MEA recordings in rat spinal cord slices for applied pharmacological investigations

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INTRODUCTION

We have developed a unique ex vivo spinal cord slice assay that can be prepared in neonate, juvenile and adult rats (up to P55) for screening pharmacological compounds.
Spontaneous single unit activity can be recorded using a Multi-Electrode Array (MEA) with electrodes placed either in dorsal or ventral region.
In the dorsal horn, the spontaneous firing activity was enhanced by capsaicin in a dose-dependent manner with an apparent EC_{50} of 101 nM.
Further, the enhanced firing activity in the presence of 100 nM capsaicin is stable over one hour of recording and this "steady-state" can be used to investigate the pharmacological efficacy of analgesic molecules.
We observed both the μ-opioid receptor agonist morphine as well as the sodium channel blocker lidocaine were able to reduce and eliminate capsaicin-induced firing activity.
Spontaneous and capsaicin-induced firing activity were also modulated by octreotide, a relatively specific agonist of somatostatin type-2 (SSTR2) receptor, used for pain-relief in patients who cannot tolerate the adverse effects of opioids.
In contrast, gabapentin and pregabalin, two anti-epileptic drugs that effectively reduce neuropathic pain in humans did not reverse capsaicin-induced firing when acutely applied.
Together, these results demonstrate the utility of MEA-based rat spinal cord assay to test the pharmacological activity of new analgesic molecules.

MATERIAL & METHODS

Preparation of acute spinal cord (SC) slices

Experiments were carried out with SC sections from P7-P11 Sprague-Dawley rats. After dorsal laminectomy, the lumbar region of the SC was cut into 350 µm thick coronal spinal sections using a vibratome (Leica). Right after cutting, slices were incubated for at least 1.5 h at 32°C in 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 mmol/L). aCSF was continously bubbled with carbogen (95% O_2, 5% CO_2).

Multi-electrode array set-up

All data were recorded using a MEA set-up commercially available from MultiChannel Systems (MCS GmbH, Reutlingen, Germany) composed of a 4-channel stimulus generator and a 60-channels amplifier head-stage connected to a 60-channel A/D card. Data acquisition and analysis was performed using MC Rack (MCS GmbH, Reutlingen, Germany).
All experiments were performed using 3-dimensional MEA (Qwane Biosciences, S.A., Lausanne, Switzerland) that consist of 60 microelectrodes arranged in a 8 X 8 layoud grid. The separation between electrodes was 100 µm.

Recording protocols

SC slices were placed on the MEA such that the array coverered the major area of one of the dorsal horns, without side distinction. The MEA chamber volume was approximately 1 mL and the flow rate was maintained at 3 mL.min^{-1}. The firing activity was continuously recorded at the 60 MEA electrodes. Data were sampled at 20 kHz.

Data analysis

The number of spikes per second recorded at each electrode was averaged for 30 s bins. Data were averaged from raw or normalized firing values, respectively. The mean value of the spikes rate (± SEM) was plotted as a function of time.

RESULTS

Capsaicin-induced firing in dorsal horn neurons

Figure 1: Rat spinal cord slices recording with MEA
A: Schematic of the rat spinal cord section in the L5 region.
B: Light microscopic image (X 5) of a rat spinal cord slice placed over a 3-dimensional MEA grid.

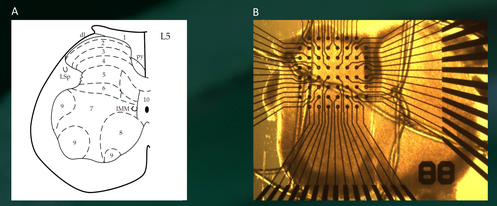


Figure 2: Capsaicin increased single unit firing activity in a dose-dependent manner
A: Dose-dependent increase in firing activity of neurons in dorsal SC in the presence of increasing concentrations of capsaicin (2 rats, 10 slices, 82 electrodes).
B: Plot of the % of increase in firing as a function of the capsaicin concentrations. Data were fitted with an empirical Hill equation (bottom and top values set to 0 and 100, respectively) to determine the apparent EC_{50} (101 nM).

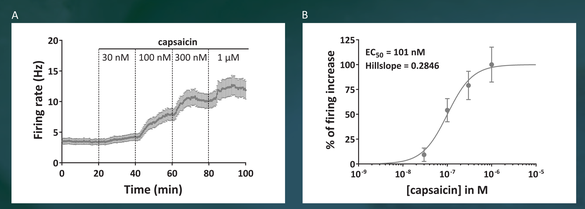
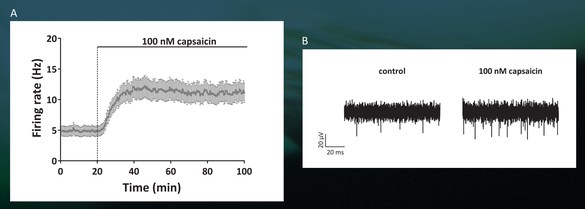
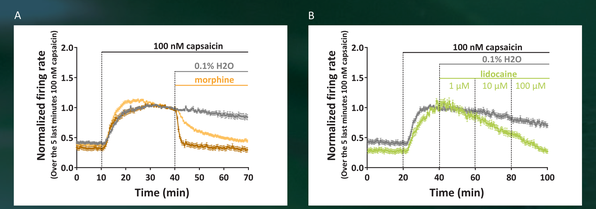


Figure 3: Stability of neuronal firing in the presence of 100 nM capsaicin
A: Following a 10 min baseline, the firing activity was increased by acute application of 100 nM capsaicin and remain stable for > 60 minutes (dark grey circles, 2 rats, 4 slices, 63 electrodes).
B: Representative traces of firing activity in control (left trace) and after exposure to 100 nM capsaicin (right trace).



Both μ-opioid receptor agonist morphine and sodium channel blocker lidocaine decreased capsaicin-induced firing

Figure 4: Dose-dependent inhibition of capsaicin-induced firing activity by morphine and lidocaine
A: Following a 10 min baseline, the firing activity was enhanced by acute application of 100 nM capsaicin (dark grey circles, 3 rats, 8 slices, 114 electrodes). Addition of 100 nM morphine substantially inhibited the capsaicin-induced firing over a 30 min period (orange circles, 2 rats, 9 slices, 153 electrodes) whereas a full inhibition was observed within 10 min with 10 µM morphine (dark orange circle, 2 rats, 6 slices, 51 electrodes).
B: Following a 10 min baseline, the firing activity was enhanced by acute application of 100 nM capsaicin (dark grey circles, 2 rats, 4 slices, 57 electrodes). Addition of increasing concentrations of lidocaine (1, 10 and 100 µM) led to a progressive and complete inhibition of the capsaicin-induced firing (green circles, 2 rats, 4 slices, 46 electrodes).



SSTR2 receptor agonist octreotide decreased both capsaicin-induced and spontaneous firing

Figure 5: Octreotide, a somatostatin receptor agonist, substantially inhibited the capsaicin-induced firing activity
A: Spinal cord section positioned over a MEA grid. The colored circles represent electrodes corresponding to traces depicted in B.
B: Recordings of firing activity corresponding to the green-dotted electrode (in lamina 2) and the blue-dotted electrode (in lamina 4-5) illustrated on the picture. Representative traces are displayed in control (left traces), in the presence of 2 µM capsaicin (middle traces) and under 2 µM capsaicin + 1 µM octreotide (right traces).
C: Time course of the effect of a high-concentration of capsaicin (2 µM) on the firing activity and its inhibition by 1 µM Octreotide (2 rats, 4 slices, 33 electrodes).

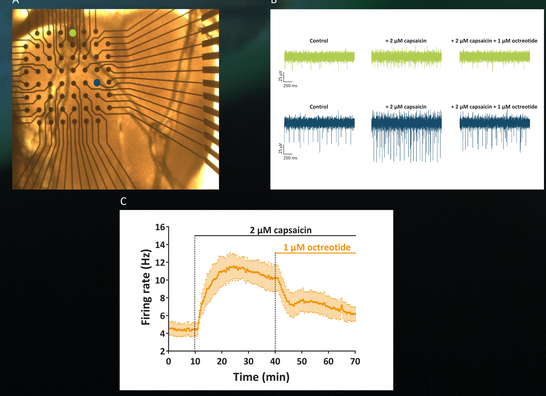
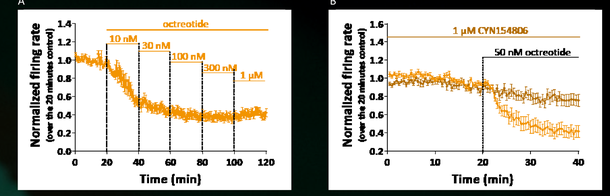
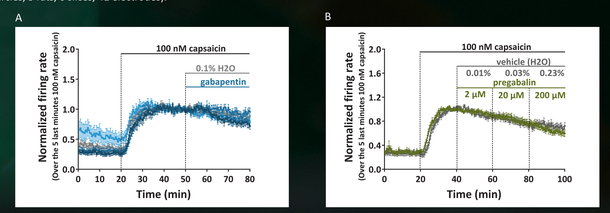


Figure 6: Octreotide inhibited spontaneous firing activity
A: Following a 20 min control period, increasing concentrations of octreotide (10 – 1000 nM) were applied every 20 min. A maximal effect was reached at 300 nM or greater concentrations of octreotide (2 rats, 8 slices, 58 electrodes).
B: 1 µM CYN154608, a selective STTR2 antagonist, inhibited the effect of 50 nM octreotide. After a 20 min baseline recording, 50 nM octreotide perfused over 20 min strongly decreased the spontaneous firing (orange circles, 2 rats, 4 slices, 27 electrodes) whereas the octreotide effect remained minor in the presence of 1 µM CYN154608 (dark orange circles, 2 rats, 5 slices, 28 electrodes).



$Alpha_2$- delta calcium channel inhibitors gabapentin and pregabalin did not reduce capsaicin-induced firing

Figure 7: Lack of effect of gabapentin and pregabalin on capsaicin-induced firing activity
A: Following a 20 min baseline recording, 100 nM capsaicin was perfused for 30 min. Then, 1 µM gabapentin (light blue circles, 1 rat, 2 slices, 10 electrodes) or 10 µM gabapentin (blue circles, 1 rat, 2 slices, 27 electrodes) or 100 µM gabapentin (2 rats, 3 slices, 29 electrodes) was acutely applied for 30 min. No effect of gabapentin was observed over this time course when compared to control slices recorded in parallel in the presence of 100 nM capsaicin + vehicle (dark grey circles, 2 rats, 3 slices, 29 electrodes).
B: Following a 20 min baseline recording, 100 nM capsaicin was perfused for 20 min. Then, 2 µM, 20 µM and 200 µM pregabalin were successively applied for 20 min for each concentration (green circles, 3 rats, 6 slices, 61 electrodes). No effect of pregabalin was observed over this time course when compared to control slices recorded in parallel in the presence of 100 nM capsaicin + vehicle (dark grey circles, 3 rats, 6 slices, 41 electrodes).



CONCLUSION

- Capsaicin-induced firing enables the investigation of putative analgesic molecules efficacy.
- Classical analgesic drugs morphine and lidocaine efficiently reduced capsaicin-induced firing activity.
- The effect of octreotide, a pain-killer used as an alternative to opioid compounds, can also be documented in the present assay.
- In contrast, gabapentin and pregabalin - two anti-epileptic drugs that effectively reduce some form of neuropathic pain - did not reverse capsaicin-induced firing over a couple of minutes. This suggests that the present model is suitable to evaluate compounds modulating acute but not neuropathic pain, or that a longer exposure time could be required depending on the compound mechanism of action.
- The present assay enable to document the pharmacological properties of assumed pain-killer over a concentration range. In addition, their mechanism of action could be confirmed by using pharmacological tools.

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