In Vitro Recordings of Gamma Oscillations with the Multi-Electrode Array for the Characterization of CNS Drugs

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INTRODUCTION

Translational biomarkers are urgently needed for many CNS Drug Discovery programs. Neuronal network **oscillations in the gamma range** contribute to normal **cognitive function**, whereas disturbances arise in certain **psychiatric disorders**, such as **Schizophrenia**.

Gamma oscillations are routinely recorded in the electro-encephalogram (EEG) of both human subjects and rodents. Earlier studies further suggest that gamma oscillations can be triggered in **acute brain slices** and recorded *in vitro* with glass electrodes.

Using **Multi-Electrode Array (MEA)** recordings, we demonstrate that Kainate (30-300 nM) and Carbachol (1-30 µM) elicit gamma oscillations in acute rat and mouse brain hippocampal slices in a **dose-dependent manner**.

Oscillations in the gamma range are characterized by calculating their **power spectra** (using a Fast Fourier Transform (FFT) algorithm, and focusing within the 20-80 Hz range) and their **amplitudes**. Here we illustrate that many drugs known for their ability to impact oscillations *in vivo* are able to modulate these same oscillations *in vitro*, demonstrating that an MEA-based assay can be a **valuable tool** for the prediction of compound activities.

MATERIAL & METHODS

Preparation of acute hippocampal slices

Experiments were carried out with Sprague-Dawley rats (3-4 week-old) and C57BL/6 mice (8-12 week-old) if specified. Hippocampal slices (350 µm for rats and 400 µm for mice) were cut with a vibratome and allowed to recover for 1.5 h in ACSF of the following composition, in mM: 126 NaCl, 10 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 0.24 ascorbate, 0.8 pyruvate, bubbled with carbogen (95% O_2, 5% CO_2).

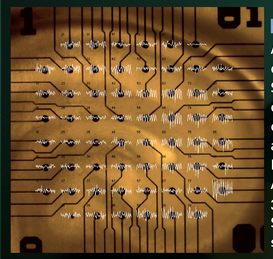


Illustration of network oscillations recorded with a MEA

Multi-Electrode Array set-up

All data were recorded with a MEA set-up commercially available from MultiChannel Systems (MCS GmbH, Reutlingen, Germany) composed of a 60-channel amplifier head-stage connected to a 60-channel A/D card. Recordings and on-line analysis are performed with the MC Rack software (MCS).

All of the experiments were carried out with 3-dimensional MEA (Qwane S.A., Lausanne, Switzerland) that consists of 60 electrodes spaced 200 µm apart.

Recording protocols

The hippocampal slice was placed on the MEA so that the array lay under the major part of the slice.

The MEA chamber volume was approximately 1 ml and the flow rate was maintained at 6 $ml.min^{-1}$. Data are sampled at 20 kHz and 3s-time slots are recorded every minute.

RESULTS

Chemically-induced gamma oscillations

Figure 1: Both carbachol and kainate trigger gamma oscillations in rat or mouse hippocampal slices.
Bottom traces show typical oscillations recorded after Carbachol (A) or Kainate exposure, in rat (B) and mouse (C) hippocampal slices. The lack of oscillatory activity before compound exposure (control period) is shown on the upper traces.

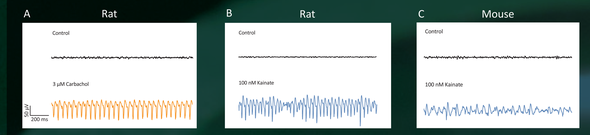


Figure 2: Data processing for determination of gamma oscillation strength.
A: Example of raw recording before (upper trace) and after exposure to the compound X (lower trace) that triggers network oscillations.
B: Power spectra both under control conditions and after compound X exposure, obtained by the mean of FFT algorithm. The hatched area is used to determine the strength of oscillations in the gamma range materialized by the Area Under Curve (AUC).
C: The strength of oscillations in the gamma range is normalized to the reference condition and presented for each condition in bar chart format ± SEM.

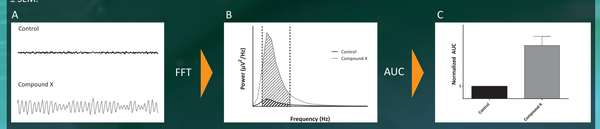
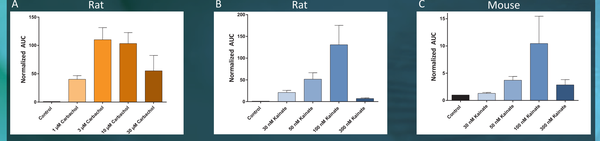
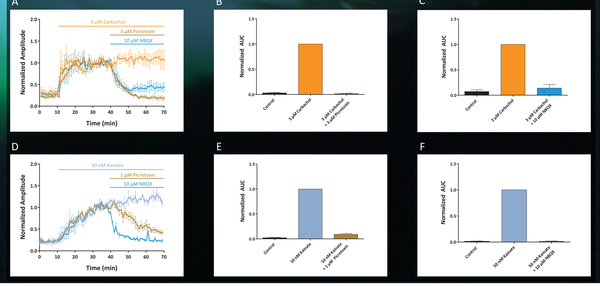


Figure 3: Kainate and carbachol dose-dependently increase gamma oscillation strength.
A: Carbachol induces gamma oscillations at all of the evaluated concentrations, with the maximum effect at 3-10 µM in rat hippocampal slices.
B, C: Kainate triggers gamma oscillations of increasing strength between 30 and 100 nM, whereas they largely decrease above 100 nM in both rat and mouse hippocampal slices.



Gamma oscillations involve both $GABA_A$ and AMPA/kainate receptors

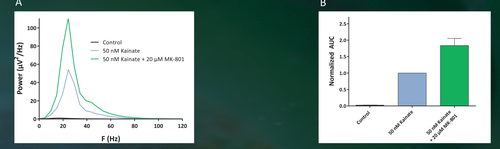
Figure 4: AMPA/kainate and $GABA_A$ receptors play a key role in gamma oscillations.
A,D: Gamma oscillation amplitude, analyzed from 3 electrodes in the CA3 region and normalized to the Carbachol or Kainate reference condition. Picrotoxin strongly inhibits both Carbachol- and Kainate-induced oscillations indicating a requirement of $GABA_A$-mediated inhibition to elicit network oscillations. NBQX strongly inhibits both Carbachol- and Kainate-induced oscillations, thereby highlighting the importance of AMPA/Kainate receptors in the gamma oscillation phenomenon.
B, C: Strength of Carbachol-induced oscillations, before and after exposure to Picrotoxin (B) and NBQX (C).
E, F: Strength of Kainate-induced oscillations, before and after exposure to Picrotoxin (E) and NBQX (F).



Block of NMDA receptors increases the strength of gamma oscillations

Figure 5: Ketamine enhances the strength of gamma oscillations.
A: 50 nM Kainate induces gamma oscillations that are stabilized after a 30-minute exposure, then 100 µM Ketamine (a NMDA receptor antagonist) is co-applied over 30 minutes. Power spectra at the end of each exposure period are presented in the left graph.
B: AUC in the gamma range are normalized to the 50 nM Kainate condition. Gamma oscillations strength is increased by about 50 % after Ketamine exposure.

Figure 6: MK-801 enhances the strength of gamma oscillations.
A: 50 nM Kainate induces gamma oscillations that are stabilized after a 30-minute exposure, then 20 µM MK-801 (a NMDA receptor antagonist) is co-applied over 30 minutes. Power spectra at the end of each exposure period are presented in the left graph.
B: AUC in the gamma range are normalized to the 50 nM Kainate condition. Gamma oscillations strength is increased by about 80 % after MK-801 exposure.



The effect of 20 µM Memantine (a low-affinity NMDA receptor antagonist) was also evaluated under the same conditions. However as Memantine requires a long pre-exposure period to inhibit NMDA receptors, no effect was observed over 30 minutes.

Dopaminergic agonist and antagonist modulate gamma oscillations

Figure 7: The dopaminergic receptor antagonist haloperidol decreases kainate-induced gamma oscillations.
A: 50 nM Kainate induces gamma oscillations that are stabilized after a 30-minute exposure, then 10 µM Haloperidol is co-applied over 30 minutes. Power spectra are calculated at the end of each exposure period.
B: AUC in the gamma range are normalized to the 50 nM Kainate condition. Gamma oscillations strength is decreased by about 70 % after Haloperidol exposure.

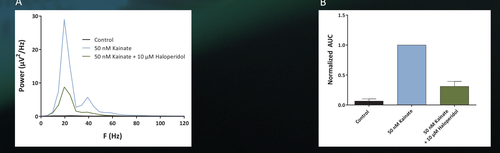
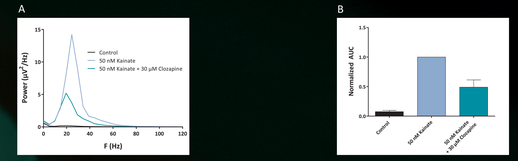


Figure 8: The dopaminergic D4 receptor agonist PD-168077 enhances kainate-induced gamma oscillations.
A: 50 nM Kainate induces gamma oscillations that are stabilized after a 30-minute exposure, then 100 nM PD-168077 is co-applied over 30 minutes. Power spectra are calculated at the end of each exposure period.
B: AUC in the gamma range are normalized to the 50 nM Kainate condition. Gamma oscillations strength is increased by about 110 % after PD-168077 exposure.

Antipsychotics decrease gamma oscillations

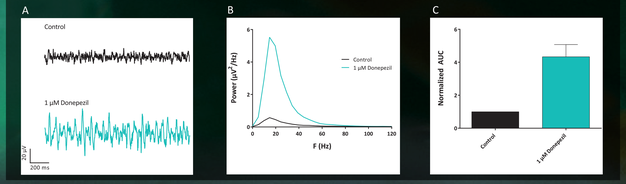
Figure 9: Antipsychotics clozapine and haloperidol both decrease the strength of gamma oscillations.
A: Kainate induces gamma oscillations that are stabilized after a 30-minute exposure, then 30 µM Clozapine (an atypical antipsychotic agent) is co-applied over 30 minutes. Power spectra are calculated at the end of each exposure period.
B: AUC in the gamma range are normalized to the 50 nM Kainate condition. Gamma oscillations strength is increased by about 50 % after Clozapine exposure.



Results obtained with the antipsychotic Haloperidol are presented in figure 7.

Donepezil, an acetylcholinesterase inhibitor, triggers gamma oscillations

Figure 10: Donepezil triggers gamma oscillations.
A: Raw traces before and 60 minutes after 1 µM Donepezil exposure.
B: Power spectra are calculated under control conditions and at the end of the 1 µM Donepezil exposure.
C: AUC in the gamma frequency range are normalized to the control conditions. 1 µM Donepezil clearly induced gamma oscillations, thereby indicating that endogenous acetylcholine is able to trigger network oscillations.



CONCLUSION

- Both GABAergic and glutamatergic transmission play a key role in the generation of gamma oscillations.
- A Dopaminergic D4 receptor agonist increases the strength of gamma oscillations.
- Antipsychotic drugs, such as Clozapine and Haloperidol, significantly reduce gamma oscillation strength *in vitro*, consistent with in vivo observations.
- Endogenous Acetylcholine is sufficient to induce gamma oscillations as revealed with an Acetylcholinesterase inhibitor.
- We have established a robust MEA-based assay to profile the activity of compounds on gamma oscillations. Gamma oscillations may represent a key element in the standard *in vitro* profiling of compounds and may potentially address important functional issues in the CNS with greater predictability.

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