

## MOLECULAR MECHANISMS OF CIRCADIAN REGULATION DURING SPACEFLIGHT

Susana Zanello<sup>1</sup> and Richard Boyle<sup>2</sup>

<sup>1</sup>*Universities Space Research Association*, <sup>2</sup>*NASA-Ames Research Center*

Disruption of the regular environmental circadian cues in addition to stringent and demanding operational schedules are two main factors that undoubtedly impact sleep patterns and vigilant performance in the astronaut crews during spaceflight<sup>1</sup>. Most research is focused on the behavioral aspects of the risk of circadian desynchronization, characterized by fatigue and health and performance decrement. A common countermeasure for circadian re-entrainment utilizes blue-green light to entrain the circadian clock<sup>2</sup> and mitigate this risk. However, an effective countermeasure targeting the photoreceptor system requires that the basic circadian molecular machinery remains intact during spaceflight. The molecular clock consists of sets of proteins that perform different functions within the clock machinery: circadian oscillators (genes whose expression levels cycle during the day, “keep the pass” of cellular time and regulate downstream effector genes), the effector or output genes (those which impact the physiology of the tissue or organism), and the input genes (responsible for sensing the environmental cues that allow circadian entrainment). The main environmental cue is light. As opposed to the known photoreceptors (rods and cones), the non-visual light stimulus is received by a subset of the population of retinal ganglion cells called intrinsically photosensitive retinal ganglion cells (ipRGC) that express melanopsin (opsin 4 -Opn4-) as the photoreceptor<sup>3</sup>. We hypothesize that spaceflight may affect ipRGC and melanopsin expression, which may be a contributing cause of circadian disruption during spaceflight. To answer this question, eyes from albino Balb/cJ mice aboard STS-133 were collected for histological analysis and gene expression profiling of the retina at 1 and 7 days after landing. Both vivarium and AEM (animal enclosure module) mice were used as ground controls. Opn4 expression was analyzed by real time RT/qPCR and retinal sections were stained for Opn4 immunofluorescence. Opn4 was decreased (abrogated in one case) in retinas that concurrently showed higher evidence of oxidative stress. 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.

1. Whitmire A, et al. Risk of Performance Errors Due to Sleep Loss, Circadian Desynchronization, Fatigue, and Work Overload; HRP Evidence Book, NASA; 2011.
2. Gooley JJ, Rajaratnam SM, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Sci Transl Med.* 2010; **2**(31): 31ra3.
3. Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, et al. Melanopsin is required for non-image-forming photic responses in blind mice. *Science.* 2003; **301**(5632): 525-7.