MULTI ELECTRODE ARRAY

CA1 neurons firing

neuroservices ALLIANCE

SUMMARY

Materials & methods

- Advantages of multipoint recording
- Experimental conditions and data analysis

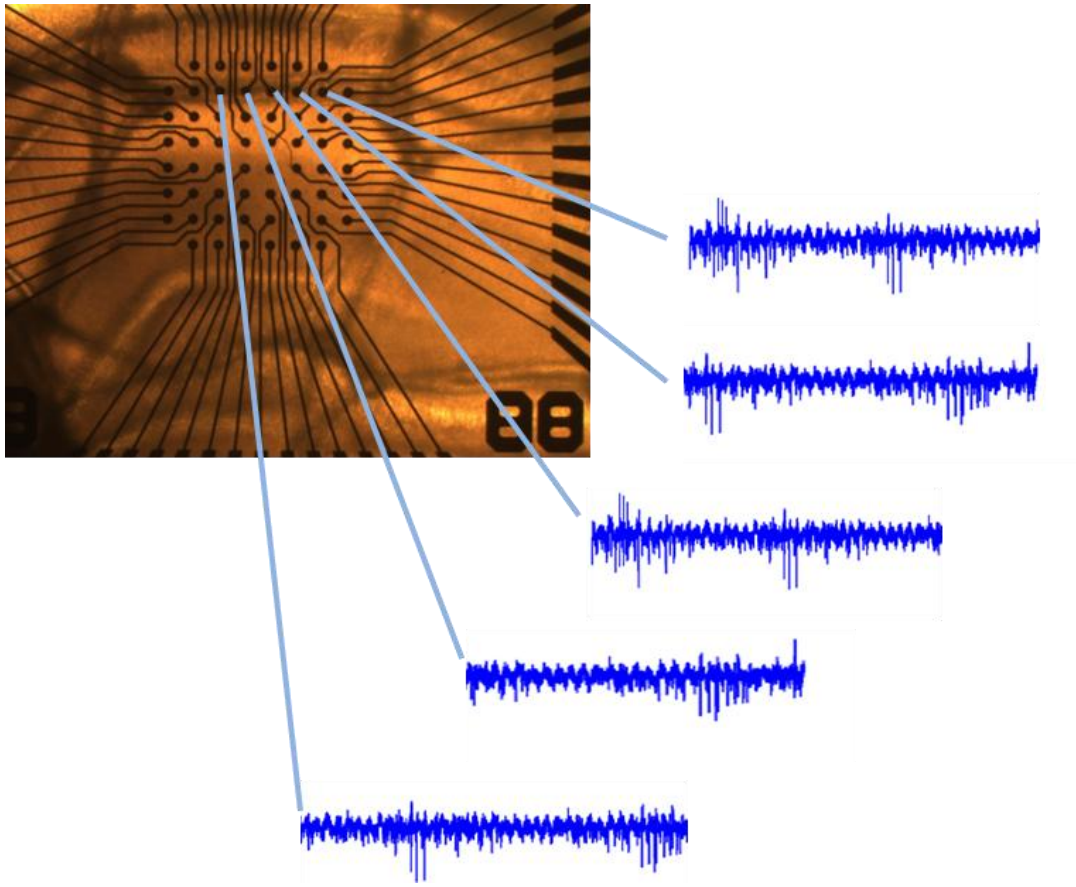
Firing modulation

- $GABA_A$ receptor – PTX, Diazepam
- NMDA receptor – NMDA, D-AP5, CIQ
- Kainate receptor – Kainate
- Cholinergic receptors – Carbachol, Pirenzepine
- Voltage-activated K^+ channels – Retigabine
- Serotoninergic receptors – 5-HT
- Somatostanin receptors – L-803,087

neuroservices ALLIANCE

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 cebebro-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.

neuroservices ALLIANCE

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 µm thickness) are cut with a vibratome

Slices perfusion

- aCSF composition: glucose 11, $NaHCO_3$ 25, NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2 in mM
- The concentration of potassium can be adjusted to 5 or 7 mM to induce firing activity of CA1 neurons

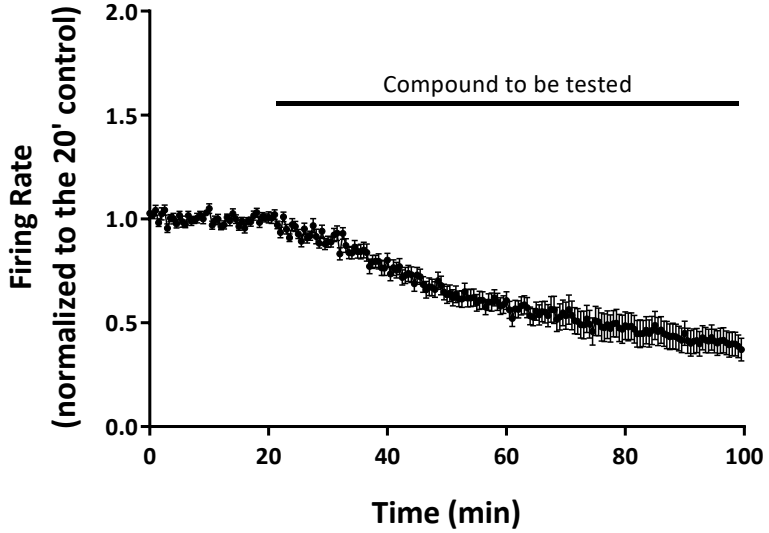
Analysis

Example of spontaneous firing

High-Pass filter
(set at 200 Hz)

Threshold detection
(-20 µV amplitude; dead time 2 ms)

Firing rate
(% of firing change - average for 30 s bins)



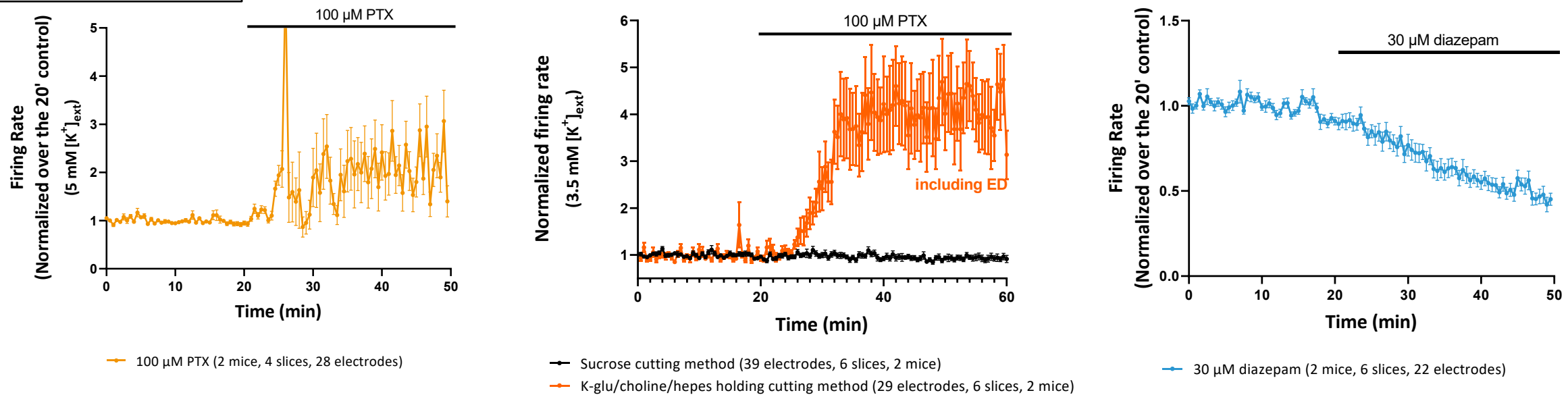
RESULTS

neuroservices
ALLIANCE

RESULTS

$GABA_A$ receptors

Picrotoxin, diazepam



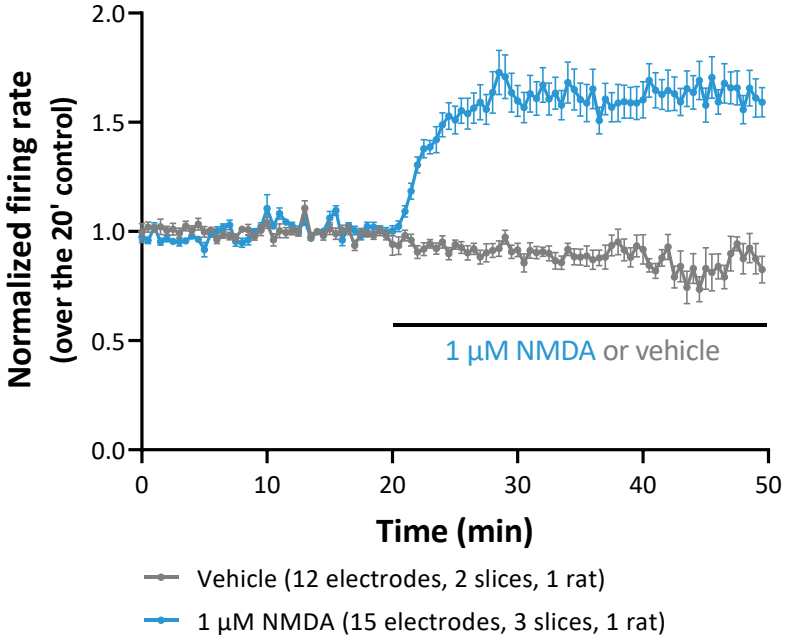
- When cutting or recordings are performed in hyperpolarizing medium (5 mM K+ or K-gluconate cutting solution), the inhibition of gabaergic interneurons with 100 µM picrotoxin (PTX) increased the firing activity of principle pyramidal neurons and also triggered epileptiform discharges after 5 minutes 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^+ aCSF).

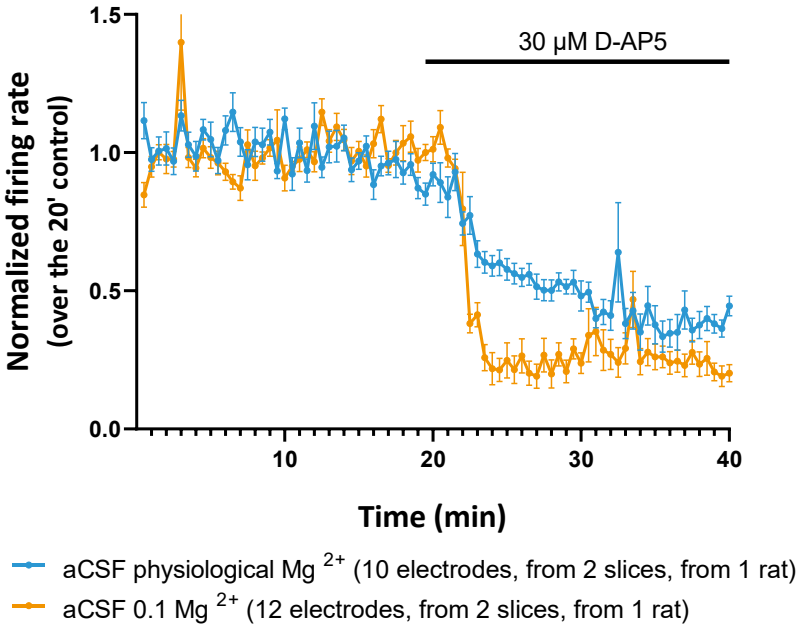
- The $GABA_A$ receptors positive allosteric modulator diazepam strongly decreased the CA1 neurons firing, in both physiological and hyperpolarizing medium.

RESULTS

NMDA receptors

NMDA, D-AP5





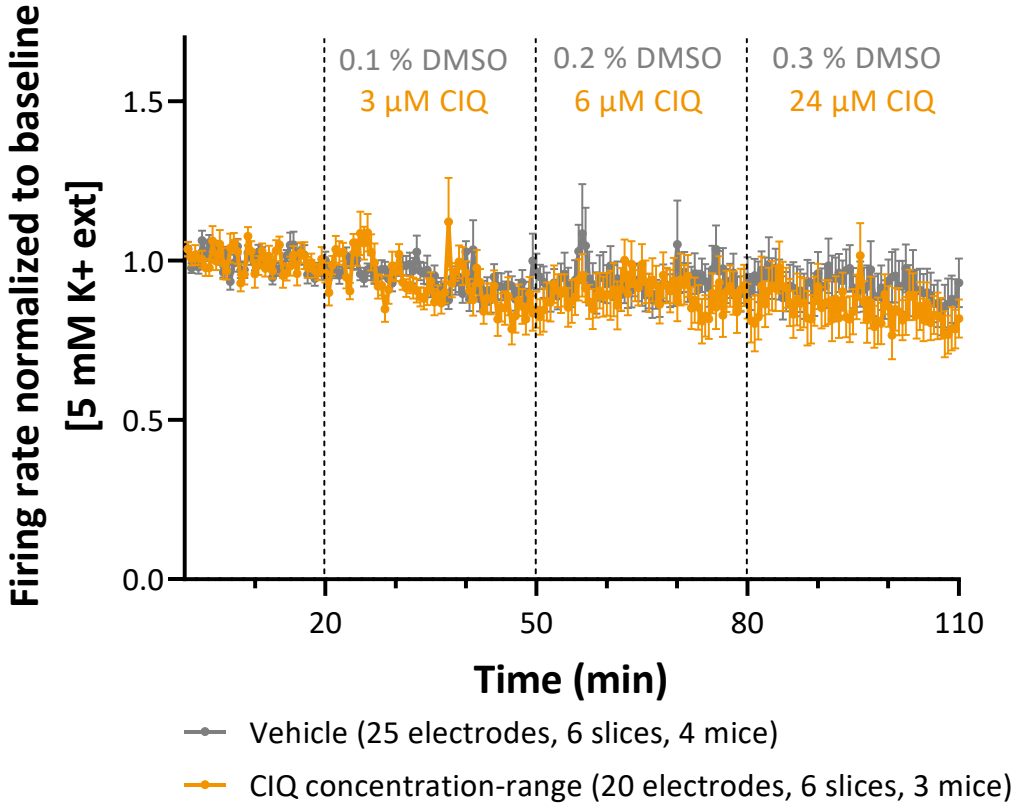
- NMDA bath applied onto the hippocampal slices strongly increased the firing rate of CA1 pyramidal neurons.

- 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.

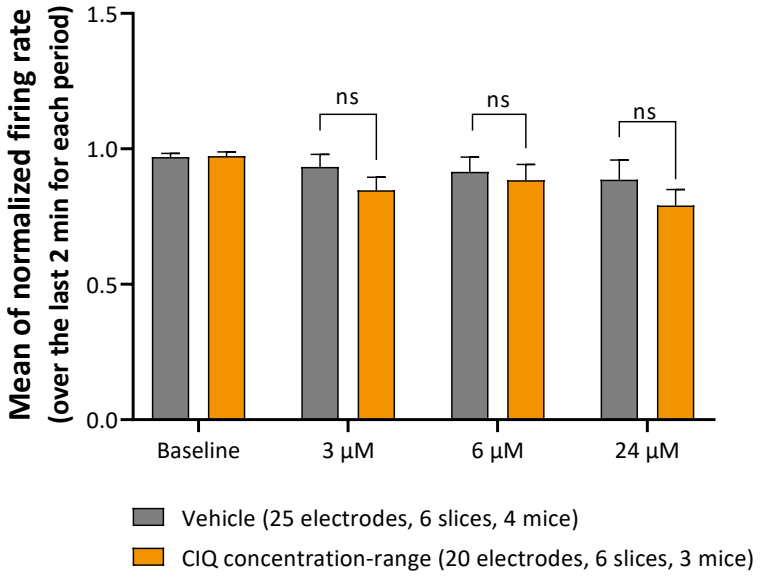
RESULTS

NMDA receptors

CIQ



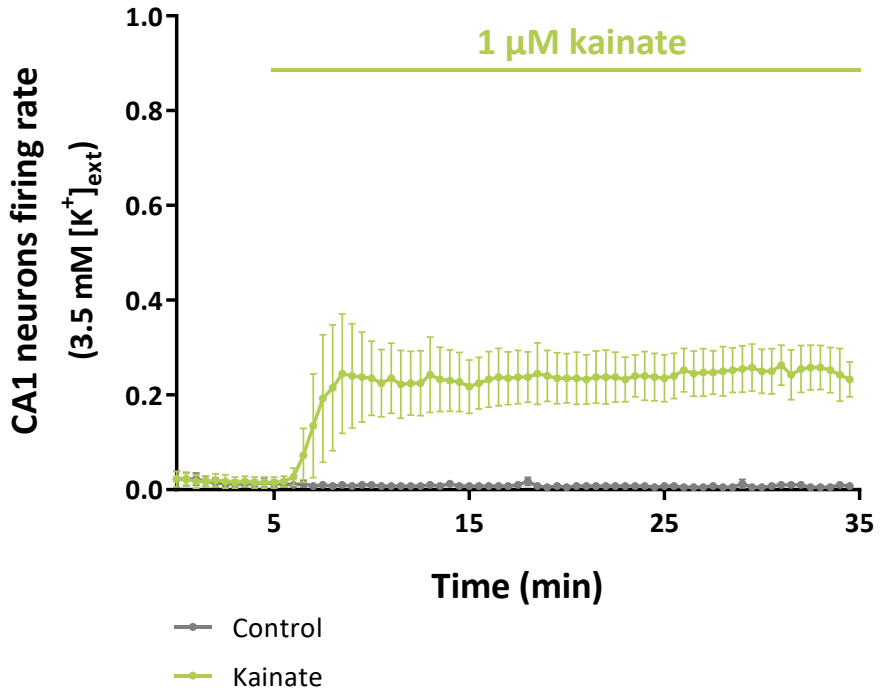
- In slices exposed to CIQ – a potentiator of NMDA receptors containing GluN2C/GluN2D sub-units - the firing rate remained comparable to vehicle slices, over the range of concentrations tested (3, 6, 24 µM).



RESULTS

Kainate receptors

Kainate

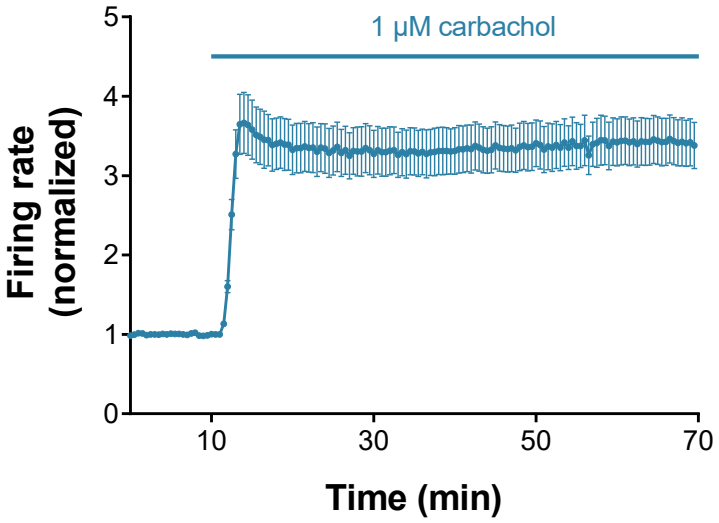


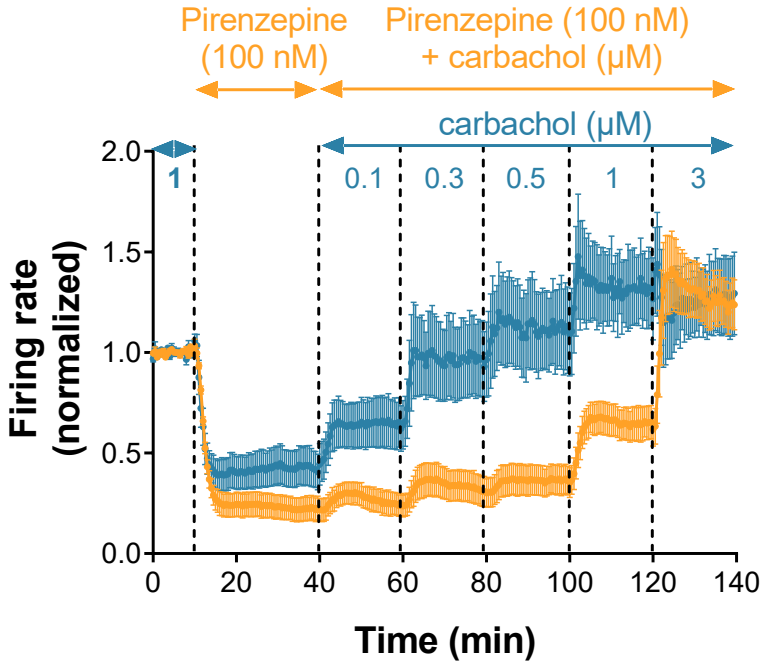
- The kainate receptors agonist kainate strongly increased the CA1 neurons firing. When applied at 1 µM over a 30-minute period, the firing rate was more than 10 times higher than in control conditions (before kainate application).

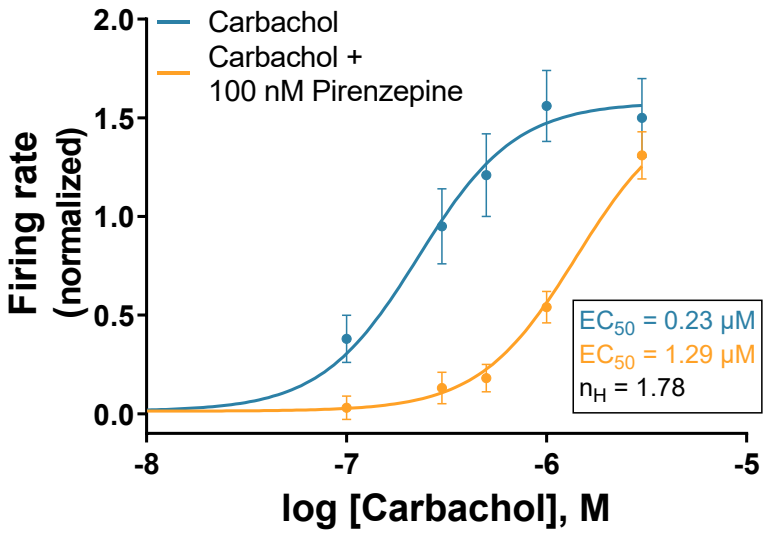
RESULTS

Cholinergic receptors

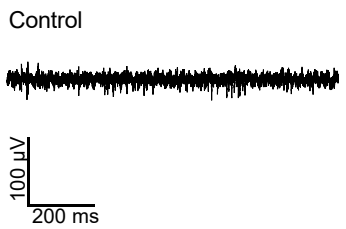
Carbachol

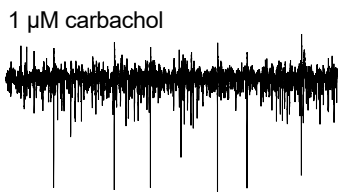


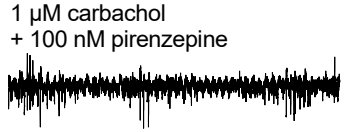




- Carbachol – a cholinergic receptors agonist – strongly increased the firing activity. Carbachol effect stabilized over the 10 first minutes of exposure and then remained steady until the end of the recording session (over 50 minutes).
- Pirenzepine – a selective M_1 antagonist – reduced the effect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



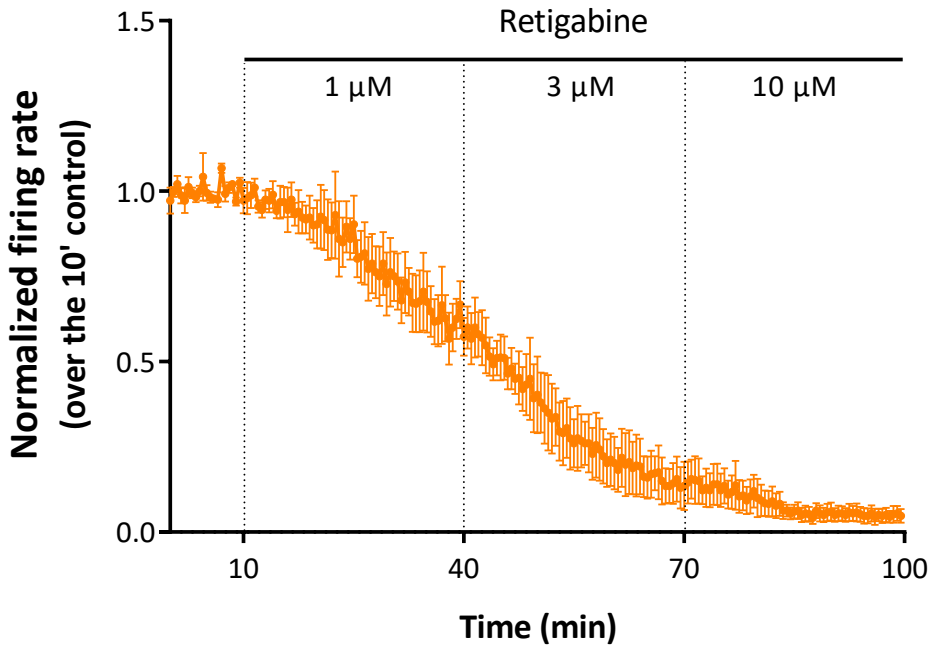




RESULTS

Voltage-activated K^+ channels

Retigabine

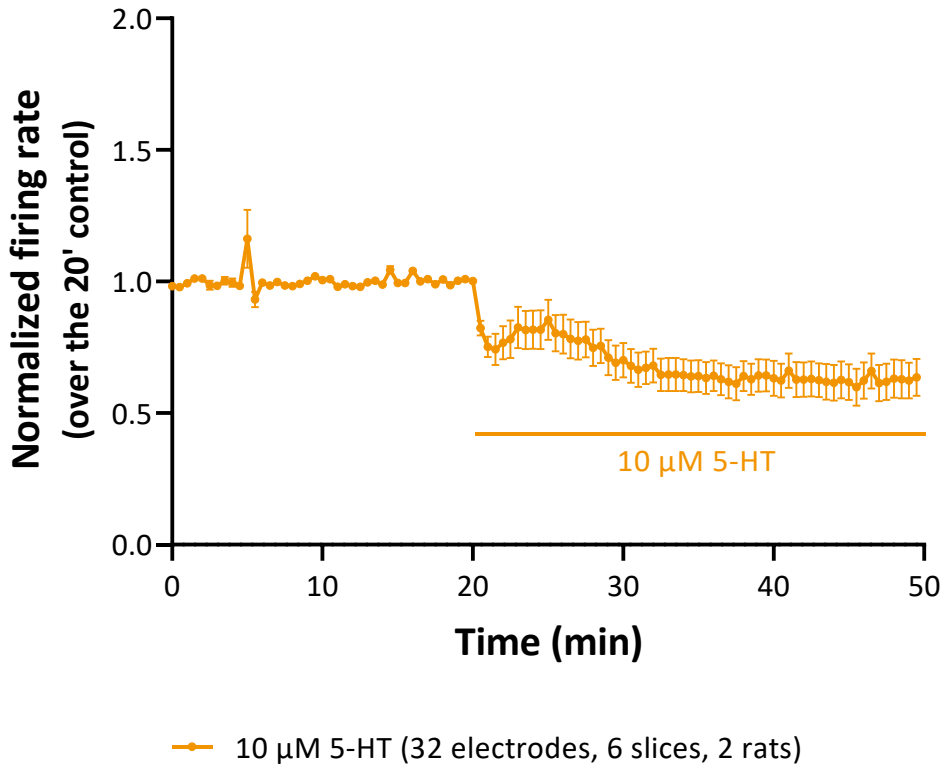


- Retigabine – a Kv7 blocker – dose-dependently reduced the firing rate from 1 µM and fully inhibited action potentials at 10 µM concentration.

RESULTS

Serotoninergic receptors

Serotonin

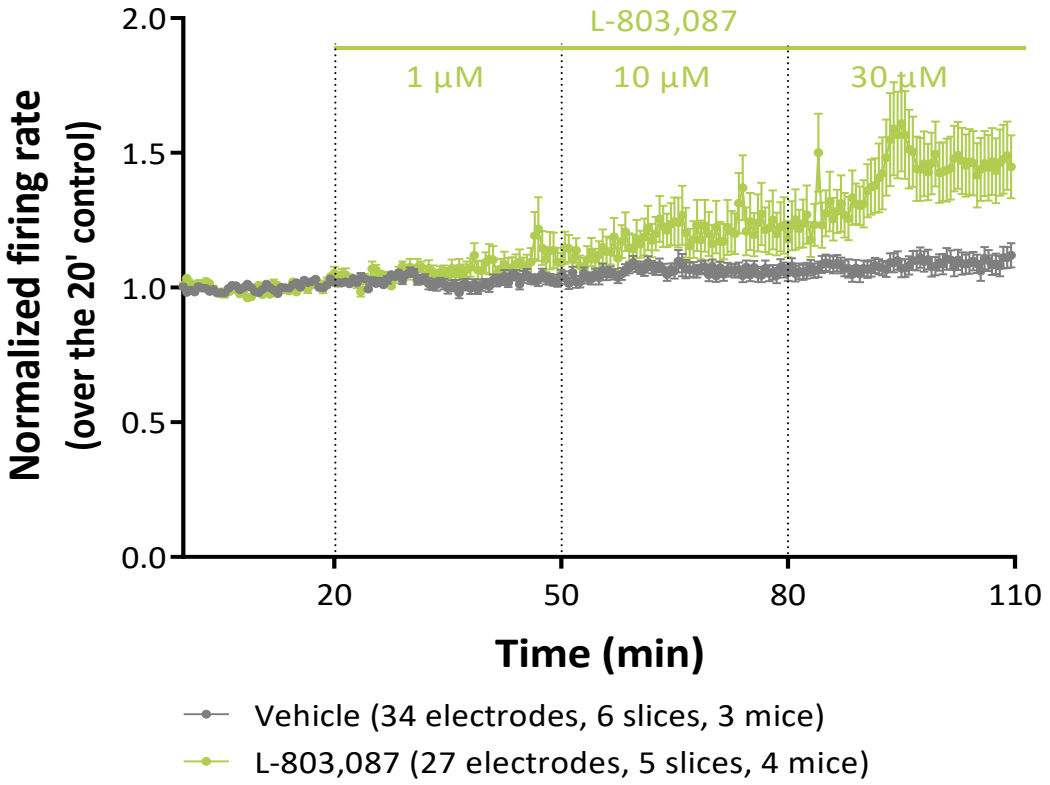


- Serotonin (5-HT) reduced the firing rate from at 10 µM concentration.

RESULTS

Somatostatin receptors

L803,087



- In slices exposed to L-803,087- a Potent and selective sst_4 agonist - , the normalized firing rate slightly increased over the application of 1 µM, 10 µM and 30 µM concentrations. Thus, the mean value of normalized firing rate was 1.12 ± 0.06 at t=50 min, 1.23 ± 0.10 at t=80 min and 1.47 ± 0.12 at t=110 min. However, it is of value to note that L-803,087 effect was variable from electrode to electrode.