In vitro neuronal activity changes under altered gravity

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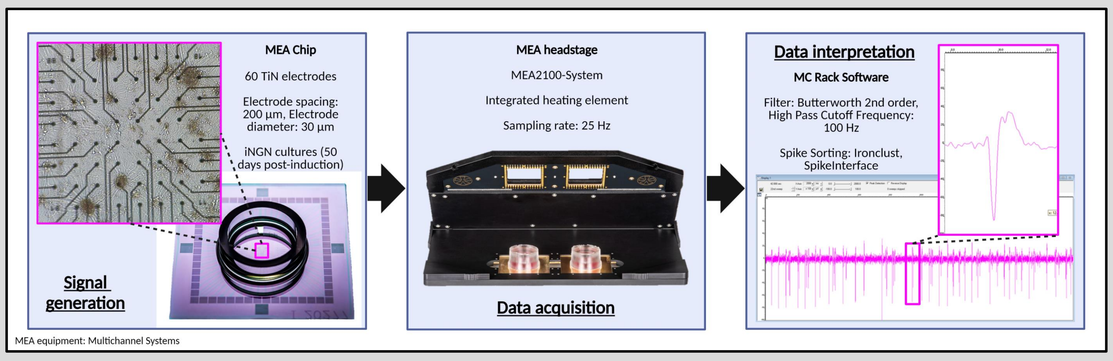
Introduction

During spaceflight, humans experience a variety of physiological changes due to deviations from accustomed Earth conditions. Specifically, the lack of gravity is responsible for many effects observed in returning astronauts, including neurological phenotypes. Thus, understanding the influence of altered gravity on neuronal activity and signal transmission on the cellular and network level is of high relevance. By using multielectrode array (MEA) technology, we advanced electrophysiological investigations covering single-cell to network level responses during exposure to decreased (micro-) or increased (hyper-) gravity conditions. Our data demonstrate that alterations in gravity levels trigger changes in neuronal activity that could cause neurological dysfunctions in space.

Methods

iNGN (induced Neurogenin) neurons were cultured into mature and functional neuronal networks atop multielectrode array (MEA) chips containing 60 recording electrodes. Integration of the MEA recording system into a custom experiment module (BIODECODER) allowed for integration into several experiment platforms. MEA chips containing cells were inserted shortly before the start of the experiment. Measurements include a 1g baseline recording before hyper-g/µ-g exposure, continuous recording during the different experiment phases followed by a 1g post-baseline recording. Filtered data was analyzed after spike sorting using the NeuroExplorer software and firing rates were used as a measure of electrophysiological activity.



Results

Hypergravity Exposure

Neuronal networks were exposed to 6g hypergravity for five minutes using the short-arm centrifuge at DLR Cologne. Cells reacted with an **elevation in firing rates during the acceleration phase** from 1g to 6g. Plotting of single unit measures showed more clearly, that **firing rates shifted toward lower frequencies in the hypergravity phase** immediately following ramp up. Notably, this effect faded after a few minutes of constant 6 g exposure, hinting at the cells being able to compensate for the initial rise in activity. The results also suggest that subgroups of cells exist which react differently to the gravity stimuli.

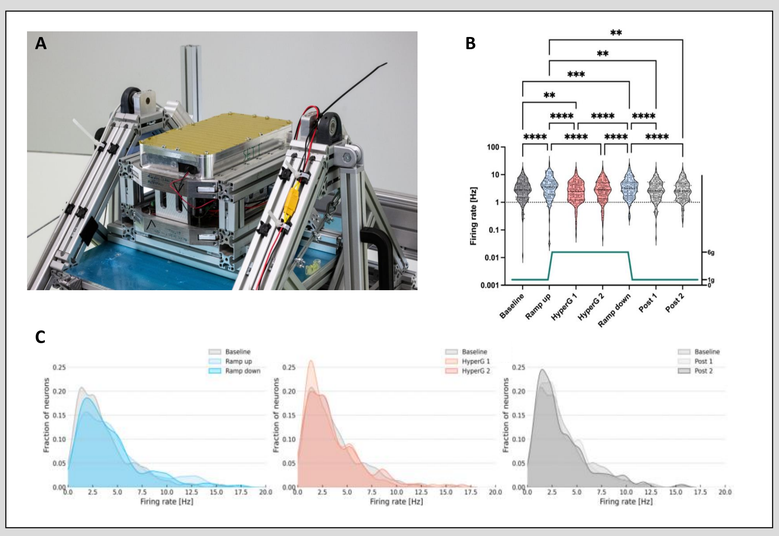


Fig. 2: (A) BIODECODER module mounted on the short-arm centrifuge at :envihab, DLR Cologne, (B) Firing rates of neuronal cultures sub-jected to 6 g hyper-gravity on a human centrifuge. n= 5 MEAs, 6 runs, 144 units. Statistics: Repeated measures one-way ANOVA and Tukey's multiple comparisons test. (C) Density plots of firing rates during the different experiment phases compared to baseline.

Microgravity Exposure (Second range)

For evaluation of potential effects of microgravity on neuronal network activity, the BIODECODER module was implemented on the ZARM Drop Tower in Bremen. Cells experienced 4.7 s of high-quality µ-g during free fall. Comparing the firing rates of the human neural networks on the MEA chips showed a **significant increase from baseline to the microgravity phase**, suggesting an increased activity of the neural network under µ-g conditions. Over the time of the post-drop baseline, the **firing rate readapted** to pre-drop baseline, indicating that cells recover from altered gravity influence.

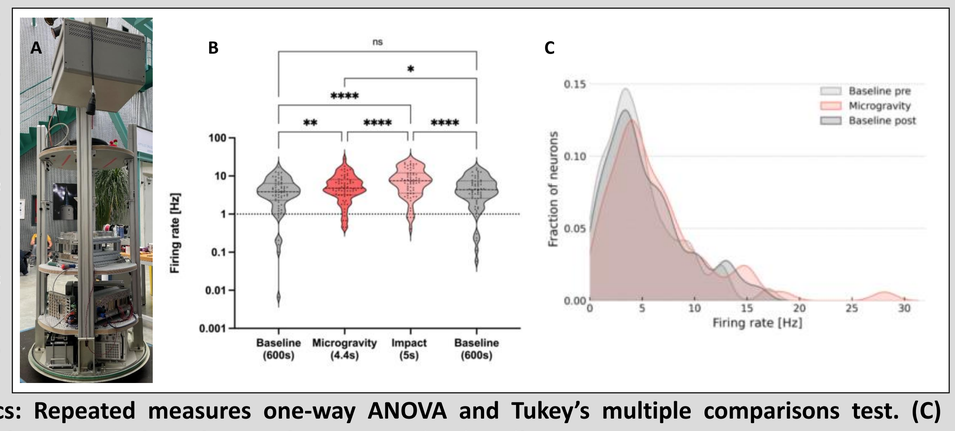


Fig. 3: (A) BIODECODER module mounted on the Drop Tower capsule, (B) Firing rates of neuronal cultures during the different phases of the drop experiment. n = 5 MEAs, 5 drops, 63 units. Statistics: Repeated measures one-way ANOVA and Tukey's multiple comparisons test. (C) Density plots of firing rates during microgravity compared to baselines.

Microgravity Exposure (Minute range)

Participation of the BIODECODER module in the MAPHEUS-13 rocket campaign yielded an exposure time of more than 5 minutes. Firing rate analysis revealed that **cells reacted with increased activity during the first seconds after onset of microgravity**. However, upon **longer exposure time, activity dropped below 1 g baseline levels**.

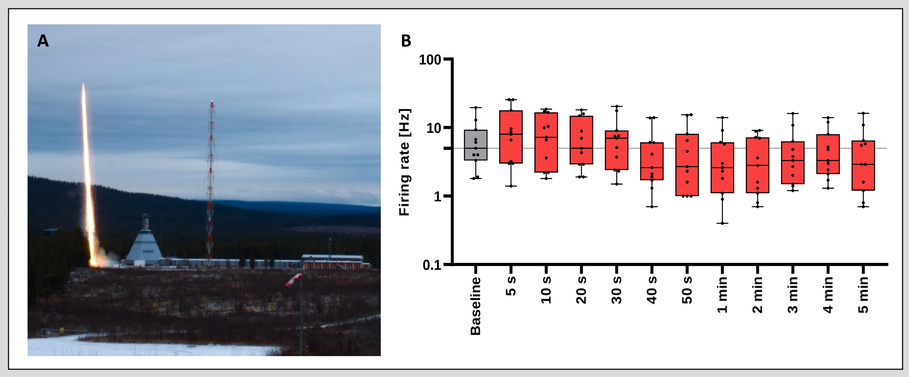


Fig. 4: (A) MAPHEUS-13 rocket launch at ESRANGE, Kiruna, Sweden (B) Firing rate changes of neuronal cultures during the microgravity phase of the rocket launch experiment. n = 2 MEAs, 1 flight, 11 units.

Conclusion & Outlook

Our results confirm the immediate impact of altered gravity conditions on the electrophysiological activity of human neurons. They suggest that sub-populations of neurons respond differently to the change in gravity and that the network as a whole is adapting in a more complex way. To get more detailed insight into this, experiments with extended research opportunities for altered gravity applications on various experiment platforms will be necessary.

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