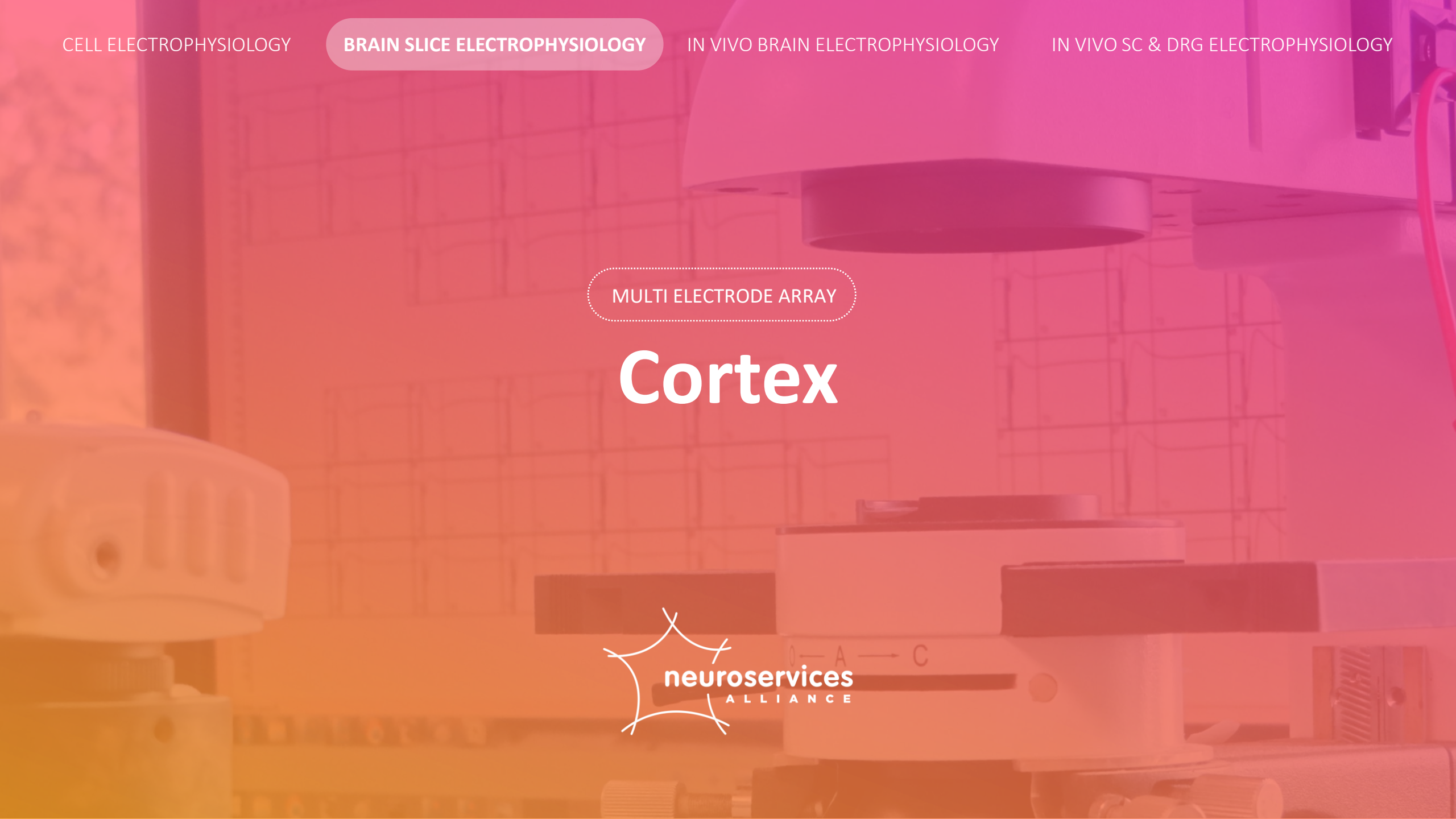

MULTI ELECTRODE ARRAY

Cortex

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MAIN SUMMARY

- ❑ Prefrontal cortex
- ❑ Enthorinal cortex
- ❑ Motor cortex
- ❑ Visual cortex

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PRE FRONTAL CORTEX

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SUMMARY – Prefrontal cortex

Main summary

Prefrontal cortex

- Materials & methods

Results

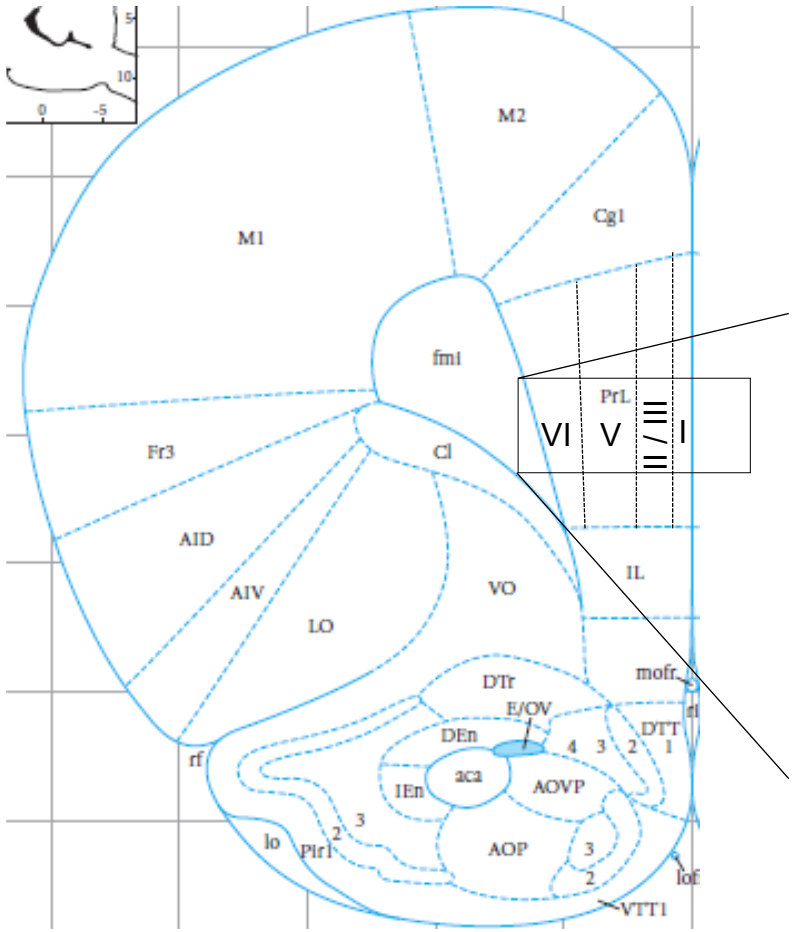
- GABA receptors – Picrotoxin
- Dopaminergic receptors – SKF38393
- AMPA/KA receptors – NBQX

Conclusion

MATERIALS & METHODS – Prefrontal cortex

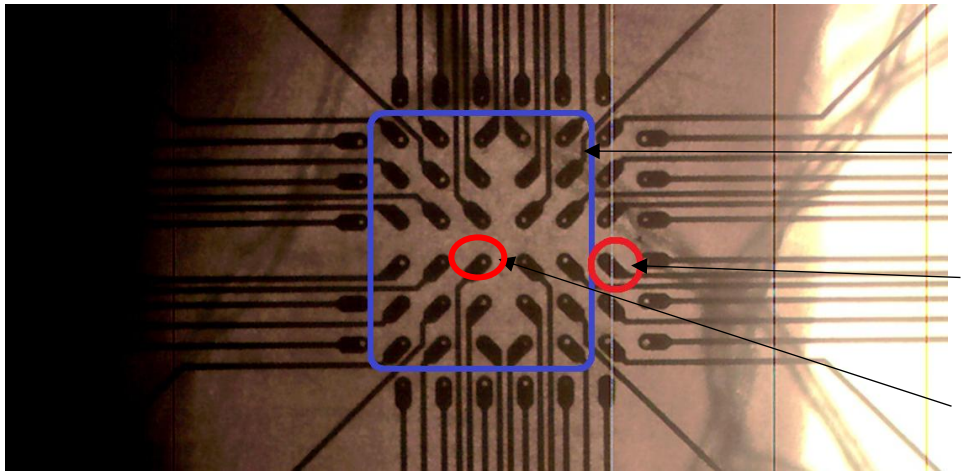
Main summary

Stimulation and recording area



Coronal rats prefrontal cortex slices

- Recording electrodes were placed to cover layer II III and V of the mPFC. Stimuli was delivered by an electrode located on the outer edge of layer V, close to the input fibers in layer II/III of the prefrontal cortex (rats) and directly winthin the layer V (mice). The stimulus consisted in a monopolar biphasic current pulse applied at 30 s intervals. Intensity was set at 40% of saturating intensity or 250 µA. fEPSP were recorded at both layer II/III and layer V.



Recorded area

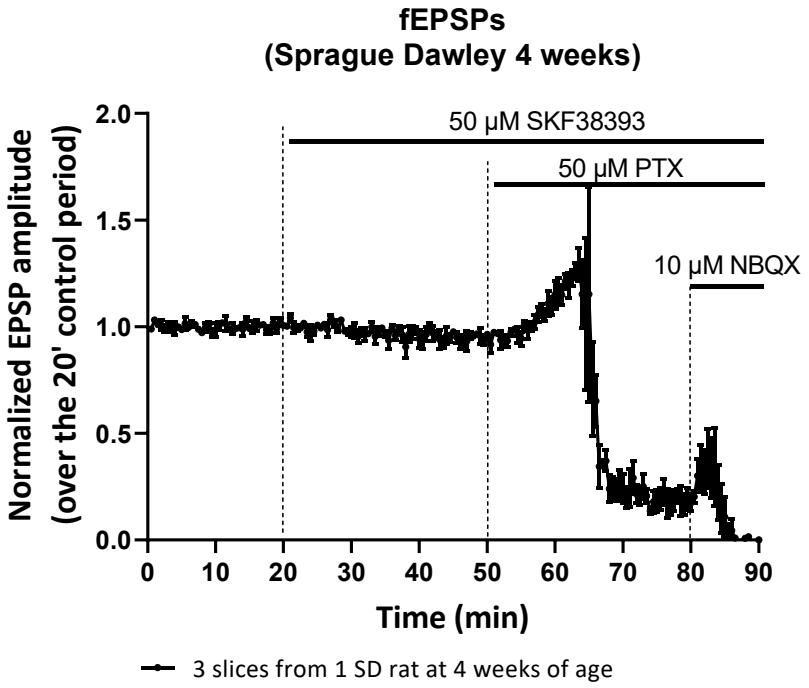
Stimulating electrode
(experiment with rats)

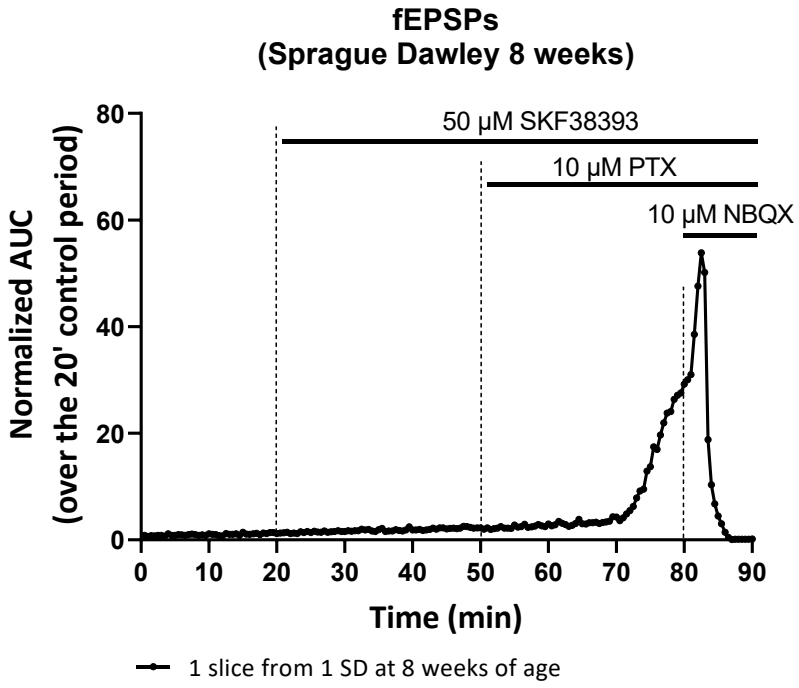
Stimulating electrode
(experiment with mice)

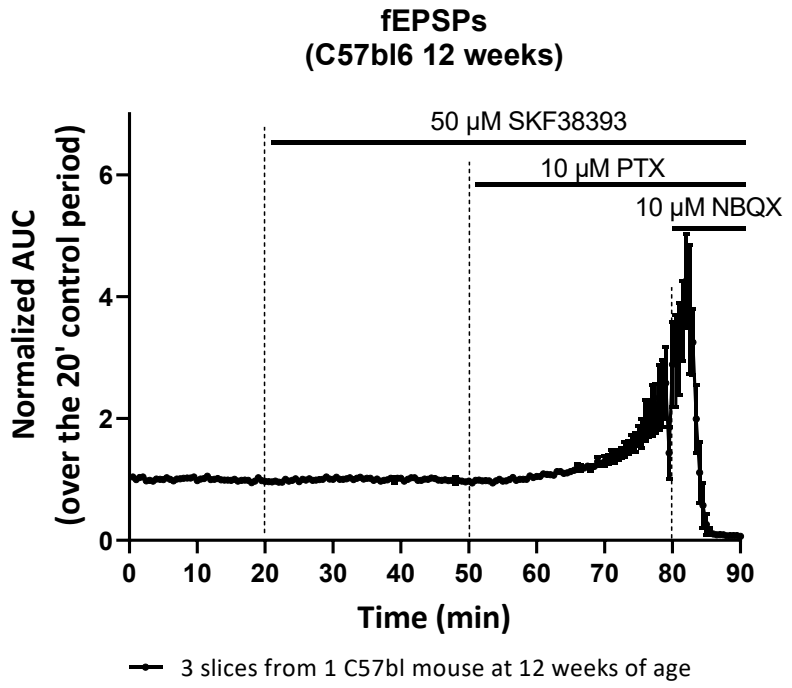
RESULTS – Prefrontal cortex

Main summary

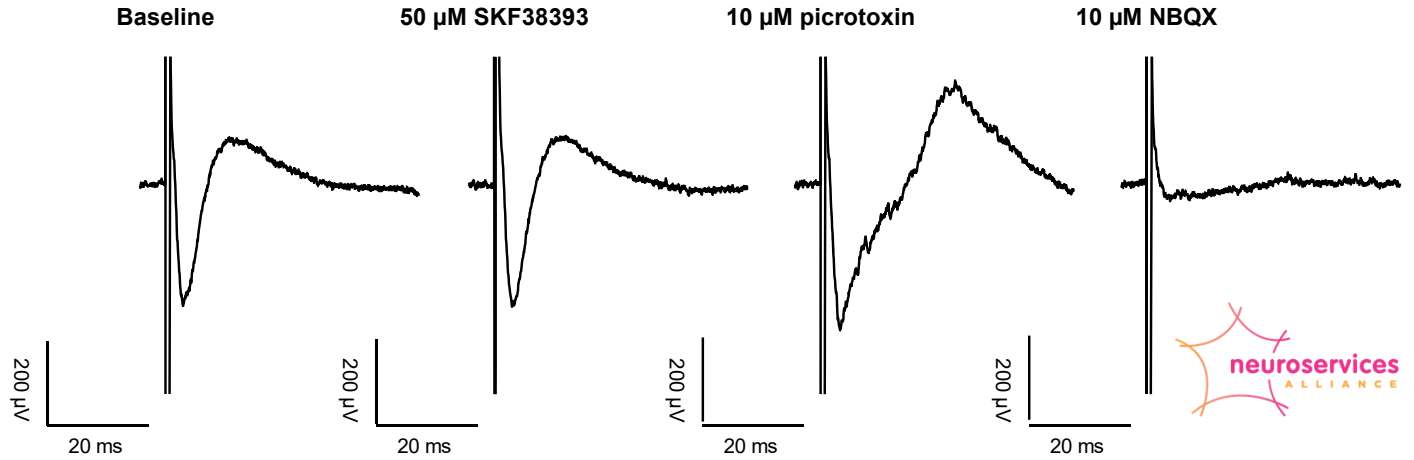
Evaluation of SKF38393 and Picrotoxin on Evoked responses in PrL







- 50 µM SKF38393 a selective D1/D5 receptor partial agonist did not modify fEPSPs recorded in the PrL of both rats and mice.
- 50 µM PTX abolished fEPSPs while 10 µM PTX modified the shape of fEPSPs turning them epileptic signals (increasing their area under curve). The steady state of 10 µM PTX effect was not reached after 30 minutes of exposure.
- 10 µM NBQX completely blocked fEPSPs confirming the glutamatergic nature of evoked-responses.



CONCLUSION – Prefrontal cortex

Main summary

- The fEPSPs can be recorded in both rats and mice when stimulating the layer II / III or directly the layer V.
- The D1 agonist (SKF SKF38393) did not modulate both the amplitude and the area under curve of fEPSPs recorded.
- Picrotoxin at 10 µM significantly increased the AUC of evoked-responses modified their shape, whereas they were abolished after 20 minutes of exposure to 50 µM picrotoxin.
- NBQX completely inhibited the fEPSPs responses confirming the glutamatergic nature of the transmission.

ENTHORINAL CORTEX

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SUMMARY – Entorhinal cortex

Main summary

Materials & Methods

- Epileptiform discharges – 4-AP induced ED ; 0 Mg 2+ / 7 mM K+ induced ED

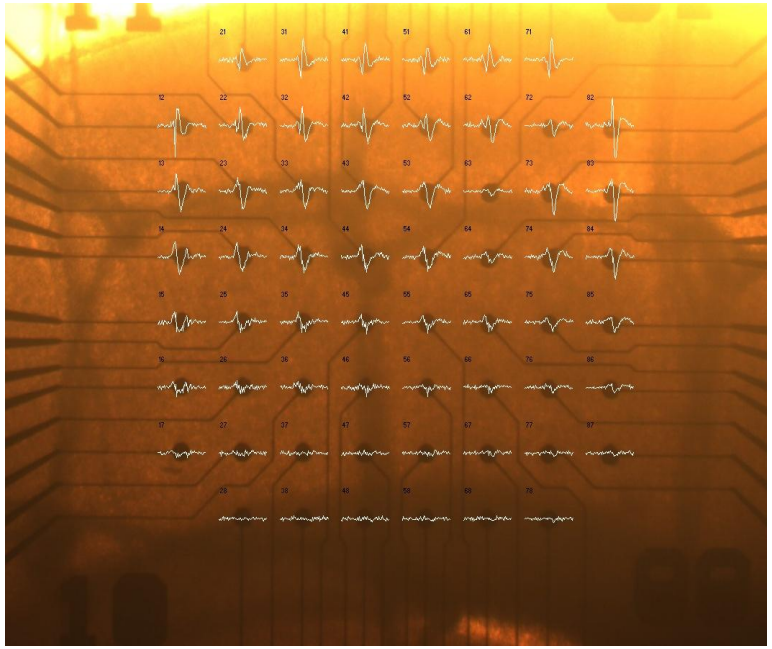
Results

- K^+ and Na^+ channels – Retigabine / Carbamazepine

MATERIALS & METHODS – Entorhinal cortex

ED in cortical slices

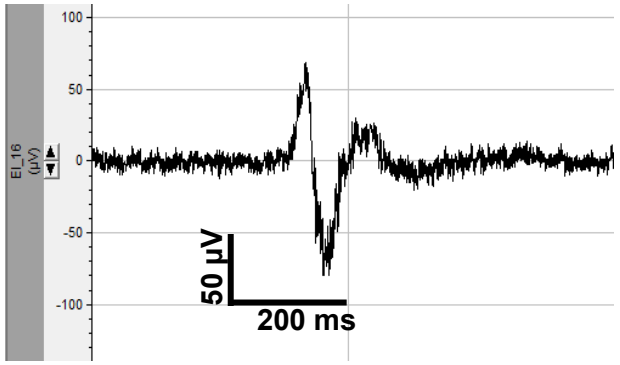
Main summary
Entorhinal cortex summary



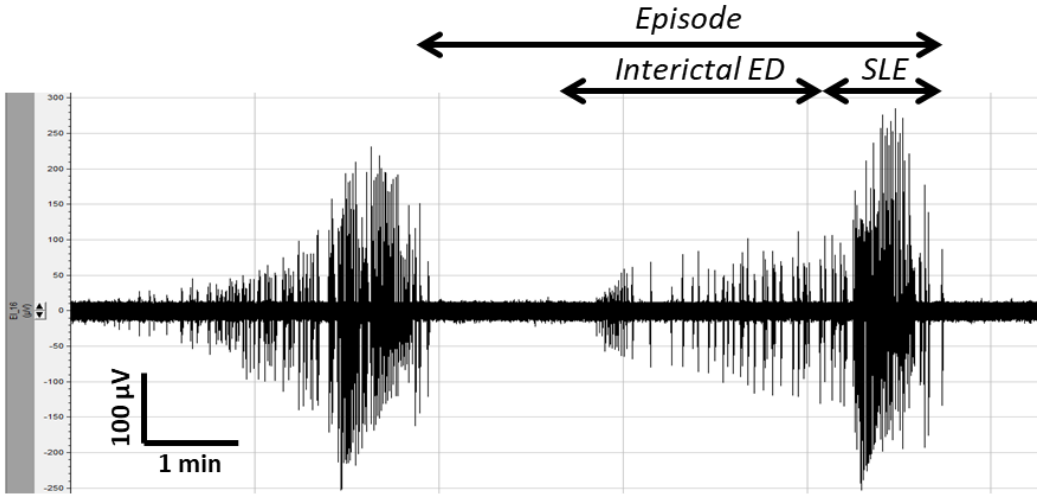
Example of EDs in a rat cortical slice in vitro.

- When cortical slices are exposed to a zero-magnesium ACSF, EDs occur synchronously over a large cortical area. The EDs frequency follows a pattern that is often different from the one recorded within the hippocampus.
- In the cortex, multiple episodes succeed, one episode consisting in silent period, interictal and ictal events (Seizure-Like Events = SLE)

Example of one interictal ED



Typical pattern of epileptiform events occurring in cortical slices in a zero-magnesium ACSF

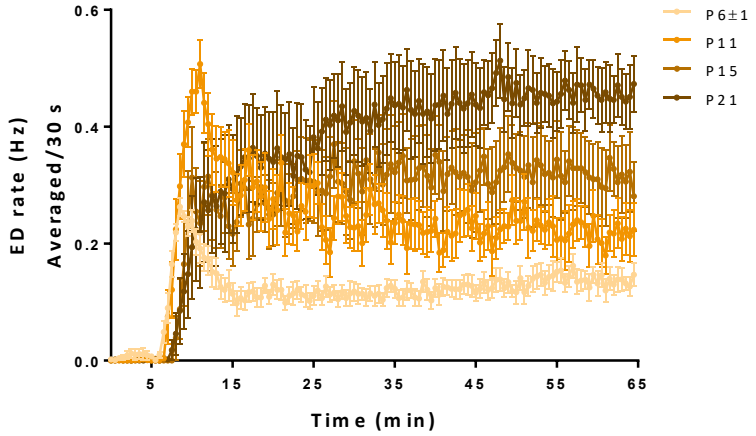


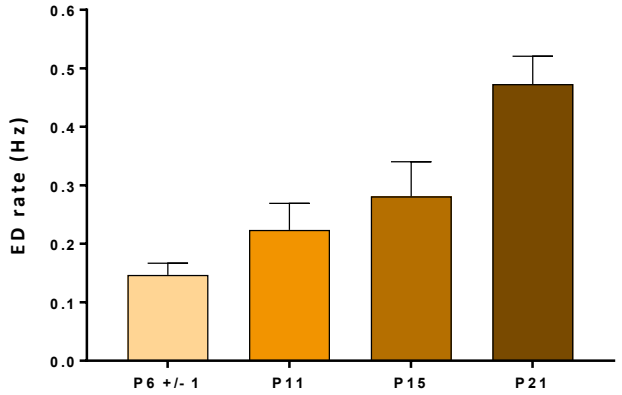
MATERIALS & METHODS – Entorhinal cortex

4-AP and zero-Mg^{2+}-induced-ED at different ages

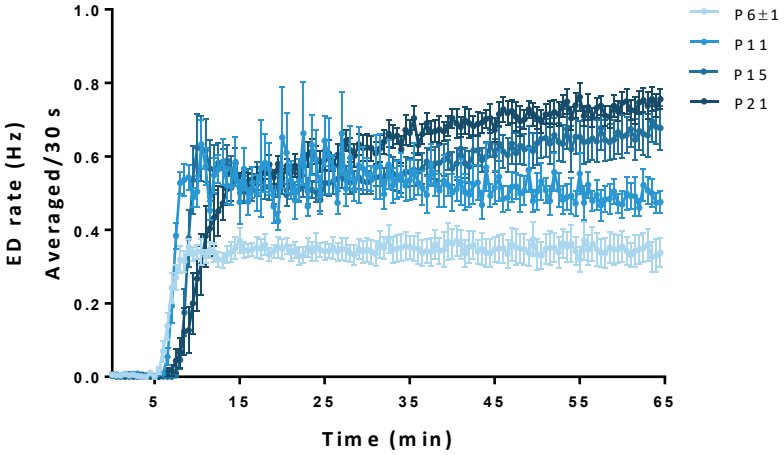
Main summary
Entorhinal cortex summary

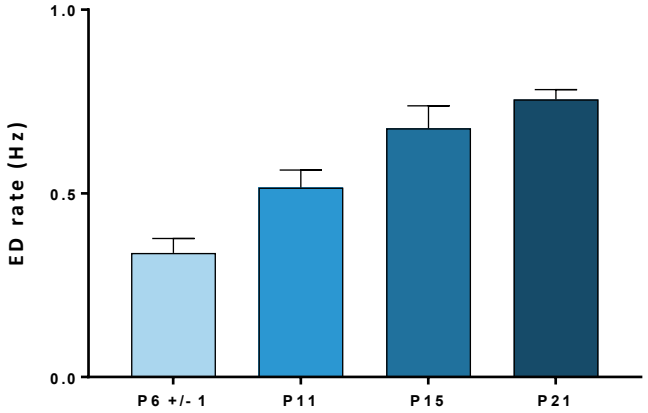
4-AP induced ED





0 Mg^{2+} / 7 mM K^{+} induced ED





- 0 Mg^{2+} aCSF and 50 µM 4-AP triggered ED in hippocampal slices from rats at all the tested ages. 50 µM 4-AP triggered a transient increase of the ED rate for P6 and P11 rats, suggesting that 4-AP concentration must be adapted according to the rat age.

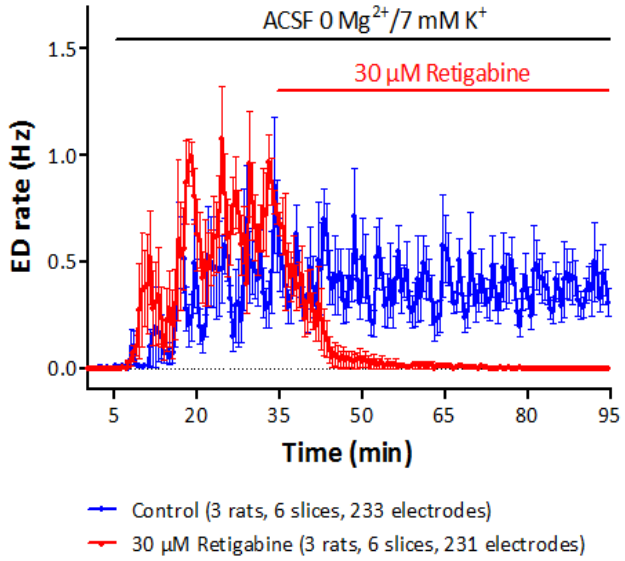
- At equivalent ages, the ED rate triggered by 0 Mg^{2+} aCSF was always higher than the ED rate triggered by 50 µM 4-AP.

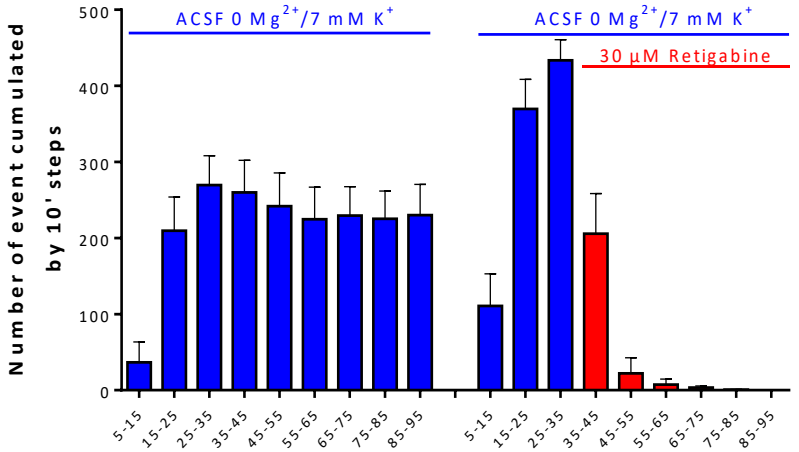
- For both 0 Mg^{2+} aCSF and 4-AP, the ED rate recorded at the end of experiment was positively correlated with the rat age.

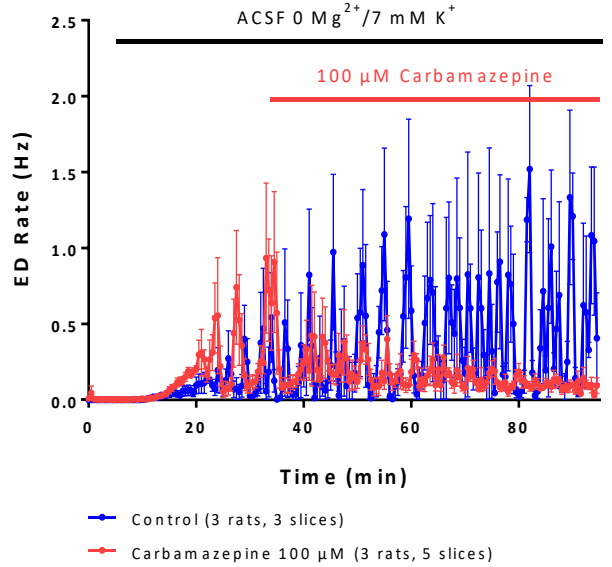
RESULTS – Entorhinal cortex

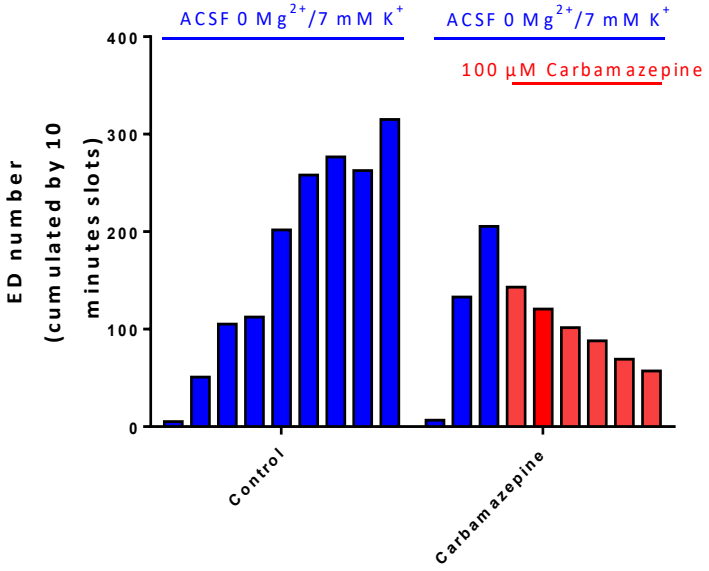
K⁺ and Na⁺ channels

Main summary
Entorhinal cortex summary









- The epileptiform activity is constituted of episodes of interictal followed by ictal events which alternate with silent period.

- 30 µM retigabine – a Kv7 channel activator - and 100 µM carbamazepine - a use-dependent Na^+ channel blocker - drastically decreased the number of zero-magnesium-induced ED in the cortex.

MOTOR CORTEX

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ALLIANCE

SUMMARY – Motor cortex

Main summary

Materials & Methods

- Motor cortex fEPSPs recording

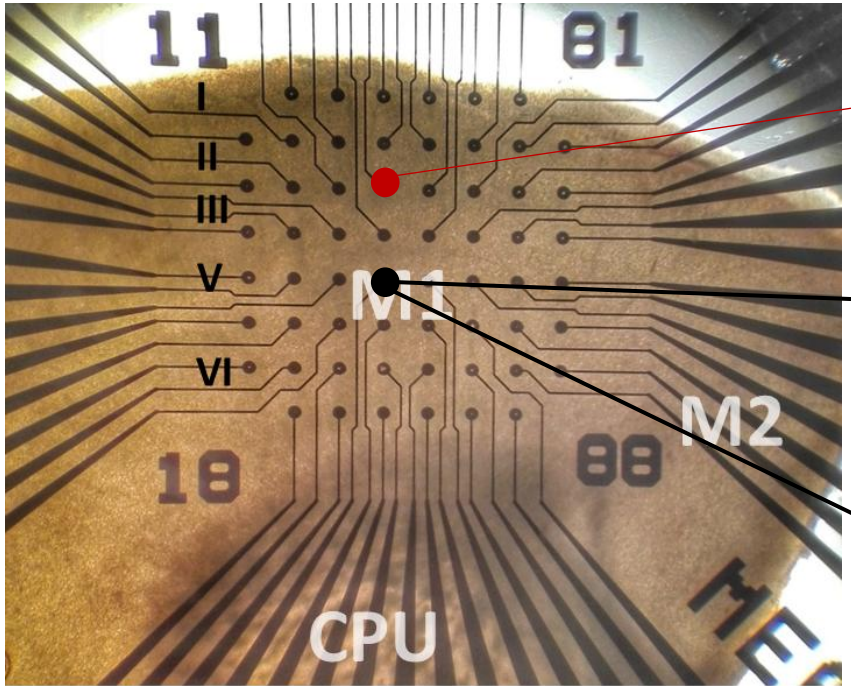
Results

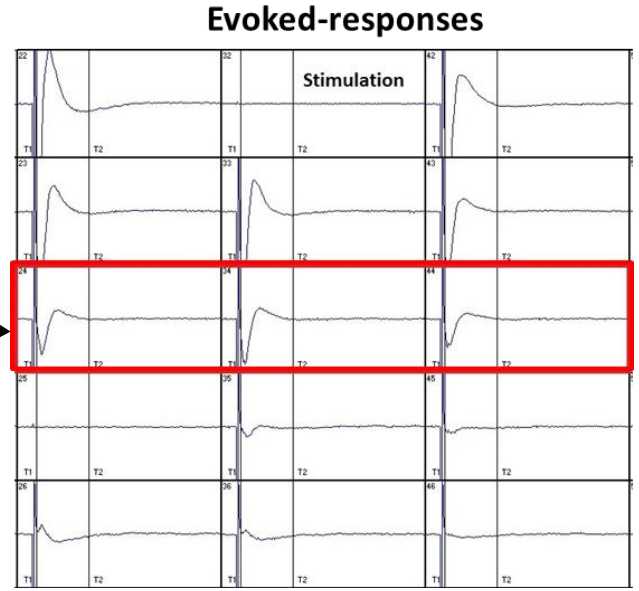
- GABA receptors – Gabazine / NNC-711 / Gaboxadol / Diazepam

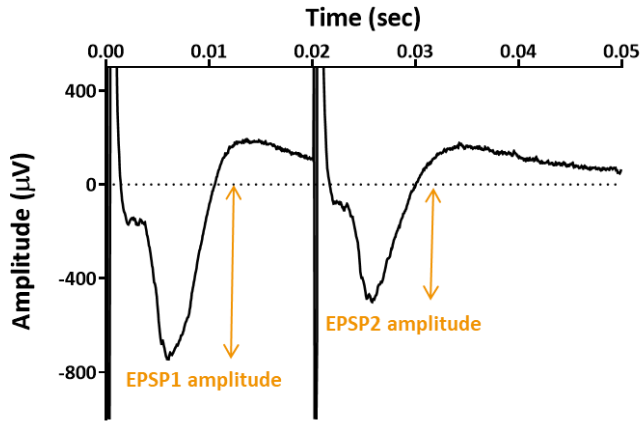
MATERIALS & METHODS – Motor cortex

Motor cortex fEPSPs recording

Main summary
Motor cortex summary







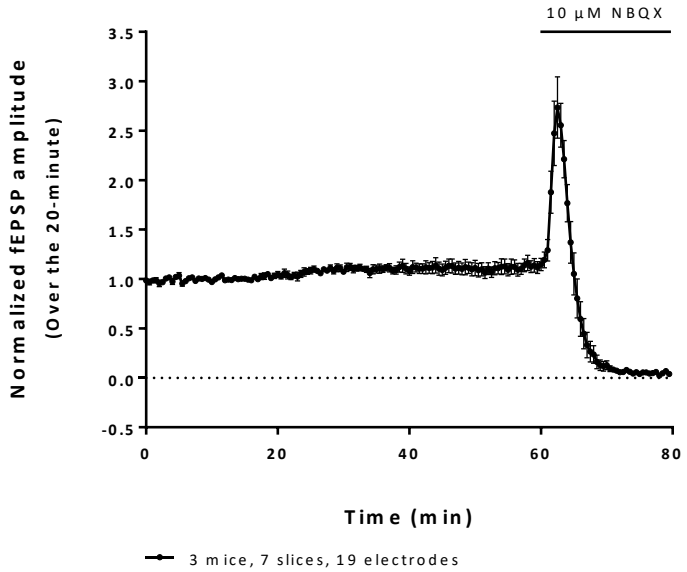
- Evoked-responses: One electrode was chosen to stimulate afferent fibers in the layer II-III. Evoked-responses were recorded in Layer the V.

- Paired-stimulation applied at 20 ms interval in layers II-III evoked paired-pulse inhibition (PPI) in the layer V of the motor cortex.

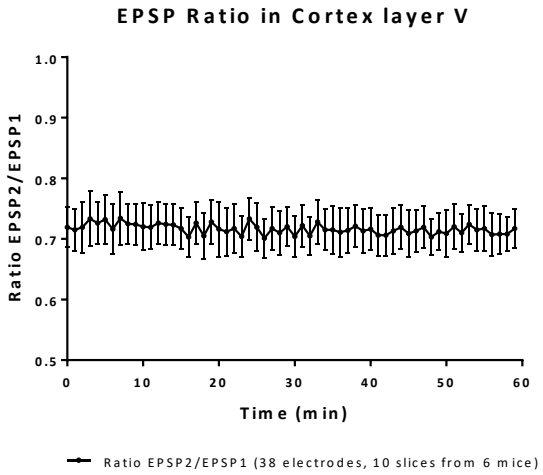
MATERIALS & METHODS – Motor cortex

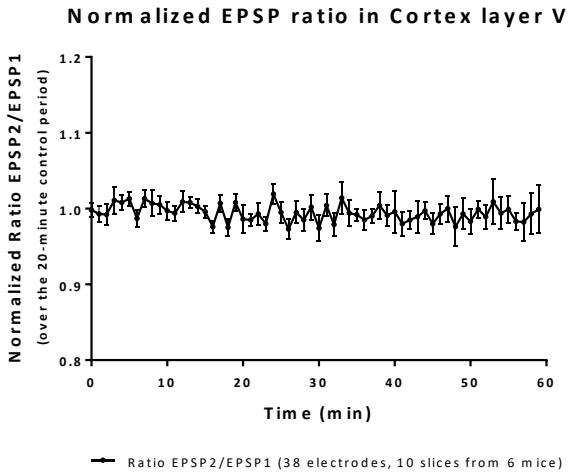
Motor cortex fEPSPs recording

Main summary
Motor cortex summary



- Stimulations applied in layers II-III evoked responses in the layer V of the motor cortex.
- Evoked-responses remained quite stable over the 60-minute of experiment (normalized EPSP amplitude was 1.12 ± 0.06 before NQBX exposure).
- 10 µM NBQX exposure completely blocked evoked-responses, and confirmed the glutamatergic nature of the recorded fEPSPs.
- On average, 3 electrodes were recorded per slice.





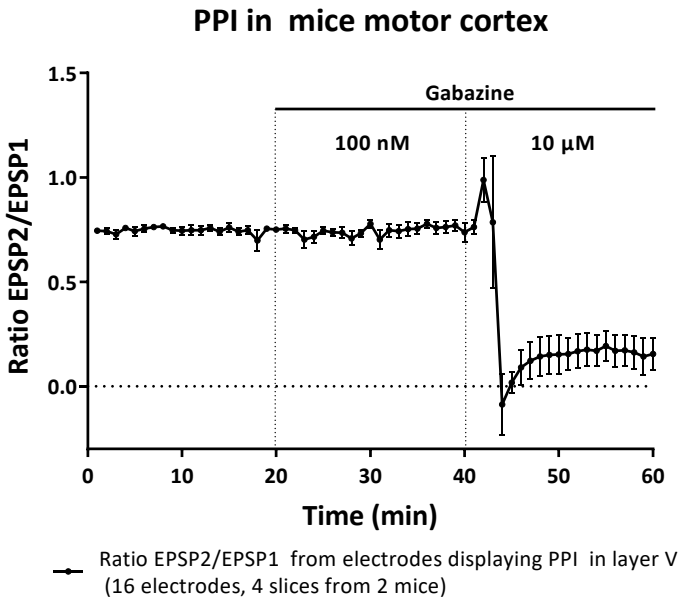
- The mean EPSP ratio (EPSP2/EPSP1) was lower than 1 (in average, EPSPs ratio was 0.72 ± 0.03 over the 60 minutes of recording), confirming that PPI could be recorded in the cortical layer V (EPSP2 was of lower amplitude than EPSP1).

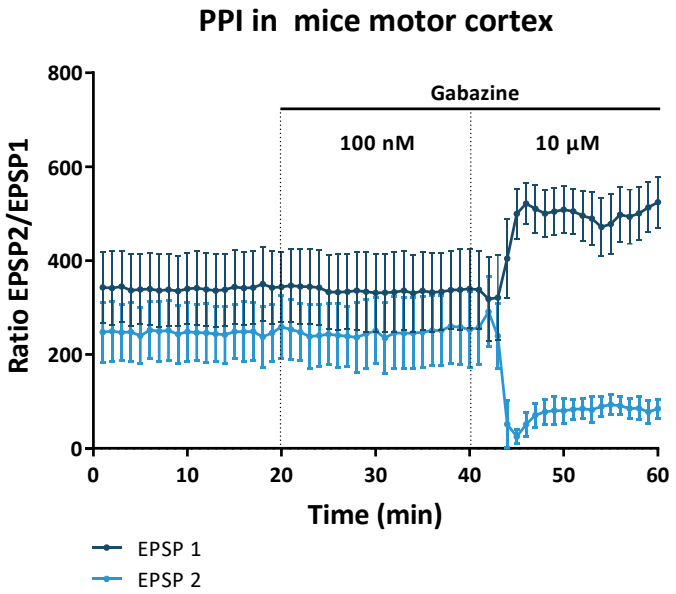
- The EPSP ratio remained stable over the 60 minutes of experiment (normalized ratio was 1.00 ± 0.03 at the end of the experiment versus 1.00± 0.01 at the beginning).

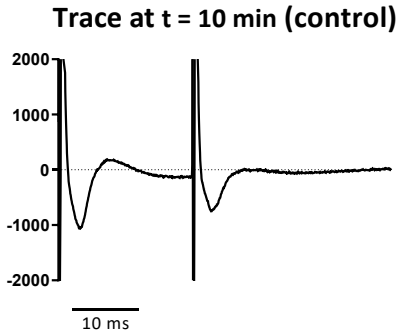
RESULTS – Motor cortex

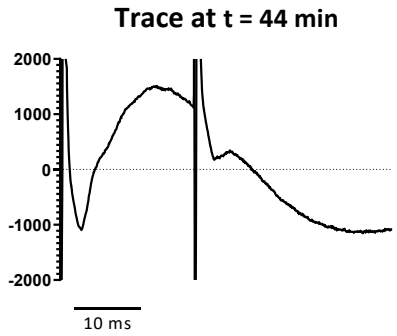
GABA receptors

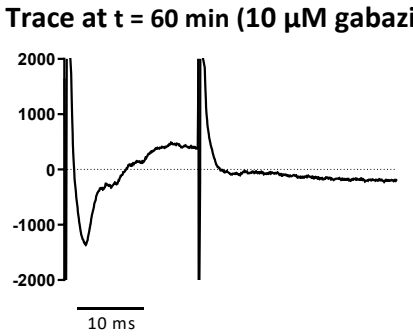
Main summary
Motor cortex summary









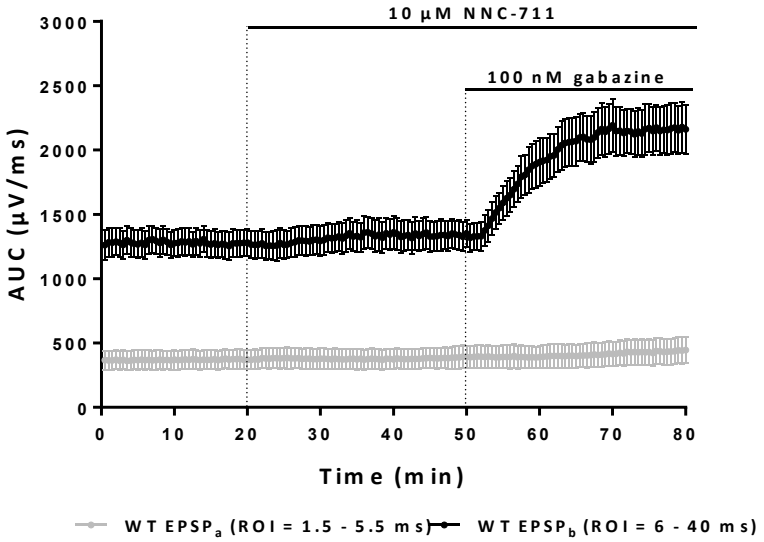


- Over the control period, the EPSP2 was lower than EPSP1 (see trace control on previous slide), thereby confirming a paired-pulse inhibition.
- 100 nM gabazine did not modify either EPSP1 or EPSP2 amplitude. Then, tonic inhibition likely does not modulate PPI in the layer V of the motor cortex.
- 10 µM gabazine increased EPSP1 amplitude, consistently with the expected suppression of gabaregic inibition on pyramidal neurons. Surprisingly, the response to the second pulse was fully inhibited after a 20-minute exposure to the GABA-A antagonist 10 µM gabazine, whereas an increase of EPSP2 amplitude was expected (see representative traces).

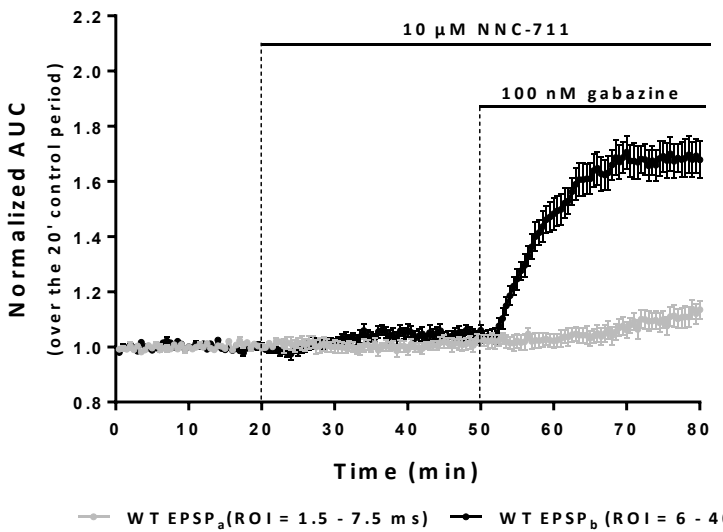
RESULTS – Motor cortex

GABA receptors

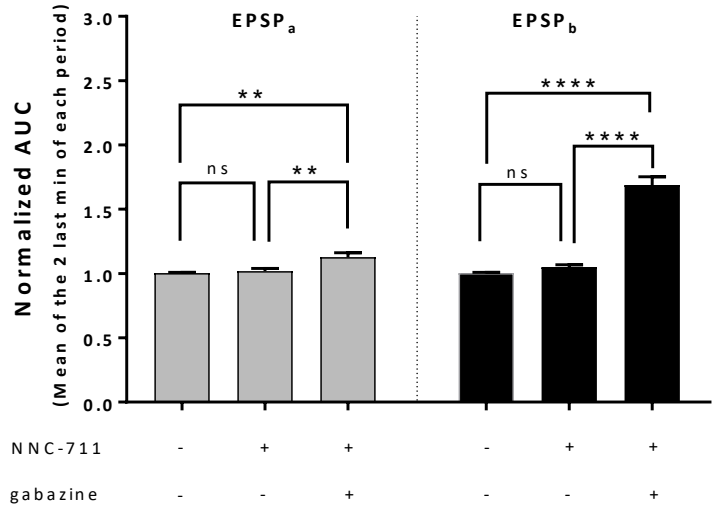
Main summary
Motor cortex summary

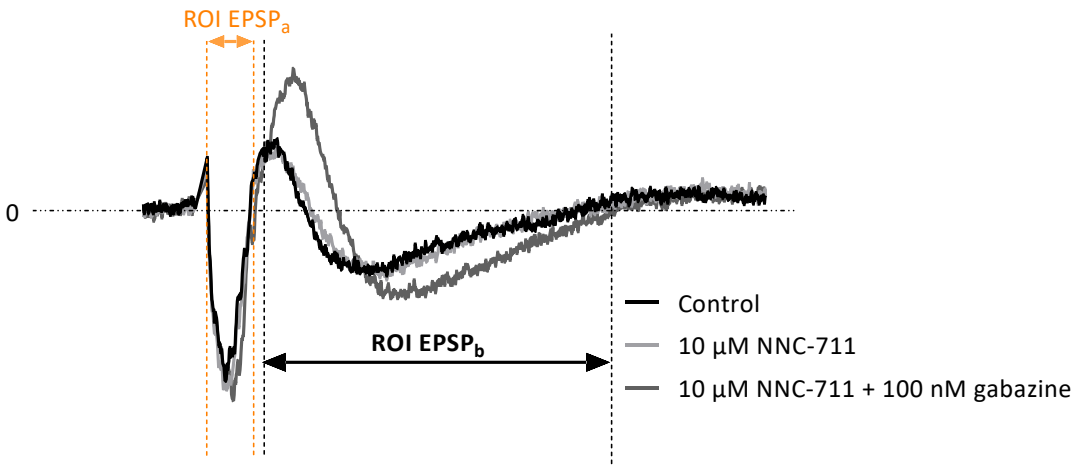


56 electrodes, 10 slices from 5 WT mice



56 electrodes, 10 slices from 5 WT mice



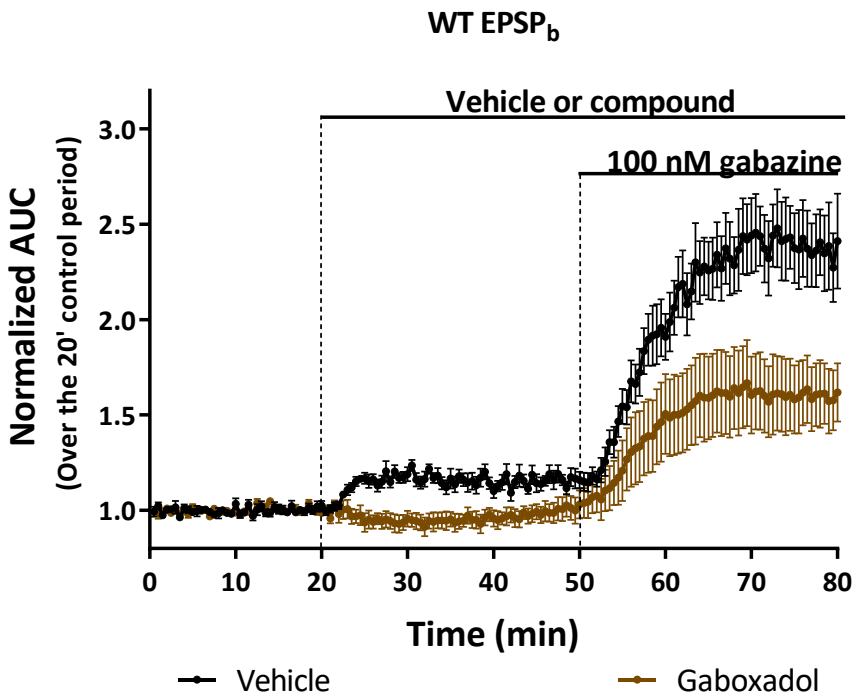


- As presented on the left panel, the area under the curve (AUC) was slightly lower than 500 µV/ms for EPSPa and higher than 1000 µV/ms for EPSPb in control conditions.
- The GABA uptake inhibitor NNC-711 did not change either EPSPa or EPSPb AUC when compared to control period (middle and right panel).
- In WT, 100 nM gabazine (concentration previously determined as preferentially blocking the tonic inhibition) largely increased AUC of EPSPb (by about 70%) and, to a lesser extend, the AUC of EPSPa (by about 15%).

RESULTS – Motor cortex

GABA receptors

Main summary
Motor cortex summary

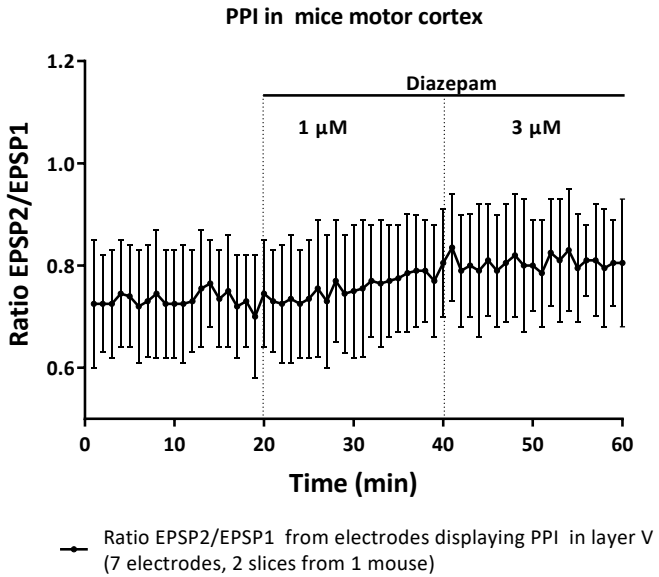


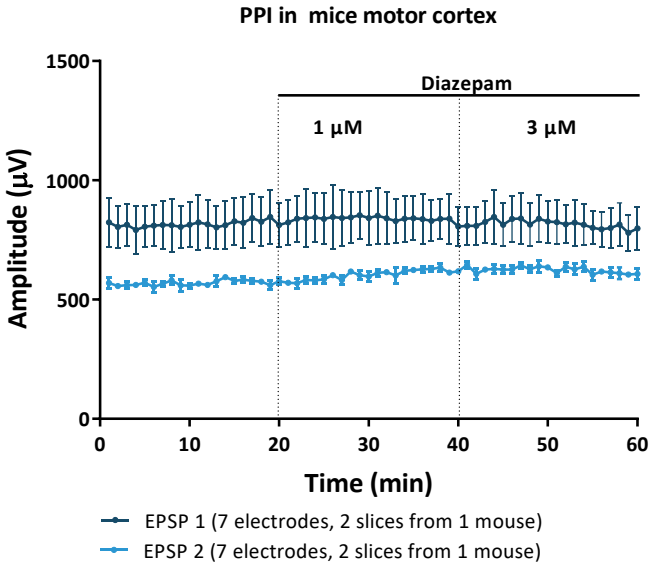
- Gaboxadol - potent agonist of GABA receptors that contain alpha4, alpha6, and delta subunits- significantly reduced the effect of 100 nM gabazine when compared with vehicle.

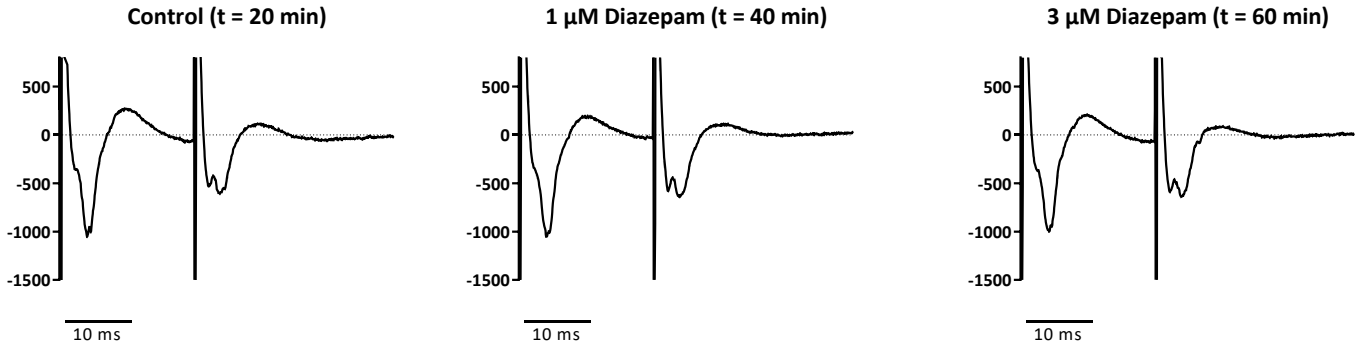
RESULTS – Motor cortex

GABA receptors

Main summary
Motor cortex summary







- Although the paired-pulse stimulation (with a 20 ms interval) evoked a lower second EPSP in the mice motor cortex, neither 1 µM nor 3 µM diazepam were able to further enhance the PPI.

Visual cortex

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ALLIANCE

SUMMARY – Visual cortex

Main summary

Visual cortex

- Spontaneous & evoked responses
- Epileptiform discharges

MATERIALS & METHODS – Visual cortex

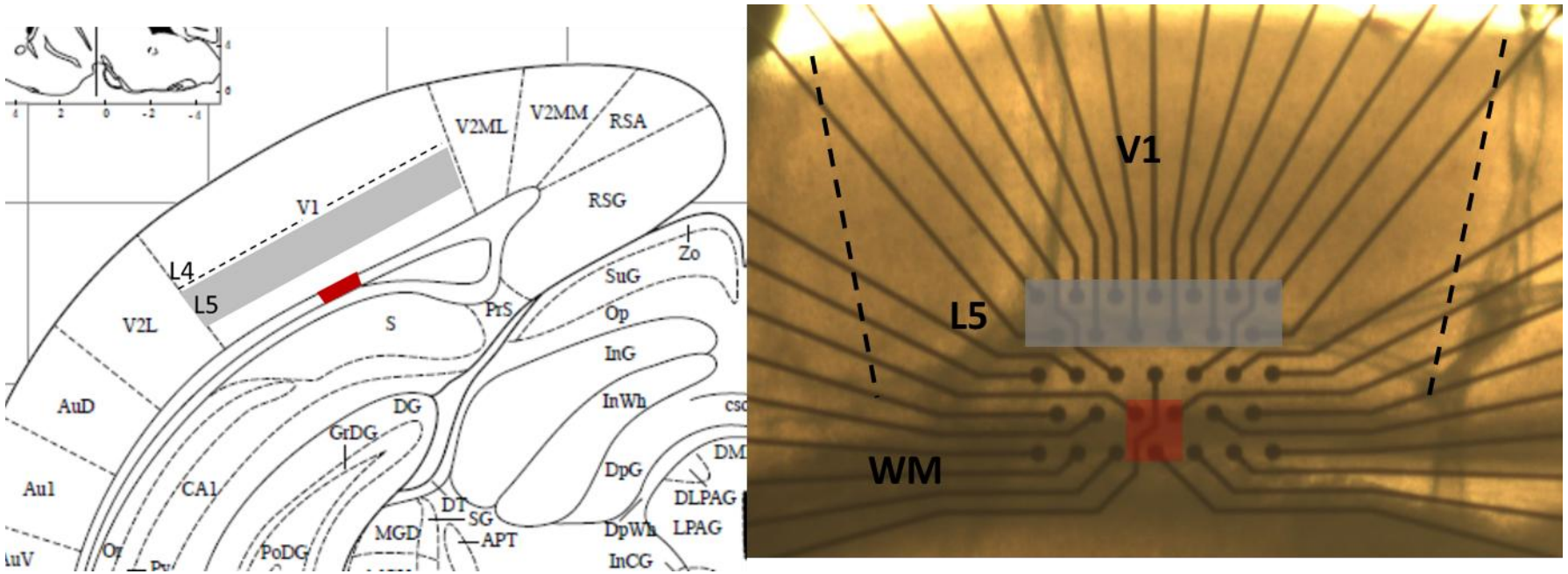
Spontaneous and evoked responses

Main summary
Visual cortex summary

Preparation of acute mice hippocampal slices

The experiments were conducted on 3-week old wild-type and Fmr1 KO mice. The method for preparing the acute slices is the one provided by Biogen and described in the commercial proposal.

Example of slice positioning



On the left, a map of the location of the visual cortex (Paxinos & Franklin, figure 59); on the right, a representative picture taken at Neuroservice with the positioning of electrodes in regards to the slice. In grey: the area corresponding to the layer 5 of the cortex, in red: electrodes used to stimulate in the white matter. Note that 13 electrodes per slice were systematically placed in the layer 5 and selected for the recording, in the aim to have the same number of electrodes recorded for both genotypes and avoid any experimental bias.

MATERIALS & METHODS – Visual cortex

Spontaneous and evoked responses

Main summary
Visual cortex summary

Analysis

For the spontaneous activity all the electrodes recorded (13 per slices) were selected for the analysis. For the evoked activity, electrodes must display on average a firing rate higher than 0.33 Hz during the evoked firing period (meaning on average 1 spike detected in the 0.3 - 3.3 s time window) and display an activity significantly higher than the one over 3.3- 29.3 s time window (see figure 1) to be validated.

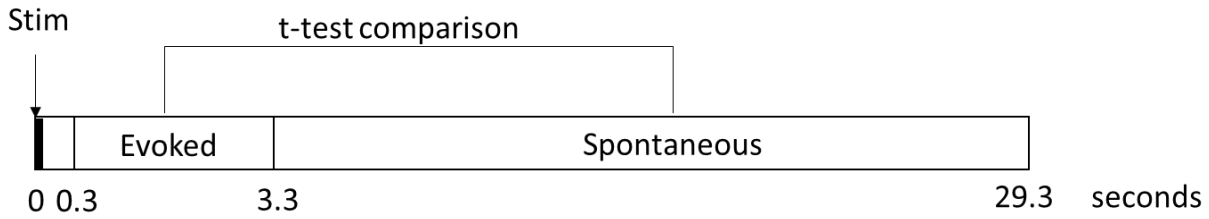


Figure 1: schematic of t-test comparison used for electrodes selection

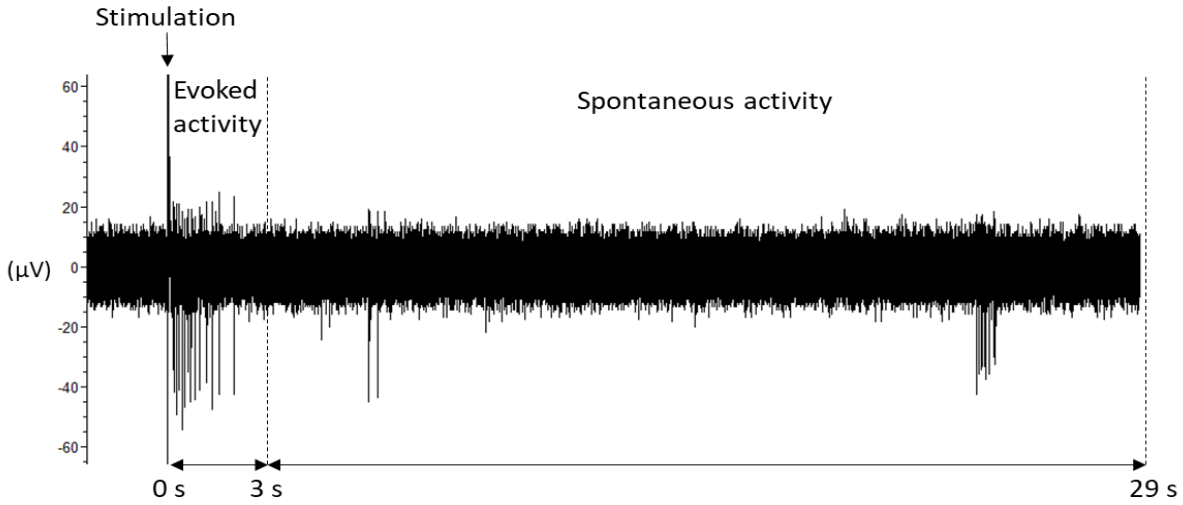
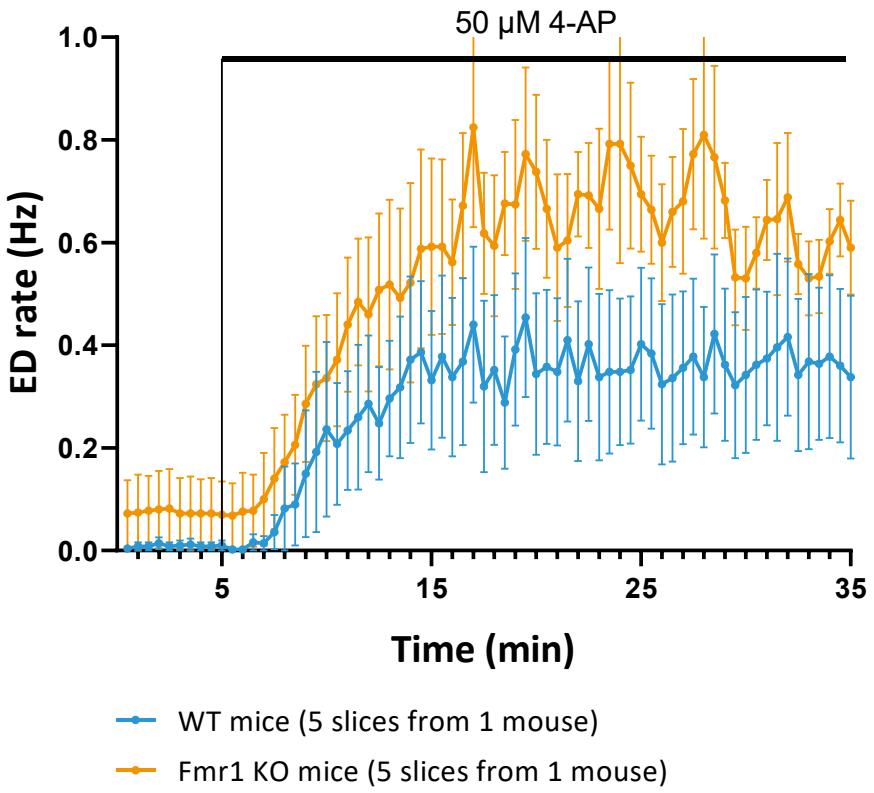


Figure 2: example of an electrode displaying evoked activity when stimulated in layer 5 of the visual cortex. The dotted lines delimit the periods considered for the evoked and spontaneous activity.

MATERIALS & METHODS – Visual cortex

Epileptiform discharges

Main summary
Visual cortex summary



Documentation of Fmr1 KO mice hyper-excitability using a model of 4-AP-induced epileptiform discharges in the hippocampus.

After 5 minutes in control aCSF, ED were triggered by the exposure of 50 µM 4-AP (a K^+ channel blocker) during 30 minutes.
The left graph shows the rate of ED as a function of time. The ED rate recorded from Fmr1 KO mouse slices (at end point, 0.59 ± 0.09 Hz, n=5) appeared to be higher than the rate recorded from WT mice (at end point, 0.34 ± 0.15 Hz, n=5).
The amplitude of ED as a function of time which seems to be comparable between both genotypes (data not shown).