sup>+</sup> or K-gluconate cutting solution), the inhibition of gabaergic interneurons with 100 μM picrotoxin (PTX) increased the firing activity of principle pyramidal neurons and also triggered epileptiform discharges after 5 minutes of PTX application. This increased of the firing rate in the presence of PTX was not observed in physiological condition (in a 3.5 mM K<sup>+</sup> aCSF).
- The GABA<sub>A</sub> receptors positive allosteric modulator diazepam strongly decreased the CA1 neurons firing, in both physiological and hyperpolarizing medium.

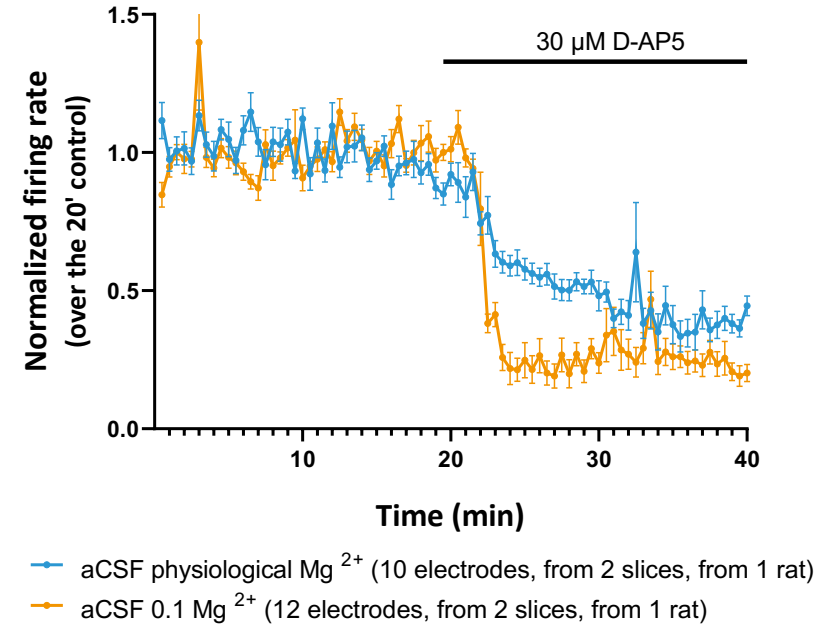
# RESULTS

## NMDA receptors

### NMDA, D-AP5



- NMDA bath applied onto the hippocampal slices strongly increased the firing rate of CA1 pyramidal neurons.



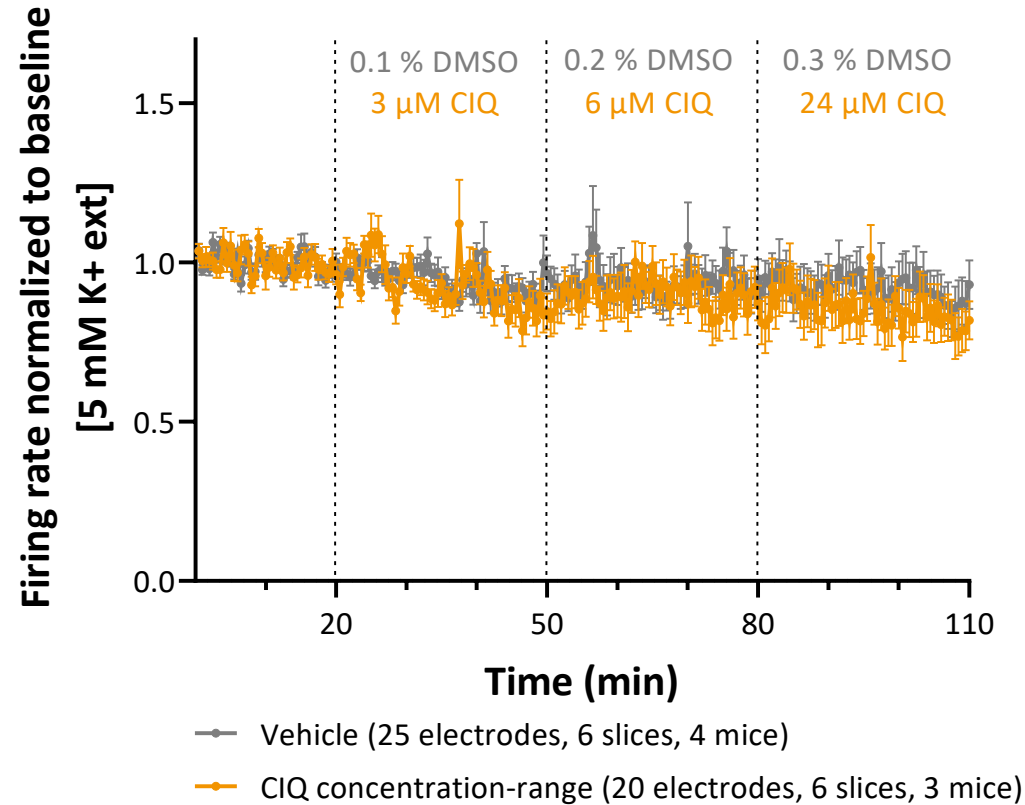
- In medium containing a physiological or a low magnesium concentration, D-AP5 – a NMDA channel blocker – substantially decreased the firing of CA1 pyramidal neurons. It is to note that D-AP5 effect was faster in a low magnesium medium.



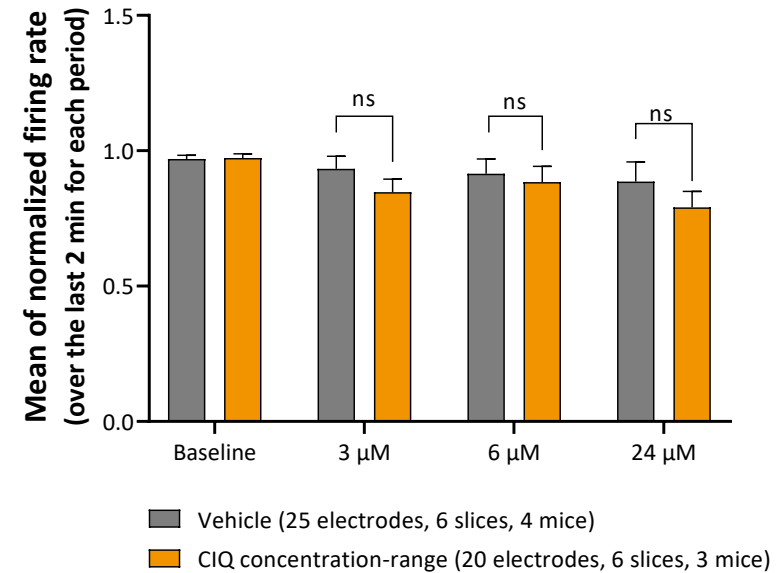
# RESULTS

## NMDA receptors

CIQ



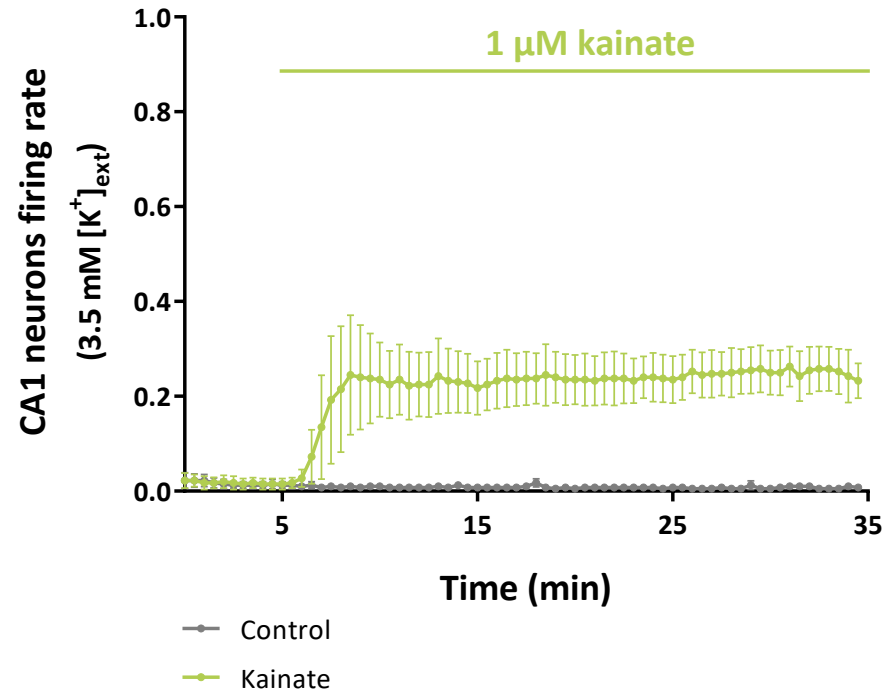
- In slices exposed to CIQ – a potentiator of NMDA receptors containing GluN2C/GluN2D sub-units - the firing rate remained comparable to vehicle slices, over the range of concentrations tested (3, 6, 24  $\mu$ M).



# RESULTS

## Kainate receptors

### Kainate

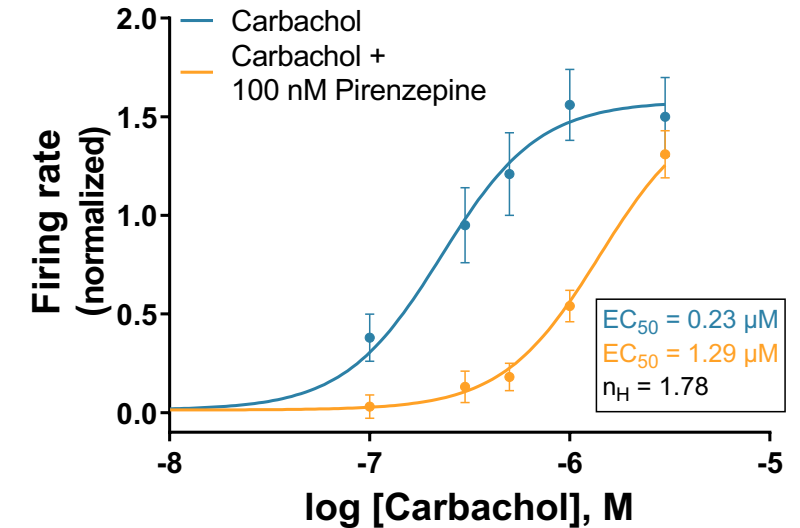
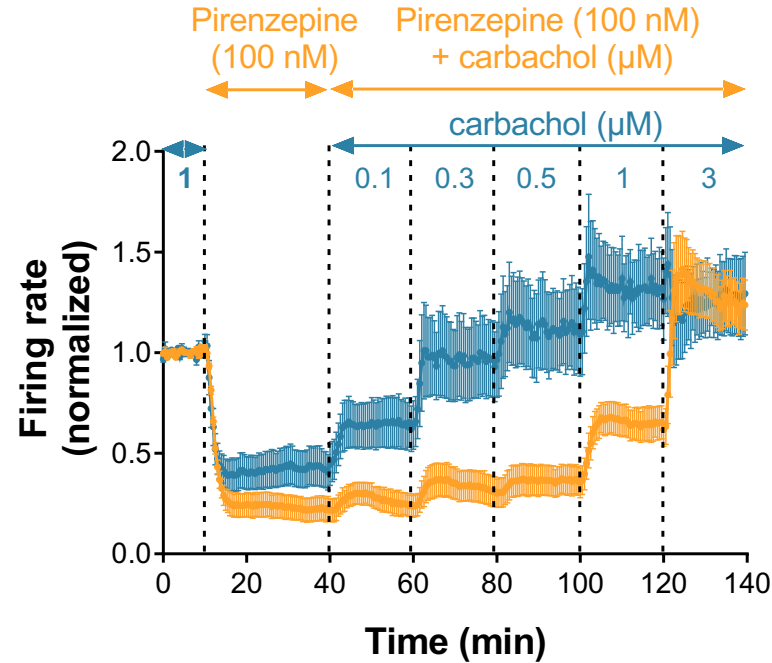
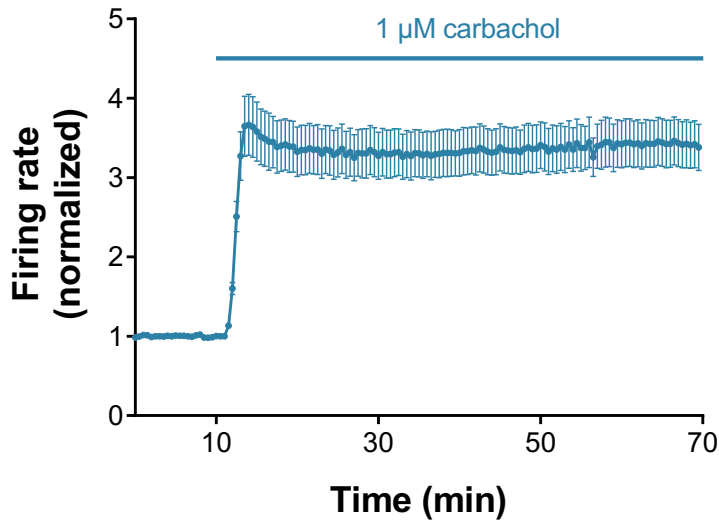


- The kainate receptors agonist kainate strongly increased the CA1 neurons firing. When applied at 1  $\mu$ M over a 30-minute period, the firing rate was more than 10 times higher than in control conditions (before kainate application).

# RESULTS

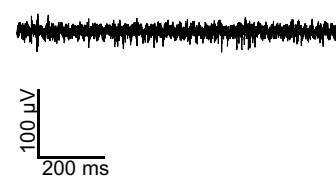
## Cholinergic receptors

### Carbachol

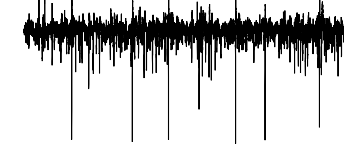


- Carbachol – a cholinergic receptors agonist – strongly increased the firing activity. Carbachol effect stabilized over the 10 first minutes of exposure and then remained steady until the end of the recording session (over 50 minutes).
- Pirenzepine – a selective  $M_1$  antagonist – reduced the effect of carbachol (right-shift of the dose-response curve).

Control



1 μM carbachol



1 μM carbachol  
+ 100 nM pirenzepine

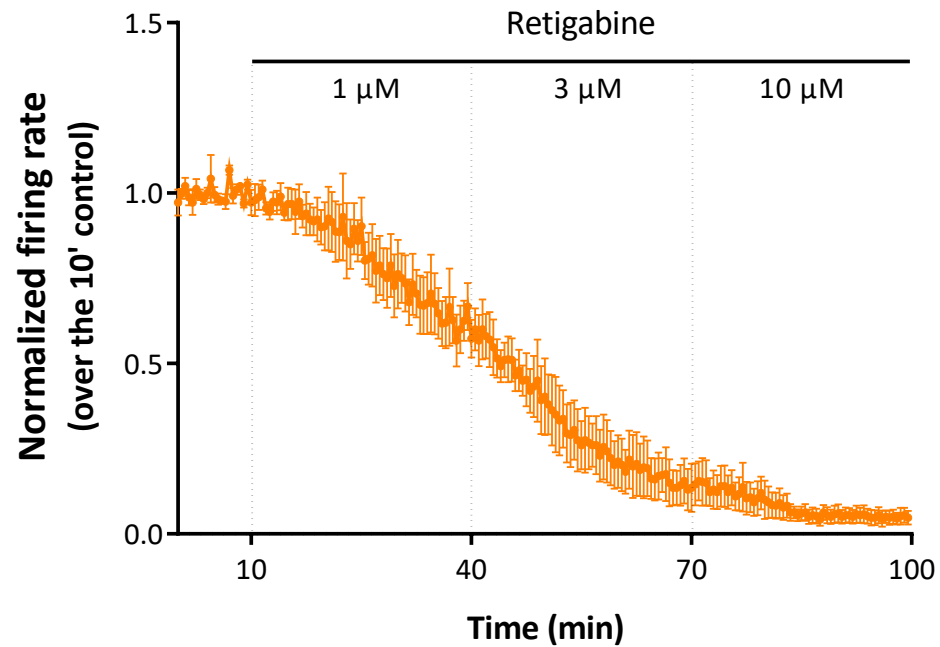




# RESULTS

## Voltage-activated K<sup>+</sup> channels

### Retigabine

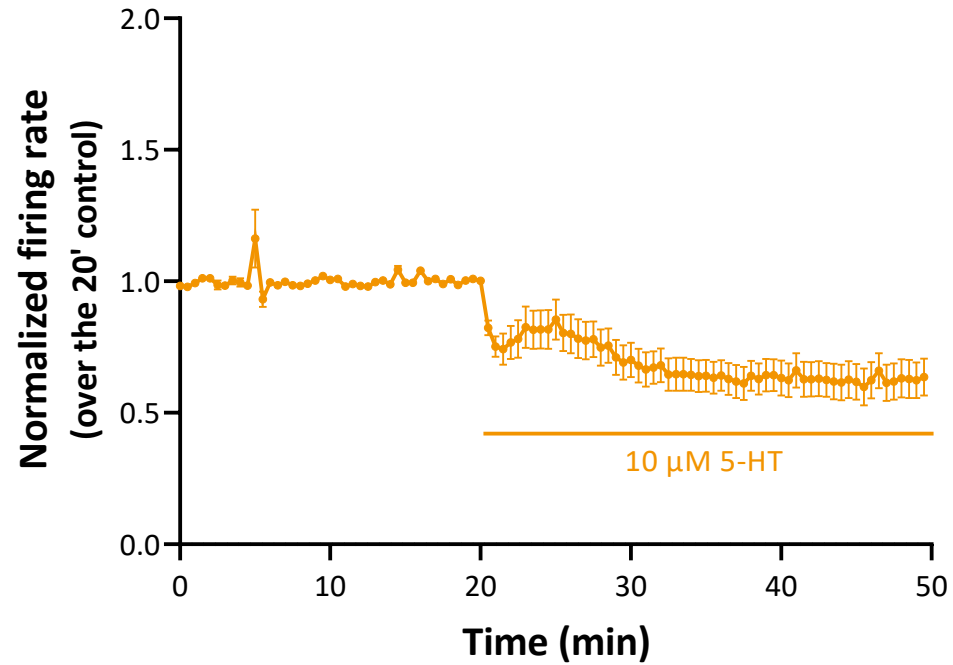


- Retigabine – a Kv7 blocker – dose-dependently reduced the firing rate from 1 μM and fully inhibited action potentials at 10 μM concentration.

# RESULTS

## Serotonergic receptors

### Serotonin



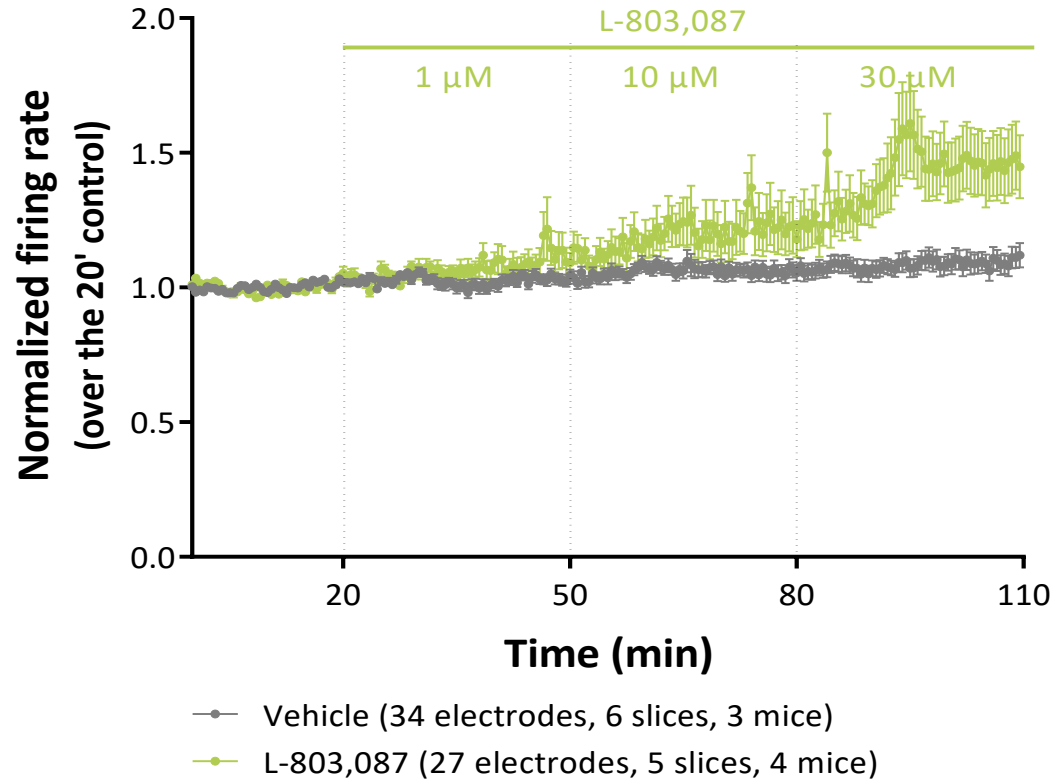
— 10 μM 5-HT (32 electrodes, 6 slices, 2 rats)

- Serotonin (5-HT) reduced the firing rate from at 10 μM concentration.

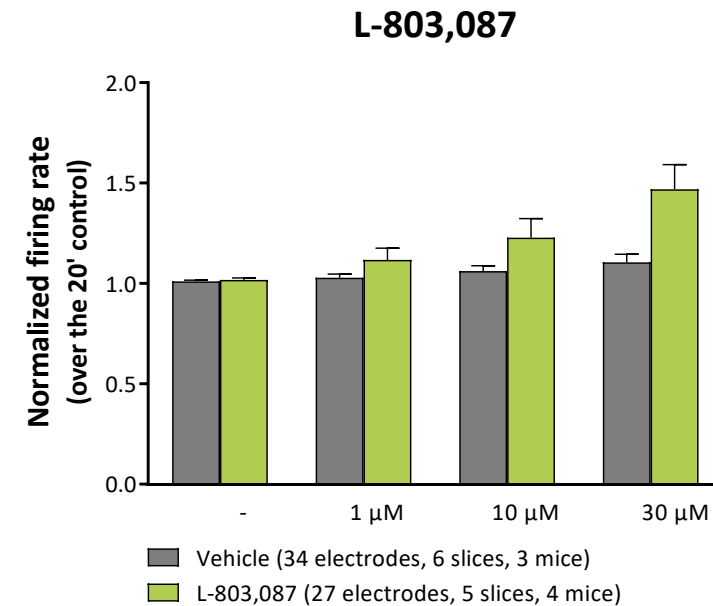
# RESULTS

## Somatostatin receptors

L803,087



- In slices exposed to L-803,087- a Potent and selective sst<sub>4</sub> agonist - , the normalized firing rate slightly increased over the application of 1  $\mu$ M, 10  $\mu$ M and 30  $\mu$ M concentrations. Thus, the mean value of normalized firing rate was  $1.12 \pm 0.06$  at t=50 min,  $1.23 \pm 0.10$  at t=80 min and  $1.47 \pm 0.12$  at t=110 min. However, it is of value to note that L-803,087 effect was variable from electrode to electrode.





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