Ketamine effect on gamma oscillations

MATERIAL AND METHODS

Preparation of acute rat hippocampal slices

Experiments were carried out with 3 to 4 week-old Sprague Dawley rats provided by Elevage JANVIER (France). Transversal hippocampal slices were cut in the horizontal plane (450 µm thickness) with a Leica VT1200S vibratome in a ice-cold oxygenated sucrose solution (saccharose 250, glucose 11, $NaHCO_3$ 26, KCl 2, NaH_2PO_4 1.2, $MgCl_2$ 7 and $CaCl_2$ 0.5 in mM). The slices were incubated 1.5 hour at 32° C in aCSF of the following composition: glucose 11, $NaHCO_3$ 25, NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2 in mM. Then, the slices were let to recover for at least 1h.

Slice perfusion and temperature control

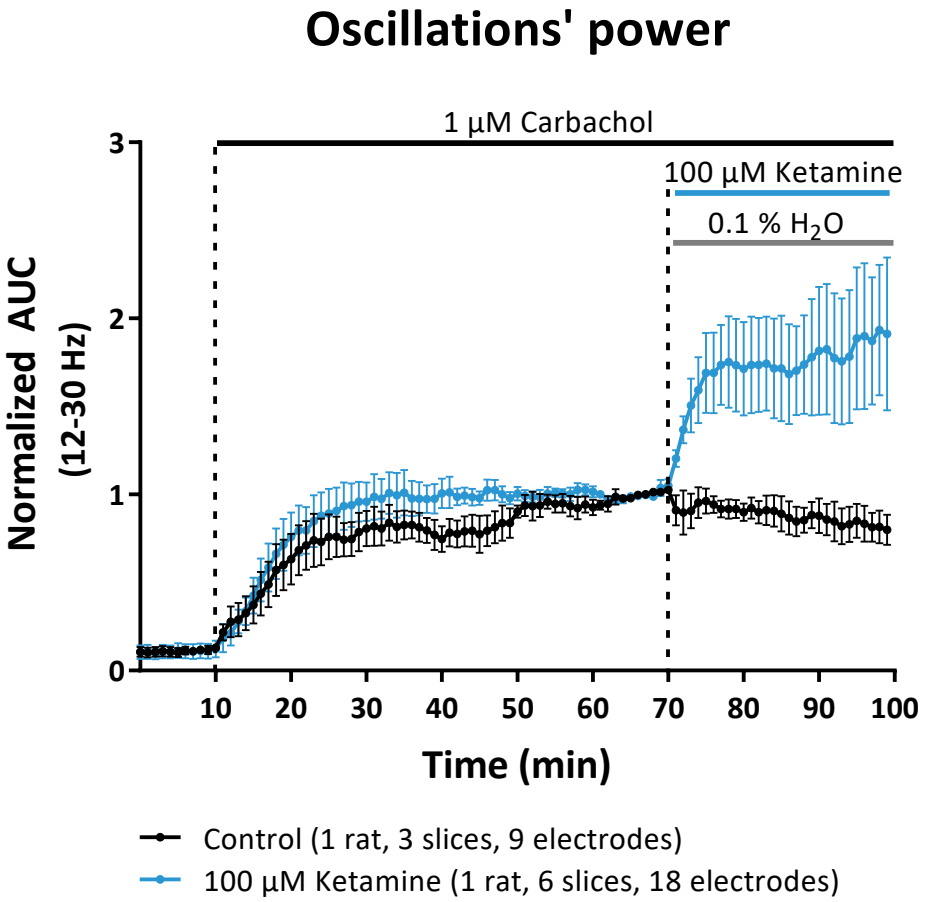
During experiments, the slices were continuously perfused with the aCSF (bubbled with 95% O_2–5% CO_2) at the rate of 6 mL/min with a peristaltic pump (MEA chamber volume: ~1 mL). Complete solution exchange in the MEA chamber was achieved 10 s after the switch of solutions. The perfusion liquid was continuously pre-heated at 32°C just before reaching the MEA chamber with a heated-perfusion cannula (PH01, MultiChannel Systems, Reutlingen, Germany). The temperature of the MEA chamber was maintained at 32 ± 0.1°C with a heating element located in the MEA amplifier headstage.

Analysis

Power spectra were calculated for 60-second-long recording segments using fast Fourier transformation. The area under the curve (AUC) of power spectra were computed for the frequency range including the predominant frequency of network oscillations i.e. between 12-30 Hz). The AUC were normalized to the AUC at the end of the kainate or carbachol-exposure period (t = 65-70 min), and represented (± EM) as a function of time. The predominant frequency of network oscillations was determined according to the maximum power value of power spectra and represented (± SEM) as a function of time

RESULTS

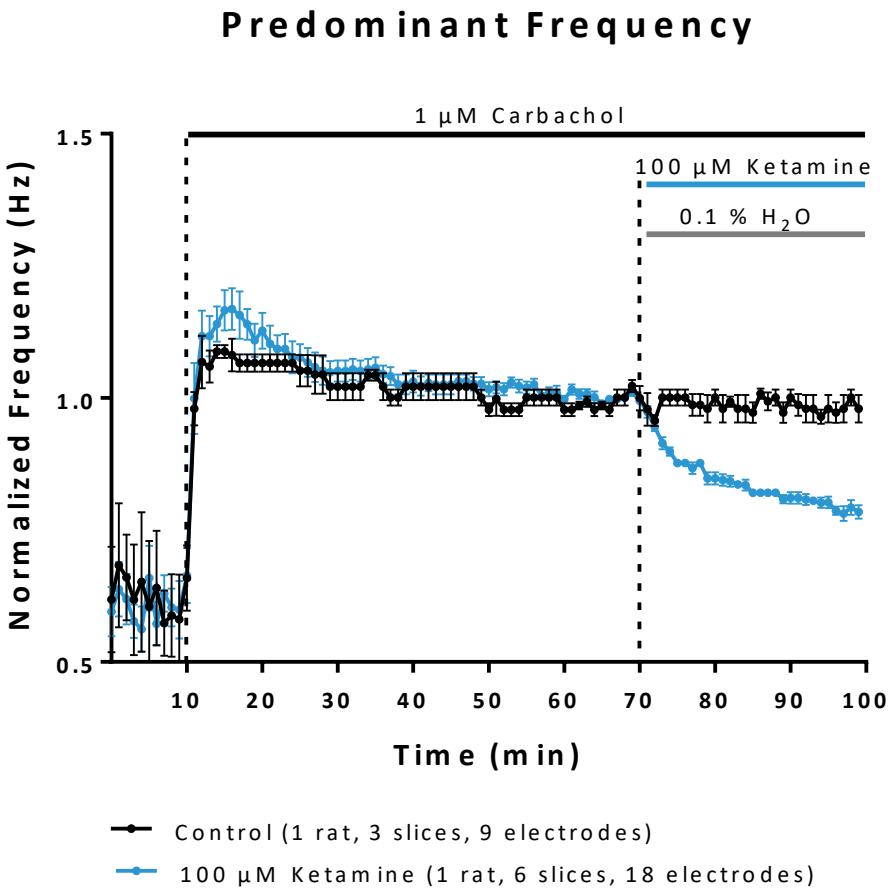
Evaluation of 100 µM ketamine on carbachol-induced network oscillations



- 1 µM carbachol triggered network oscillations that stabilized after about 30 minutes.
- In control slices (vehicle only, black trace), the strength of carbachol-induced oscillations was not substantially modified over the last 30 minutes of recording (a slight decrease of the normalized AUC was observed, it was 0.80 ± 0.09 at t = 100 minutes. However, please note that data were averaged from 3 slices only).
- In ketamine-exposed slices (blue trace), the strength of oscillations was almost doubled over the 30-minute exposure to 100 µM ketamine. Thus, the normalized AUC was 1.79 ± 0.37 at t = 100 minutes.

RESULTS

Evaluation of 100 µM ketamine on carbachol-induced network oscillations



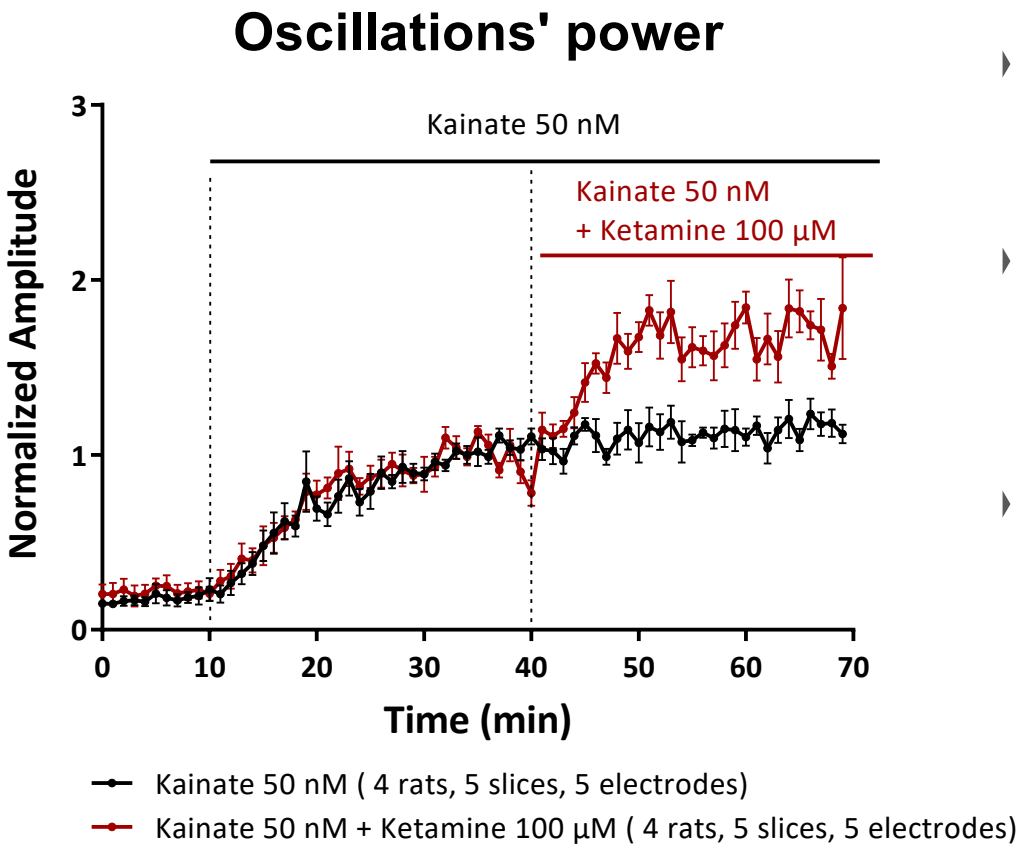
- In control slices (vehicle only, black trace), predominant frequency rapidly increased after carbachol exposure and remained stable until the end of experiment (the predominant frequency at t = 70 min, 19.12 Hz, was close to the one at endpoint, 18.31 Hz).

- Exposure to 100 µM ketamine decreased the predominant frequency of carbachol-induced oscillations by about 20%. Indeed, the predominant frequency at endpoint, 17.09 Hz, was much lower than the one at t = 70 min, 22.04 Hz).

Due to the low sample of slices for control conditions, the predominant frequency over Carbachol exposure was not equivalent for control slices and those exposed to 100 µM Ketamine afterward. Thus, the predominant frequency has been normalized (at t = 70 min) to compare the steadiness of this parameter in control and compound-exposed slices, and highlight the effect of Ketamine.

RESULTS

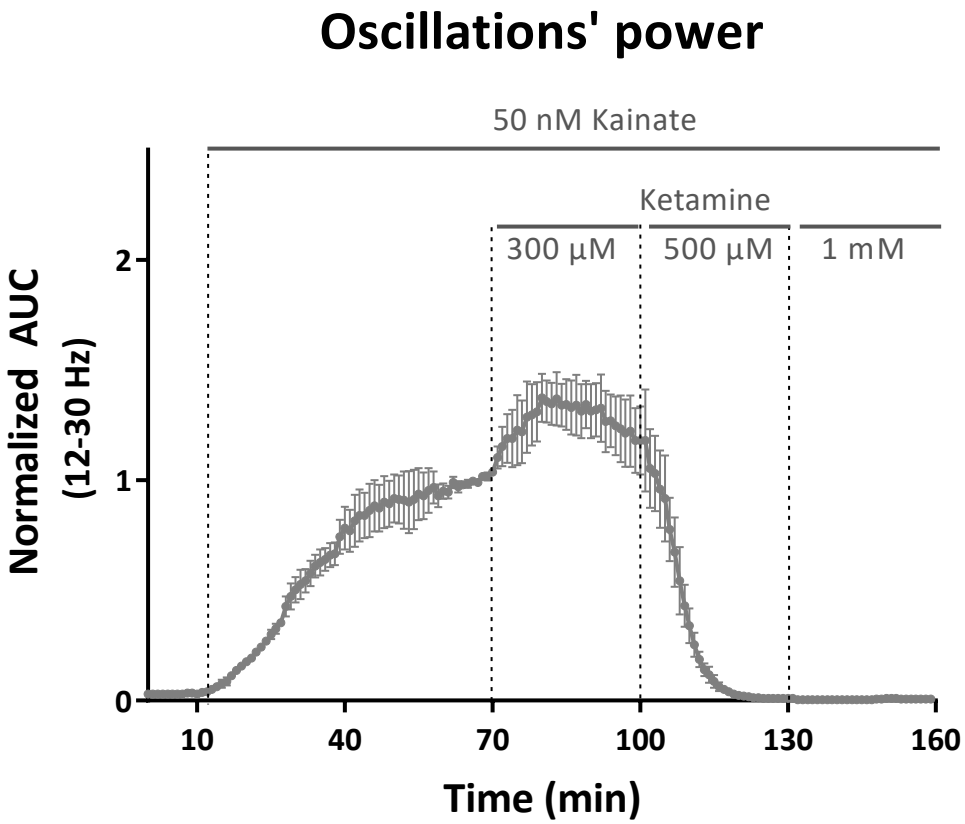
Evaluation of 100 µM ketamine on kainate-induced network oscillations



- The NMDA receptors antagonist ketamine (100 µM) clearly enhances the kainate-induced oscillations amplitude.
- In control slices (vehicle only, black trace), the amplitude of kainate-induced oscillations was not substantially modified over the last 30 minutes of recording and reach 1.12 ± 0.05 a t = 70 minutes.
- In ketamine-exposed slices (red trace), the amplitude of oscillations was almost doubled over the 30-minute exposure to 100 µM Ketamine. Indeed, the normalized AUC reached 1.84 ± 0.29 at t = 70 minutes.

RESULTS

Evaluation of 300, 500 and 1000 µM ketamine on kainate-induced network oscillations



Concentration-range of ketamine (1 rats, 3 slices, 9 electrodes)

- 300 µM ketamine transiently increases the power of network oscillations to reach 1.34 ± 0.11 after 20 minutes of application. Then, the power of network oscillations slightly decreased over the last 10 minutes of 300 µM ketamine application to reach 1.18 ± 0.15 a t = 100 minutes.
- From 500 µM concentrations, the oscillations were rapidly and completely inhibited by Ketamine.

CONCLUSION

- On both kainate and carbachol-induced oscillations, 100 µM ketamine, applied for 30 minutes, strongly increased the strength of network oscillations at the same time it strongly decreased their predominant frequency. Moreover, the ketamine effect appeared to be in the same range in both models of oscillations induction.

- 300 µM ketamine also increased the power of oscillations and decreased their frequency (data not shown). However, this effect started to reverse 20 minutes after the application of 300 µM Ketamine. Then, from 500 µM, Ketamine fully inhibited the network oscillations. These results suggest a bell-shaped-effect of ketamine with a reversal around 300 µM.