ORAL 34

Hypergravity Attenuates Reactivity in Primary Murine Astrocytes

Yannick Lichterfeld[1], Laura Kalinski[1], Sarah Schunk[1], Theresa Schmakeit[1], Sebastian Feles[1], Timo Frett[2], Harald Herrmann[3], Ruth Hemmersbach[1] and Christian Liemersdorf[1]

[1] German Aerospace Center, Institute of Aerospace Medicine, Department of Gravitational Biology, Cologne, Germany; yannick.lichterfeld@dlr.de; laura.kalinski@dlr.de; sarah.schunk@dlr.de; theresa.schmakeit@dlr.de; sebastian.feles@dlr.de; ruth.hemmersbach@dlr.de, [2] German Aerospace Center, Institute of Aerospace Medicine, Department of Muscle and Bone Metabolism, Cologne, Germany; timo.frett@dlr.de. [3] University of Erlangen, Institute of Neuropathology, harald.herrmann-lerdon@uk-erlangen.de

Introduction

Neuronal activity is the key modulator of nearly every aspect of behavior, affecting cognition, learning and memory as well as motion. Alterations or even disruptions of the transmission of synaptic signals are the main cause of many neurological disorders. Lesions to nervous tissues are associated with phenotypic changes mediated by astrocytes becoming reactive. Reactive astrocytes form the basis of astrogliosis and glial scar formation. Astrocyte reactivity is often targeted to inhibit axon dystrophy and thus promote neuronal regeneration. Here, we use increased gravitational (mechanical) loading induced by hypergravity to identify a potential method to modify key features of astrocyte reactivity. We exposed primary murine astrocytes as a model system closely resembling the reactivity phenotype in vivo on custom-built centrifuges for cultivation as well as for livecell imaging under hypergravity conditions in a physiological range (2*g* and 10*g*). This resulted in significant changes to astrocyte morphology, behavior and reactivity phenotypes, with the ultimate goal being to enhance neuronal regeneration for novel therapeutic approaches.

Morphological features and dynamics related to glial scarring are influenced by hypergravity

We revealed cell spreading rates to be strongly diminished under 2*g* hypergravity exposure, with the initial spreading after seeding of the cells being 45% lower than under normal gravity. Long-term hypergrqavity exposure in an incubator centrifuge over two or more days led to a consistent 20% decrease in cell spreading under 2*g*.

Employing astrocyte wound-healing assays, we could show that the migration velocity of astrocytes can be decreased by hypergravity exposure. Livecell microscopy under hypergravity enabled the identification of an up to 35% lower cell migration velocity as compared to 1*g*. Stopping and starting of the centrifuge while imaging resulted in the observation of until now unknown adaptatation and re-adaptation phases to hypergravity in astrocytes, with their migration velocity lagging behind the actually perceived gravity phase.

In contrast to the changes in morphology and migration, proliferation and apoptosis rates were not affected by 2*g* hypergravity exposure over several days.

Livecell actin fluorescence microscopy under hypergravity supplemented by STED imaging revealed changes in actin filament and microtubule dynamics

Livecell imaging of LifeAct-GFP astrocytes under 1*g* and 2*g* revealed changes in actin-related cellular structures under hypergravity. STED microscopy of cells fixed after hypergravity exposure confounded these findings, adding changes in the microtubule networks of the hypergravity exposed cells.

Hypergravity attenuated reactivity induction in primary murine astrocytes.

Exposure of primary murine astrocytes to hypergravity did not only influence cell morphology, behavior and cytoskeletal dynamics. By immunostaining of reactivity related proteins, we could show, that astrocyte reactivity induction was attenuated under hypergravity conditions. Our findings revealed a novel role of gravitational loading conditions on neuronal cell behavior. We plan on further investigating the underlying mechanisms of these changes, to identify the key pathways and induce these via pharmacological means, in order to treat patients suffering from the adverse effects of glial scarring.