MULTI ELECTRODE ARRAY

Network oscillations

neuroservices ALLIANCE

SUMMARY

Network oscillations

- Information about network oscillations
- Materials & Methods - Go
- *In vitro* Models - Go
- From *in vitro* to *in vivo* - Go

Reference data

- AMPA/Kainate and $GABA_A$ receptors – Picrotoxine, NBQX
- NMDA receptors – Ketamine, MK-801, CIQ
- Sodium channels – Carbamazepine
- Dopaminergic receptors – Haloperidol, PD-168077
- Antipsychotics (Clozapine) – Clozapine
- Serotoninergic receptors – 5-HT, mCPBG
- Adrenergic receptors – Norepinephrine
- Somatostatin receptors – L-803,087
- Cholinergic receptors – Scopolamine, Anatabine
- Opioid receptors - Fentanyl
- Neuregulin-ERBb4 pathway – NRG-1β
- SK channels – TEA

neuroservices ALLIANCE

INTRODUCTION

Brain rhythms are periodically fluctuating waves of neuronal activity. Such rhythms reflect the synchronized activity of large numbers of neurons because synchronous currents sum together to generate large-amplitude fluctuations.

Different types of brain rhythms are observed in humans, primates, as well as rodents and occur in many cortical and subcortical regions. Each type of rhythm is observed during particular behaviors, generated by specific mechanisms, associated with characteristic neuronal firing properties and are thought to perform distinct functions.

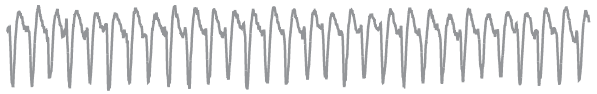
Different brain regions display various oscillatory activities, for instance slow oscillation (<1 Hz), delta (1-4 Hz), theta (4-12 Hz), beta (12-30 Hz), gamma oscillations (20-80Hz) and sharp waves ripples (150-250 Hz).

Within the hippocampus, theta, gamma oscillations and sharp wave ripples could be observed.

- Theta oscillations (4-12 Hz) arise during active exploration and rapid eye movement (REM) sleep
- gamma oscillations (20-80 Hz) are associated with cognitive function
- Sharpe wave ripples (150-250 Hz) are observed in animals during waking immobility and during slow wave sleep. Sharp waves ripples consist in a replay of previous activity in a temporally compressed manner, which is necessary for memory consolidation.

The most ubiquitous rhythms are gamma oscillations. This rhythms also represent a relevant model of investigation in CNS drug discovery as it is often impaired in disorders involving cognitive deficits, such as schizophrenia, Alzheimer's disease, and depression.

INTRODUCTION



Extracellular recording of local field potential

(E) Excitatory, (I) inhibitory, (PSCs) postsynaptic currents (P) pyramidal cell, (B) basket interneuron (Buzsáki and Wang, 2012)

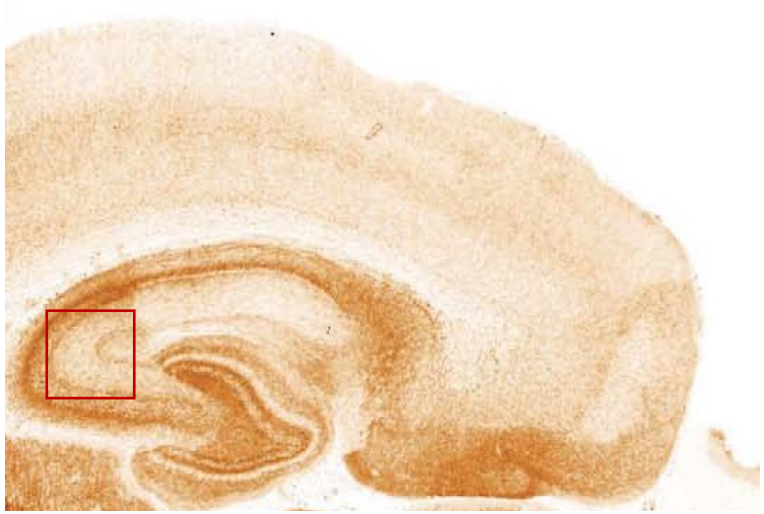
Gamma oscillations mechanisms

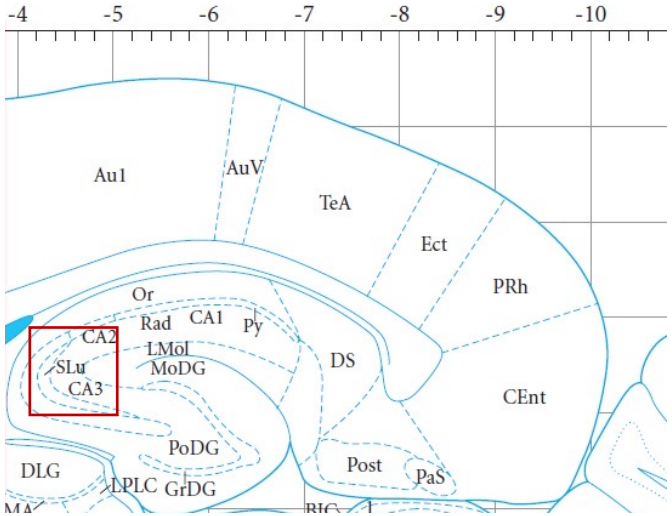
Gamma oscillations are generated by reciprocal connections between interneurons and pyramidal cells. Particular perisomatic-targeting interneurons - parvalbumin-positive basket cells - play a critical role in synchronizing the activity of numerous pyramidal cells by fast rhythmic inhibition (Bartos et al, 2007).

To summarize, excitatory cells provide feedback excitation to the circuit, while interneurons contribute phasic drive to pace the oscillations.

MATERIALS & METHODS

Hippocampal slices – CA3 recording (1/3)





Area of recording

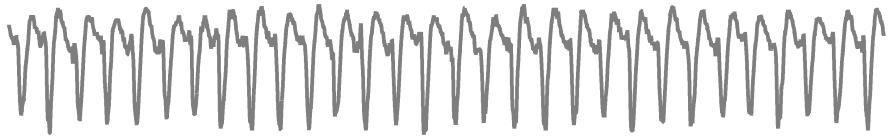
MATERIALS & METHODS

Data analysis (2/3)

Extracellular recording of local field potential

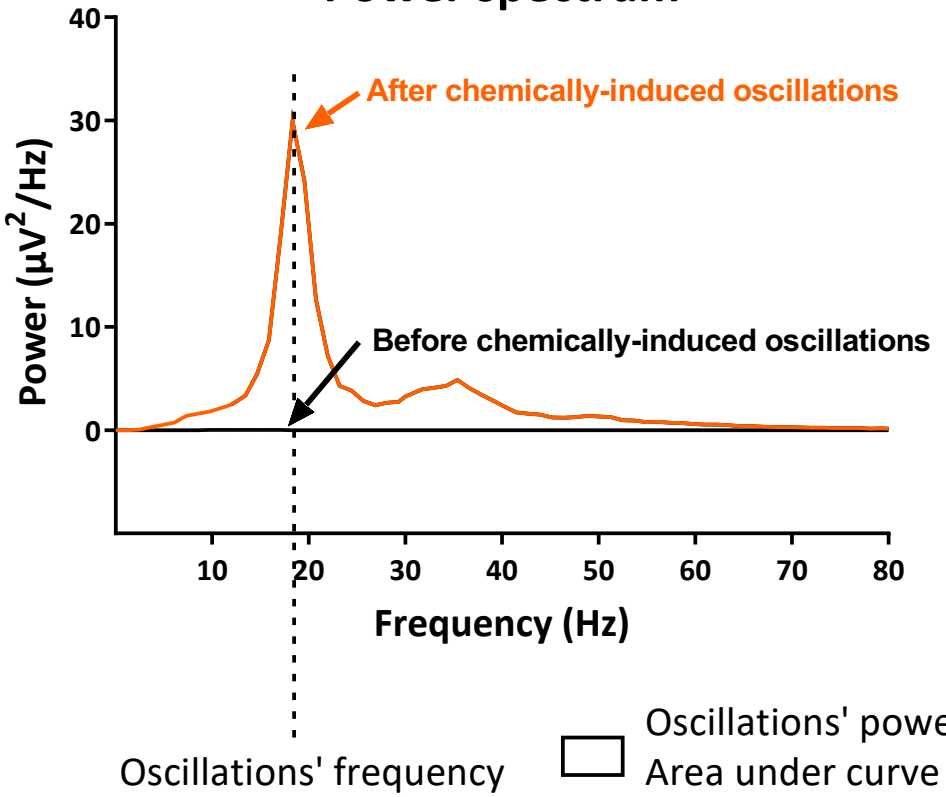
Baseline

Carbachol- or kainate-induced oscillations



Fast Fourier Transform
(1 minute bin)

Power spectrum



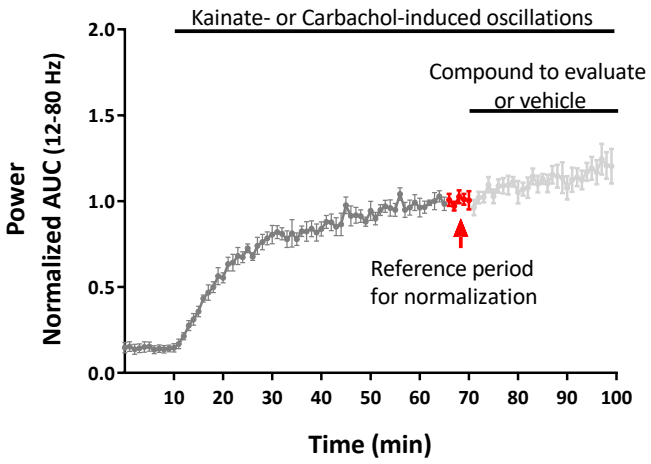
neuroservices ALLIANCE

MATERIALS & METHODS

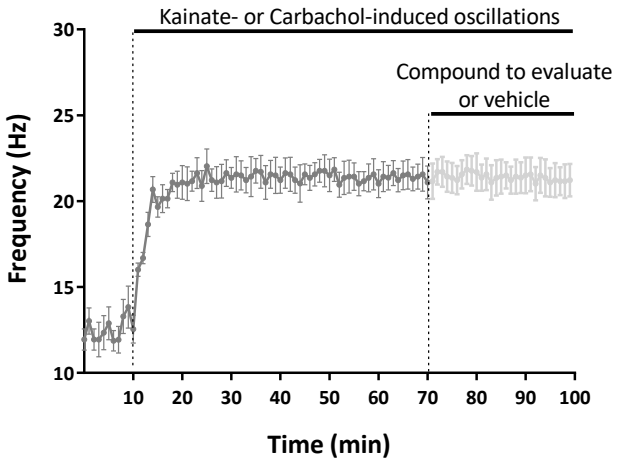
Data analysis (3/3)

Compound testing – paired comparison

Assessment of the power of oscillations as a function of time

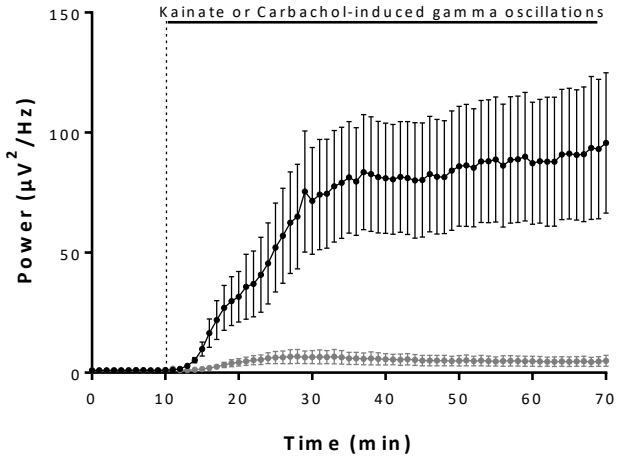


Assessment of the predominant frequency of oscillations as a function of time

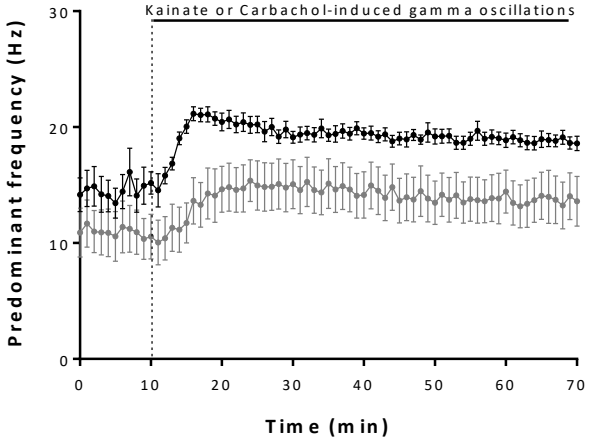


Phenotyping – unpaired comparison

Assessment of the power of oscillations as a function of time



Assessment of the predominant frequency of oscillations as a function of time



neuroservices ALLIANCE

NETWORK OSCILLATIONS

In vitro models (1/4)

Network oscillations in hippocampus

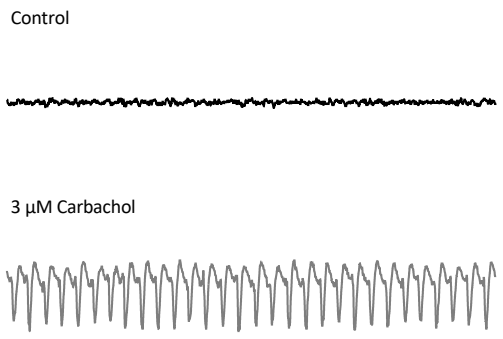
Gamma oscillations arise in the cortex as well as in the hippocampus. However, several features of the hippocampus facilitate the study of such oscillations.

- Gamma oscillations are an emergent property of the circuitry in the hippocampus .
- The hippocampus contains densely packed neurons that generate large local field potentials and, in turn, generate large rhythms. This is especially true for CA3 and CA1 regions, because the pyramidal cell dendrites are aligned in parallel. Because of this, synaptic currents flow in the same direction and sum together to produce large amplitude signals that are easily detected in local field potential recordings.
- Much is known about hippocampal interneurons that are important for rhythm generation.
- Persistent gamma oscillations (up to 2hours of recording) can be induced *in vitro* in both rat and mouse. Either ionotropic glutamatergic (kainate model) or muscarinic acetylcholine (carbachol model) receptors activation allow for the circuit to oscillate without external inputs.

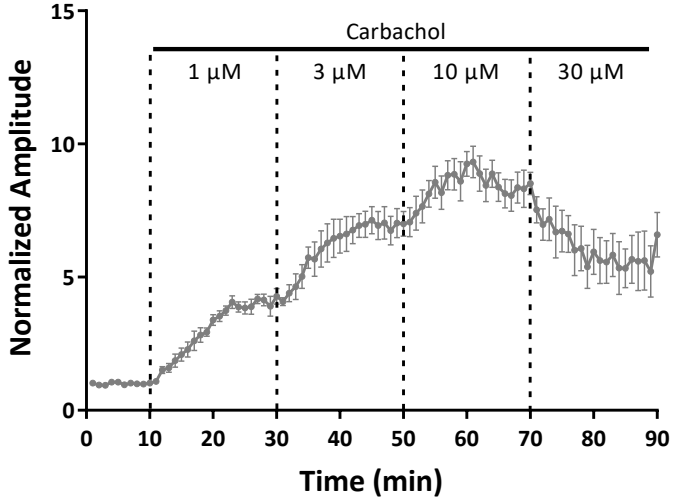
NETWORK OSCILLATIONS

In vitro models (2/4)

Carbachol-induced oscillations

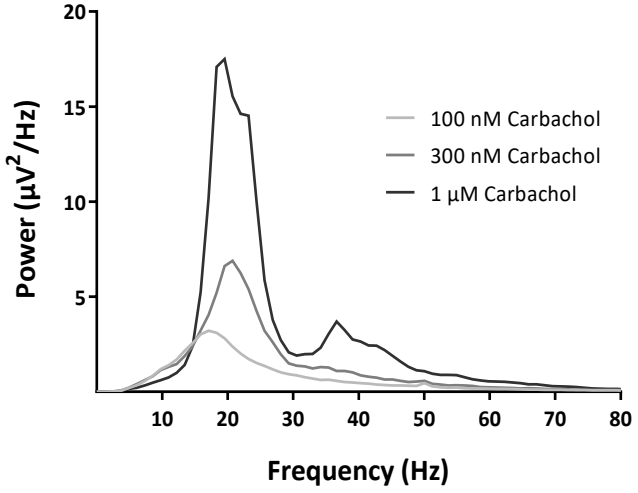


Carbachol dose-range (rat hippocampal slices)

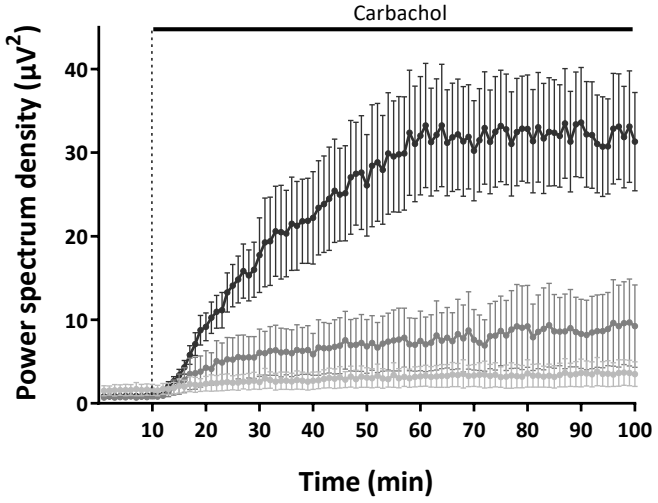


Carbachol induced oscillations by mimicking cholinergic input to the hippocampus via the septum during exploratory behavior *in vivo*. Oscillations are generated by alterning cycles of AMPA receptor mediated recurrent excitation followed by feedback inhibition from perisomatic-targeting interneurons. (Traub et al., 2000, Mann et al., 2005)

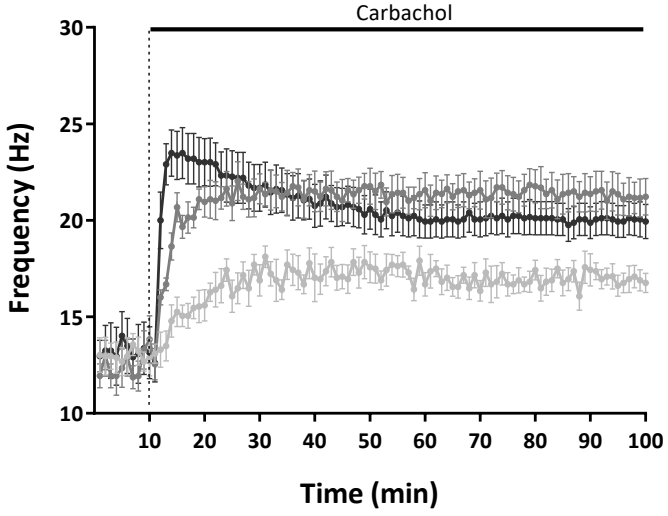
Power spectra



Power



Frequency



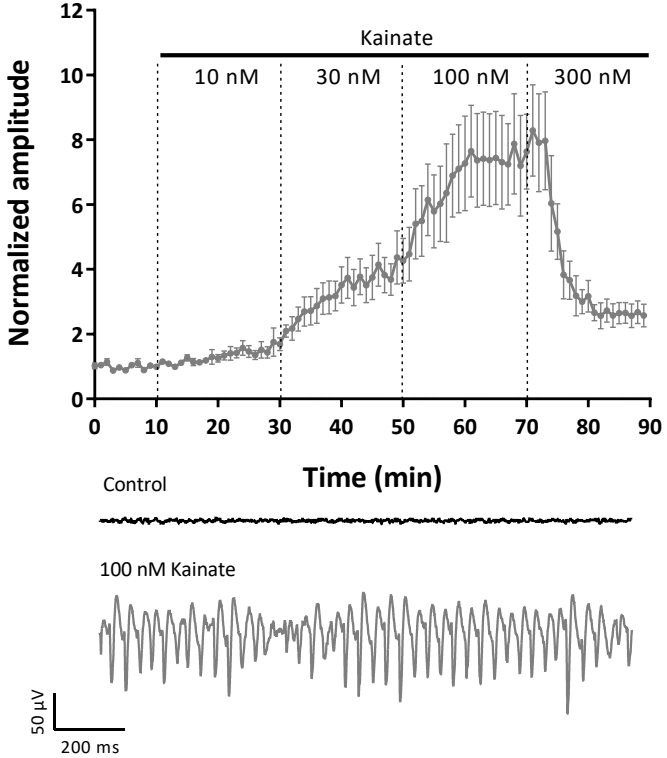
neuroservices ALLIANCE

NETWORK OSCILLATIONS

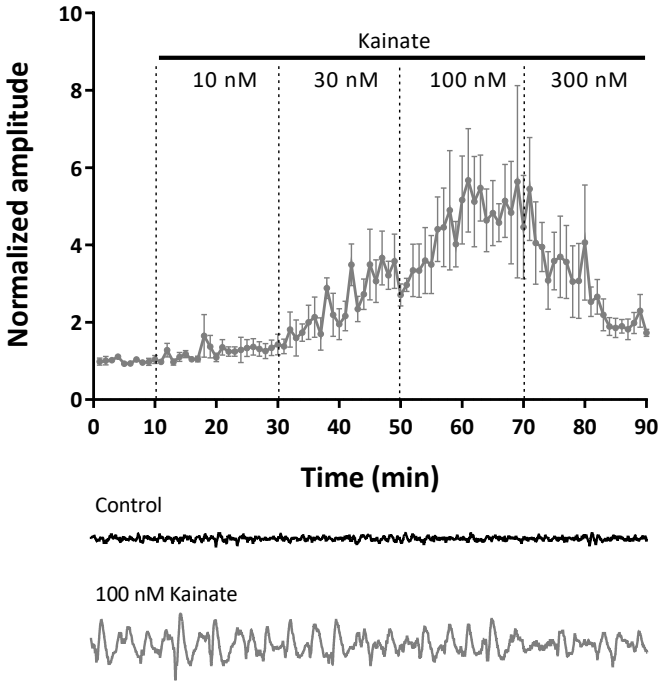
In vitro models (3/4)

Kainate-induced oscillations

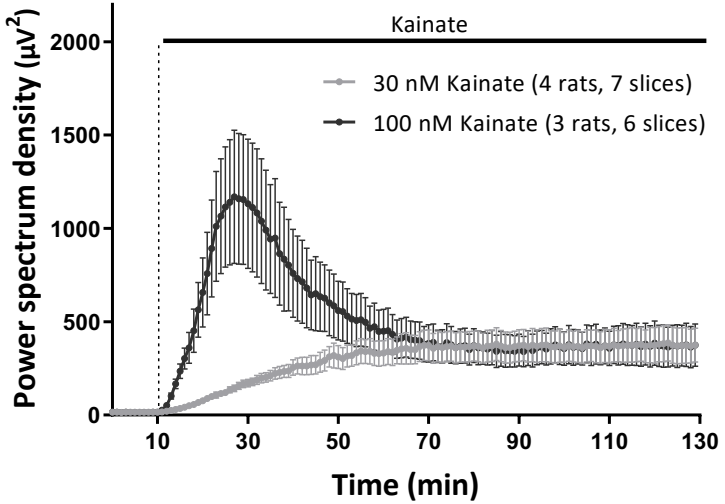
Kainate dose-range (rat hippocampal slices)



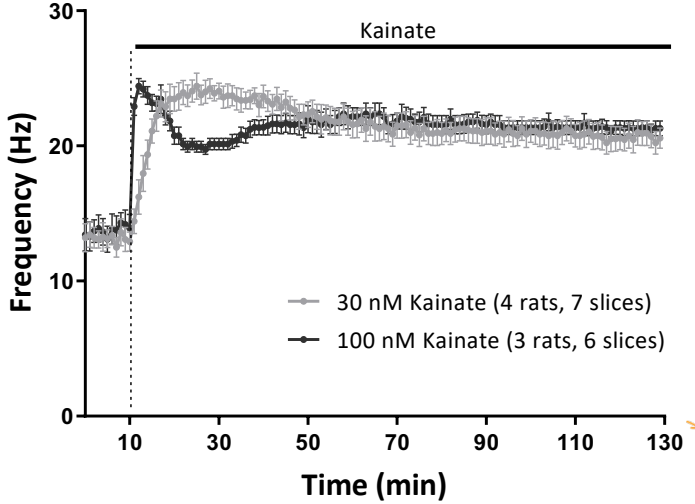
Kainate dose-range (mouse hippocampal slices)



Power



Predominant Frequency



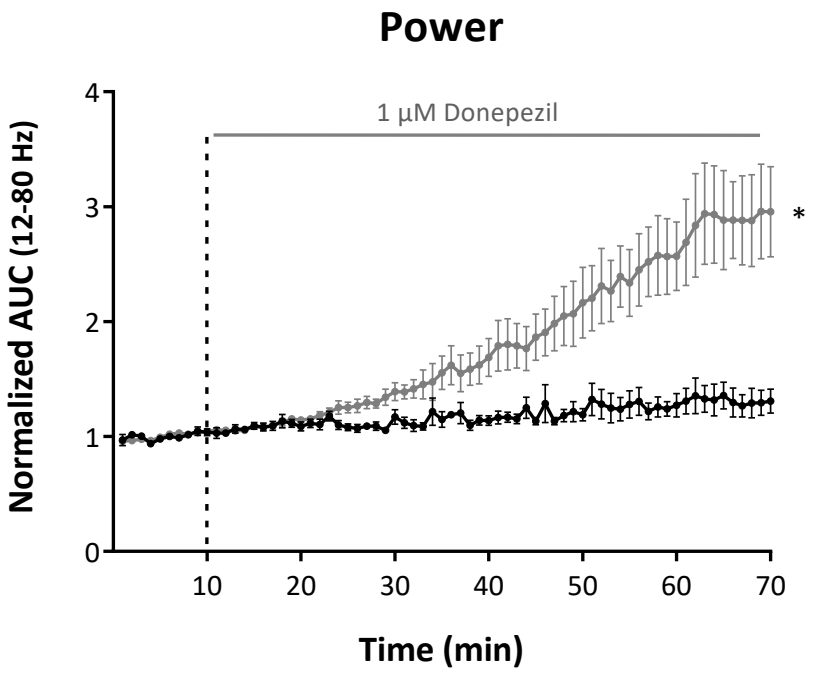
Kainate induces oscillations by providing tonic excitation via ionotropic glutamate receptors activation to both excitatory and inhibitory neurons in the hippocampus (Clarke et al., 1997; Fisahn et al., 2004). Network oscillations are induced in both rat and mouse hippocampal slices with maximal effect reached at 100 nM concentration.

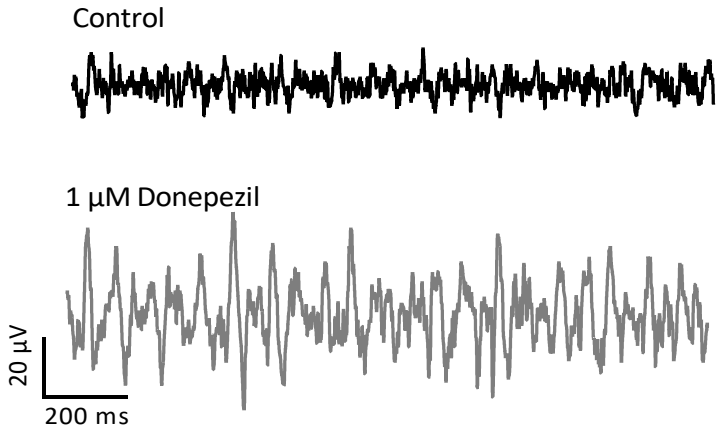
neuroservices ALLIANCE

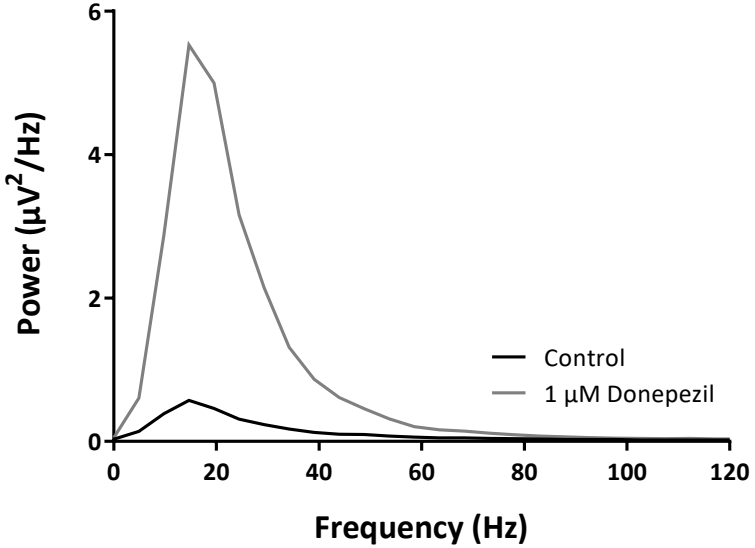
NETWORK OSCILLATIONS

In vitro models (4/4)

Donepezil-induced oscillations







Donepezil – an acetylcholinesterase inhibitor approved for symptomatic treatment of patients with mild-moderate AD – is capable of inducing network oscillations. The power of donepezil-induced oscillations continuously increased and did not reach stability after one hour of 1 µM donepezil exposure.

neuroservices ALLIANCE

NETWORK OSCILLATIONS

From *In vitro* to *in vivo* (1/2)

Mechanisms

- Gamma oscillations can be recorded both *in vivo* using Electro-encephalogram (EEG) or field potentials recordings and *in vitro* using extracellular recordings. Both *in vitro* models (carbachol and kainate) are based on the phasic GABAergic inhibition onto pyramidal cells to generate gamma oscillations. The genesis of gamma oscillation *in vivo* is also based on phasic GABAergic inhibition. Moreover, Basket cells fire action potentials that are phase-locked to gamma oscillations and play a critical role in the oscillations genesis both *in vivo* and *in vitro*. To summarize, gamma oscillations *in vivo* and *in vitro* mechanisms are quite similar and based on phasic GABAergic inhibition primarily mediated by parvalbumin-positive basket cells.

Temperature susceptibility

- While gamma oscillations *in vivo* display a peak frequency of around 30-40 Hz, it is slightly lower *in vitro* as the frequency is very susceptible to temperature (Lu et al., 2012). Since recording for a long period of time at physiological temperatures causes cell death, recording temperature is kept at 32° C. As the consequence the predominant frequency of gamma oscillations recorded *in vitro* is shifted to around 20 Hz.

neuroservices ALLIANCE

NETWORK OSCILLATIONS

From *In vitro* to *in vivo* (2/2)

Consistent results between *in vivo* and *in vitro*

- **NMDA antagonists:** in accordance with literature, ketamine and MK-801 increase the power of gamma oscillations induced by either kainate or carbachol (Neuroservice data). *In vivo*, ketamine and MK-801 increase the power of cortical oscillations in the gamma range in rodent and humans (Hakami et al., 2009 ; Kocsis et al., 2012 ; Hiyoshi et al., 2014, Sanacora et al., 2014).

- **Antipsychotic drugs:** *In vitro*, clozapine – serotoninergic & dopaminergic antagonist – and haloperidol – dopaminergic antagonist – decrease the power of oscillations induced by kainate (Neuroservice data). *In vivo*, clozapine and haloperidol depressed the power of cortical oscillations in the gamma range (Jones et al., 2012).

- **Neurotransmitters**: 5-HT reduces gamma oscillations recorded *in vitro* (Neuroservice data) and *in vivo* in the prefrontal cortex (Puig, 2010). Norepinephrine also decreases gamma oscillations recorded *in vitro* (Neuroservice data) and *in vivo* (Brown et al., 2005) in the hippocampus.

neuroservices ALLIANCE

RESULTS

neuroservices
ALLIANCE

RESULTS

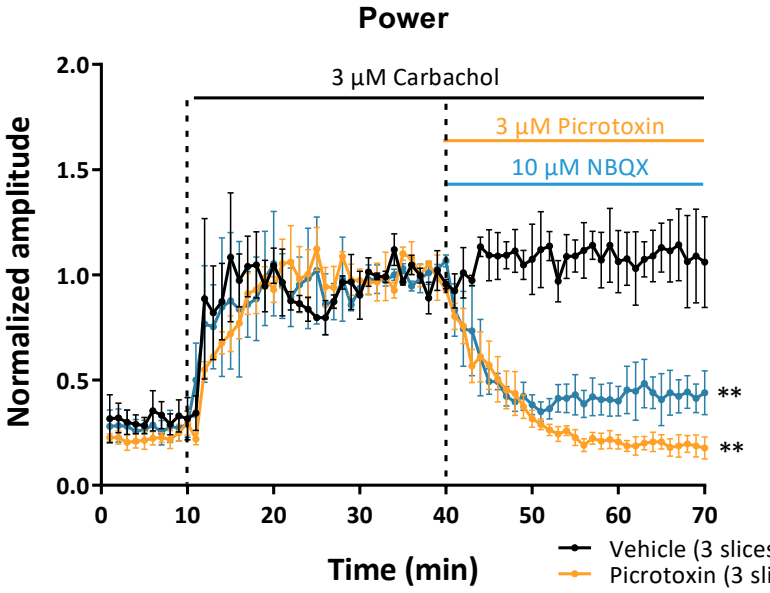
AMPA/Kainate and $GABA_A$ receptors

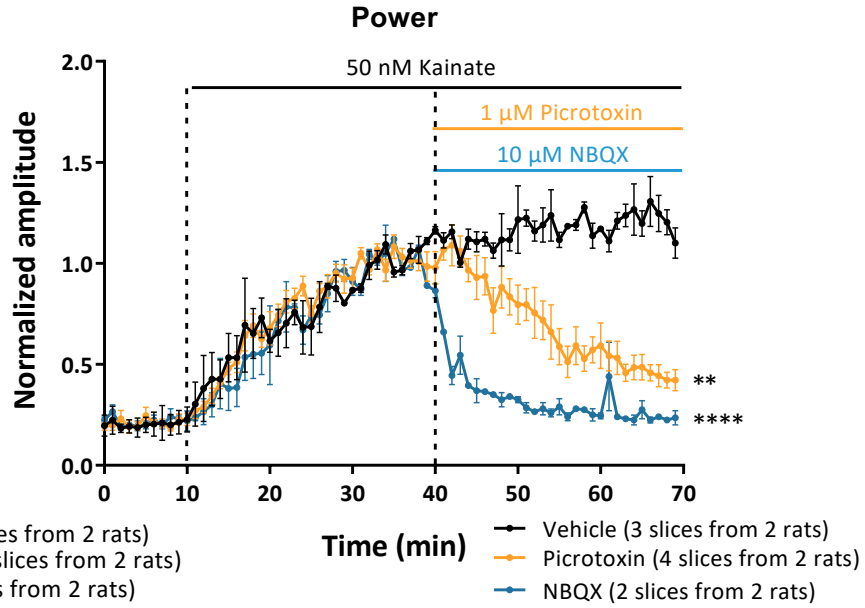
Summary
Materials and methods

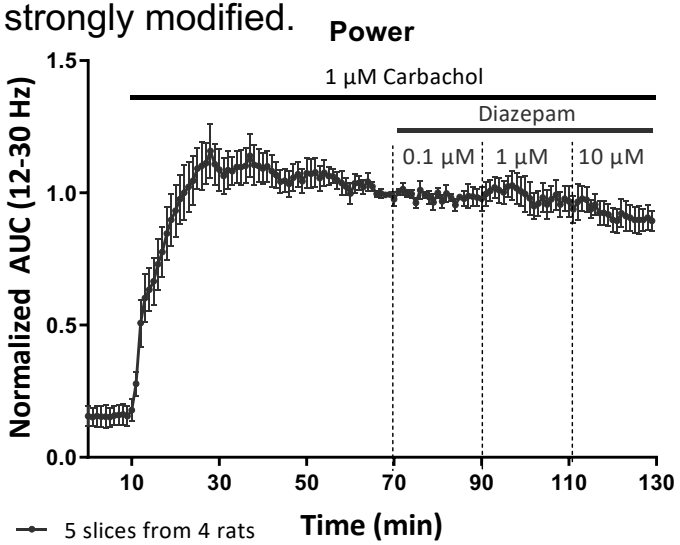
Picrotoxin, NBQX, diazepam

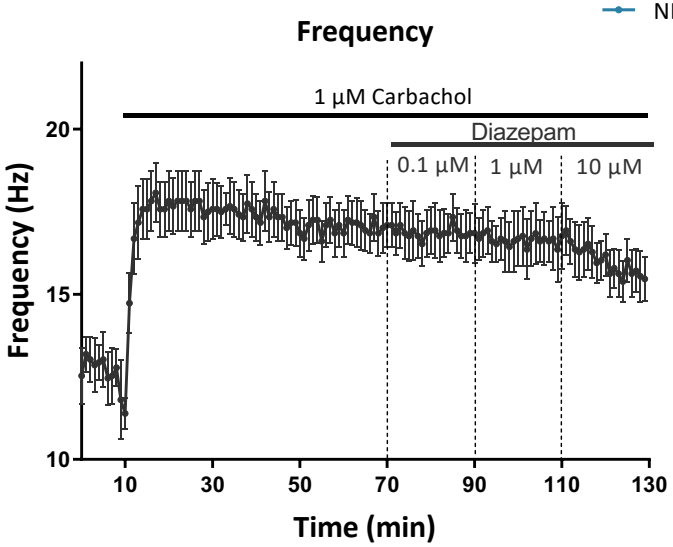
- Picrotoxin strongly inhibited both carbachol- and kainate-induced oscillations indicating a requirement of $GABA_A$-mediated inhibition within the neuronal network to observe oscillations. NBQX strongly inhibited both carbachol- and kainate-induced oscillations indicating the importance of AMPA/Kainate mediated transmission in this process.

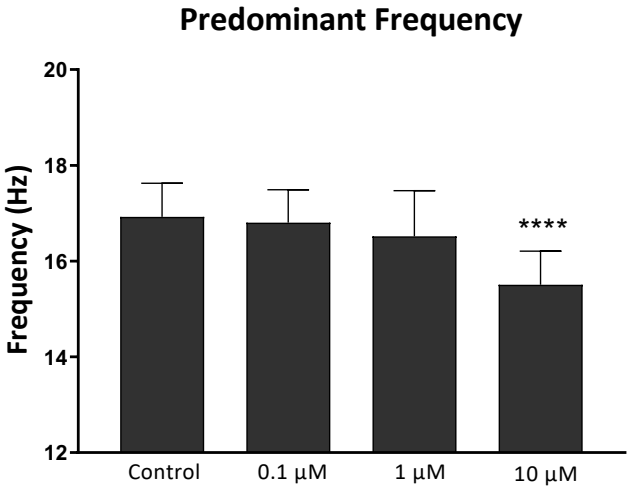
- 10 µM diazepam application reduced the frequency of network oscillations. The power of network oscillations was not strongly modified.









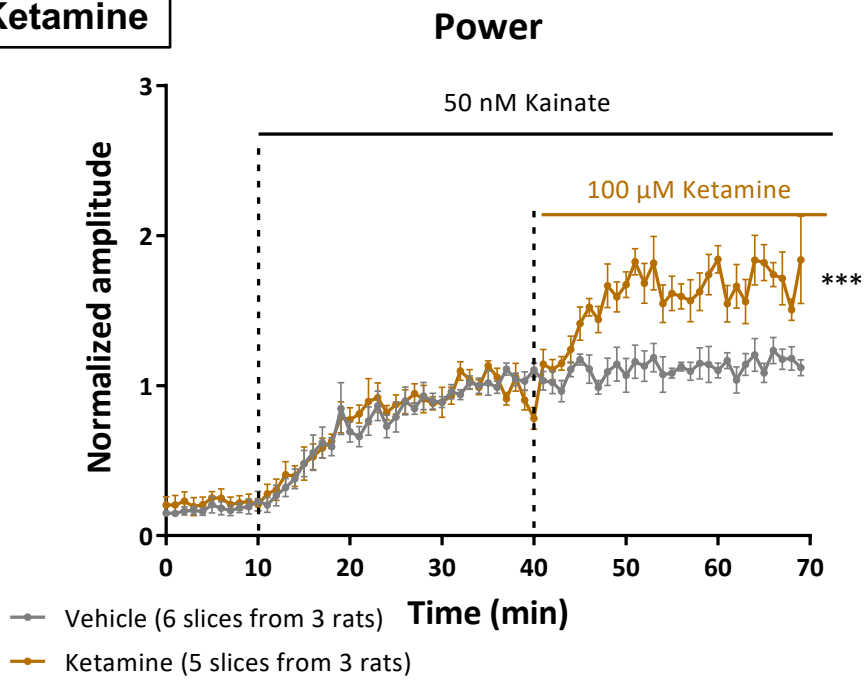


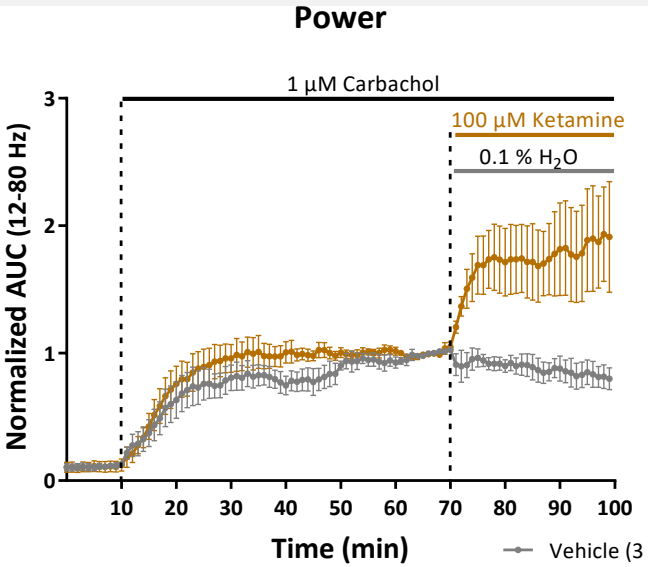
RESULTS

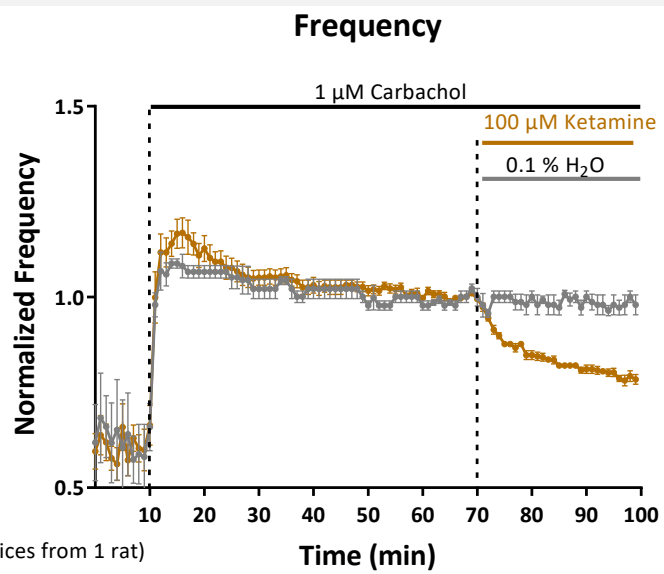
NMDA receptors(1/2)

Summary
Materials and methods

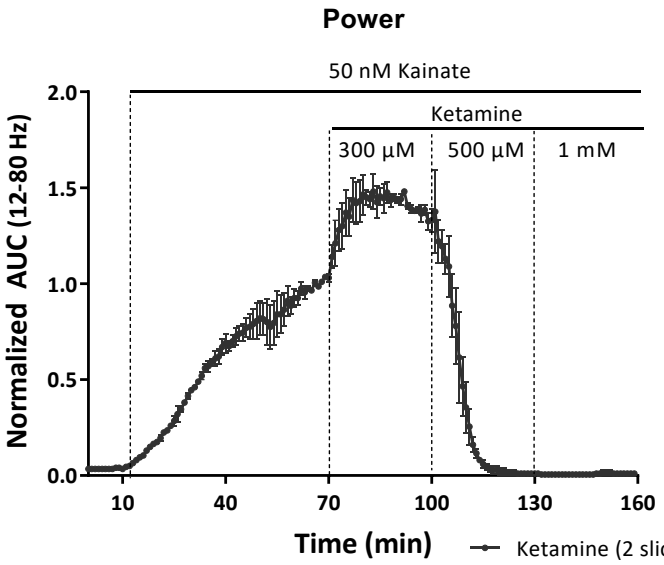
Ketamine

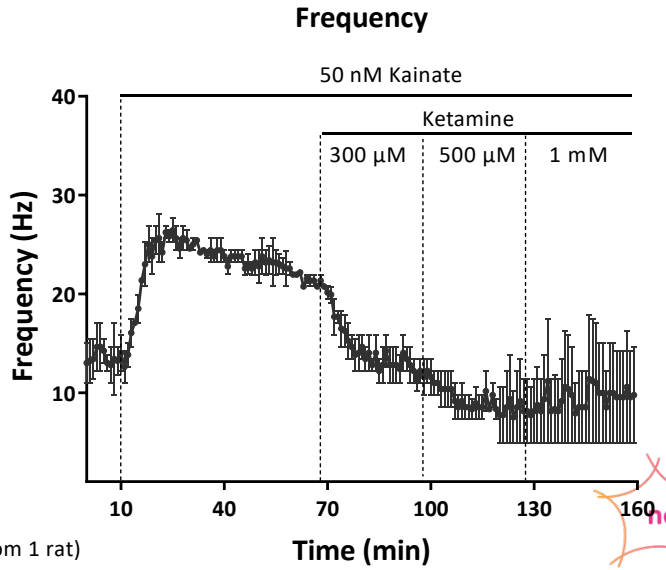






Vehicle (3 slices from 1 rat)
Ketamine (4 slices from 1 rat)





Ketamine (2 slices from 1 rat)

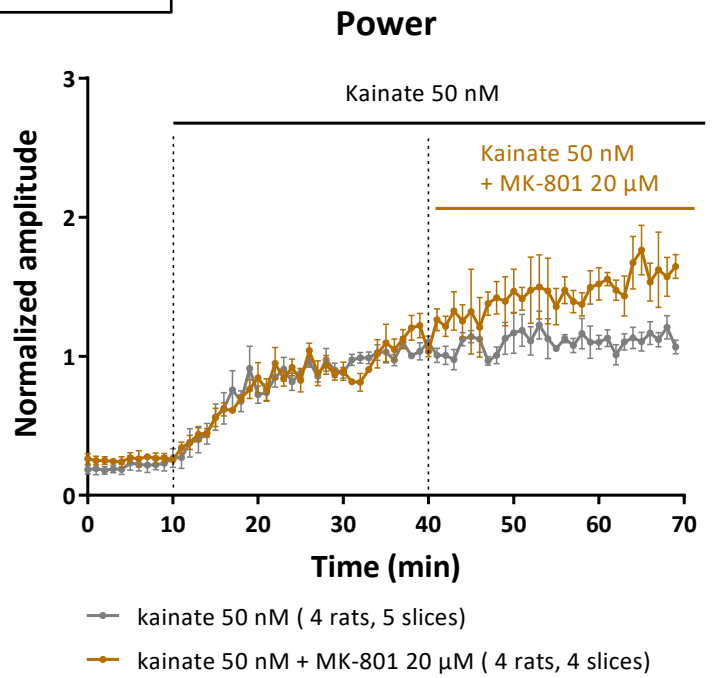
- Ketamine – a NMDA receptor antagonist – applied at 100 µM concentration increased the power and decreased the frequency of kainate and carbachol-induced oscillations. From 500 µM concentration, ketamine abolished gamma oscillations.

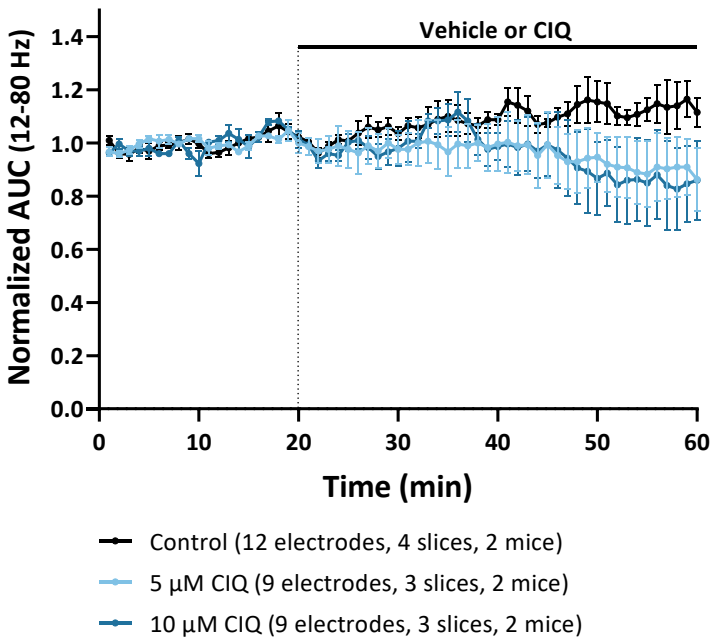
RESULTS

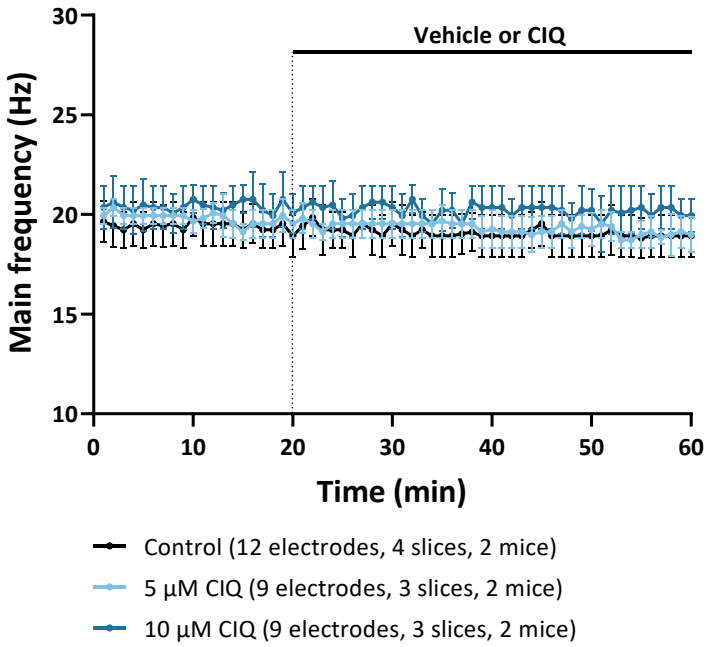
NMDA receptors (2/2)

Summary
Materials and methods

MK-801, CIQ







- The MK-801 – a NMDA receptor antagonist – slightly increased the power of network oscillations
- In slices exposed to CIQ – a potentiator of NMDA receptors containing GluN2C/GluN2D - the power and the frequency of oscillations was not modified.

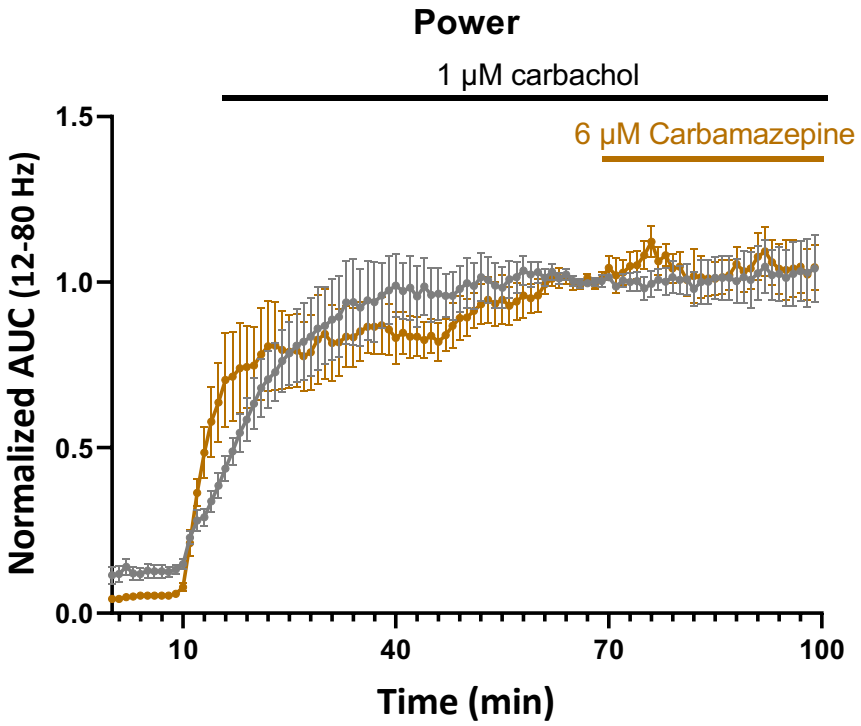
neuroservices ALLIANCE

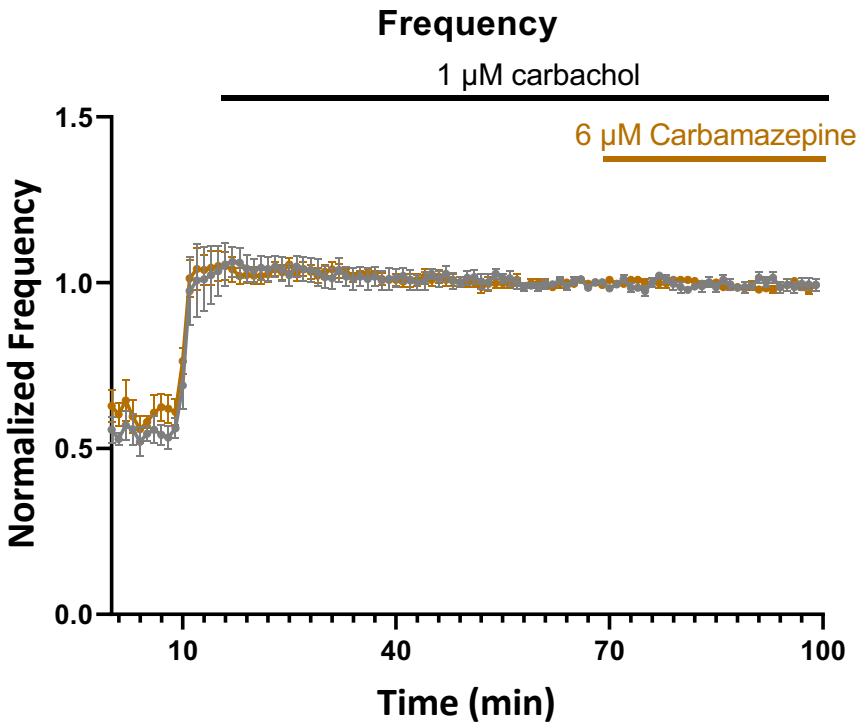
RESULTS

Sodium channels

Summary
Materials and methods

Carbamazepine





Vehicle (6 slices from 2 rats)
Carbamazepine (6 slices from 2 rats)

- Carbamazepine – a sodium channel inhibitor – did not modulate both the power and the frequency of carbachol-induced network oscillations when applied at 6 µM.

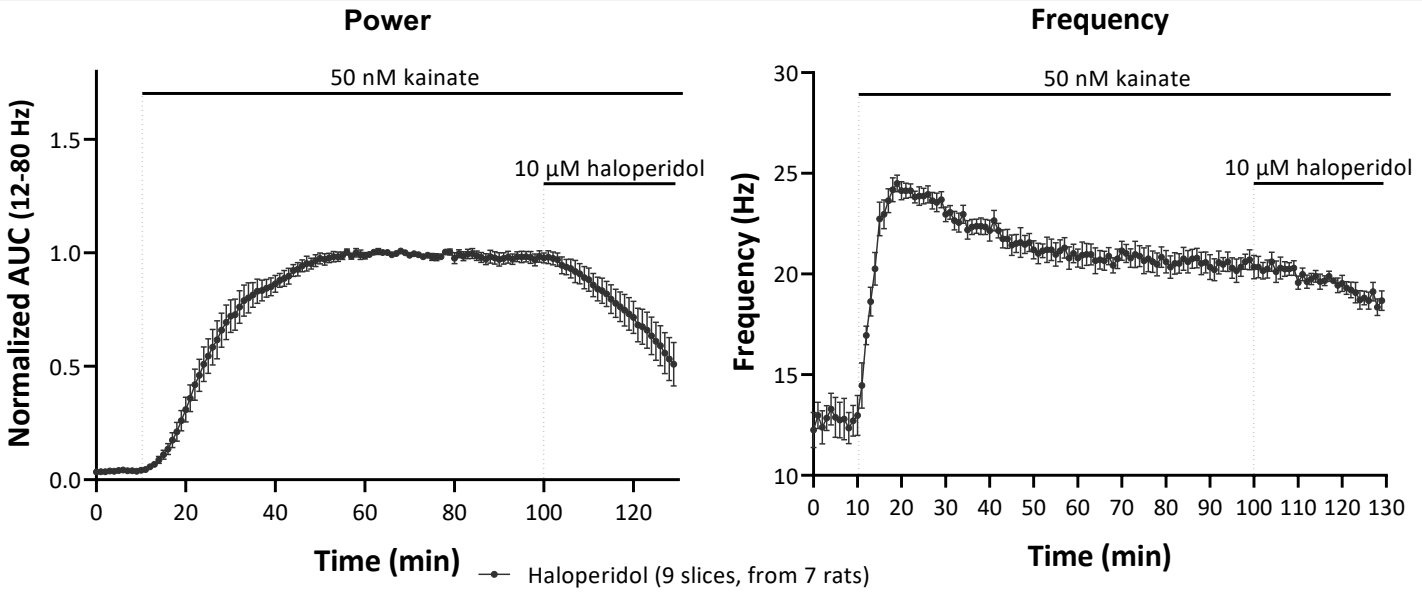
neuroservices ALLIANCE

REFERENCE DATA

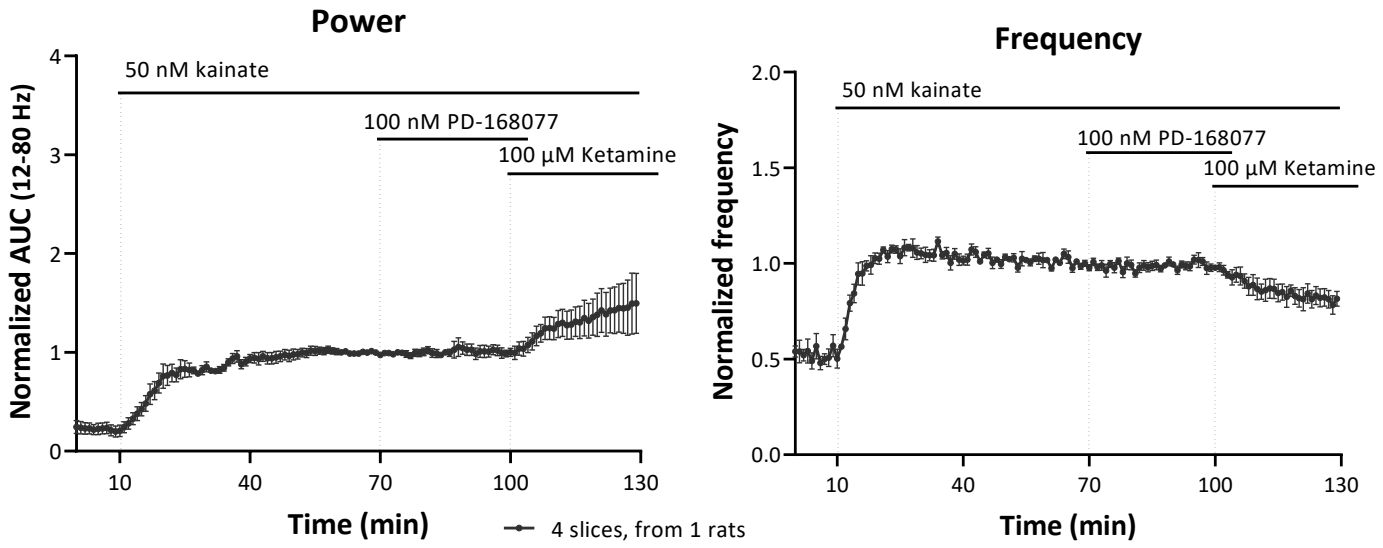
Dopaminergic receptors

Summary
Materials and methods

Haloperidol
PD-168077



- Haloperidol – a dopaminergic receptor antagonist – applied at 10 µM concentration decreased the power and the frequency of oscillations.



- PD-168077 – a dopaminergic receptor D4 agonist – applied at 100 nM concentration did not modify the power and the frequency of network oscillations. As expected, ketamine applied at 100 µM increased the power and decreased the frequency of network oscillations.

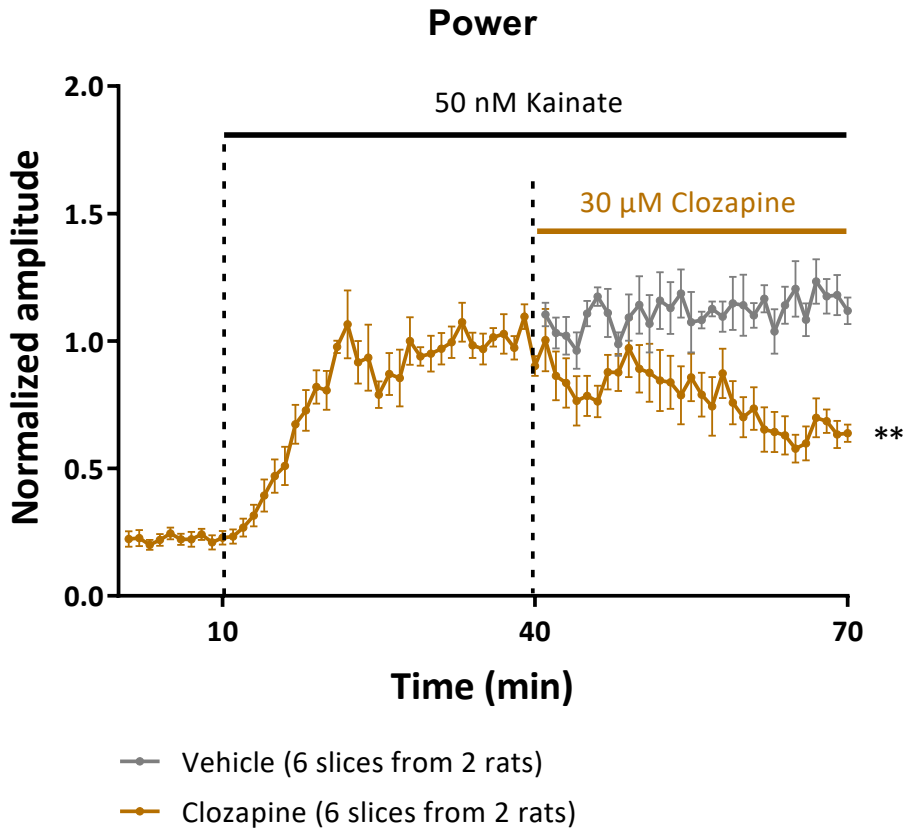
neuroservices ALLIANCE

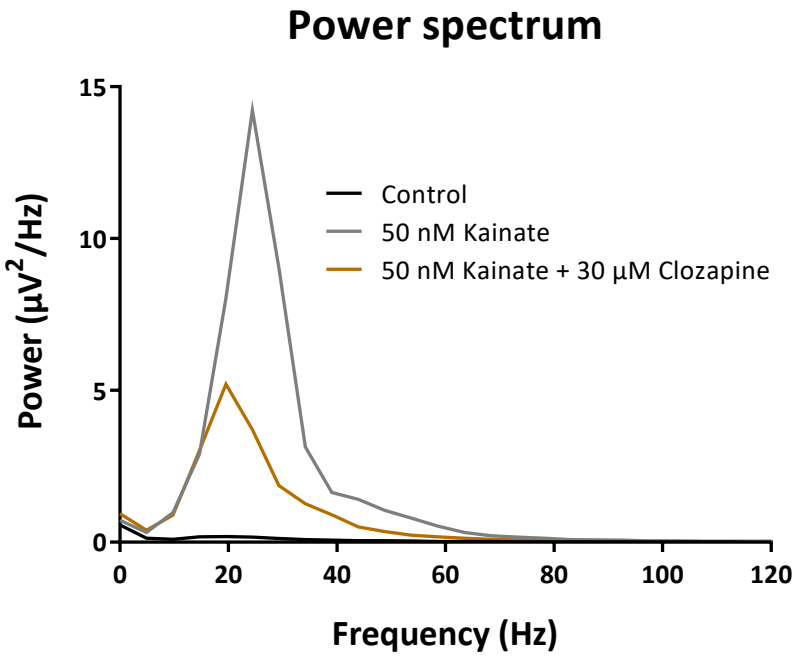
RESULTS

Antipsychotic

Summary
Materials and methods

Clozapine





- Clozapine – a serotonin and dopamine receptors antagonist used as antipsychotic agent – applied at 30 µM concentration decreased the power of network oscillations.

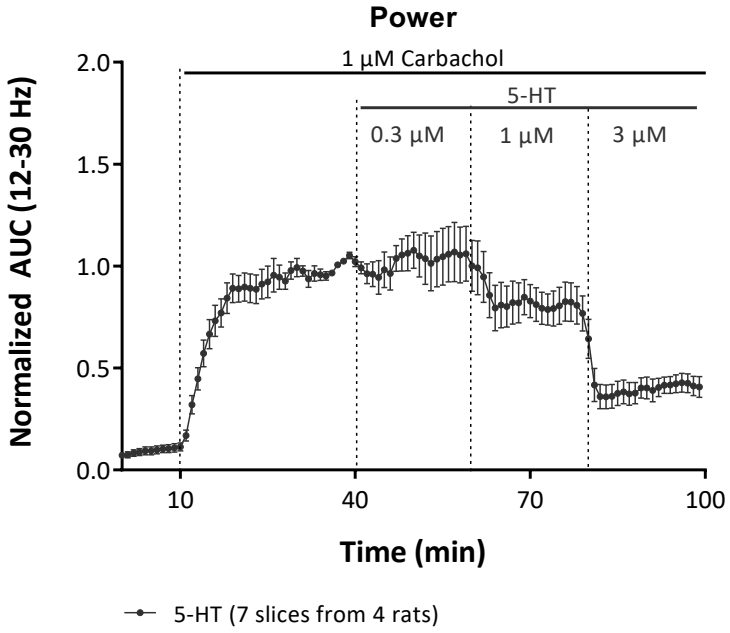
neuroservices ALLIANCE

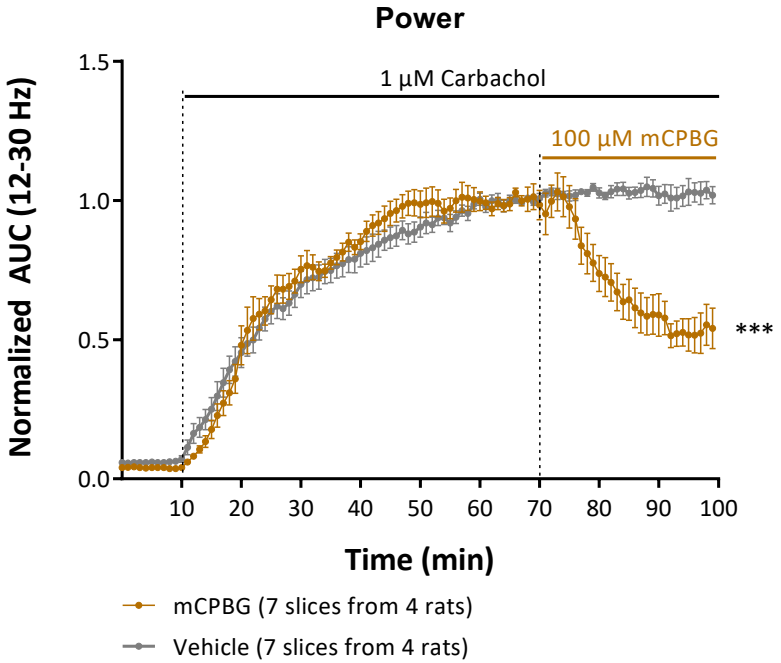
RESULTS

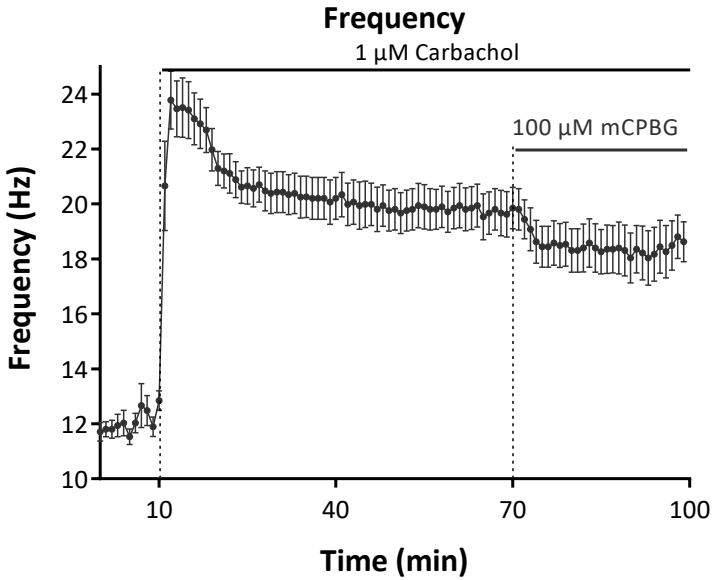
Serotoninergic receptors

Summary
Materials and methods

5 HT, mCPBG







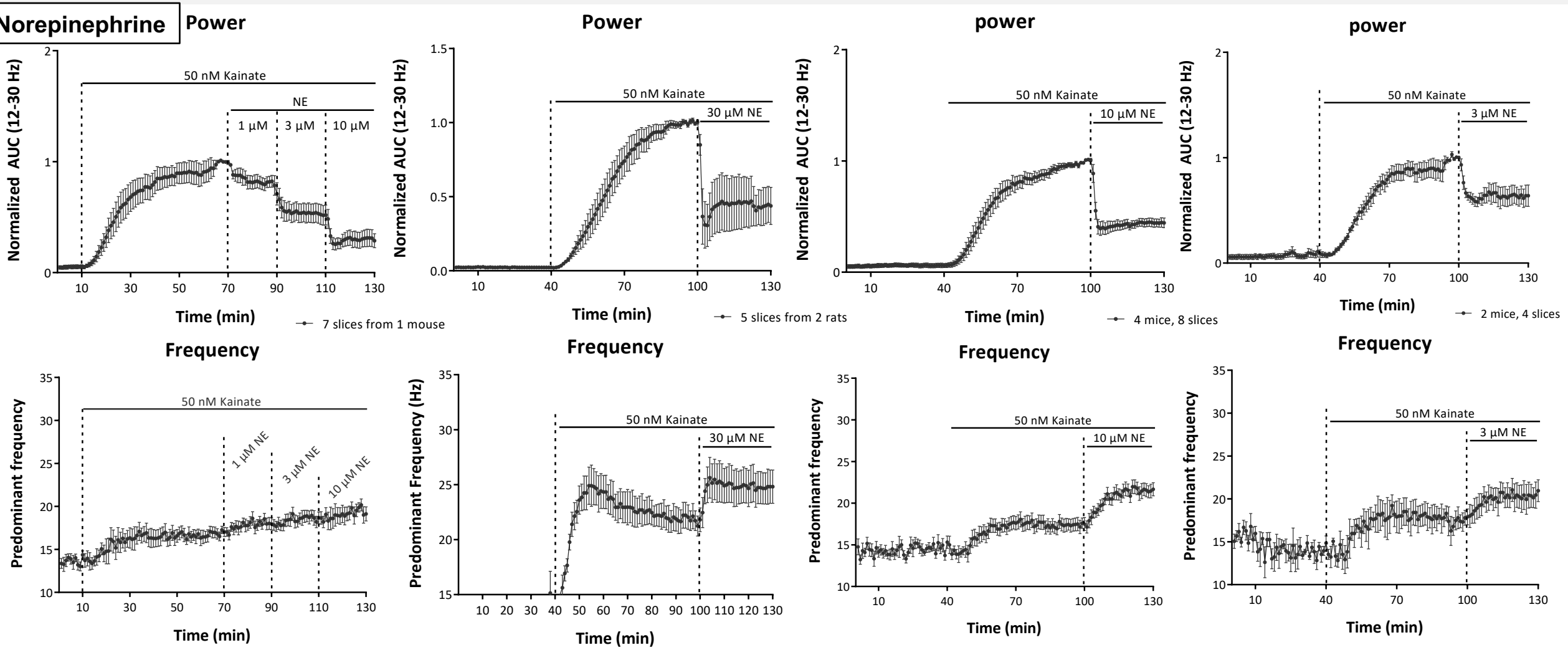
- 5-HT dose-dependently decreased the power of network oscillations. Similarly, mCPBG – a 5-HT_3 agonist – applied at 100 µM concentration decreases the power and the frequency of oscillations .

RESULTS

Adrenergic receptors (1/2)

Summary
Materials and methods

Norepinephrine



- Norepinephrine (NE) decreased the power and increased the frequency of kainate-induced network oscillations in both rat and mouse hippocampal slices.

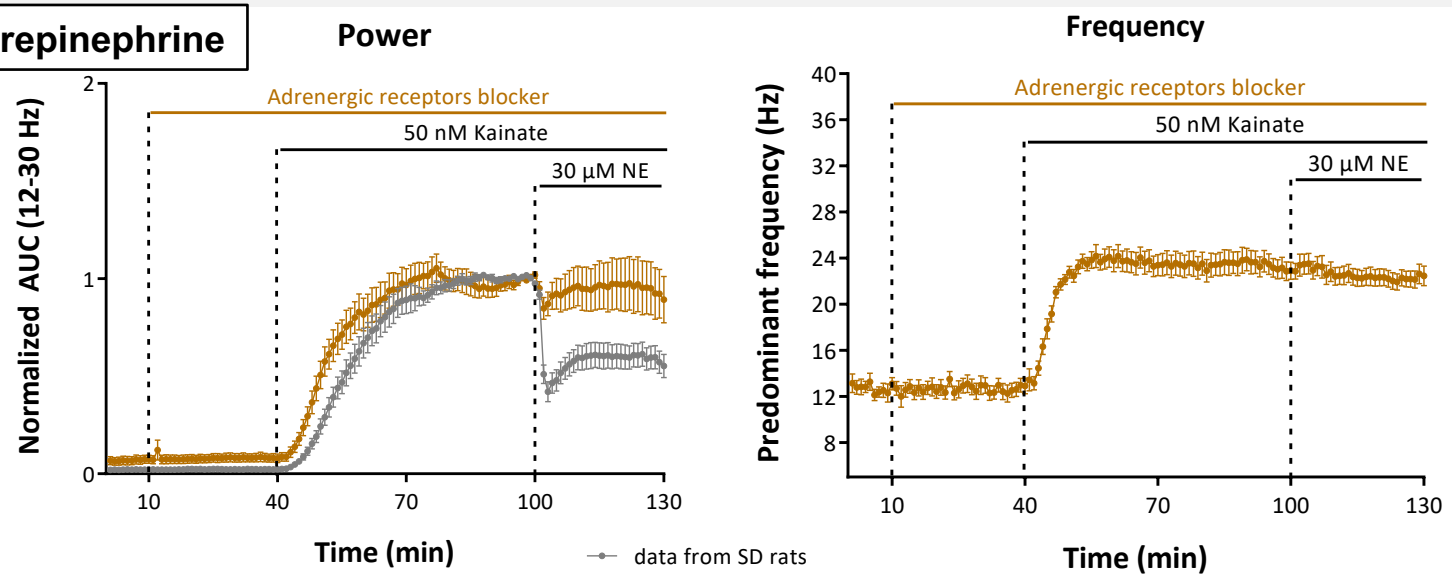
neuroservices ALLIANCE

RESULTS

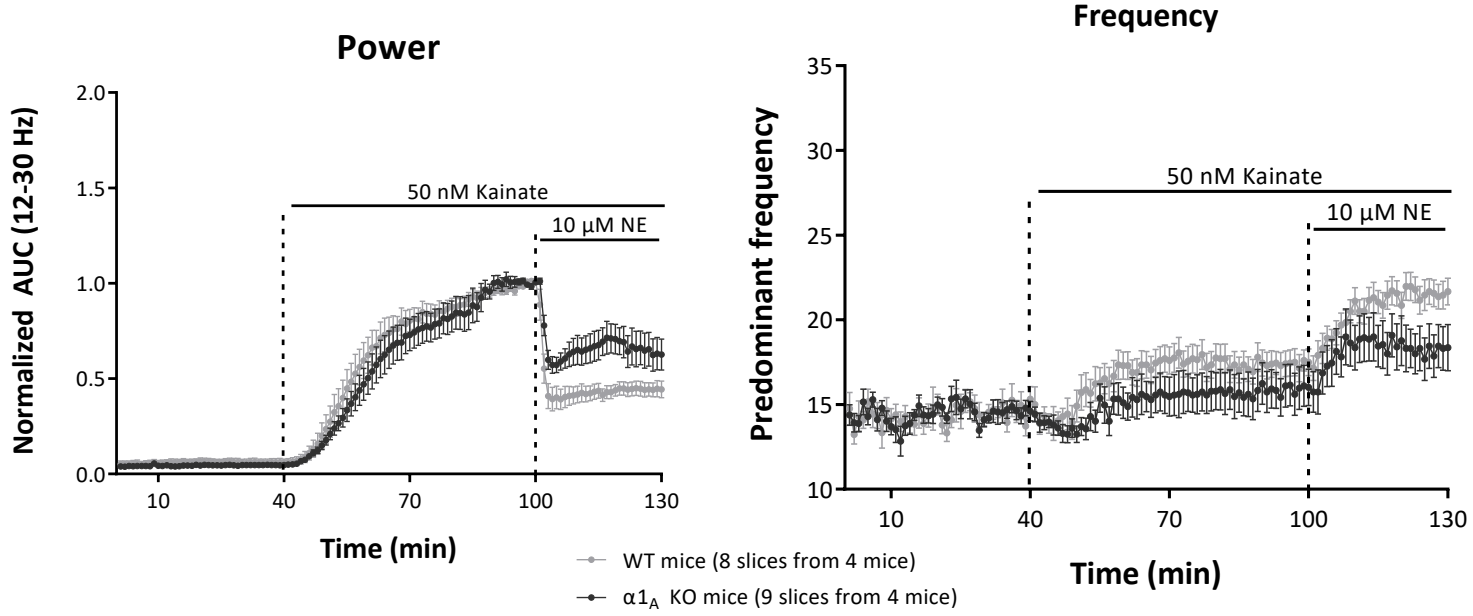
Adrenergic receptors (2/2)

Summary
Materials and methods

Norepinephrine



- In the presence of an Adrenergic receptors blocker, norepinephrine did not substantially modulate kainate-induced network oscillations in rat hippocampal slices.



- In kainate-induced network oscillations from α1A KO mice, the effect of NE was reduced in comparison with the one in WT mice.

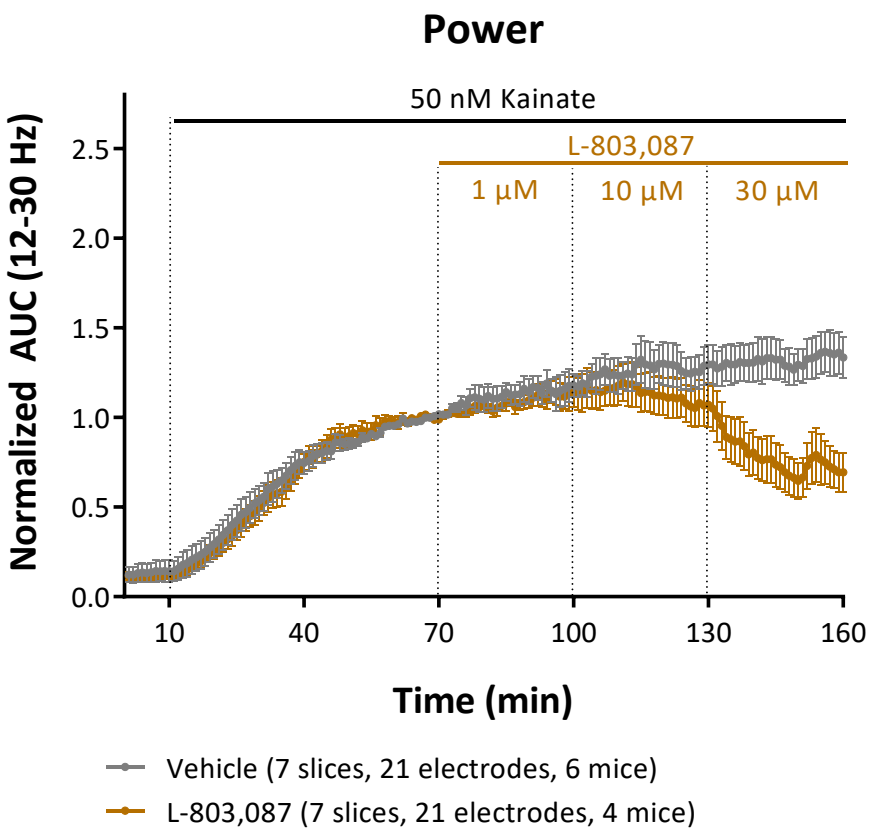
neuroservices ALLIANCE

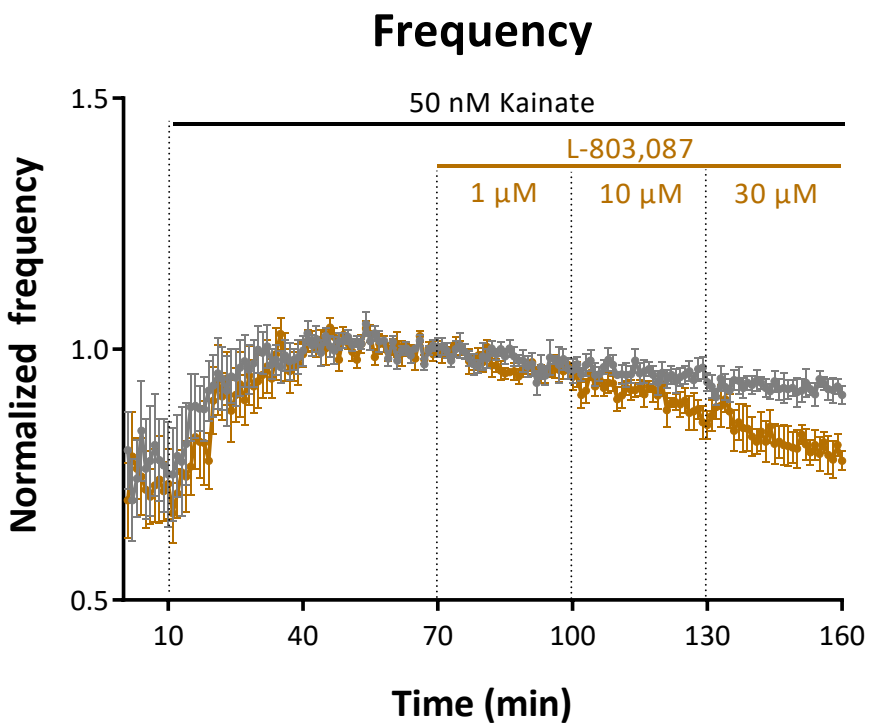
RESULTS

Somatostatin receptors

Summary
Materials and methods

L803,087





- L-803,087 – a potent and selective SST4 agonis t– decreased both the power and the frequency of network oscillations when applied at 30 µM.

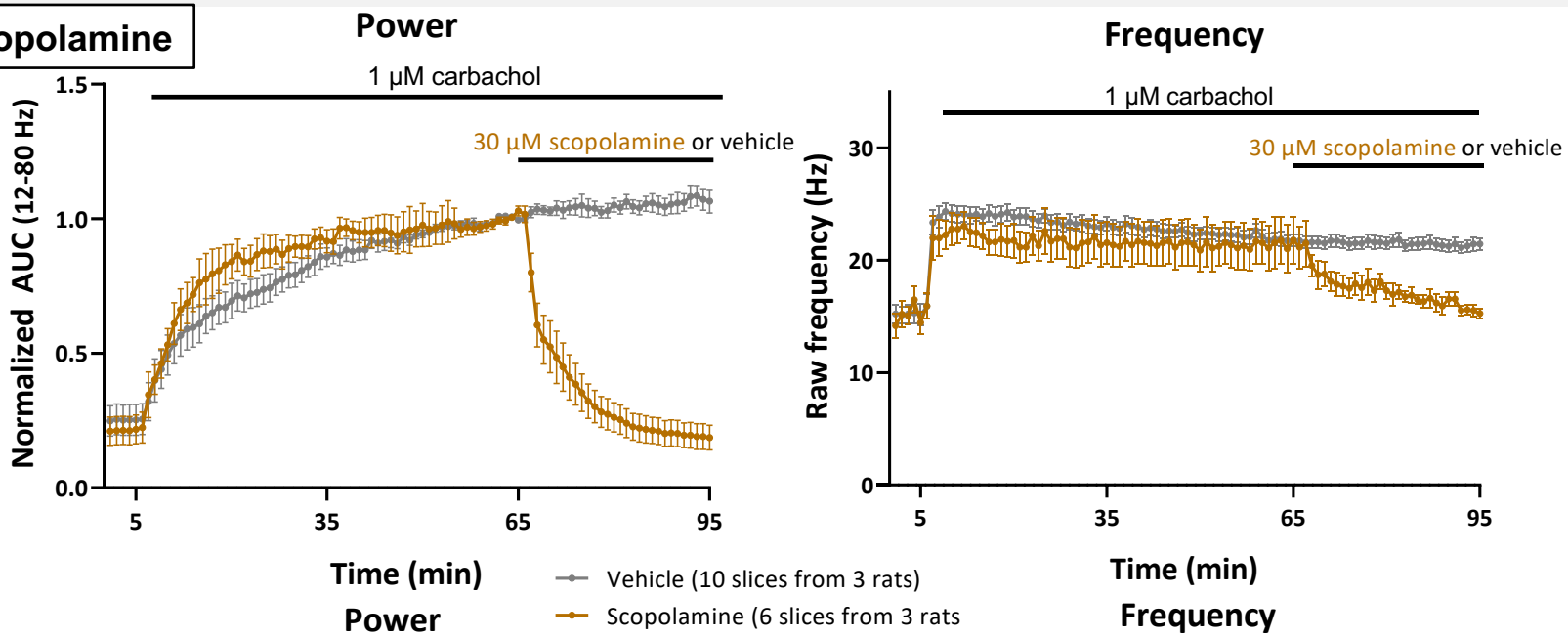
neuroservices ALLIANCE

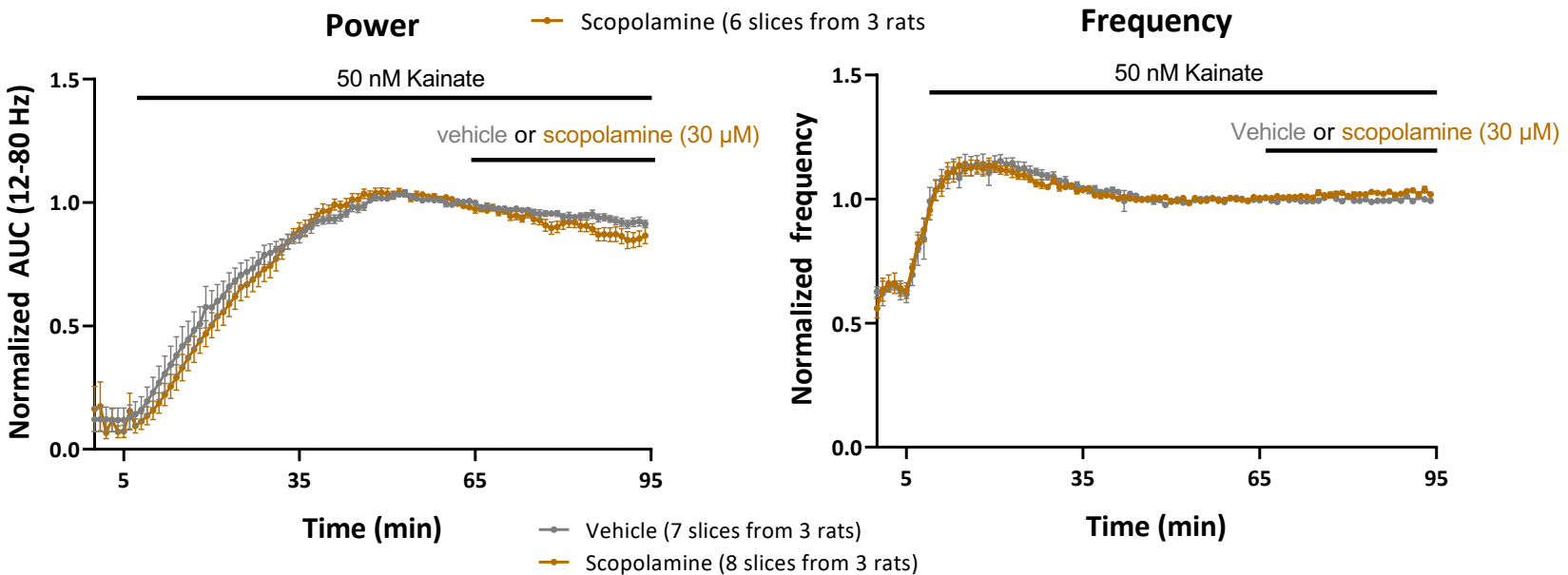
RESULTS

Cholinergic receptors (1/2)

Summary
Materials and methods

Scopolamine





- Scopolamine – a non-selective muscarinic antagonist – blocked the network oscillations induced by carbachol but did not modify oscillations induced by kainate.

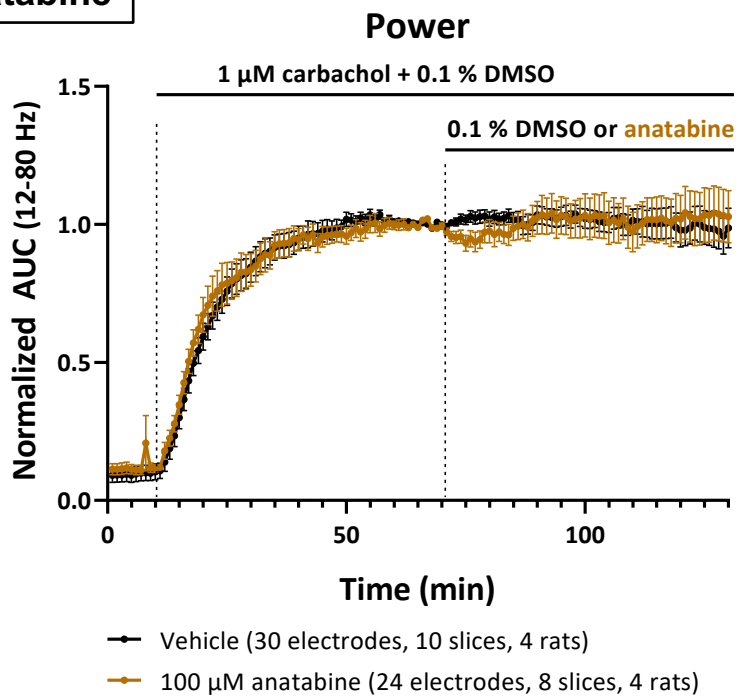
neuroservices ALLIANCE

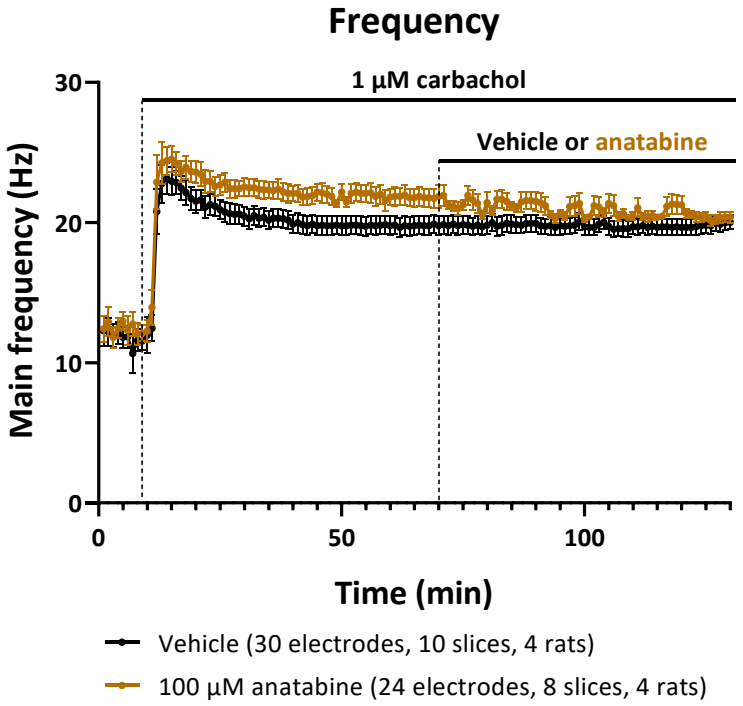
RESULTS

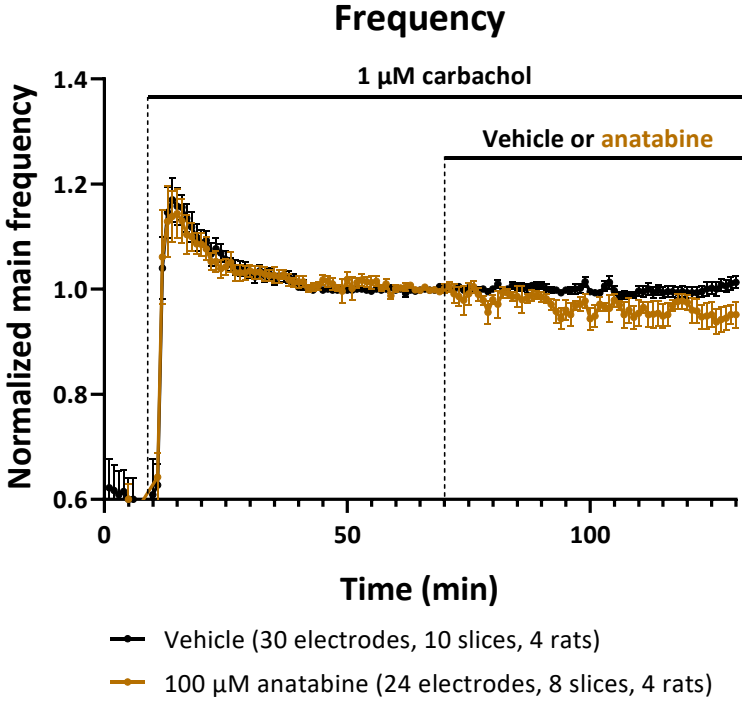
Cholinergic receptors (2/2)

Summary
Materials and methods

Anatabine







- Anatabine – a cholinergic agonist – did not modulated the power of carbachol-induced oscillations but did slightly reduced the frequency.

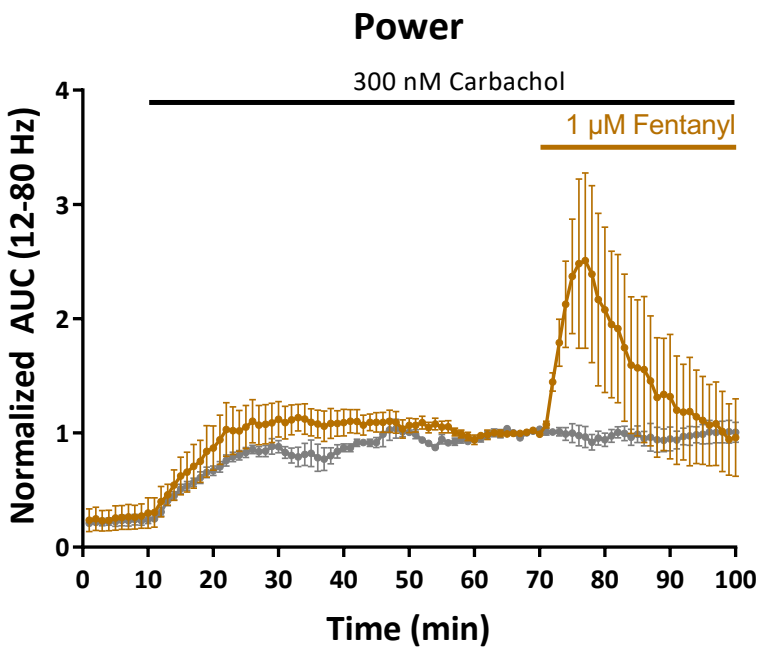
neuroservices ALLIANCE

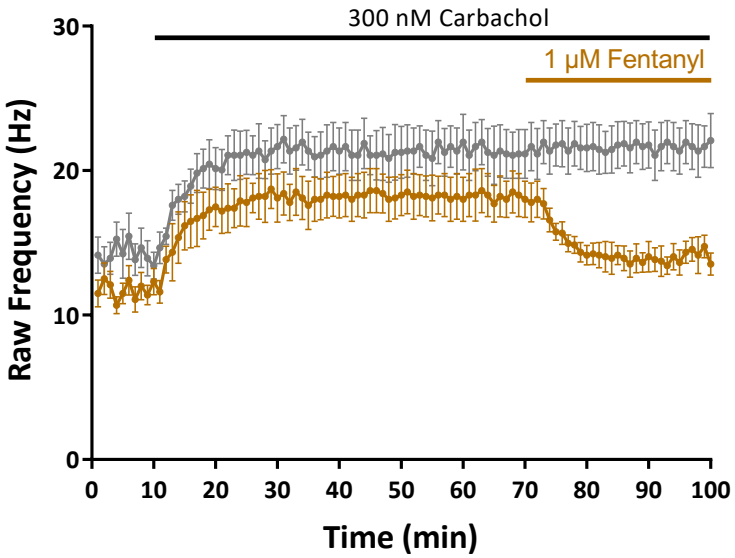
RESULTS

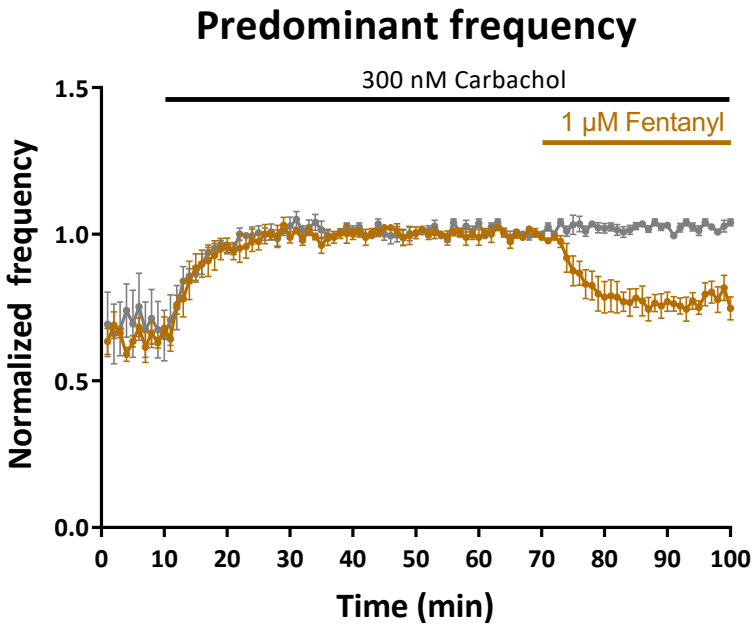
Opioid receptors

Summary
Materials and methods

Fentanyl







Fentanyl (4 slices from 2 rats)
Vehicle (4 slices from 2 rats)

- Fentanyl – a µ-opioid agonist – applied at 1 µM modulated carbachol-induced network oscillations in rat hippocampal slices by transiently increasing their power and decreasing their frequency.

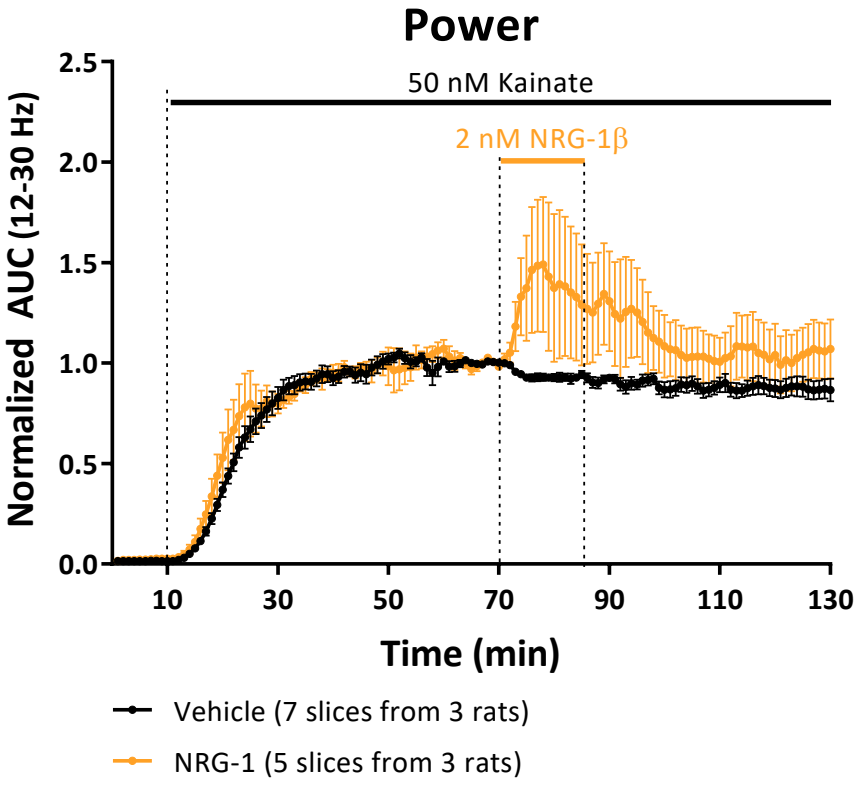
neuroservices ALLIANCE

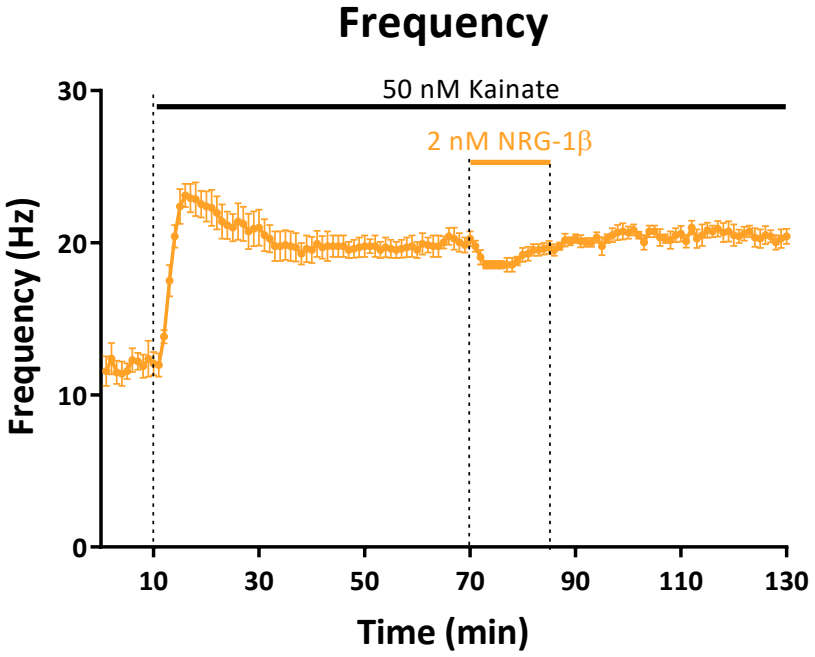
RESULTS

Neuregulin-ERBb4 pathway

Summary
Materials and methods

NRG-1β





- NRG-1β – an epidermal growth factor receptor (EGFR) agonist – increased the power and decreases the frequency of kainate-induced oscillations.

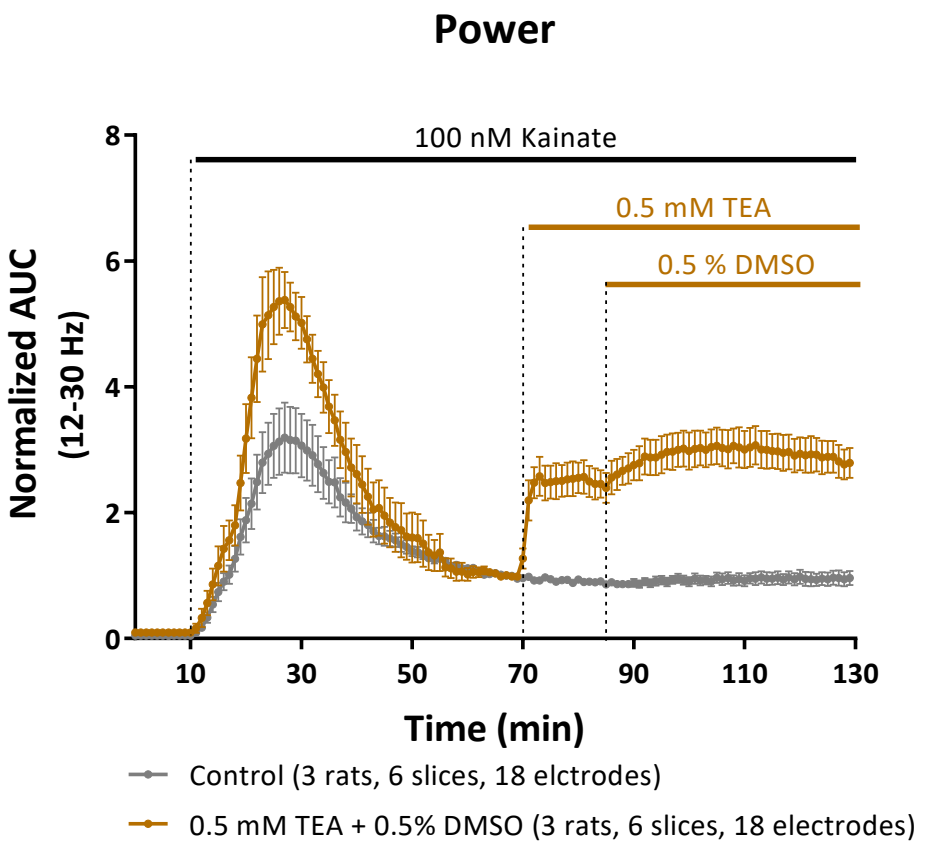
neuroservices ALLIANCE

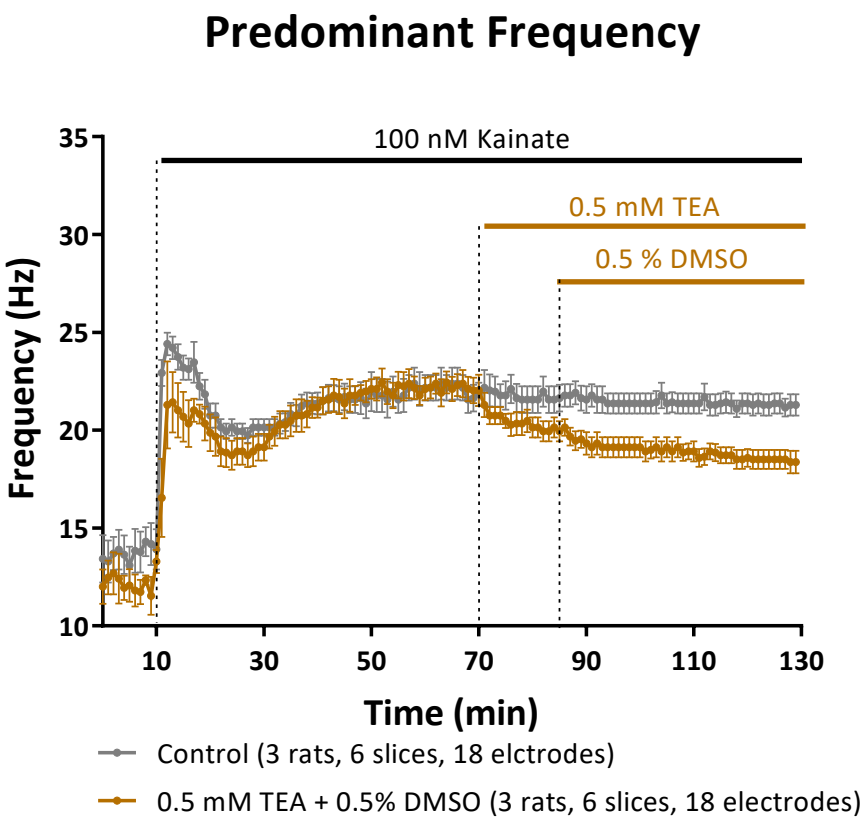
RESULTS

SK channels

Summary
Materials and methods

TEA





- The application of 0.5 mM Tetraethylammonium (TEA) – an inhibitor of Ca^{2+}-activated K^+ (SK) channels - significantly increased the AUC of network oscillations and decreased their frequency.

neuroservices ALLIANCE

Contact us

Raymond Price, PhD, EMBA
Chief Business Officer
Raymond.price@neuroservices-alliance.com
Mobile - +1 (858) 649 9403

Jeffrey Hubbard, PhD
CSO, Brain Slice Electrophysiology
Jeffrey.hubbard@neuroservices-alliance.com
Mobile - +33 442 991 220