Impact of MicroRNAs Based Biomarkers On The Psychopathologies Of Depression Under Simulated Space Complex Environmental Model

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Abstract

Long-term space travels impact deleterious biological and psychological effects on astronauts' health, resulting in serious behavioral and physiological abnormalities. Depression is one of the mood disorders experienced by 60% of astronauts during space travel, which negatively impacts their brain functioning, disturbing synaptic plasticity, trophic factors, neuron morphology, and neurogenesis. It is believed that space induces serve neurological changes in hippocampus through various epigenetic factors. miRNAs as a negative regulator of genes are known to regulate various hippocampus-dependent functions in response to stress, but still the underlying mechanism involved in regulating neurological changes is unclear. Therefore, the present study is designed to investigate the role of candidate miRNAs implicating in the neuropsychiatric changes using simulated space model. Out of 20 rats, 10 rats were exposed to simulated space environmental complex model (SSE) for 21 days and 10 rats as control. Behavioral tests were executed to analyze depressive behavior through body weight, sucrose preference rate, open field test score and force swimming scores. Hippocampus samples were used for the quantification of miRNAs expression levels and targets were analyzed through *in-silico* analysis. Our results showed significantly reduced body weight, sucrose preference rate, open field score and forced swimming scores in SSE model. The oxidative test presented significant differential values in SSE model than control group. SSE group displayed upregulated miR-16 and miR-132 expression than control. Through *in-silico* analysis, we identified various biological functions influenced by miR-16 and miR-132, including memory, behavior, nervous system development, axon extension, growth, and neuron migration etc. Furthermore, our *in-silico* study provided a landscape of potential miR-16 and miR-132 targets and relevant canonical pathways related to axonal guidance and P13K-Akt pathway. In conclusion, this study provided an avenue for the future use of miRNAs as potential biomarkers for the early diagnosis of mood disabilities and neurological abnormalities, thereby providing a great insight for future health sciences and space health care

Introduction

Astronauts' health has always been a major concern during space travel. Harsh space environment poses deleterious effects on astronauts' physical and mental health that awaken the need for comprehending the alteration of brain function in space for mission propulsion. Microgravity is one of the crucial factors in the space environment, causing the astronauts' physiological function damages. According to previous studies, microgravity leads to the reduction of spatial learning and memory and elevated cognitive demand of human sensorimotor performance [1]. It has also been reported that simulated microgravity can cause mental disorders [2] and induce the human mood changes (Figure-1a). Various models were used to simulate the microgravity on the ground National Aeronautics and Space Administration (NASA) approved standard operating procedures for hind-limb unloading in rats used to simulate microgravity, and the reduced lumbar weight-bearing and normal cervical forces in the hindlimb unloading model well simulated the headward transfer of body fluids (Figure 1b). [3]. Besides microgravity, other stressful environmental factors in space, such as noise, confinement, circadian rhythms disorders, radiation, and vibration are reported to trigger behavioral disorders such as increased anxiety, mood changes and depression (Figure-1a) [4]. So far, all these studies reported physiological changes and underlying mechanisms governing these changes is still mystery.

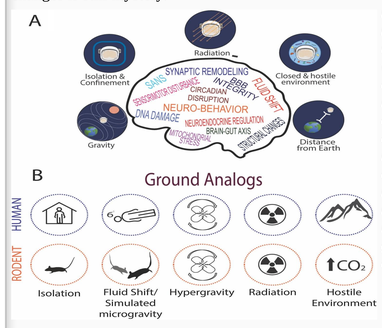


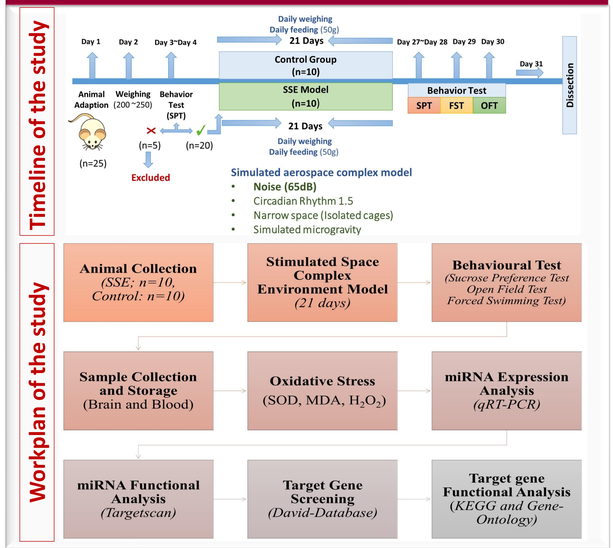
Figure-1: Overview of spaceflight hazards and ground-analog models used to study these hazards. *The various hazards in the spaceflight environment such as altered gravity, isolation or confinement, radiation, closed and hostile environment, and distance from earth, affect the health of the brain. Ground analogs are used to simulate some of these hazards on Earth and study their impacts on physiology and behavior. Here we have only highlighted ground analogs for human studies and the equivalent rodent models.*

MicroRNAs (miRNAs) are increasingly becoming recognized as major systemic regulators of responses to stressors, including microgravity, oxidative stress, radiation, and DNA damage [5]. Various studies reported that miRNAs play an important role in CNS and psychiatric related diseases with the potential of utilizing miRNAs as biomarkers and therapy. Research has also shown many miRNAs to be heavily involved in development and maintenance of normal neuronal function [6]. This has led researchers to hypothesize that key miRNAs might be driving neurodevelopmental disorders, neuropsychiatric disorders, and neurodegenerative disorders. For example, miR-16 induces a depression-like phenotype in rats. Unfortunately, research on actual spaceflight model neural system research does not exists much due to which neural miRNA profiles of microgravity models are non-existent that hindered researchers to uncover biological mechanisms and as such, researchers currently have to rely on CNS related miRNA research to provide insight about CNS related health risks that might occur in space.

Objective of the study

1. The present study is designed to investigate the effects of earth-based simulation for 21 days on the rat hippocampus and examine differential expression of miR-16, miR-132 and miR-124 in the hippocampus.
2. The differential miRNAs are probed to identify targets involved in regulating space induced underlying biological mechanisms involved in the neuropsychology of depression.

Methodology



Results

During 21 days of simulated-space model, feeding pattern and body weight of SSE and control group were measured. After 21 days, we observed significant weight-loss in SSE rats than control. However, no change in feeding pattern of both groups was found as shown in Figure-2.

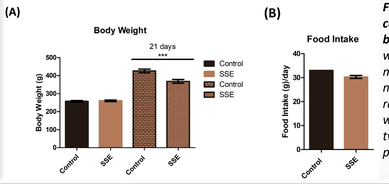


Figure-2:Impact of 21 days simulated complex space environment on rat's body weight and food intake. *(a) Body weight before and after 21 days of SSE modeling (SSE group, n=10; Control, n=10). (b) Average food intake of each rat. Data plotted as mean ± SEM. One way ANOVA (p*<0.005) and unpaired, two tailed student t-test was performed for comparison.*

After 21 days of simulated space model, behavioral tests were performed to analyze depressive behavior among rats. We found SSE rats exhibit reduced affinity for sucrose (Figure-3a), high immobility time in force swimming tests (Figure-3b) and lower curiosity in the open field tests (Figure-3c-f). These behaviors tests indicate that SSE rats exhibit depressive behavior.

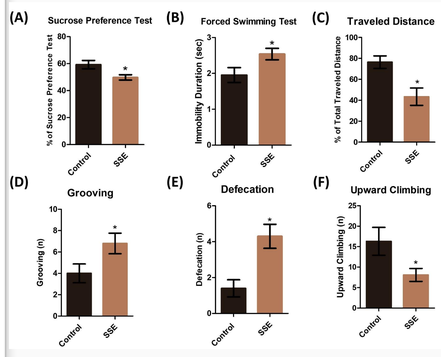


Figure-3: Impact of 21 days of the simulated complex space environment on rat's behavior (SSE: n=10, control: n=10) *(a) Sucrose preference test. (b) Forced Swimming Test (c) Open field test: Traveled distance (d) Grooving (e) Defecation (f) Upward climbing. Data plotted as mean ± SEM. Unpaired, two-tailed student t-test was performed for comparison. Values in the range of *p<0.05 were considered statistically significant.*

After behavior tests, rats were sacrificed and hippocampus from both groups were obtained. To analyze underling biological changes, oxidative stress in both groups were measured, SSE group showed higher oxidative stress than control (Figure-4).

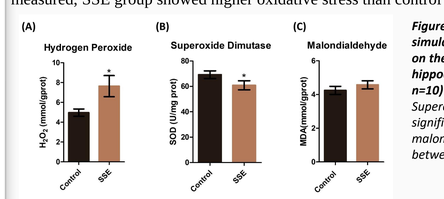


Figure-4: Impact of 21 days of simulated complex space model on the oxidative stress in rat hippocampus (SSE, n=10; Control, n=10) *(a) Hydrogen peroxide (b) Superoxide Dimutase (c) No significant difference in malondialdehyde was found between two groups.*

On analyzing miRNA expression level, we observed upregulated miR-16 and miR-132 in the hippocampus of SSE than control, while no difference was observed in the expression level of miR-124 (Figure-5)

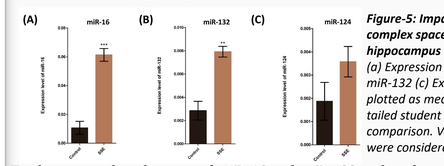


Figure-5: Impact of 21 days of simulated complex space model on the miRNAs in rat hippocampus (SSE, n=10; Control, n=10) *(a) Expression of miR-16 (b) Expression of miR-132 (c) Expression of miR-124. Data plotted as mean ± SEM. Unpaired, two-tailed student t-test was performed for comparison. Values in the range of *p<0.05 were considered statistically significant.*

Further we predicted targets of miR-16 and miR-132 and performed functional analysis

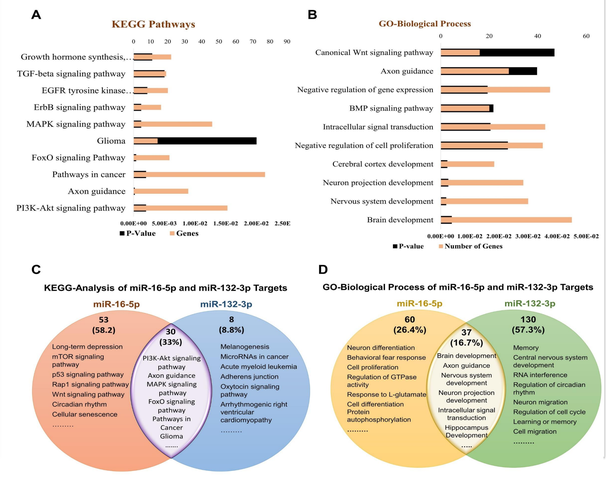


Figure-6: KEGG and Gene-Ontology of dysregulated targets of miR-16 and miR-132. (a) KEGG pathways (b) Gene Ontology (c) Venn diagram showing common KEGG pathways of miR-16 and mIR-132 targets (d) Venn diagram showing common Gene-ontology-Biological processes of miR-16 and miR-132 targets

After functional analysis, we found that miR-16 and miR-132 coregulate P13K-Akt pathway and axon guidance pathway and share common targets that regulate neuronal synaptic connection and neuron morphology. Thus, we proposed that dysfunctional expression of miR-16 and miR-132 in space simulated model might dysregulated P13K-Akt pathway and axon guidance pathway, which further disturbs synaptic connections and promote neuronal apoptosis, thus contributing to the pathophysiology of depression (Figure-7)

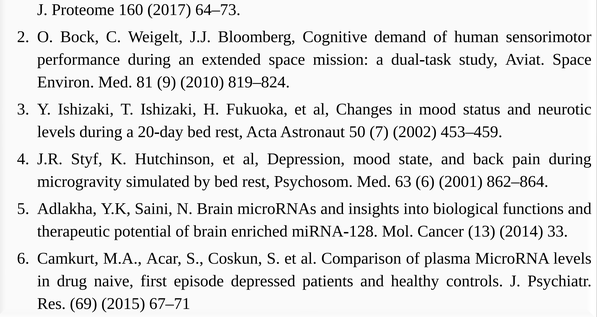


Figure-7: miR-16 and miR-132 targets coregulating P13K-Akt pathway and Axon Guidance pathway *(a) Venn diagram showing targets coregulating P13K-Akt pathway and axon guidance (b) Landscape of miR-16 and miR-132 targets regulating P13K-Akt pathway and axon guidance and impart role dysfunctional synaptic connection and neuronal apoptosis to promote depressive symptoms.*

Conclusion

Overall, this study showed that simulated space model induced depressive symptoms in the SSE rats that causes upregulated expression of miR-16 and miR-132 in the hippocampus. Further analysis showed that both miRNAs coregulate P13K-Akt and axon guidance pathway, which further regulate other neuronal pathways and promote in regulating synaptic connections and neuronal atrophy. However, any disturbance in these pathways may significantly contribute to the psychopathology of depression. So far, the study is at infancy and on-board space neuronal model are required to investigate real consequences of these dysregulated expression of miRNAs and future health benefits.

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