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NEUROSERVICE DATA
Pro – Anti convulsive

MULTI ELECTRODE ARRAY

SUMMARY

Pro – Anti convulsive models

- Epileptiform discharges
- Population spikes

Results Epileptiform discharges

- Anti epileptic drugs (AEDs) - **Phenytoin, Midazolam, Perampanel, Lamotrigine, Topiramate, Tiagabine**, **Carbamazepine, Retigabine, Phenobarbital**
- Pro-convulsive compounds - **Bicuculline, PTX**, **PTZ, Strychnine**, **Caffeine**, **Theophylline, Aminophylline, Eserine, Pilocarpine**
- Neutral compounds - **Acetaminophen, Ascorbate, Fluoxetine**

Results Population spikes

- PTZ
- Somatostatine, Bicuculline

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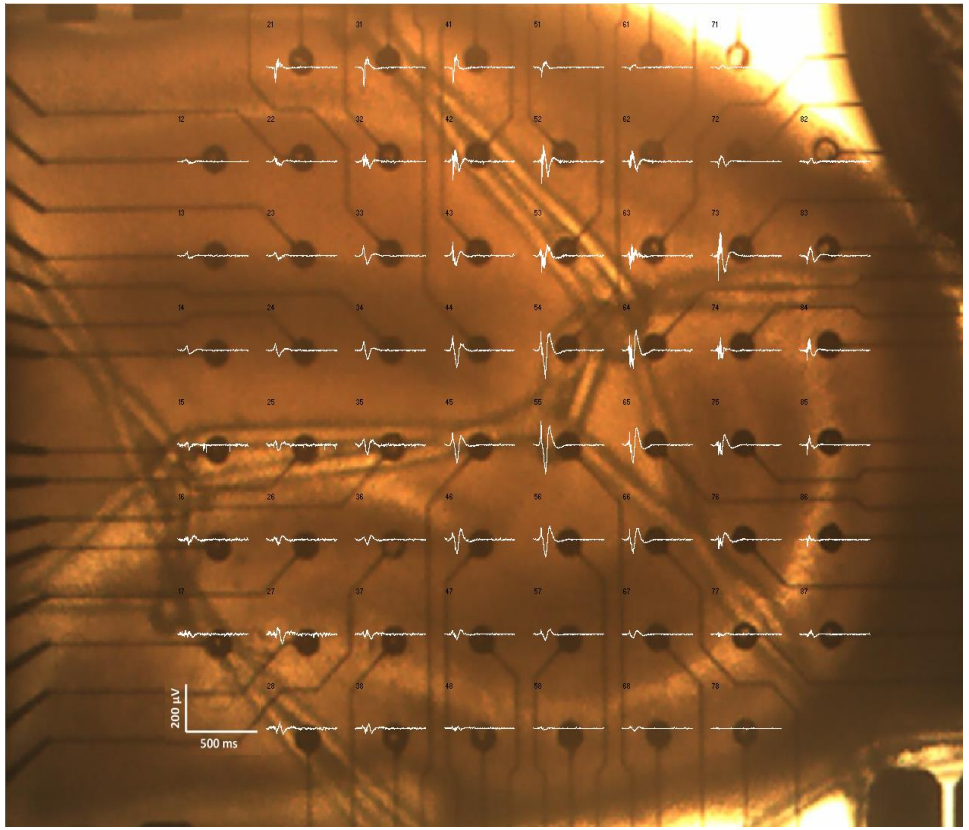
PRO – ANTI CONVULSIVE MODELS

Epileptiform discharges



Example of EDs in a rat hippocampal slice in vitro.

- ED correspond to a massive and synchronous discharge of a group of neurons over a large brain area, in the absence of any electrical stimulation (see nearby an example of 4-AP-induced ED in a hippocampal slice in vitro).
- ED are observed in the EEG of 90% of patients suffering of epilepsy, whereas they are observed in less than 5% of in the EEG of non-epileptic patients.
- Comparable ED can be observed in hippocampal slices exposed to certain pro-convulsive compounds.
- ED can be reduced or suppressed by anti-epileptic drugs (AED) in vivo as well as in vitro

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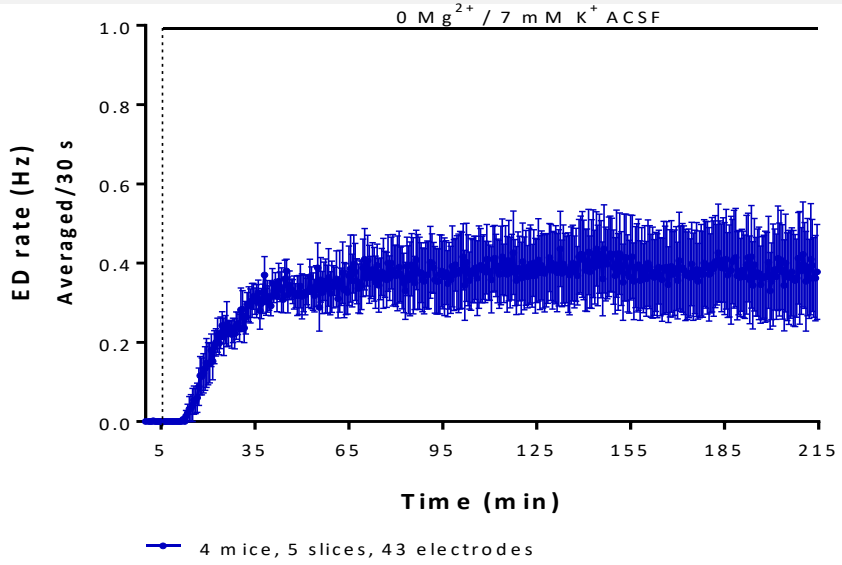
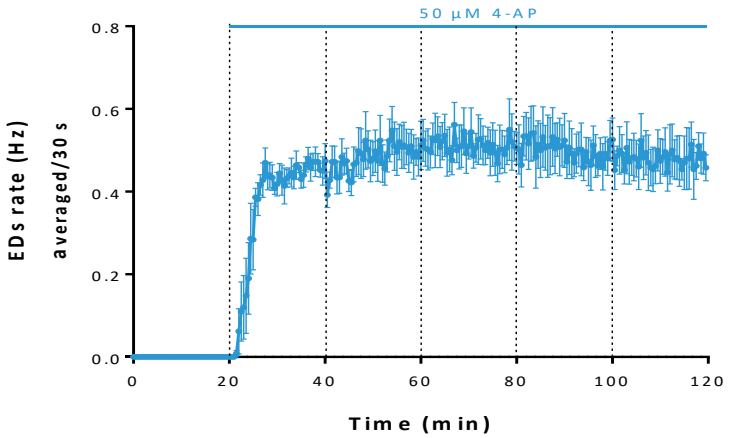
PRO – ANTI CONVULSIVE MODELS

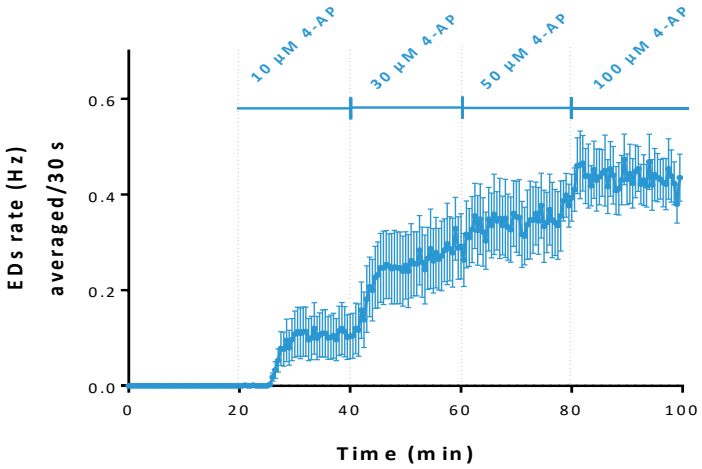
Epileptiform discharges

4-AP as well as 0 Mg^{2+} / 7 mM K^{+} aCSF induced epileptiform discharges

- 4-Aminopyridine (4-AP) is widely used in Epilepsy models. 4-AP is a non-selective voltage-dependent K+-channel blocker, which blocks the intrinsic neuronal potassium currents IA and ID.
- 4-Aminopyridine (4-AP) elicits epileptiform activity, in vitro, in vivo and clinically.
- 4-AP enhances both excitatory and inhibitory synaptic transmission. The presence of inhibition confers some specific characteristics on the synchronization of the neuronal population after 4-AP exposure.
- Low magnesium concentration coupled with High potassium concentration induced epileptiform discharges.







- The rate of ED triggered by a zero-magnesium ACSF remains quite steady over a ~3-hour period, around 0.4 Hz.
- The rate of ED triggered by 50 µM 4-AP remains quite steady over a 120 min period, around 0.4/0.5 Hz.
- 10 µM 4-AP rapidly triggers EDs which stabilizes around 0.1 Hz. Then, application of increasing concentration of 4-AP shows a dose-response effect.

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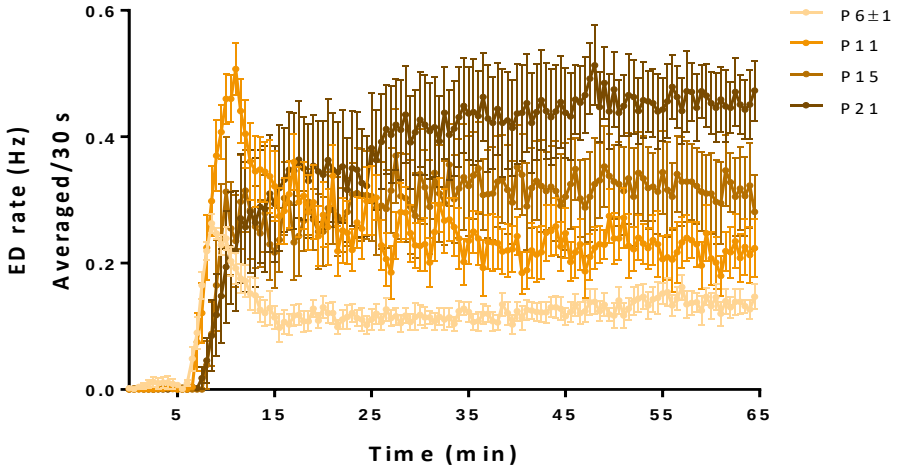
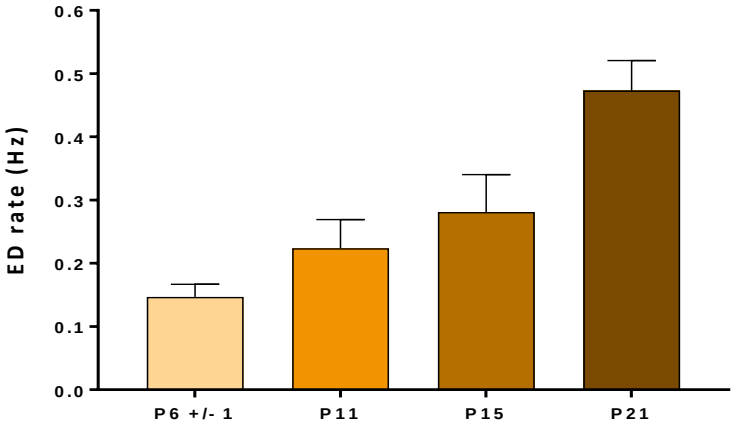
PRO – ANTI CONVULSIVE MODELS

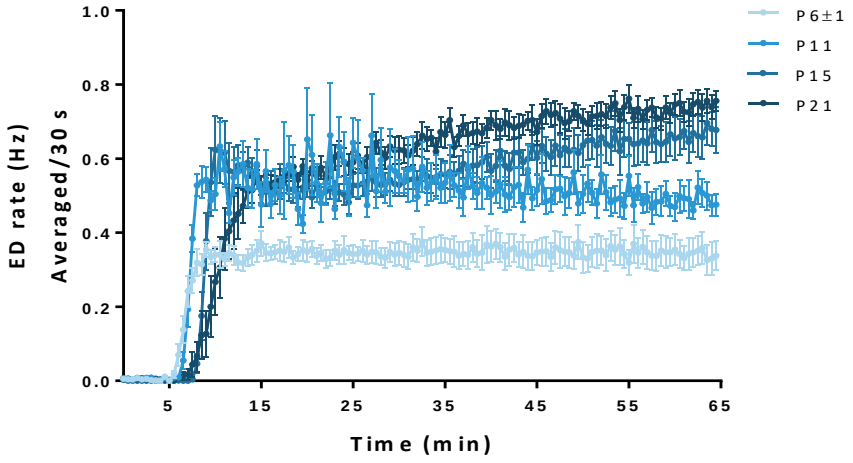
Epileptiform discharges

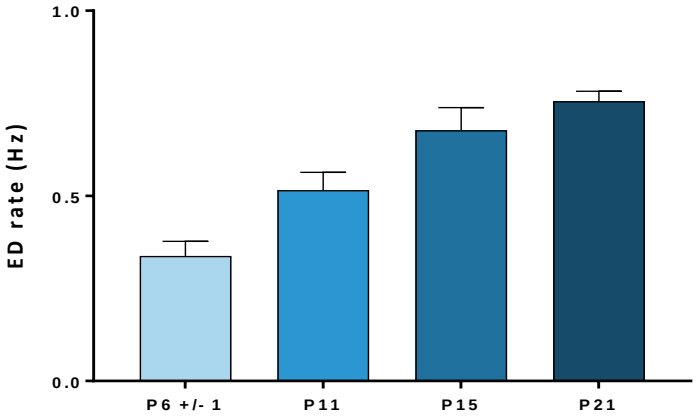
4-AP induced ED





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





- 0 Mg2+ 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 Mg2+ aCSF was always higher than the ED rate triggered by 50 µM 4-AP.

- For both 0 Mg2+ aCSF and 4-AP, the ED rate recorded at the end of experiment was positively correlated with the rat age.

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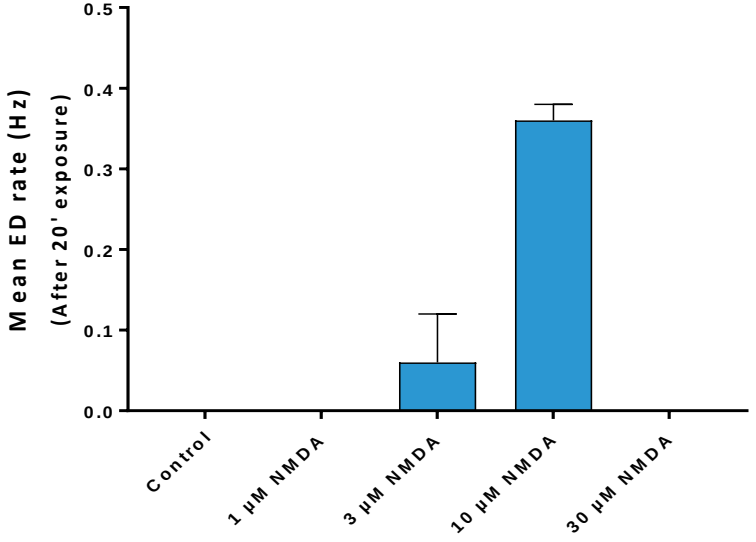
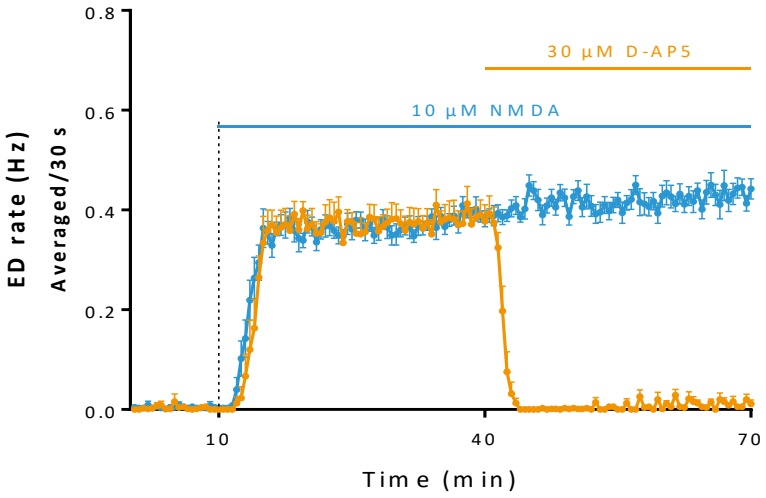
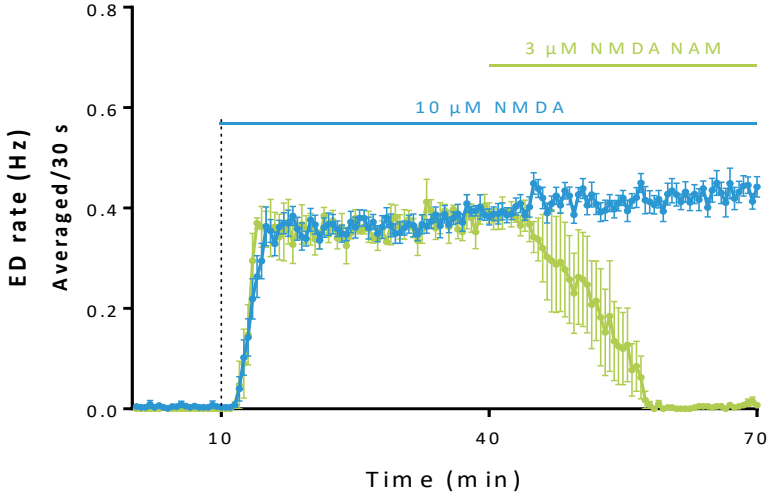
EPILEPTIFORM DISCHARGES

Epileptiform discharges

NMDA overactivation induced epileptiform discharges







- 10 µM NMDA (79 electrodes, 9 slices, 2 rats)
- 10 µM NMDA + 30 µM D-AP5 (59 electrodes, 7 slices, 2 rats)

- 10 µM NMDA (79 electrodes, 9 slices, 2 rats)
- 10 µM NMDA + 3 µM NMDA NAM (36 electrodes, 4 slices, 1 rat)

- NMDA triggers epileptiform discharges (ED) in rat hippocampal slices from 3 to 10 µM. The ED stabilized around 0.4 Hz after 10 minutes and remained steady. D-AP5 – NMDA channel blocker – and a NAM NMDA abolished the NMDA-induced ED

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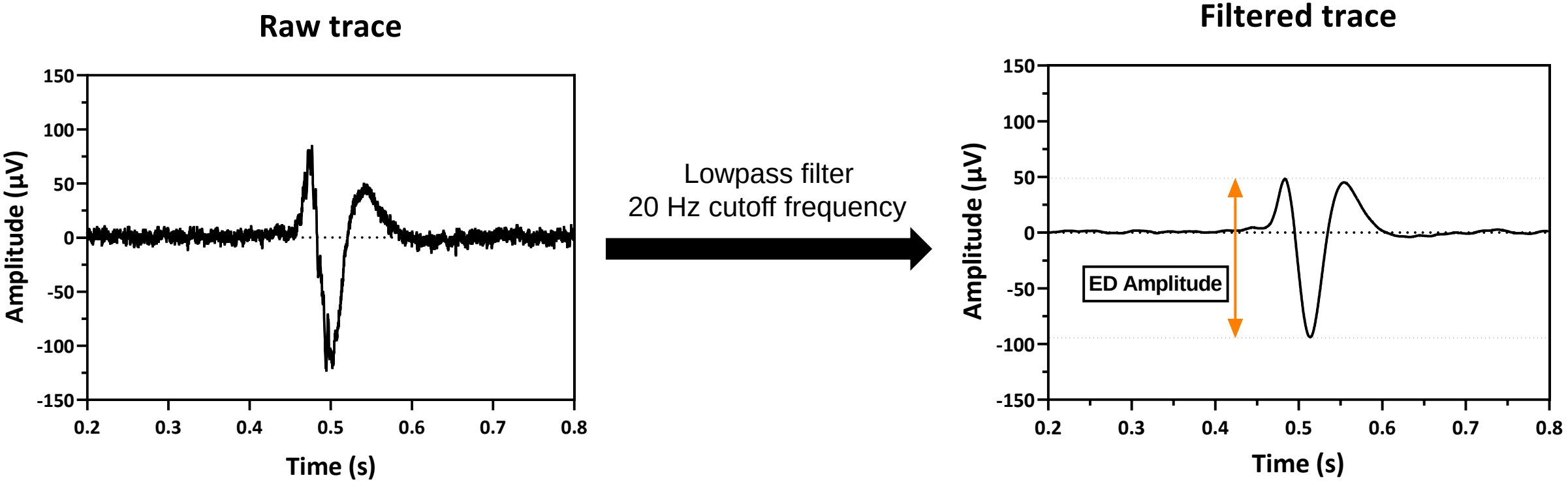
EPILEPTIFORM DISCHARGES

ANALYSIS



Validation criteria

- Epileptiform Discharges (EDs) have to be higher than 15 µV to be counted.

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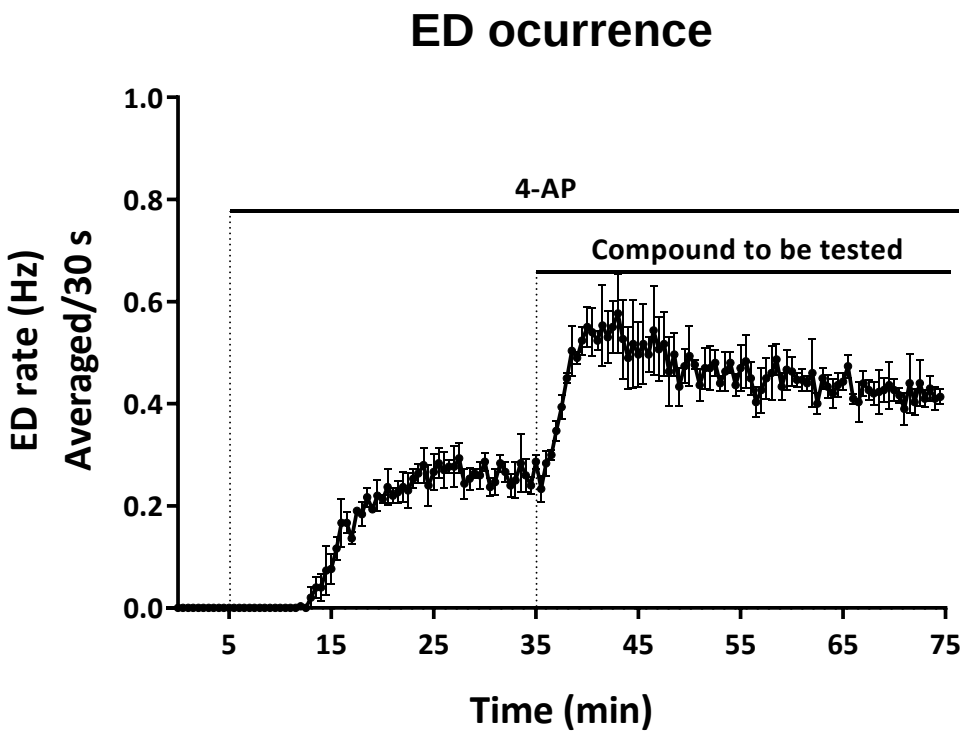
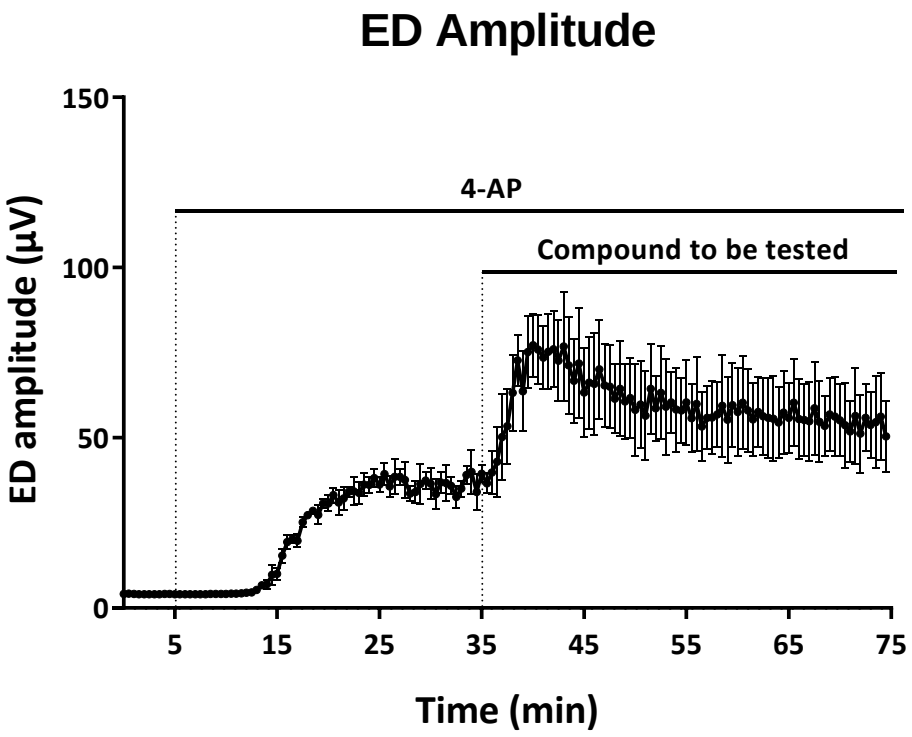
EPILEPTIFORM DISCHARGES

Epileptiform discharges

READOUT





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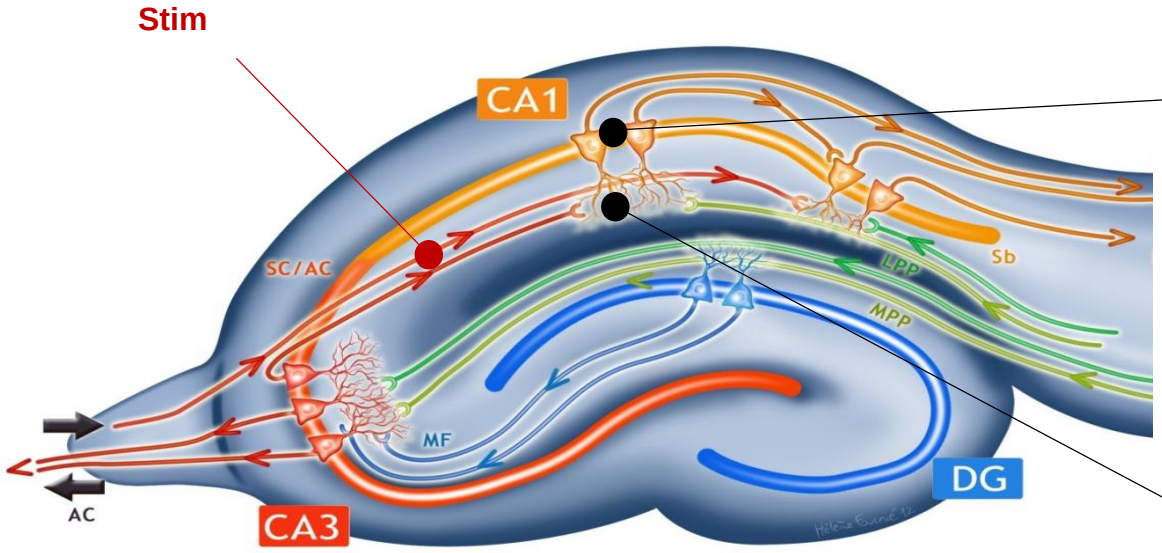
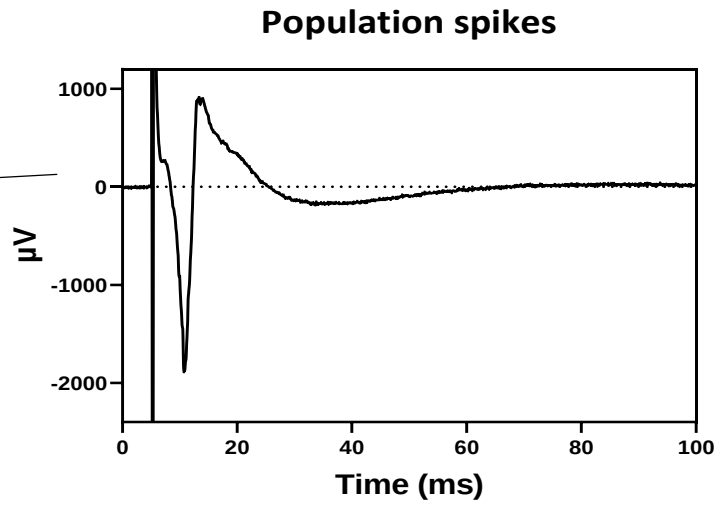
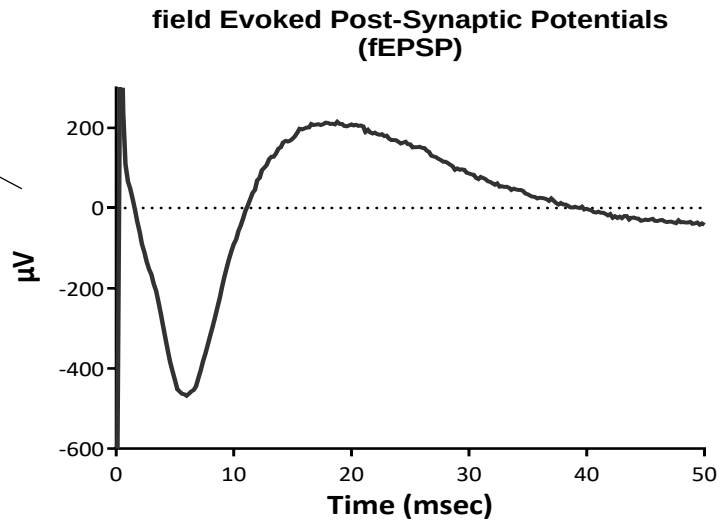
PRO – ANTI CONVULSIVE MODELS

Populations spikes – Area Under Curve (AUC)







- **Evoked-responses (excitatory post-synaptic potentials, EPSP) reflect the synaptic activity and population spikes (PS) reflects the firing induced by the synaptic activity. Both can be recorded in brain slices with Multi-Electrode Arrays (MEA).**

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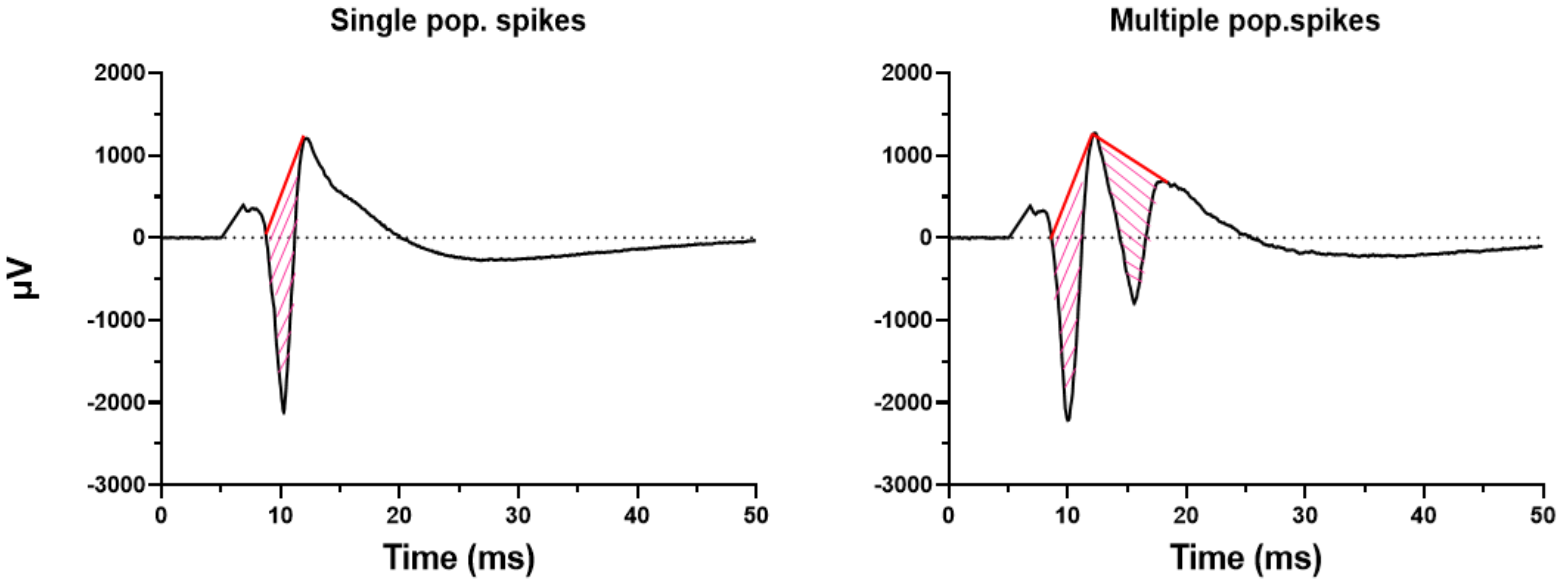
PRO – ANTI CONVULSIVE MODELS

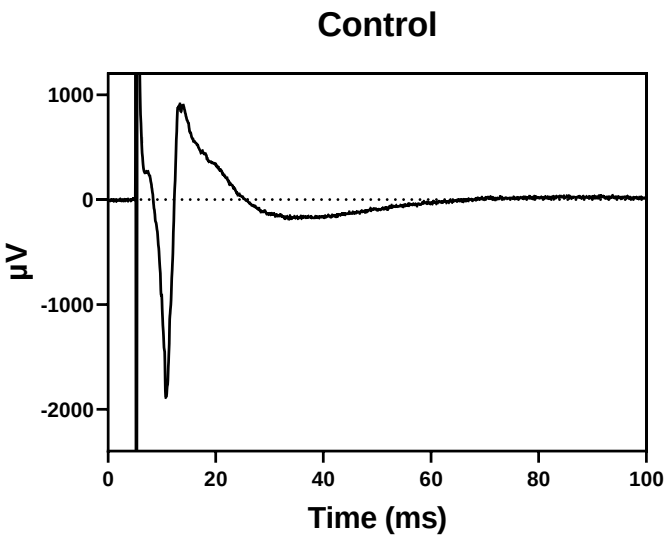
Populations spikes

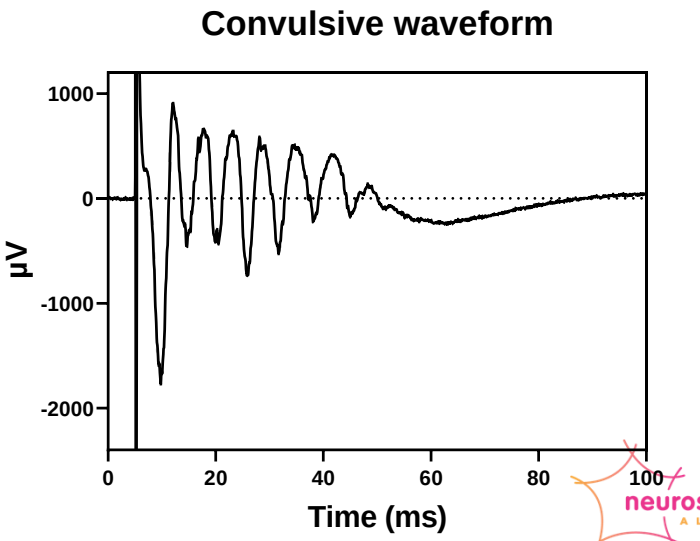
Area Under Curve (AUC)



Schematic of AUC calculation

When the normal excitatory / inhibitory balance is disrupted by treating the slice with picrotoxin ($GABA_A$ receptor blocker), a single stimulation elicits recurrent excitation, manifested by multiple pop spikes and an increase area under the curve.





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RESULTS
Epileptiform discharges

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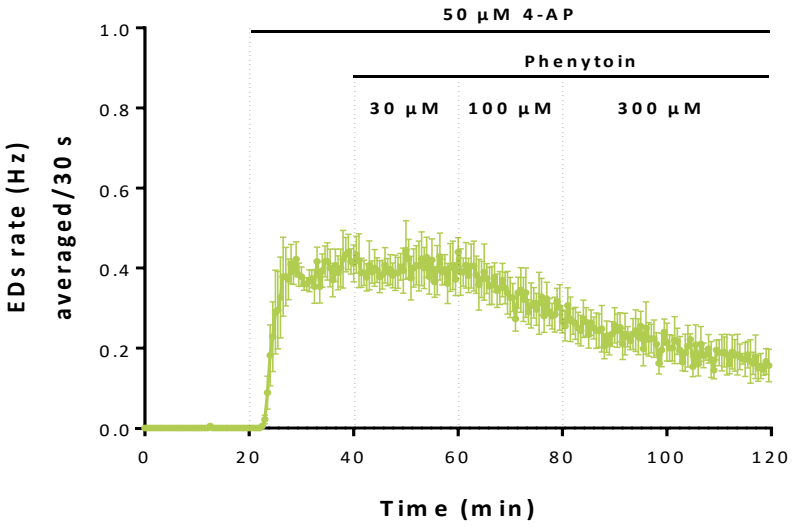
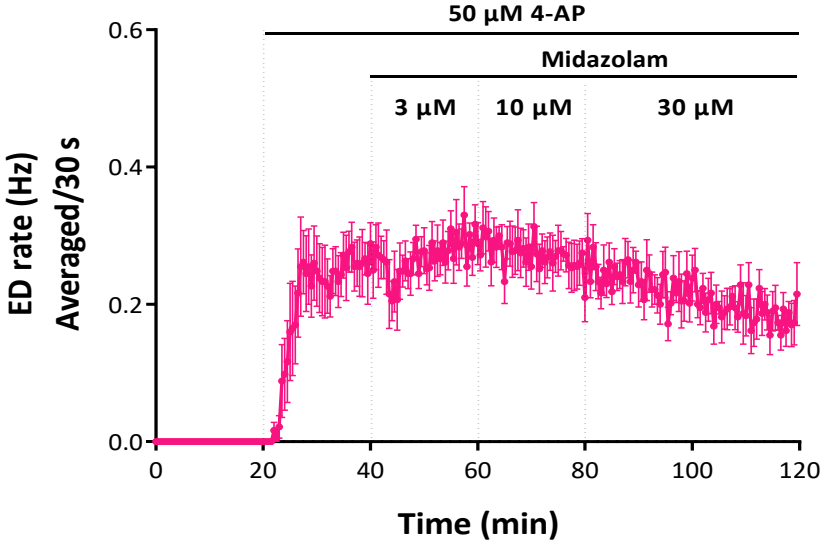
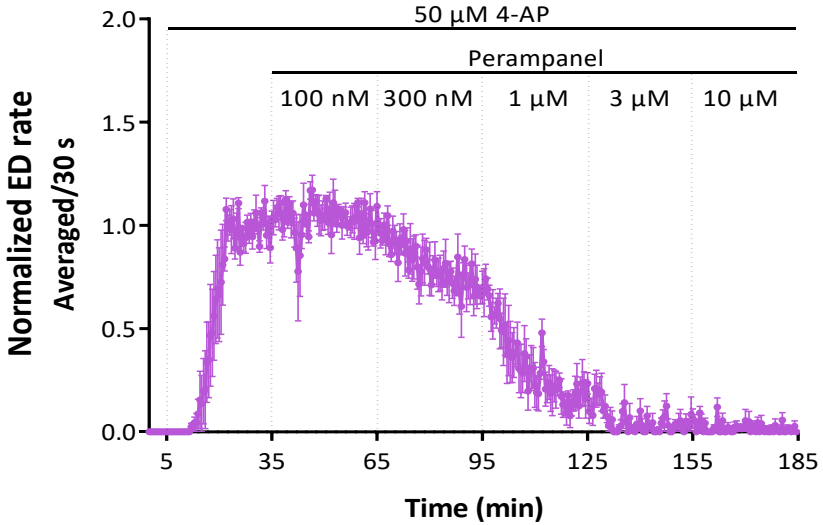
RESULTS_Epileptiform Discharges

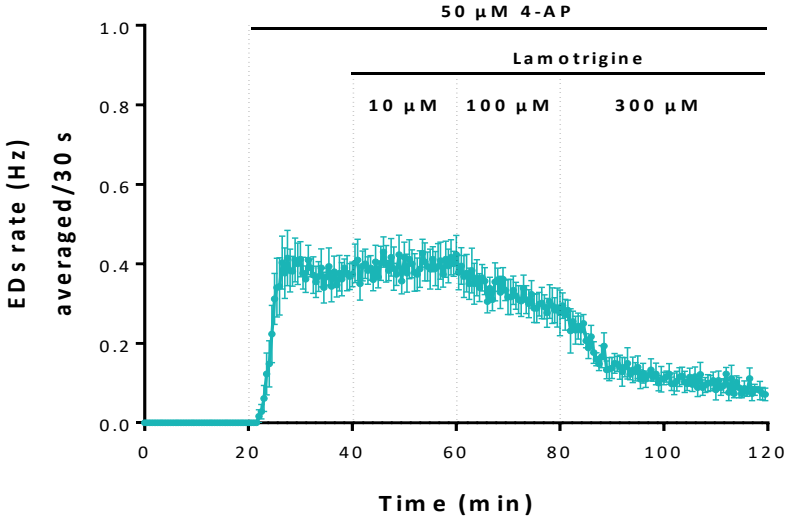
Anti epileptic drugs (AEDs)

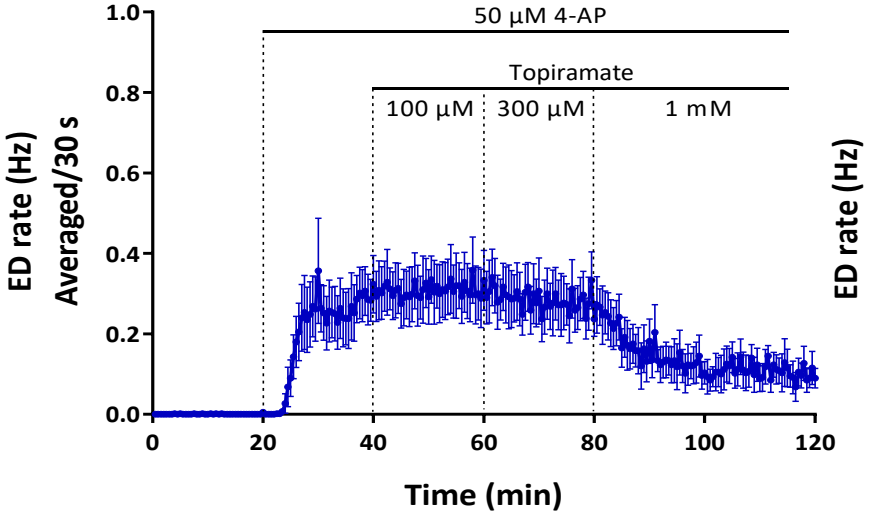
Phenytoin, Midazolam, Perampanel, Lamotrigine, Topiramate, Tiagabine

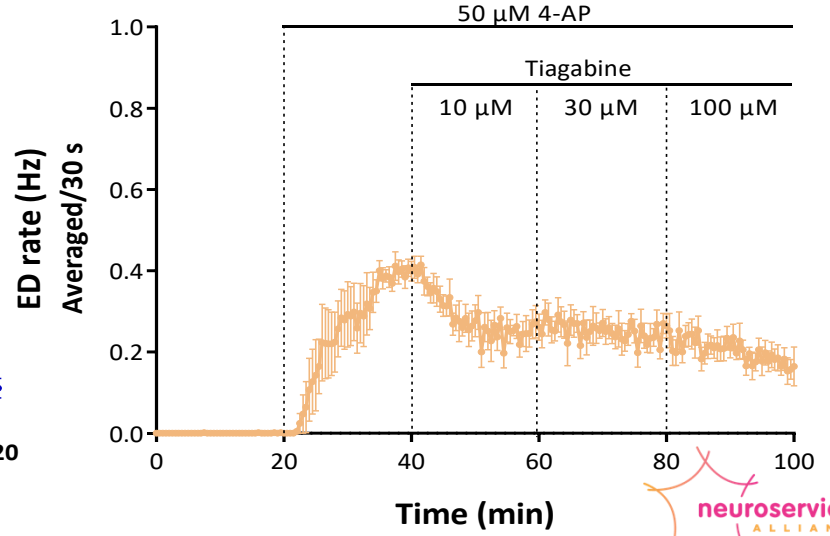












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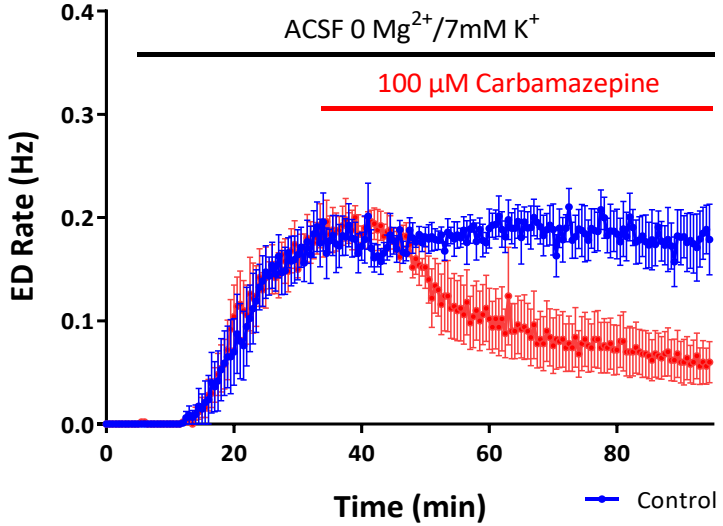
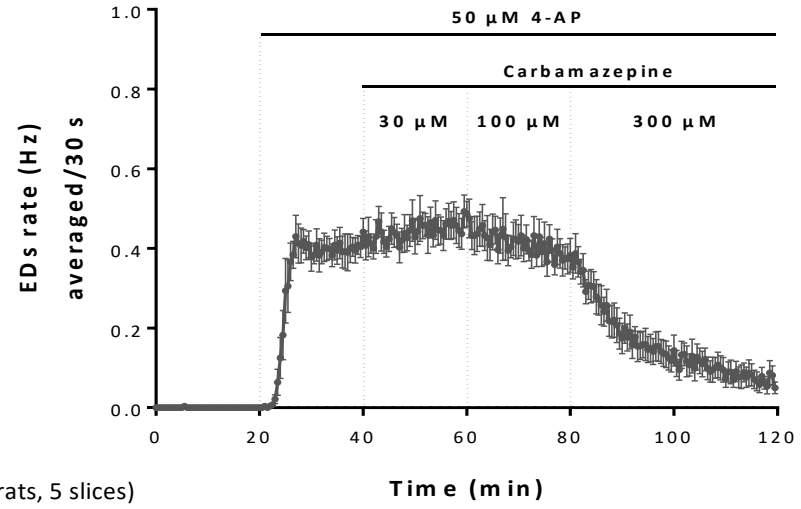
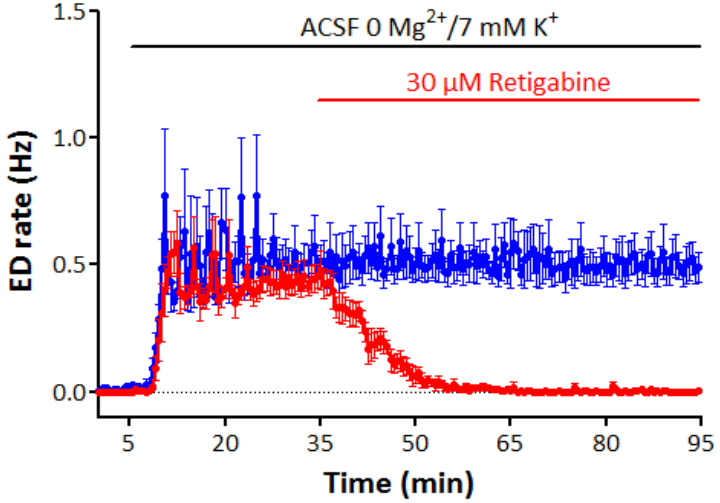
RESULTS_Epileptiform Discharges

Anti epileptic drugs (AEDs)

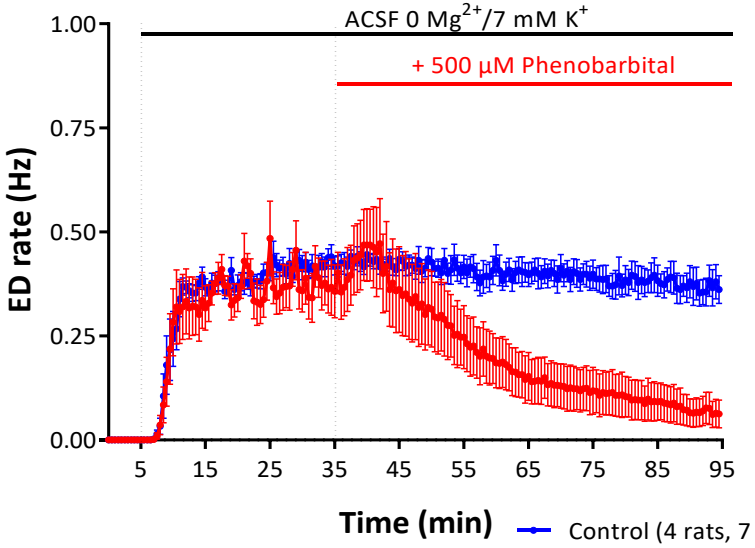
Carbamazepine, Retigabine, Phenobarbital

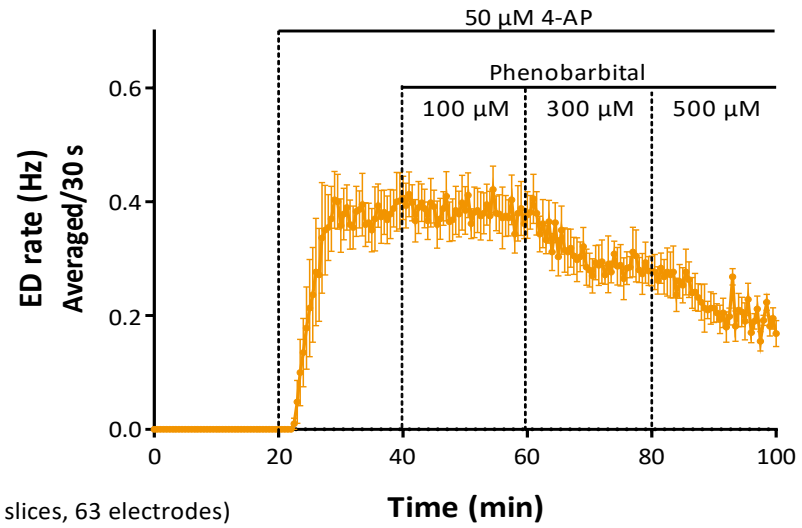




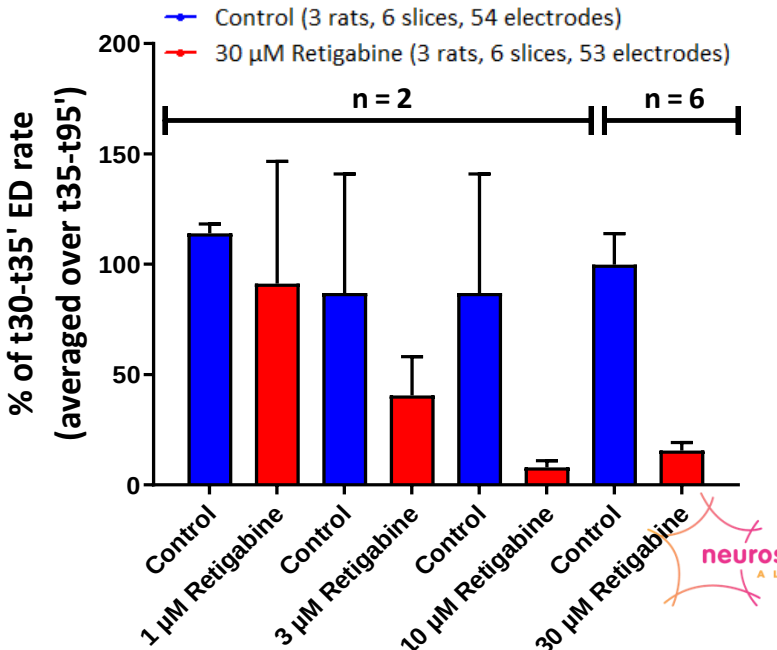


Control (3 rats, 5 slices)
Carbamazepine 100 µM (3 rats, 5 slices)





Control (4 rats, 7 slices, 63 electrodes)
500 µM Phenobarbital (4 rats, 7 slices, 63 electrodes)



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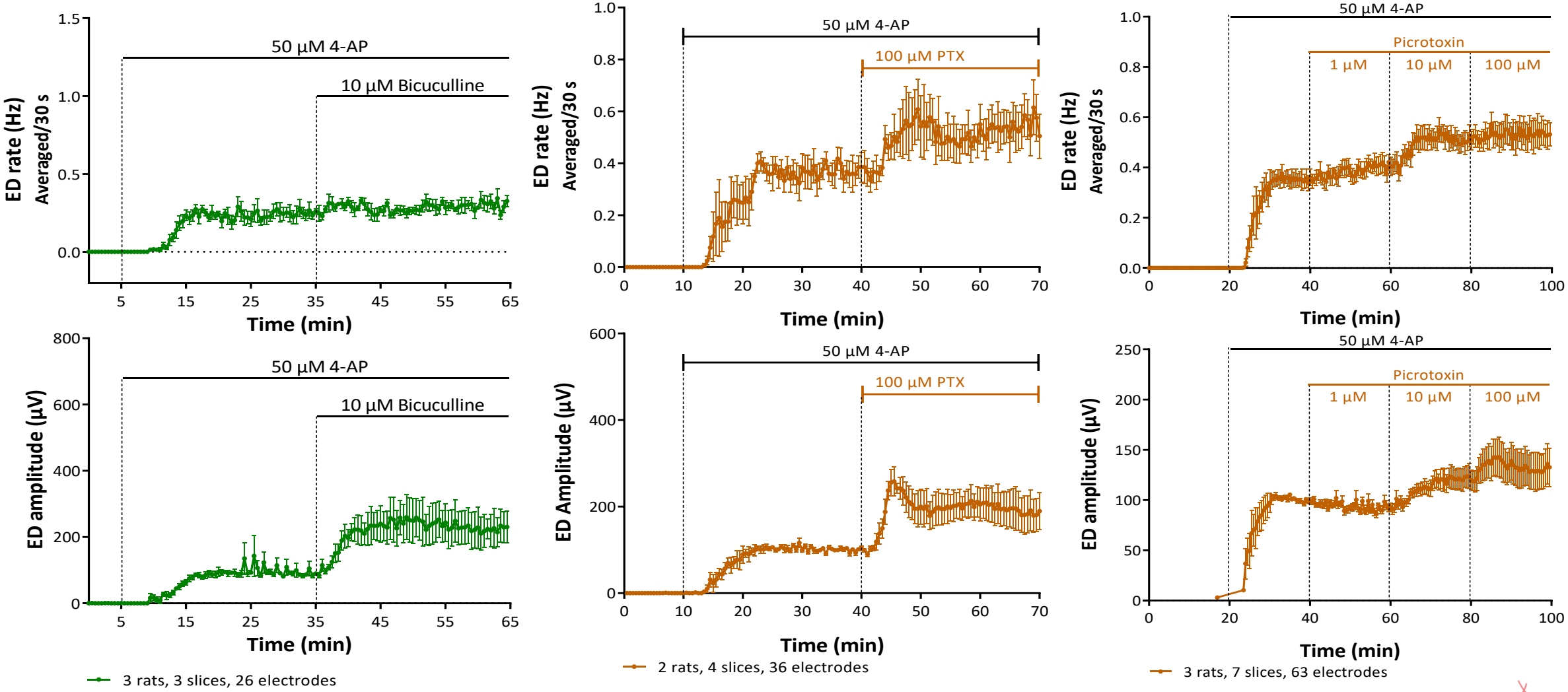
RESULTS_Epileptiform Discharges

Pro-convulsive compounds

Bicuculline, PTX



- Bicuculline and picrotoxin, $GABA_A$ antagonists, majorly increased the ED rate and amplitude. Bicuculline is a reference pro-convulsive compound and displays convulsive properties in the zebrafish model. Picrotoxin displays convulsive properties in rat brain slices, zebrafish, mouse EEG and mouse spontaneous seizure models.

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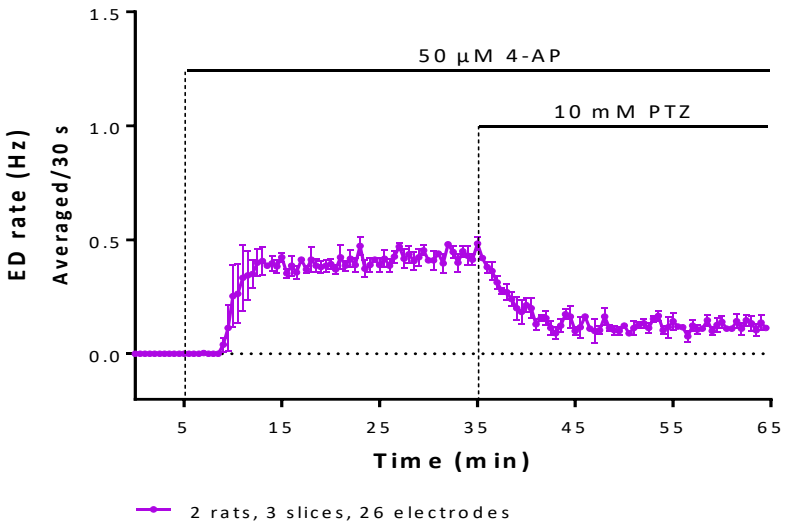
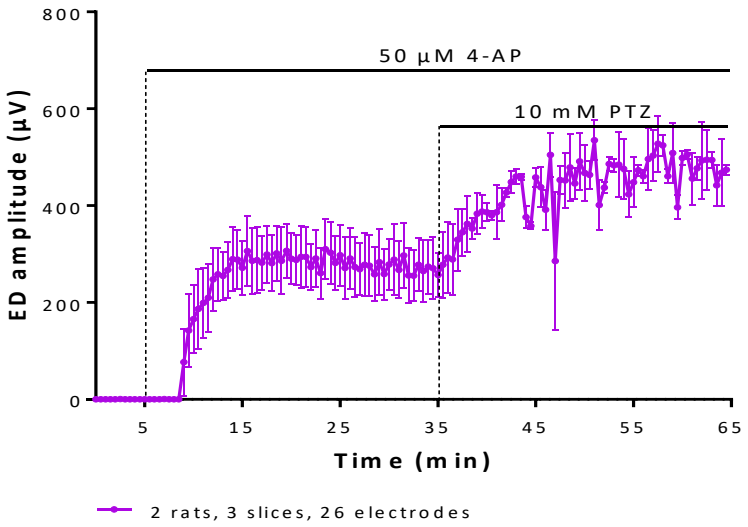
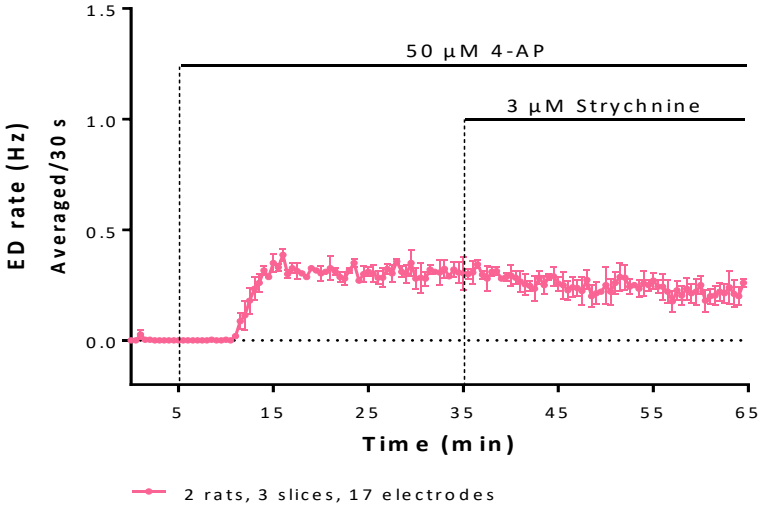
RESULTS_Epileptiform Discharges

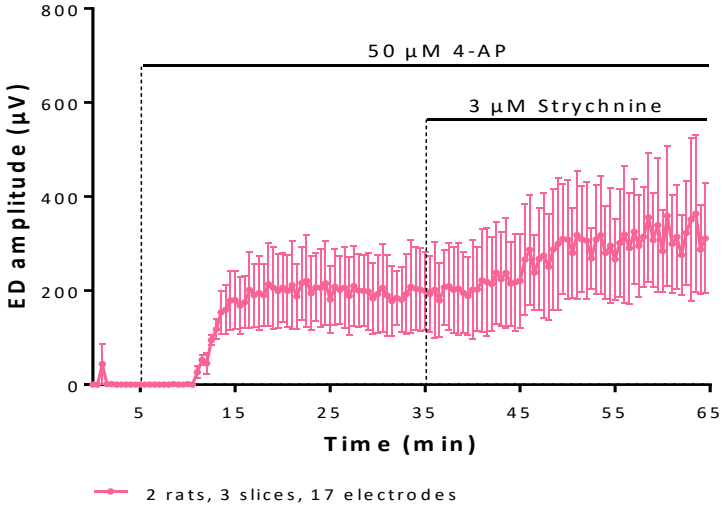
Pro-convulsive compounds

PTZ, Strychnine









- Pentylenetetrazole (PTZ), a $GABA_A$ antagonist compound that also alters excitatory transmission, decreases the rate of 4-AP-induced ED at the same time it largely increases their amplitude. PTZ is a compound widely use in seizure liability assays, displaying convulsive properties in rat brain slices, zebrafish, mouse EEG and mouse spontaneous seizure models.

- Strychnine, a glycine antagonist, slightly decreases the rate of 4-AP-induced ED at the same time it increases their amplitude (selectively, close to the Stratum Pyramidale). Strychnine displays pro-convulsive properties in rat brain slices, zebrafish, mouse EEG and mouse spontaneous seizure models.

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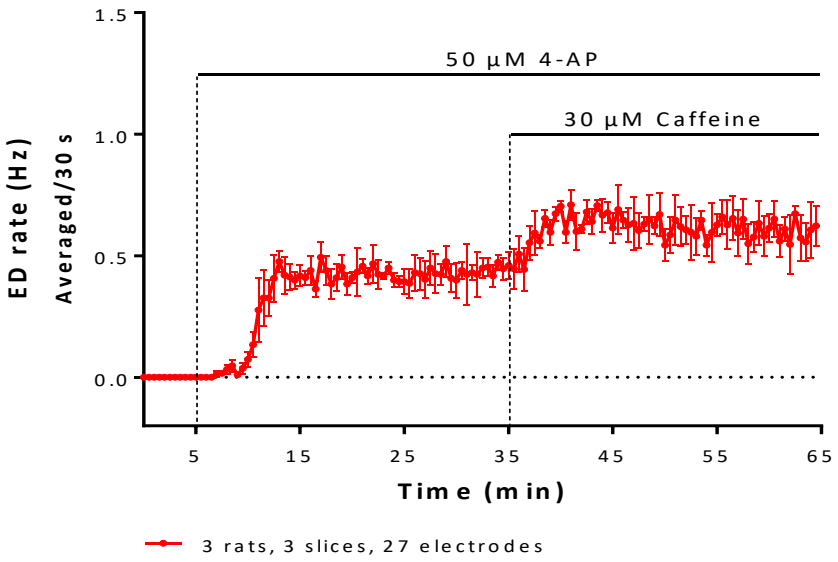
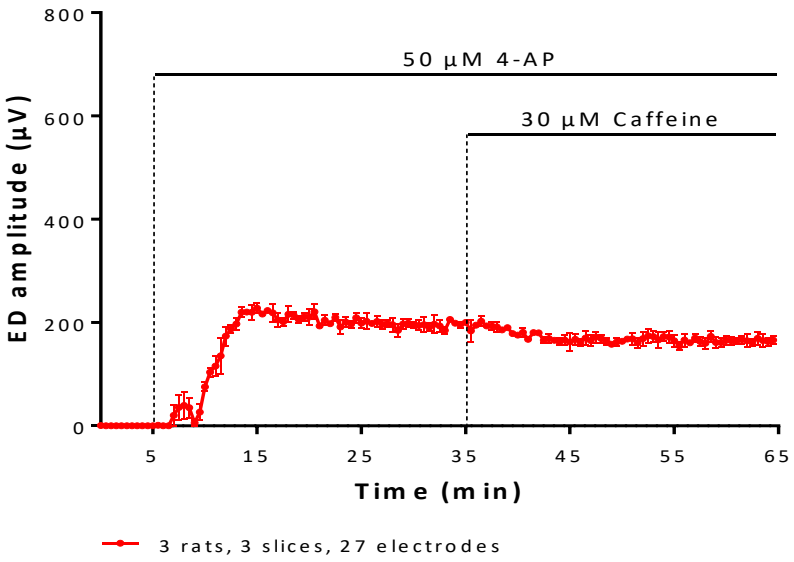
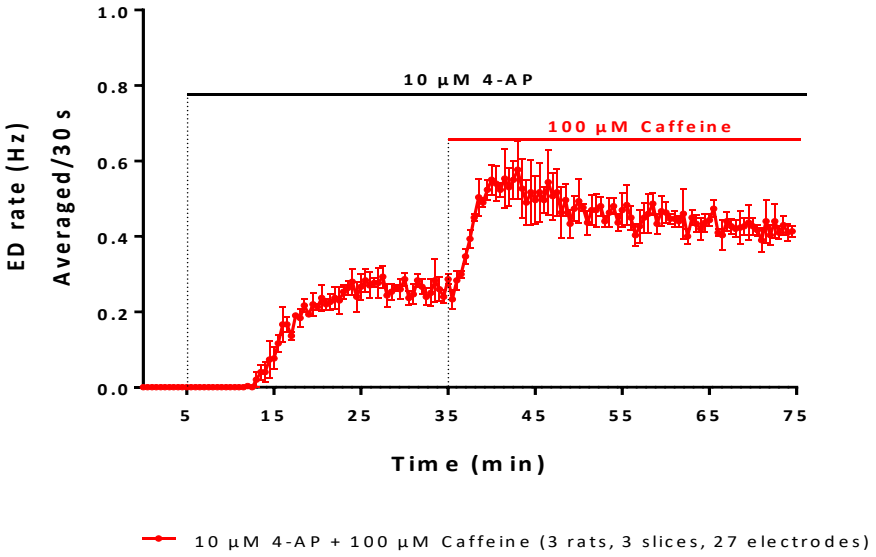
RESULTS_Epileptiform Discharges

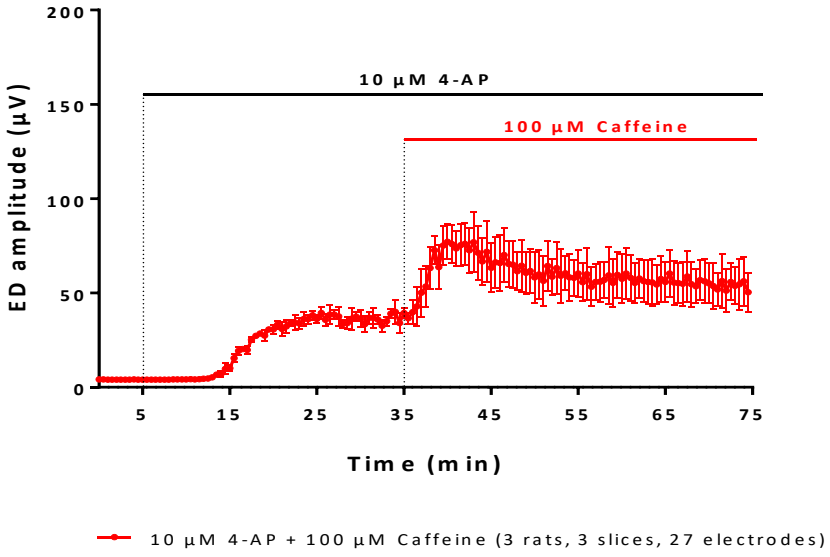
Pro-convulsive compounds

Caffeine









- Caffeine, a wide spectrum adenosine receptors antagonist, increases both 4-AP-induced ED and amplitude.
- The effect of 30 µM Caffeine on the ED rate, is lower than the one observed with 100 µM Caffeine. Dose-ranges of compounds could be documented with the present assay, to determine the threshold concentration eliciting pro-convulsive effects.

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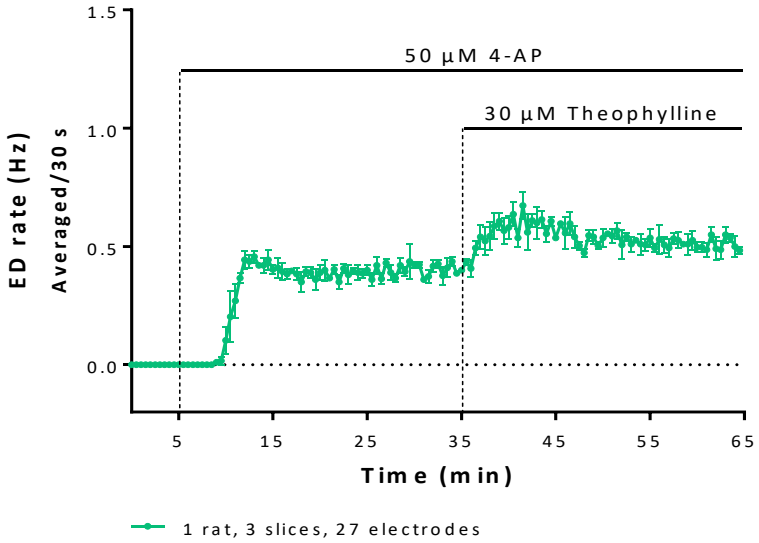
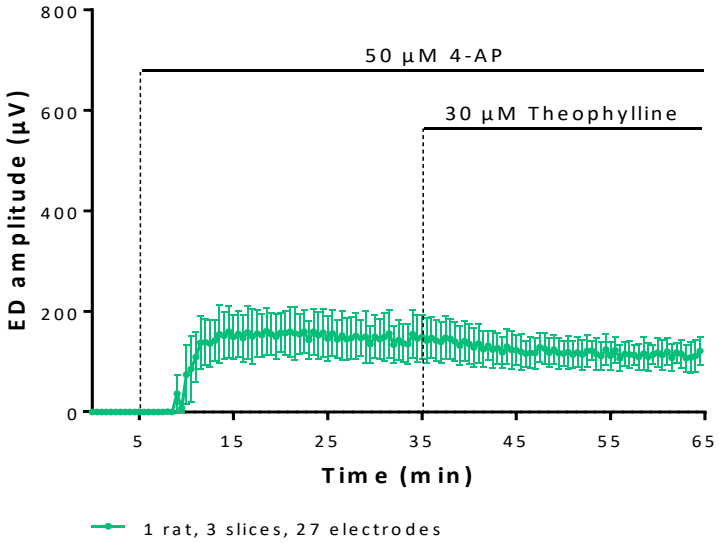
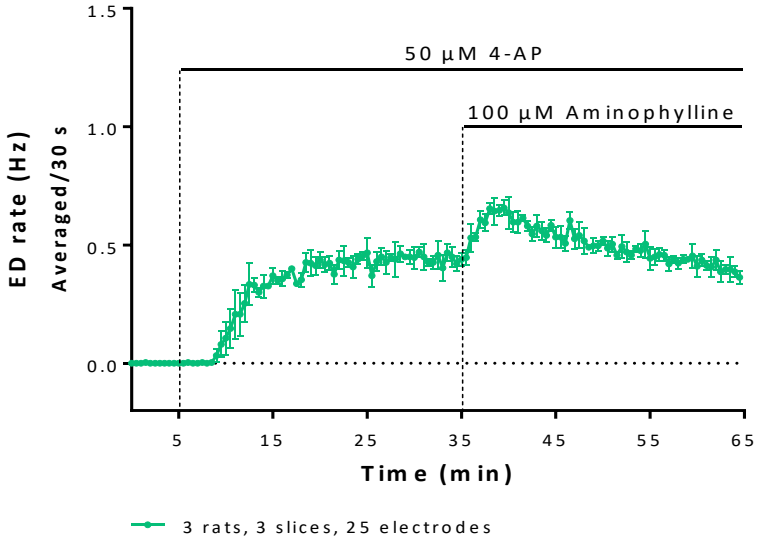
RESULTS_Epileptiform Discharges

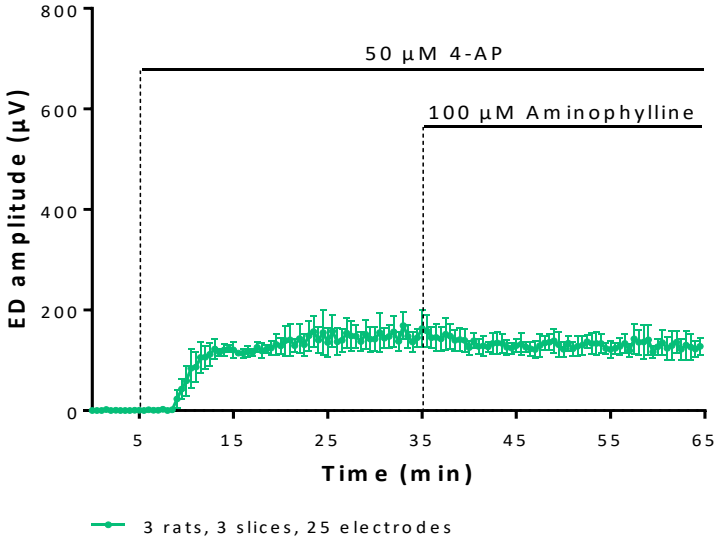
Pro-convulsive compounds

Theophylline, Aminophylline









- Theophylline, a wide spectrum Phosphodiesterase (PDE) inhibitor and an antagonist of A1, A2 and A3 adenosine receptors, increases 4-AP-induced ED, and slightly decreases the ED amplitude. Theophylline is used as a bronchodilator for respiratory diseases as asthma. It reduces the efficacy of the anti-epileptic drug Phenytoin in humans and is a reference pro-convulsive compound.

- Aminophylline, a wide spectrum Phosphodiesterase (PDE) inhibitor and a non-selective adenosine receptors antagonist, transiently increases 4-AP-induced ED, and slightly decreases the ED amplitude. Aminophylline is used as a bronchodilator for respiratory diseases. It reduces the efficacy of the anti-epileptic drugs Topiramate in humans and displays pro-convulsive properties in rat brain slices, zebrafish, mouse EEG and mouse spontaneous seizure models.

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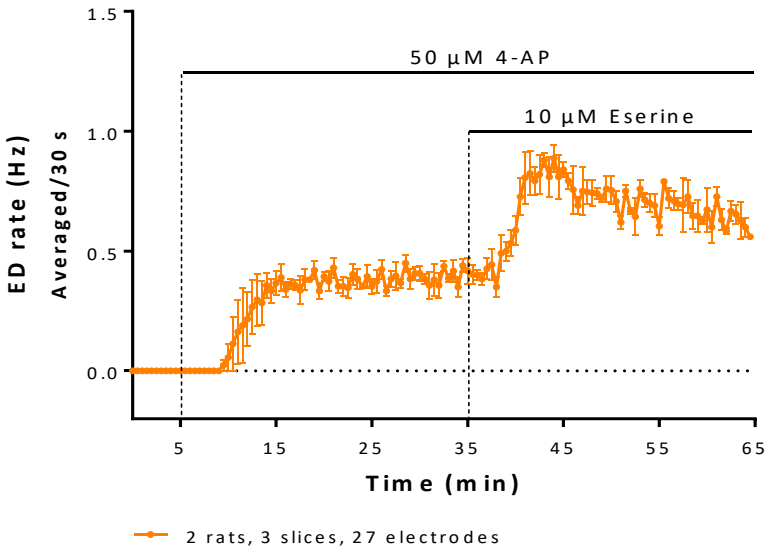
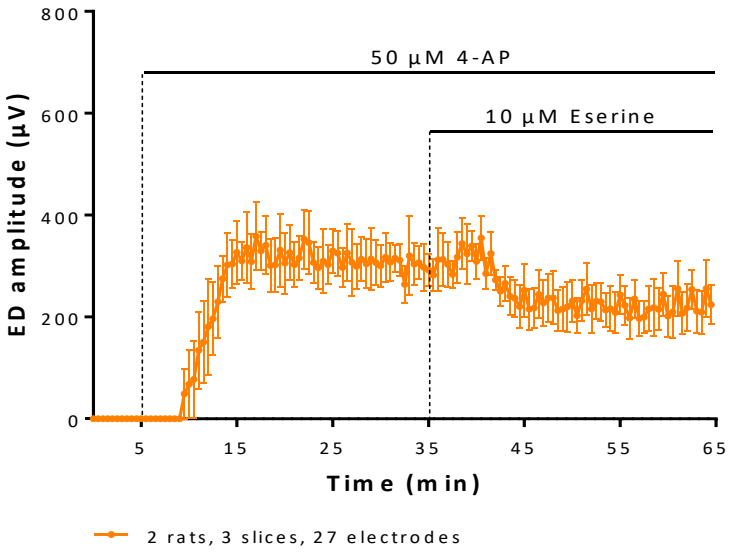
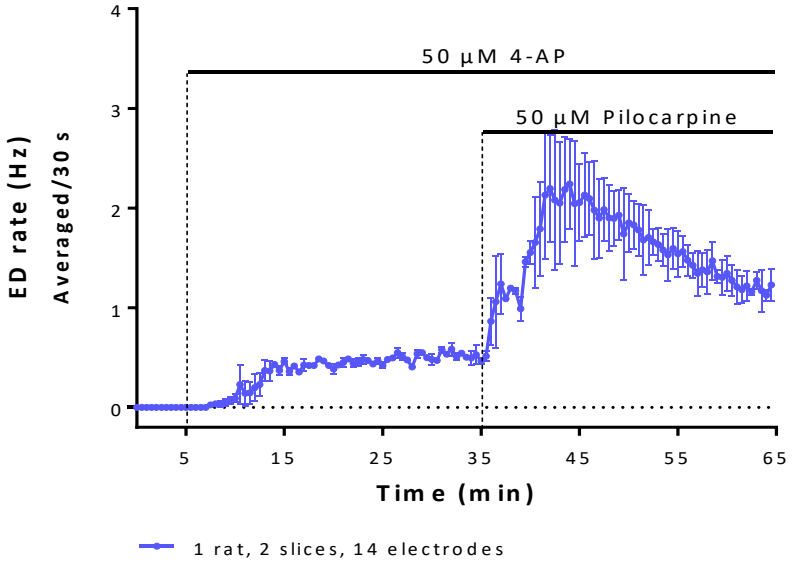
RESULTS_Epileptiform Discharges

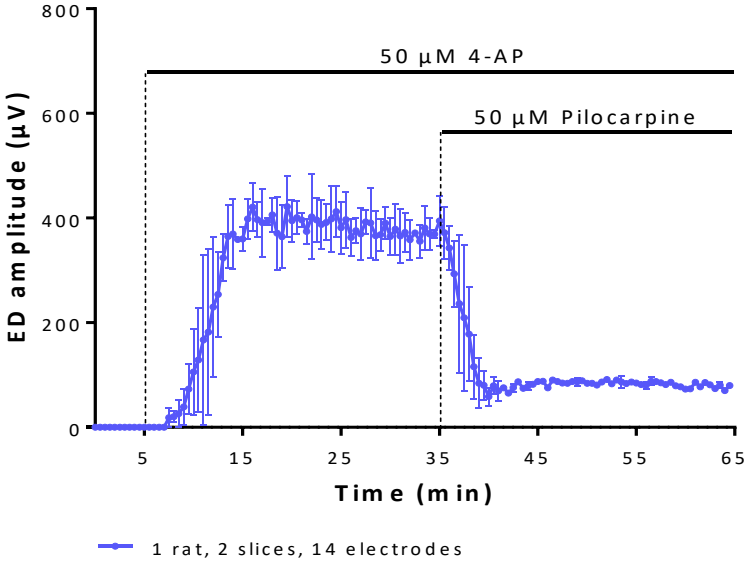
Pro-convulsive compounds

Eserine, Pilocarpine









- Eserine, a cholinesterase inhibitor, largely increases 4-AP-induced ED and decreases the ED amplitude. Eserine is a reference pro-convulsive compound.
- Pilocarpine, a non-selective muscarinic receptor agonist, largely increases the rate of 4-AP-induced ED at the same time it largely decreases their amplitude. Pilocarpine is a compound widely use to induce chronic epilepsy in rats, and displays convulsive properties in mouse EEG and mouse spontaneous seizure models.

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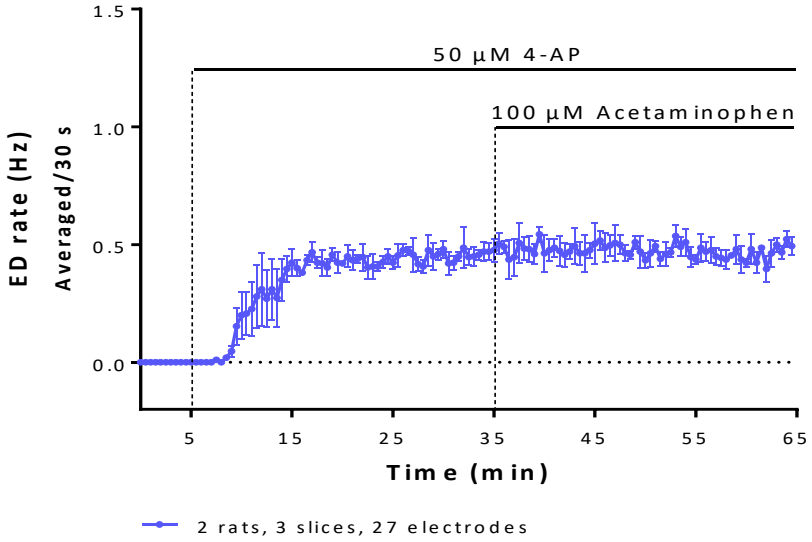
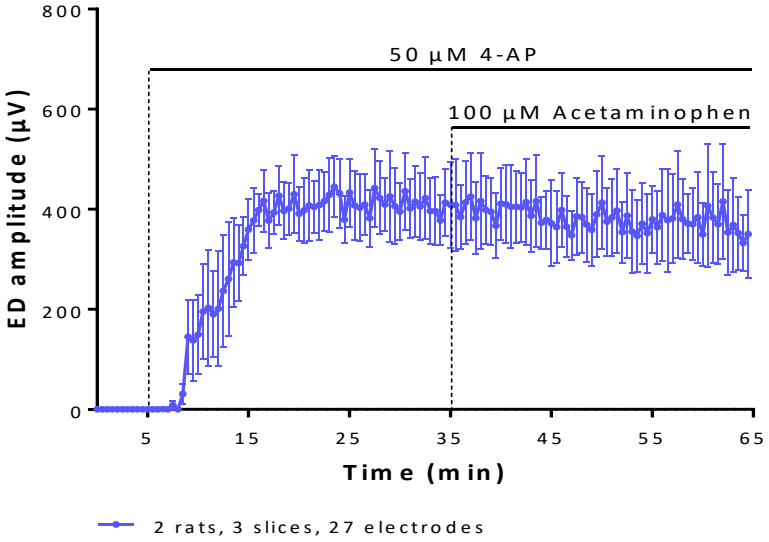
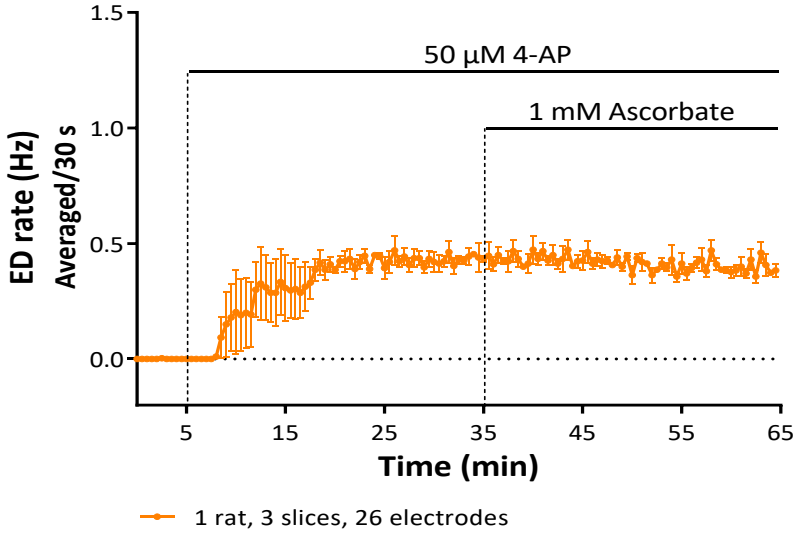
RESULTS_Epileptiform Discharges

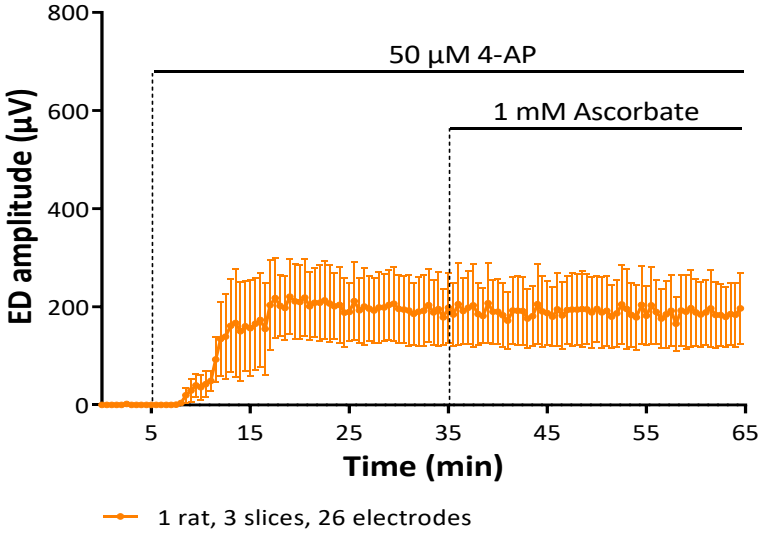
Neutral compounds

Acetaminophen, Ascorbate









- Acetaminophen and ascorbate, negative control, does not modify the rate or the amplitude of 4-AP-induced ED.
- Acetaminophen (paracetamol), a COX-2 inhibitor, is devoid of pro-convulsive properties in the therapeutic window.
- Ascorbate (Vitamin-C), an organic compound with anti-oxidant properties, is devoid of pro-convulsive properties.

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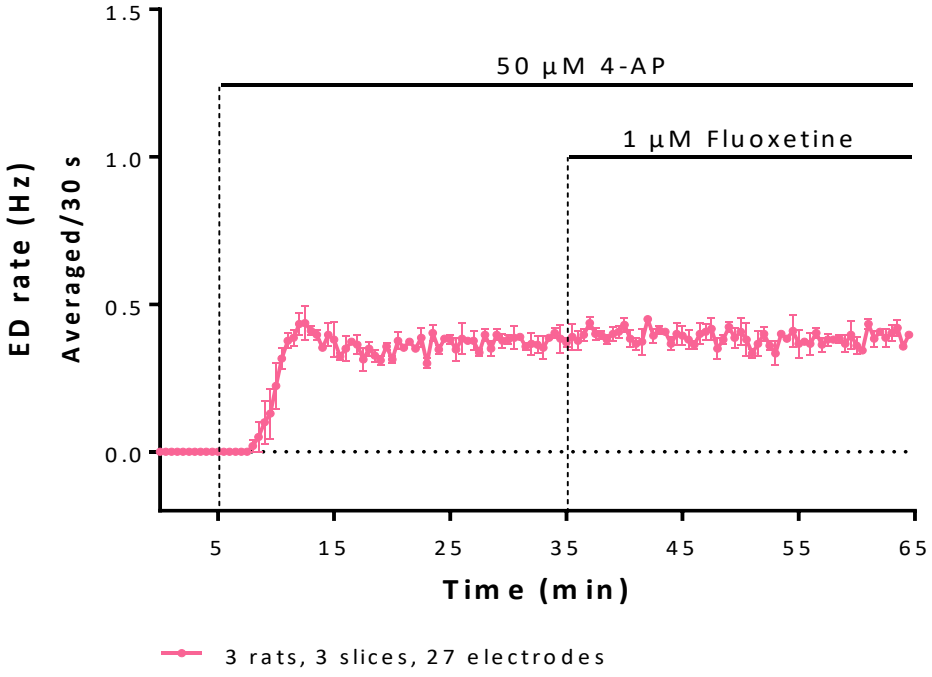
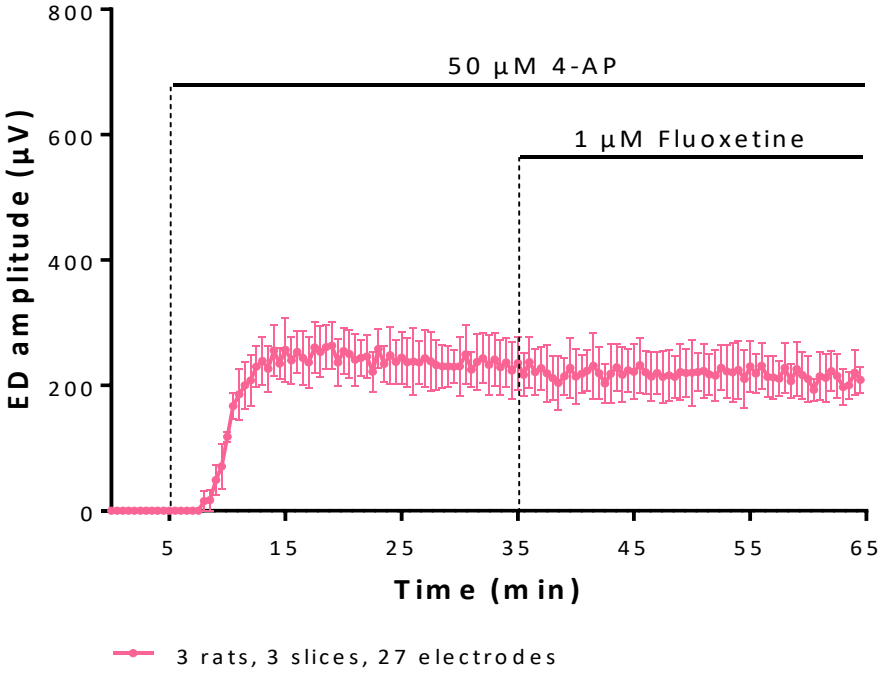
RESULTS_Epileptiform Discharges

Neutral compounds

Fluoxetine





- Fluoxetine (Prozac®), a Selective Serotonin Re-uptake Inhibitor, (SSRI), does not modify the rate or the amplitude of 4-AP-induced ED.
- Tricyclic Antidepressant (TCA), that non selectively inhibit serotonin, norepinephrine and dopamine were discovered in the 60's, but displayed intolerable adverse side effects, including seizures. Unlike TCA, SSRI such as Fluoxetine were developed in the 80's and have low propensity to cause seizures. Fluoxetine is still a widely used antidepressant and is devoid of pro-convulsive properties in the therapeutic window.

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RESULTS
Population spikes

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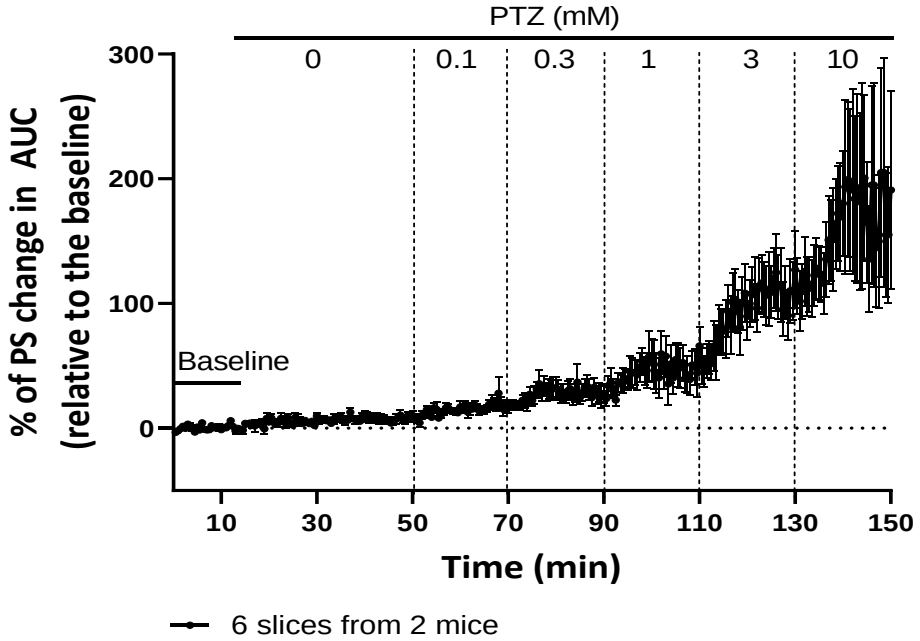
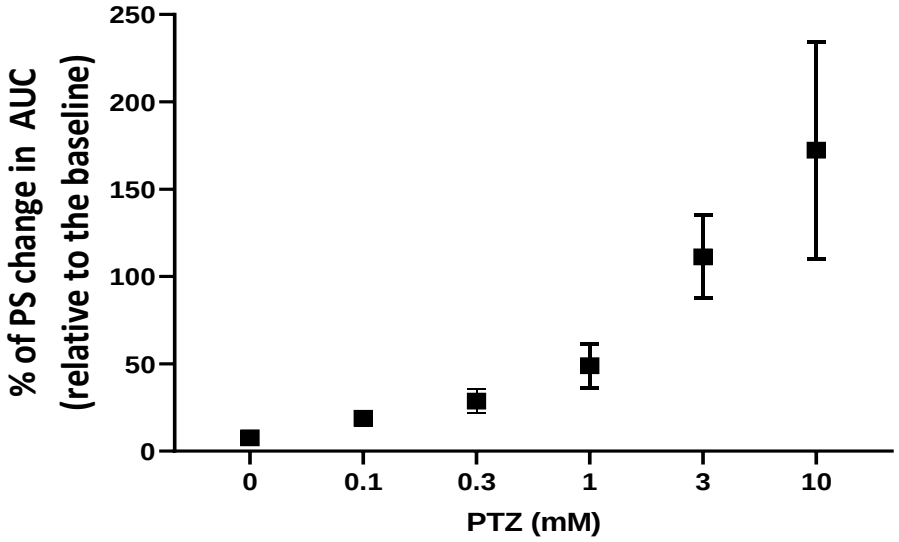
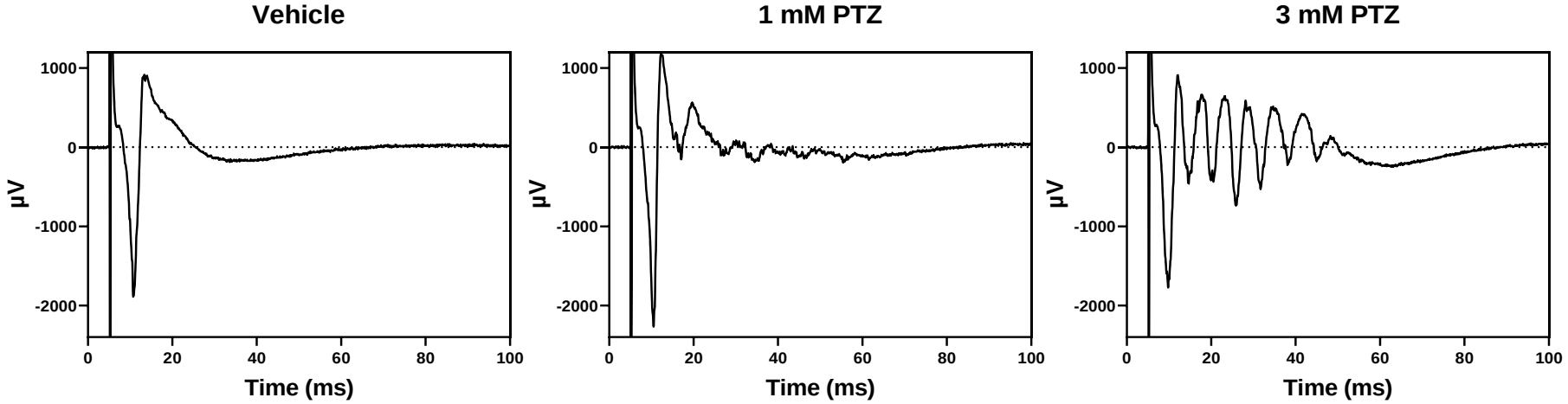
RESULTS_Population spikes

Pentylenetetrazole dose-dependently induced convulsive waveform of pop. Spikes PTZ





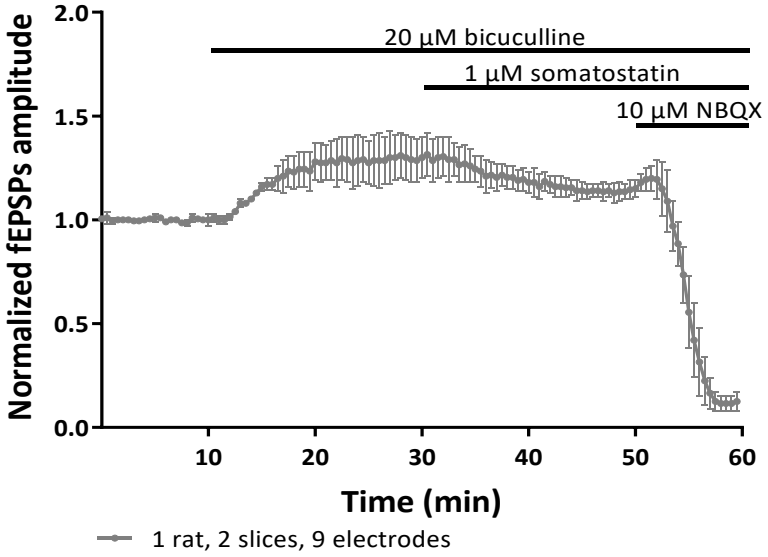


RESULTS_Population spikes

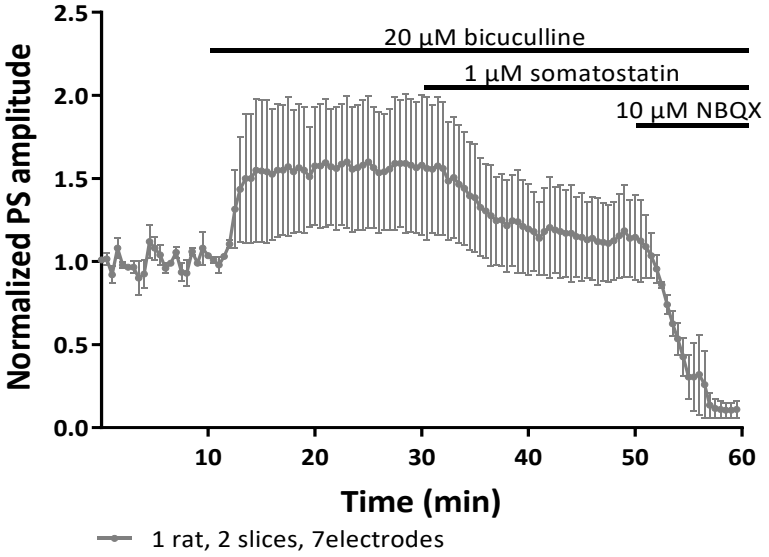
Somatostatin reverses convulsive waveform induced by bicuculline

Somatostatine, Bicuculline

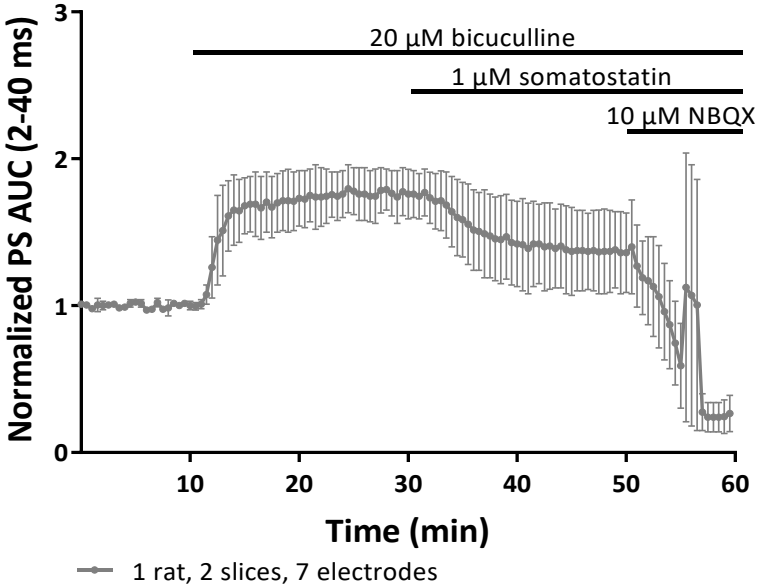
fEPSPs Amplitude



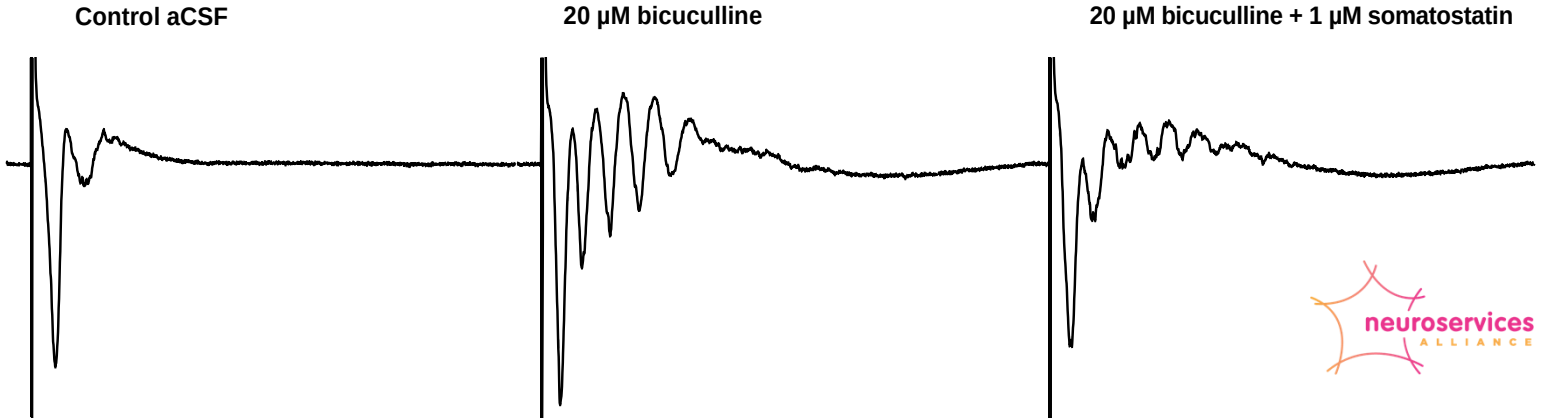
Population spikes Amplitude



Population spikes AUC



- Bicuculline – a GABAA antagonist – increase EPSP and PS amplitude and modify their waveforms. Somatostatin reverse the effect of bicuculline.



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