MOLECULAR MECHANISMS OF CIRCADIAN REGULATION DURING SPACEFLIGHT

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INTRODUCTION

The physiology of both vertebrates and invertebrates follows internal rhythms coordinated in phase with the 24-hour daily light cycle. This circadian clock is governed by a central pacemaker, the suprachiasmatic nucleus (SCN) in the brain. However, peripheral circadian clocks or oscillators have been identified in most tissues. How the central and peripheral oscillators are synchronized is still being elucidated.

Light is the main environmental cue that entrains the circadian clock. Under the absence of a light stimulus, the clock continues its oscillation in a free-running condition. In general, three functional compartments of the circadian clock are defined:

1. Molecular clock (SCN)
2. Output: circadian effects, gene expression, behavior, metabolism
3. Peripheral clock

In spaceflight, the circadian rhythm disruption (SDR) and prolonged space flight (STS-133) induced photoreceptor cell death. Loss of photoreceptors is a major cause of vision loss after extended spaceflight.

RESULTS

Our immunofluorescence results are in agreement with the description of the distribution of ipRGC. RGC positive for melanopsin were found uniformly distributed in the RGC layer throughout the retina, with occasional crowding along the periphery. Virtually no immunoreactive cells were found in retina samples from mice aboard STS133 after one day upon return; however, several positive cells were seen in samples from mice after flight on R+7. Likewise, both vivarium and AEM ground controls showed evidence of ipRGC.

Melanopsin (Opn4) gene expression levels in retina samples from BALB mice in the STS133 experiment, measured by real time qPCR. Y axis: logarithmic scale of comparative gene expression level normalized to housekeeping genes. The numbers in green indicate the mouse # in the experiment. For #13, the expression levels were closest to zero, as pointed by the arrow (qPCR amplification curved shifted to the right, below threshold, so the value that appears in the graph is actually an artificial value close to zero that was necessary to be added in the calculations process).

In order to investigate the levels of oxidative stress in the samples, the DNA damage oxidative stress marker 8OHdG was qualitatively measured in each immunostained retinal sections. The figure below shows representative fields of retinal sections comparing the DNA damage due to oxidative stress. RGC appear to be a target of damage in flight samples, whereas photoreceptors are more affected in vivarium samples. AEM ground controls showed the lowest incidence of oxidative stress.

CONCLUSIONS

In conclusion, the number of melanopsin-immunoreactive RGC as well as melanopsin gene expression were decreased in flight samples immediately after flight but this change was attenuated in flight sample 7 days after return. Retinal ganglion cells are a target of the effects of oxidative stress induced by spaceflight, based on immunohistochemistry of 8OHdG in eyes samples. We propose that oxidative stress can lead to a decrease in melanopsin expression, likely via ipRGC loss or impairment, and thus, it can be a contributing factor to circadian disruption during spaceflight.

Countermeasures contemplating the use of light should therefore be complemented with melanopsin expression maintenance and/or reduction in oxidative stress.

There is previous published evidence suggesting that the central clock is susceptible to oxidative stress4, often associated with aging, and that DNA repair mechanisms and circadian clocks share regulatory pathways.

Future questions to be answer include: a) is the decrease in melanopsin expression observed after spaceflight due to RGC loss or to RGC impaired gene expression?; b) are other clock genes also affected?; c) is the local retinal clock output affected?; d) does the decrease in melanopsin translate into a significant alterations in the signaling to the SCN to contribute to circadian rhythm disruption?; e) which retina-specific cellular rhythms might be affected by a local circadian clock disruption?

BIBLIOGRAPHY


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