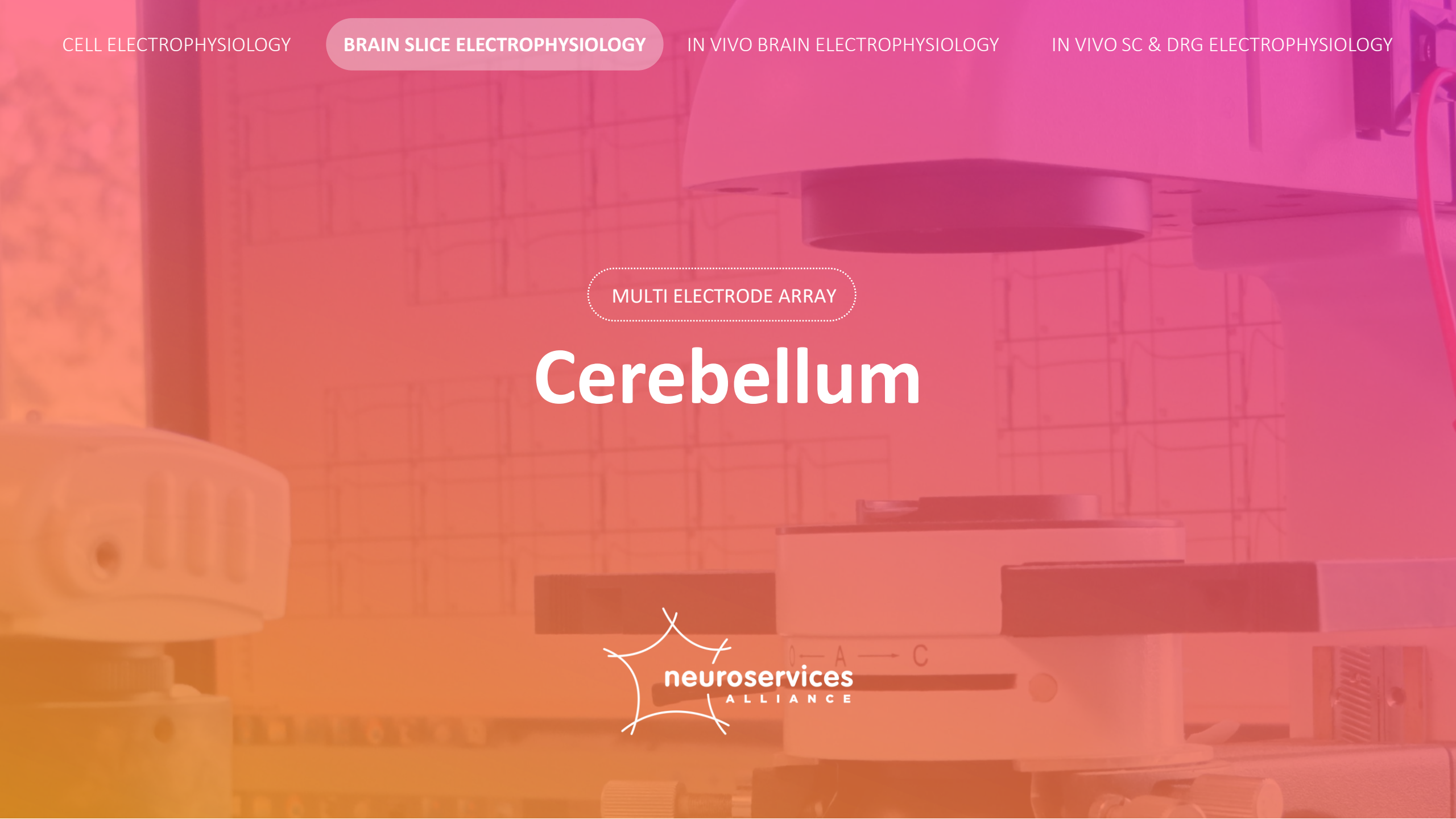

MULTI ELECTRODE ARRAY

Cerebellum

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SUMMARY

Introduction

Materials & methods

- Cellular organization
- Long-term depression (mossy fiber – granular layer)
- Long-term depression (parallel fiber – purkinje)
- Purkinje neurons firing

Results

- Sodium channel – TTX, Lidocaine
- $GABA_A$ – Bicuculline, Isoguvacine
- β-amyloid peptide - Aβ(1-40), Aβ(20-40), Aβ(1-19)

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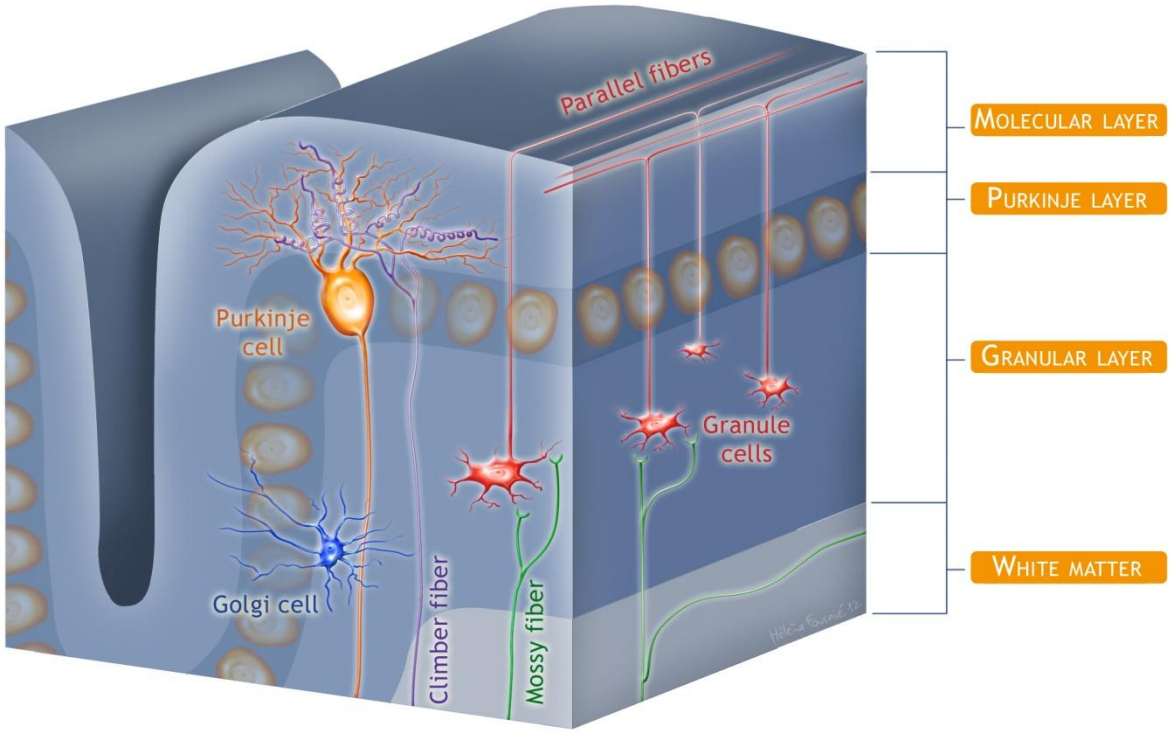
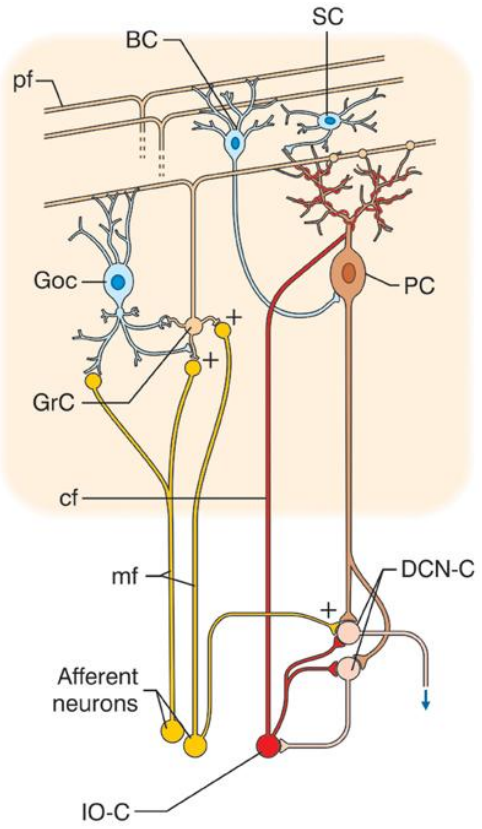
INTRODUCTION

Cellular organization

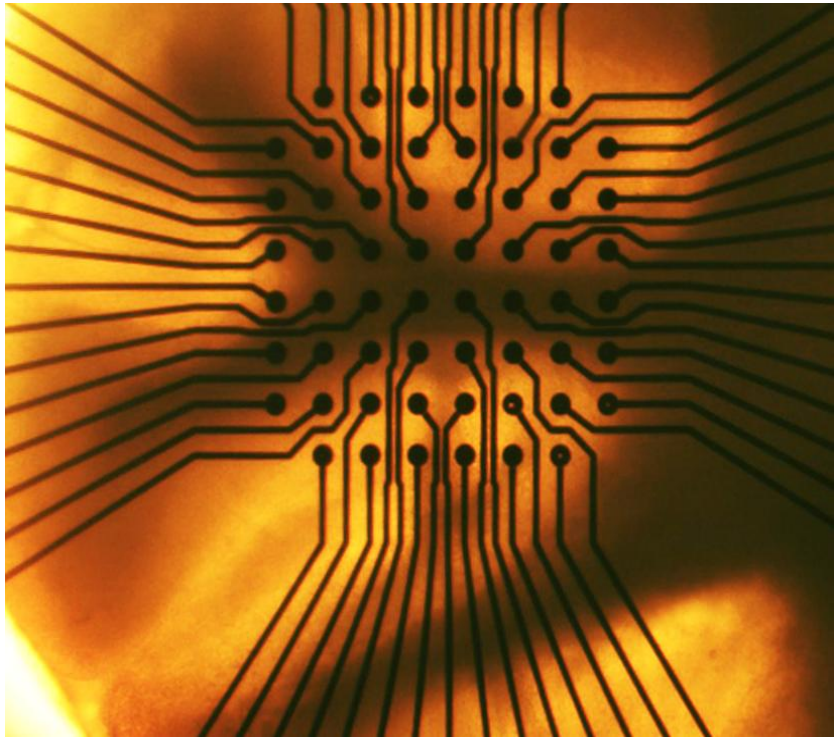


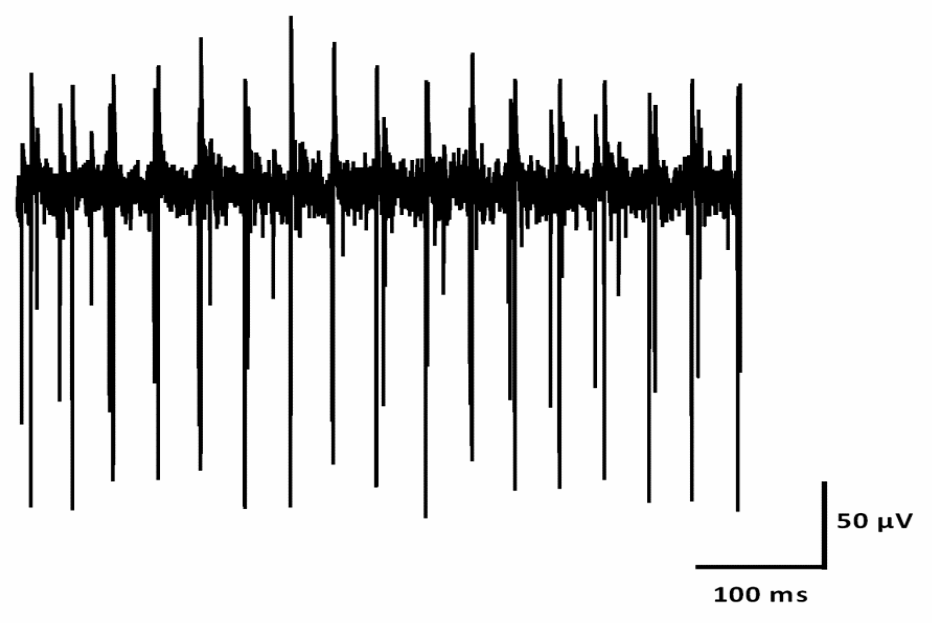


- Purkinje neurons (PN) are directly innervated by climbing fibers originating from the inferior olive. Granule cells also project parallel fibers onto the Purkinje cell dendritic trees. Gabaergic interneurons from the Moleculare Layer (Basket Cells and Stellate cells) modulate these excitatory synaptic inputs. PN axons are the sole output of the cerebellar cortex, projecting to the deep cerebellar nuclei.

INTRODUCTION

Purkinje neurons (PN) spontaneous firing





- Purkinje neurons (PN) are intrinsically active: they fire action potentials in absence of synaptic input. This intrinsic pacemaking activity is regular and fast, and originates from resurgent sodium and potassium conductances.

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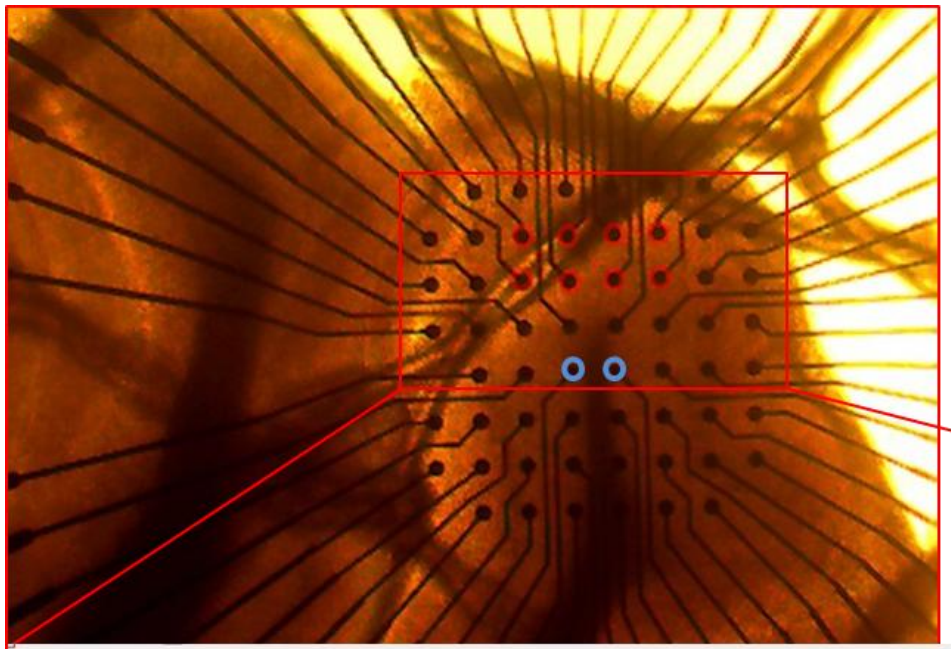
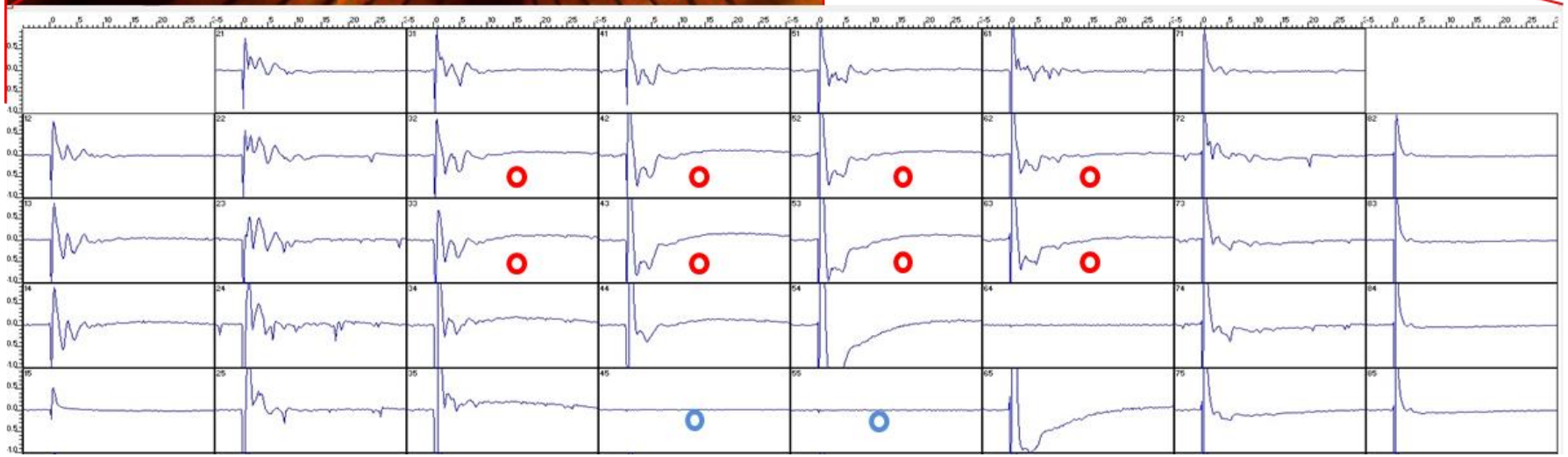
MATERIALS & METHODS

Long-term depression (mossy fiber – granular layer)

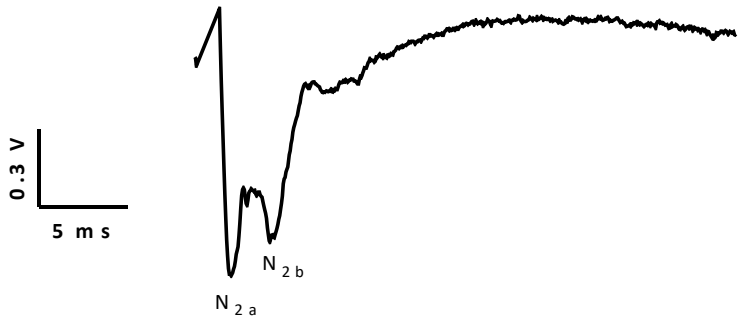


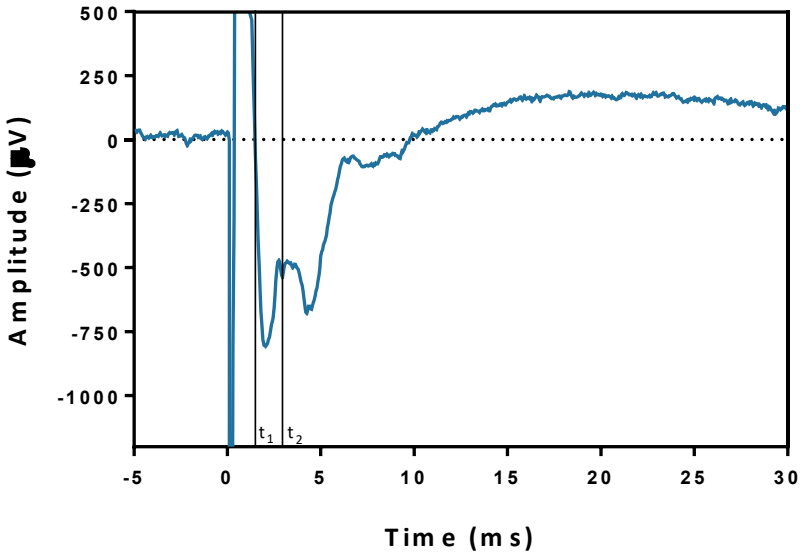
- The Long-Term Depression (LTD) is recorded in the granular layer (GL)when stimulating the mossy fiber (MF). The stimulus consisted in a monopolar biphasic current pulse (−200 µA for 60 µs followed by +200 µA for 60 µs) applied at 30 s intervals. The synaptic plasticity is induced by 900 pulses applied at 1 Hz, with an intensity set to 600 µA (low frequency stimulation = LFS).
- The pair of electrodes used to selectively stimulate the mossy fiber (MF) are surrounded in blue. The electrodes surrounded in red display evoked-responses in the granular layer (GL). Signals recorded at the electrodes within the red frame are shown below the picture of the slice.



MATERIALS & METHODS

Long-term depression (mossy fiber – granular layer)





- Typical evoked-responses recorded from the GL with focal stimulation between two neighboring electrodes located at the tip of the MF bundle.

- The amplitude of the EPSP presented in the next slides are the one from the first peak. The region of interest to determine the EPSP amplitude was set between t1 = 1.5 ms and t2 = 3 ms (focus on the N2a peak).

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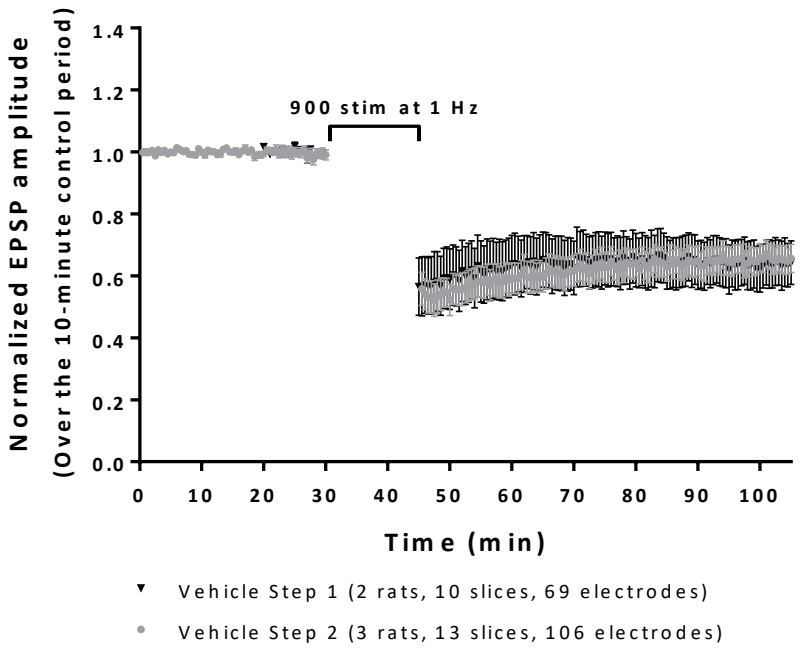
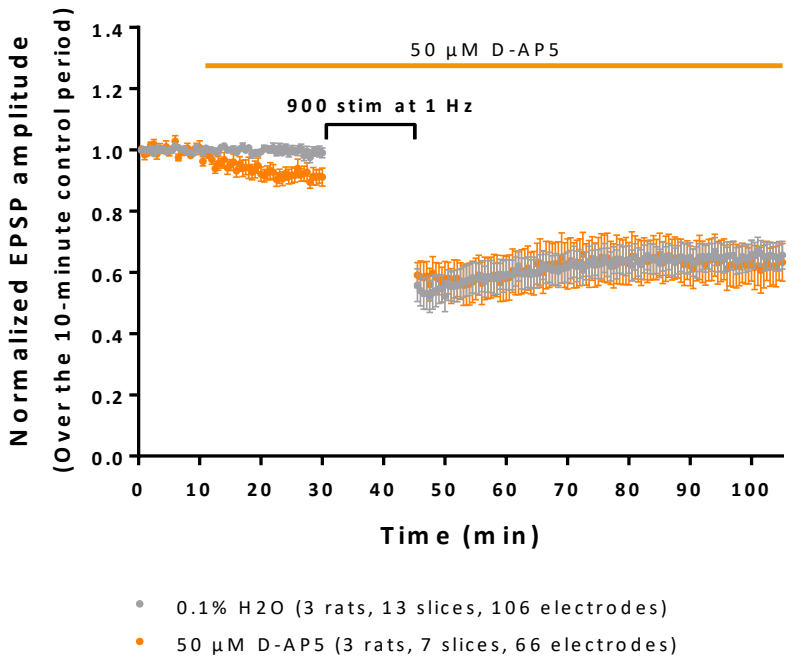
MATERIALS & METHODS

Long-term depression (mossy fiber – granular layer)





Reproducibility

The LTD was induced by a low frequency stimulation (LFS) that consisting in 900 stimulations at 1 Hz for both set of experiment with similar results.

+/- D-AP5

D-AP5 slightly decreased the basal synaptic transmission but did not inhibit the LTD induced by the LFS protocol. This indicates that the plasticity is not NMDA-dependent. The D-AP5 effect observed on the basal synaptic transmission revealed the NMDA component in the basal transmission.

RESULTS

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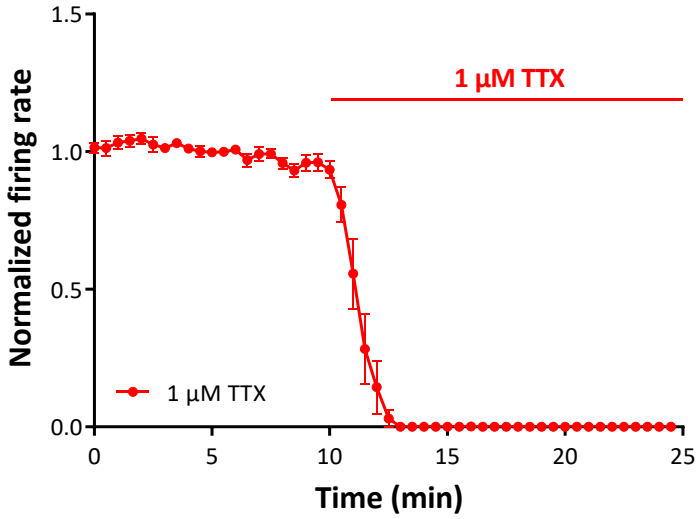
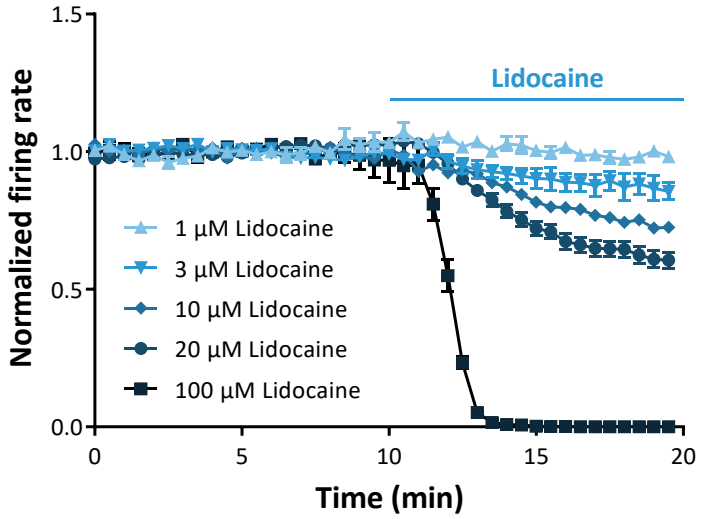
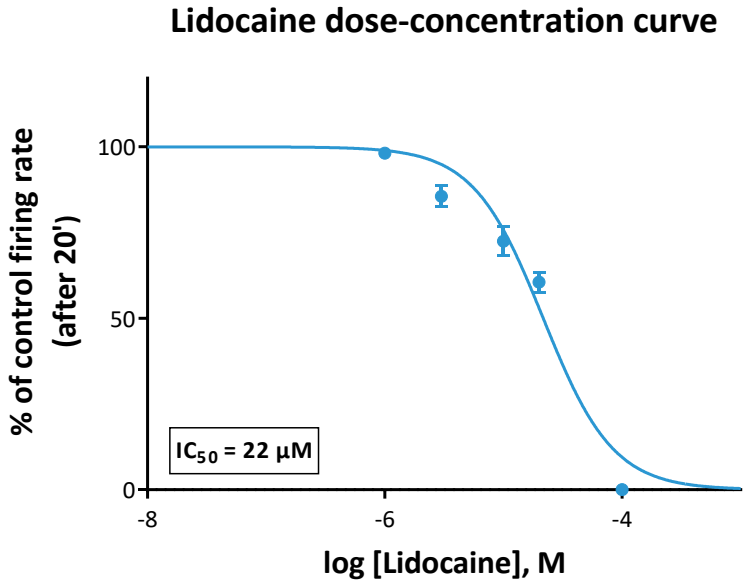
RESULTS

Sodium channel

TTX, lidocaine





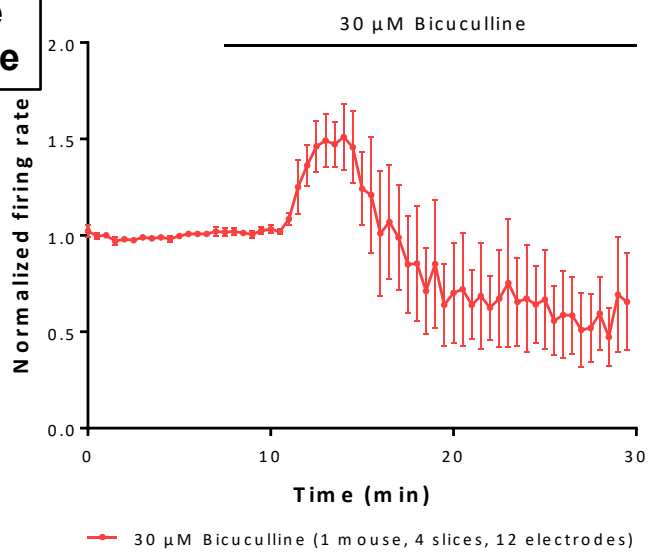


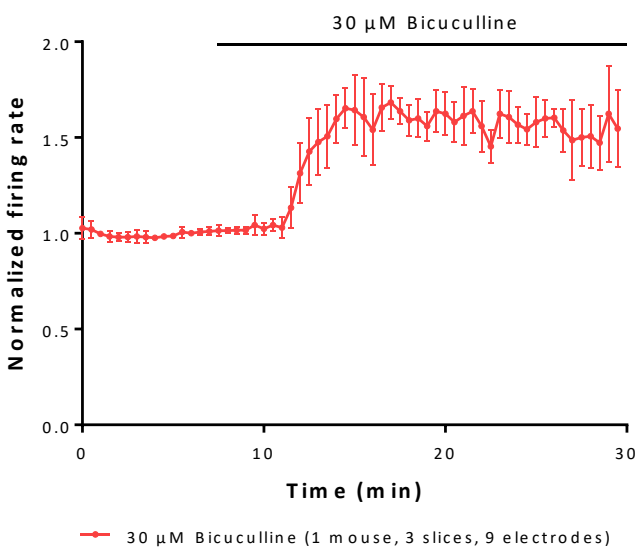
- Any compound modifying AP genesis or propagation will change Purkinje neurons spikes rate (as do sodium channel antagonists tetrodotoxin (TTX) and lidocaine).
- Dose-range of compounds could be evaluated to determine their IC_{50}

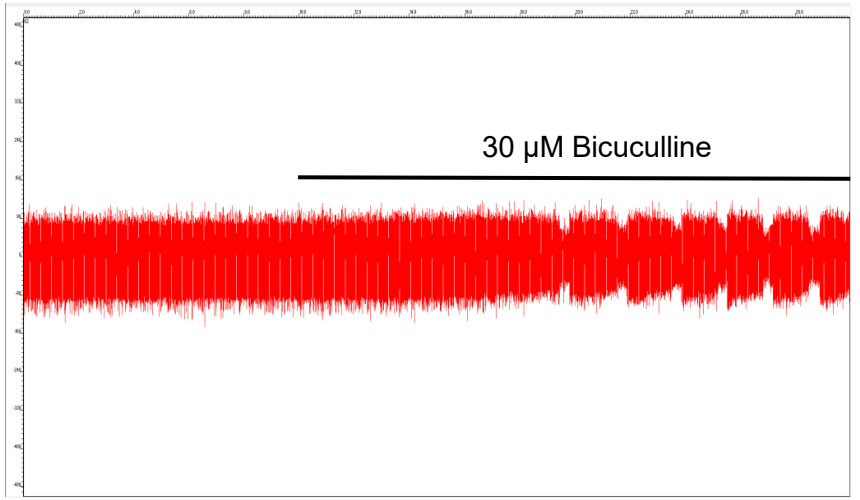
RESULTS

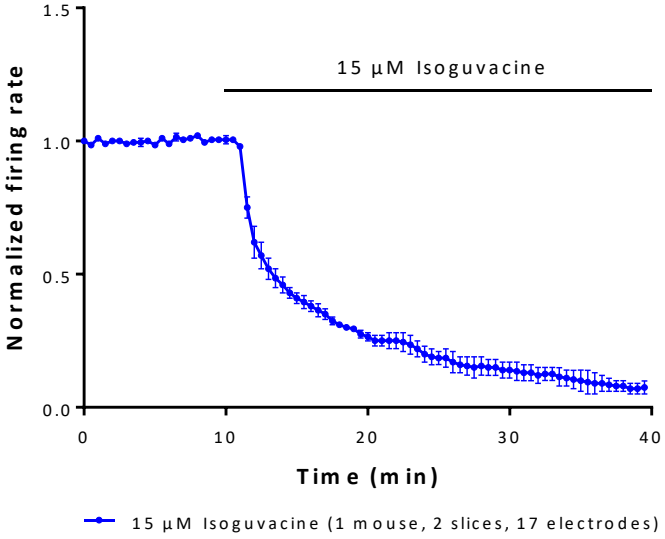
$GABA_A$

Bicuculline
Isoguvacine









- Bicuculline – a $GABA_A$ antagonist – , clearly modulates the PN firing rate. The effect differs depending on the electrodes and can be divided in 3 types:
  - ~50% shows first an increase of the firing rate which then rapidly decrease
  - ~35% shows an increase of the firing rate
  - ~15% shows a modification of their firing pattern switching from a continuous firing to a “bursting” firing.
- Isoguvacine – a $GABA_A$ agonist – rapidly and drastically decreases the PN firing rate. That effect is very consistent over all the recording electrodes.

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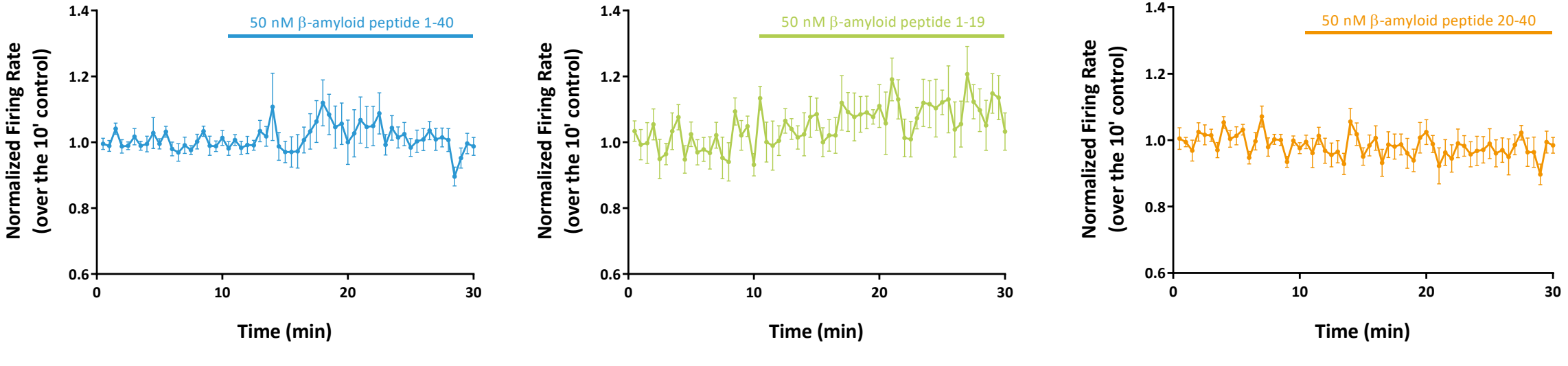
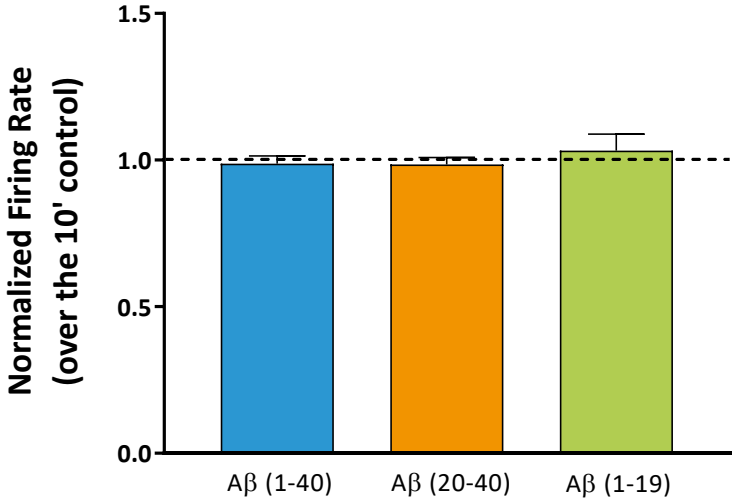
RESULTS

β-amyloid peptide

β-amyloid peptide





- The Purkinje neurons firing rate was not modulated by the β-amyloid peptides 1-40, 20-40, and 1-19.