Astrocyte Reactivity can be Modulated by Altered Gravity

Yannick Lichterfeld, Sarah Schunk, Laura Kalinski, Theresa Schmakeit, Zoe Meerholz, Maximilian Sturm, Patrick Lau, Sebastian Feles, Timo Frett, Ruth Hemmersbach and Christian Liemersdorf

German Aerospace Centre (DLR), Institute of Aerospace Medicine, Gravitational Biology, Cologne, Germany

Introduction

Astrocytes are the most prevalent type of glial cells in the brain. These cells show a high impact on the regulation of neuronal signaling and play a key role in neuronal regeneration. The regeneration of neuronal projections is modulated by astrocytes that change into a reactive state (reactive astrogliosis). This mechanism of astrocyte reactivity on the one hand prevents the proliferation of inflammatory signaling and infection, but on the other hand inhibits neuronal regeneration through glial scarring.

Aim of the Project

Project NeuroSpace aims to investigate pathways regulated in primary astrocytes by altered gravity conditions to identify novel targets for pharmacological interventions to specifically modulate astrocyte reactivity and further promote neuronal regeneration.
For the assessment of both simulated micro- and hypergravity the ground-based facilities at the DLR Institute of Aerospace Medicine in Cologne, Germany were employed.

Multi Sample Incubator Centrifuge (MuSIC)

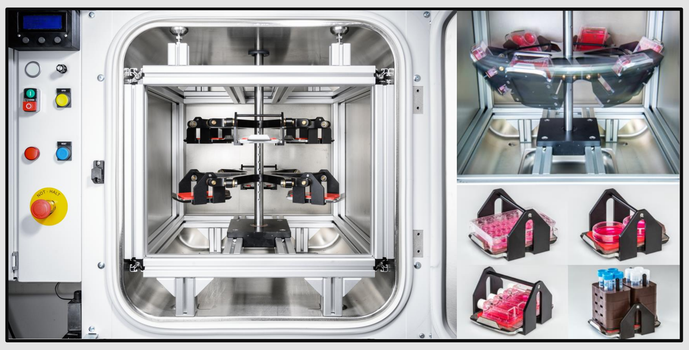

Fig. 1 | The Multi Sample Incubator Centrifuge (MuSIC) provides variable hypergravity profiles for various cell culture applications.

The Hyperscope

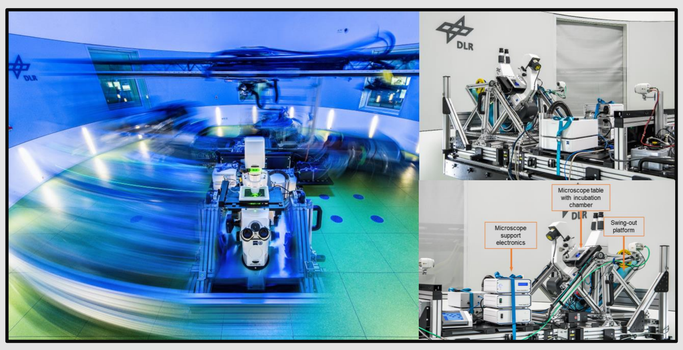

Fig. 2 | The Hyperscope is a fully automated epifluorescence live-cell imaging microscope on the DLR human centrifuge.

2D-Clinostats

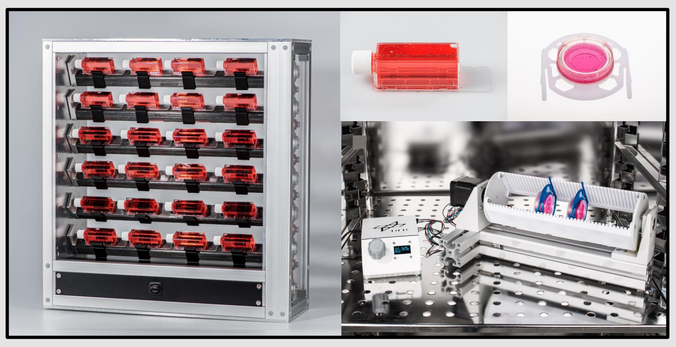

Fig. 3 | Clinostats are custom-designed to simulate microgravity for various cell biology applications. Several clinostats are available e.g., for adherent cells in slide flasks, cell culture dishes or MEA chips.

Results

1. Decreased Spreading Capabilities of Astrocytes under Hypergravity and Simulated Microgravity

Over the course of 48 hours, astrocytes exposed to 2*g* hypergravity showed an area reduction of approx. 25%. Simulated microgravity exposure through clinorotation led to 18% smaller cells.

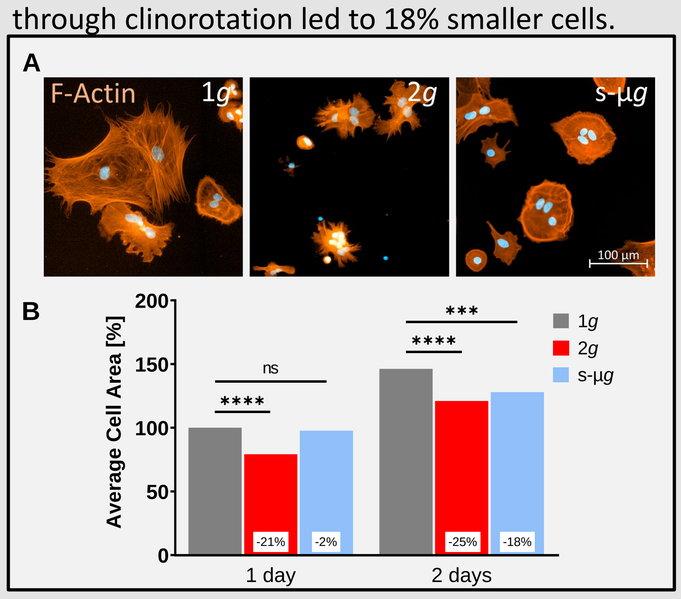

Fig. 4 | Cell spreading of astrocytes is reduced after 2*g* hypergravity and simulated microgravity (clinorotation) exposure.

2. Astrocyte Migration Speed is significantly regulated by Altered Gravity

Live-cell imaging on the Hyperscope platform revealed a regulation of the migration speed of astrocytes under different gravity levels.
Hypergravity (2*g*) reduced the migration rate by approx. 35% over the course of 48 hours, and still by 15% over 5 days (Fig. 5 A). Upon gravity transition the cells showed a recurrent “lag phase” of 1-2 h before the effects were stabilized (Fig 5 B). Astrocytes exposed to simulated microgravity (s-µ*g*) on the clinostat displayed a significant increase in migration velocity by approx. 21% over the course of 6 days (Fig. 5 A and C).

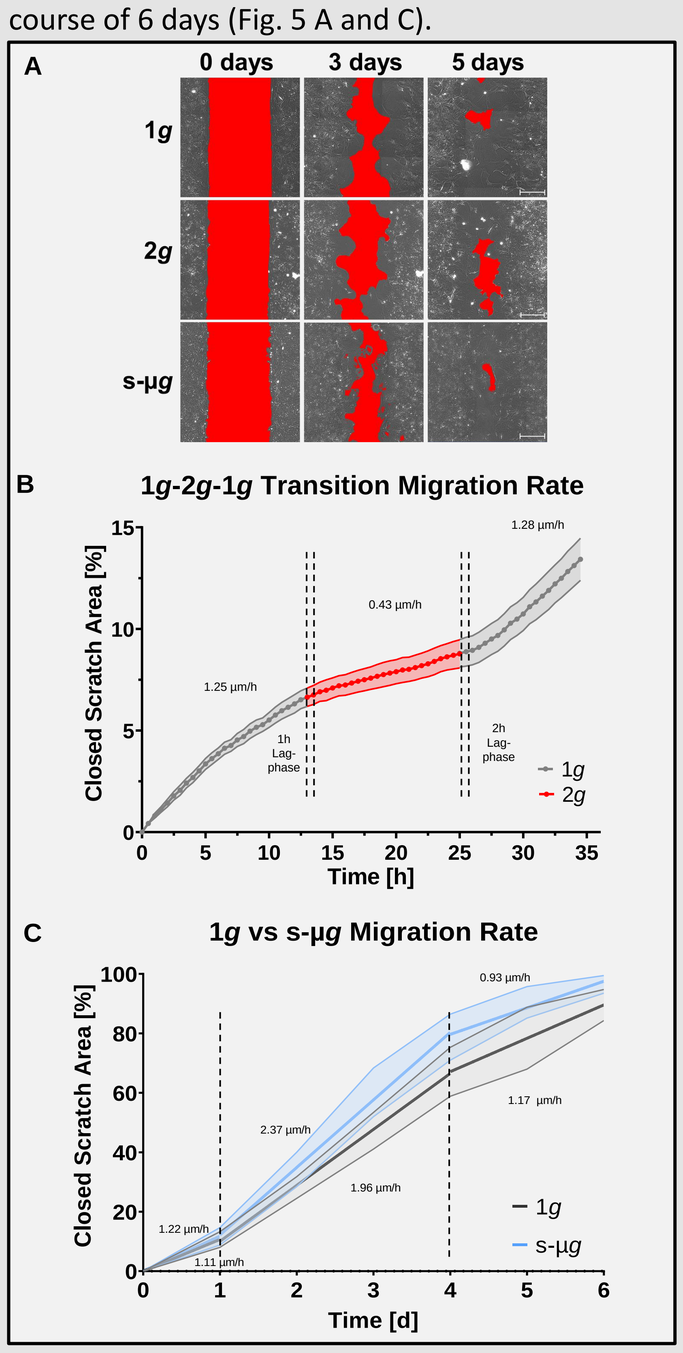

Fig. 5 | Increased gravitational loading of 2*g* reduces astrocyte migration speed with 1-2h-long (re-)adaptation lag phases. Mechanical unloading through clinorotation has an opposite effect and cells migrate faster.

3. Altered Gravity affects Key Genes involved in Reactive Astrogliosis

Increased gravitational loading by centrifugation (2*g*) led to a reduction of astrocyte reactivity markers whereas gravitational un-loading by clinorotation (s-µ*g*) increased the amount of reactive (GFAP up-regulated) cells (Fig. 6).

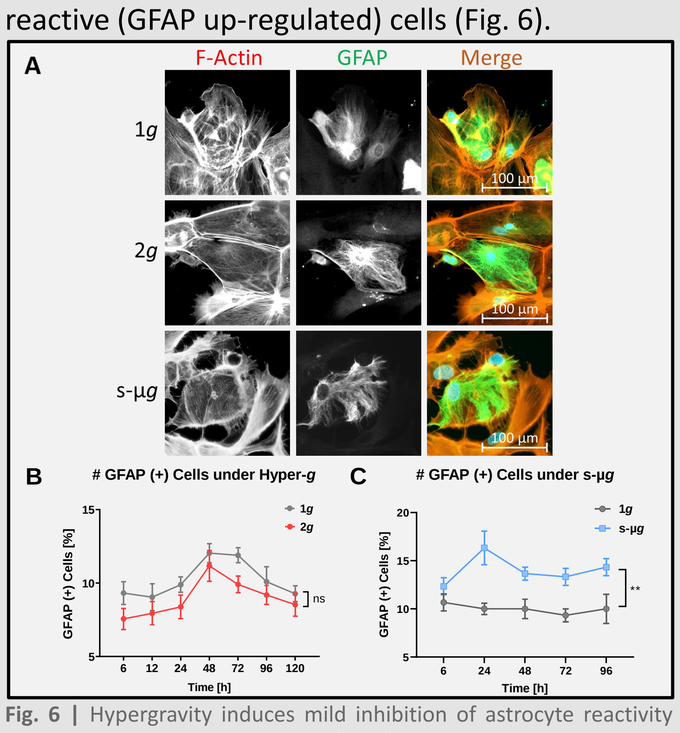

Fig. 6 | Hypergravity induces mild inhibition of astrocyte reactivity markers, whereas clinorotation (s-µ*g*) increases the number of reactive astrocytes. GFAP visualized via antibody staining, F-actin was stained using Phalloidin.

4. Proteomic Analysis of Clinorotated Astrocytes

Protein mass spectrometry was performed on astrocytes which were exposed to simulated microgravity on a clinostat for up to 72 hours (Fig. 7). Compared to the 1*g* controls, several proteins were expressed significantly different. Especially metabolism and cytoskeleton-related proteins increased after 72h clinorotation compared to a time-matched 1*g* control.

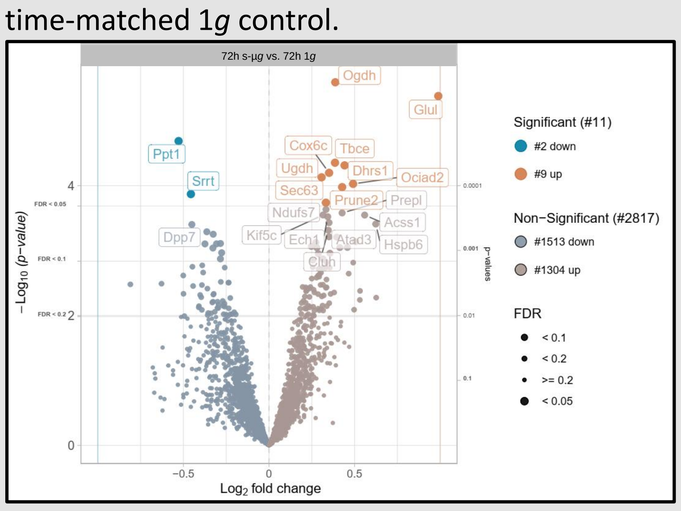

Fig. 7 | Volcano plot showing fold changes of protein levels in astrocytes after 72h of clinorotation (s-µ*g*) compared to a time-matched 1*g* control.

Conclusion

Increased mechanical loading by hypergravity in the physiologically relevant range of 2*g* attenuated various traits of reactive astrogliosis in primary murine astrocytes *in vitro*. In contrast, clinorotation induced gene expression changes related to reactive astrogliosis. Migration speed of astrocytes was reduced in hypergravity and increased in simulated microgravity. Proteomic analysis of clinorotated astrocytes indicates altered metabolism and cytoskeletal rearrangements in these cells.
Validation of the simulation approach in real microgravity and identification of the underlying mechanisms and target genes or pathways involved in these regulatory processes will be among future investigations. This might aid the development of pharmacological treatments which shall reduce reactive astrogliosis and thus enhance neuronal regeneration *in vitro* and in future also *in vivo* in patients.

Reference

Lichterfeld, Y.; Kalinski, L.; Schunk, S.; Schmakeit, T.; Feles, S.; Frett, T.; Herrmann, H.; Hemmersbach, R.; Liemersdorf, C. *Hypergravity Attenuates Reactivity in Primary Murine Astrocytes*. Biomedicines 2022, 10, 1966. https://doi.org/10.3390/biomedicines10081966

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Contact
Sarah Schunk
Email: Sarah.Schunk@dlr.de
Phone: +49 2203 601 3540

DLR Institut für Luft- und Raumfahrtmedizin

NeuroSpace