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. Photoreceptor(s)
3. Output: circadian effects, gene expression, metabolic and functional pathways

The vertebrate retina contains endogenous clocks that control many aspects of retinal physiology, including retinal sensitivity to light, neurohormone synthesis (melatonin and dopamine), rod disk shedding, signalling pathways and gene expression.

Neurons with putative local circadian rhythm generation are found among all the major neuron populations in the mammalian retina. In the mouse, clock genes and function are more localized to the inner retinal and ganglion cell layers. The photoreceptor, however, secretes melatonin which may still serve an important circadian signal.

The reception and transmission of the non-visual photic stimulus resides in a specialized population of retinal ganglion cells, the intrinsically photosensitive retinal ganglion cells (ipRGC). Melanopsin peak absorption is at 420 nm and all the axons of the ipRGC target the SCN.

We hypothesize that spaceflight may affect ipRGC and melanopsin expression, which may be a contributing cause of circadian disruption during spaceflight.

OBJECTIVE
To compare the ipRGC population and melanopsin expression in retinas from mice flown aboard STS-133 with ground controls.

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.

Analysis of melanopsin mRNA levels by real time PCR revealed a drop in melanopsin gene expression in flight samples upon return to Earth, compared to ground controls, but control levels are recovered after 7 days after landing.

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 80HdG in eye 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 contemplated 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 stress, 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?

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