Neurological, Cardiovascular and Behavioral Consequences of Lunar Exploration using *Drosophila melanogaster* - Artemis III mission

NASA is exploring crewed lunar missions with plans to commercialize low-Earth orbit, thus laying the foundation for human exploration and expansion to Mars, yet we have limited information on the effects of long-duration spaceflight on human physiology. Responses to spaceflight factors such as gravitational changes, high-energy ionizing radiation, altered circadian rhythms, and isolation have been observed in astronauts marking back to the Apollo Era, including 1-2% loss of bone mineral density[1], higher rates of cardiovascular-related deaths[2], increased cataract risks[3], neurological[4], and immune responses such as reactivation of viruses[5]. While the International Space Station (ISS)- and ground-based studies have been instrumental in assessing the effects of spaceflight, it is imperative that we study long-term implications of exposure to lunar conditions to gain a better understanding of how it affects astronauts' health and operational abilities in deep space. However, it would require complex animal experiments beyond low-Earth orbit, which is challenging to conduct due to major limitations on payload size and crew time. Therefore, we propose an automated biological payload containing a genetically well-characterized model *Drosophila melanogaster* (fruit fly). *Drosophil*a allows the use of large statistical sample sizes with well-characterized and manipulatable genetics to be monitored for multigenerational growth while using limited spaceflight resources. Furthermore, once established and tested, this high-throughput autonomous payload system would be able to house other model organisms (*C. elegans,* yeast, bacteria, among others) with minimal need for adaptation and further developed to contain personalizable human organ models as well.

Drosophila is a well-established model organism that has been used for over a century to study physiological and molecular responses to the manipulation of genetics, the environment, diet, and effects of aging. Most cellular and molecular mechanisms are highly conserved between flies and mammals, with over 60% of human genes and ~75% of human disease-causing genes being represented in the fly[6,7]. For example, the circadian rhythm of flies is similar to humans, with an analogous molecular mechanism of circadian clock discovered in flies, which received a Nobel prize in 2017; in fact, 6 Nobel prizes have been awarded for research using the *Drosophila* model. The fly is ideal for studies designed to understand the unknown biological effects of the deep space environment given its short generation time and lifespan, limited genetic redundancy, and wealth of genetic tools, plus the availability of suitable flight hardware, ease of fly maintenance, and ability to propagate large population sizes within the limited volume and mass requirements available in space. Further, the function of a number of organ systems is conserved between flies and humans. For example, several genes involved in neurodevelopmental processes, synaptic mechanisms, and neural structure and signaling are highly conserved between flies and humans, making *Drosophila* an excellent model for neurological studies[8–10]. Also, cardiac and skeletal muscle gene function and cell structure and function are highly conserved between human and fruit fly hearts[11–14]. *Drosophila* is also a powerful model organism to understand the function of the innate immune system in organisms ranging from flies to humans. In space research, *Drosophila* has been established as a valuable model for understanding the effects of microgravity, hypergravity, and radiation in spaceflight. Current literature indicates the significance of behavioral alterations and longevity[15–18], immune changes[19–21], oxidative stress response[22,23], cardiac and skeletal muscle changes[24] and neurological changes[25,26] in spaceflight conditions that are measurable in fruit flies.

Recent advances in spaceflight hardware, such as the Techshot Multi-use Variable-gravity Platform (MVP), have made it possible to segregate different generations of flies, thus preserving age-matched adult flies reared in space, permitting the characterization of the acclimation of multiple organ systems to long-term space travel as well as re-acclimation to ground conditions. Importantly, MVP is equipped with an "onboard" centrifuge that provides an inflight 1g control (mimicking Earth's gravity), presenting

a gravity-controlled environment in space. Further technologies to be developed for use in this platform for Artemis missions are habitats that accommodate high-quality, high-speed video data collection and telemetry, and high-speed fluorescence imaging in an automated manner at the resolution of an individual fly.

In addition, *Drosophila* genetics enables in-flight tracking of observable phenotypes allowing genetic tagging of visible markers to follow survival, behavioral changes, and organ/cellular phenotypes of both mutant and wild type control flies. Assessment of these aspects can be automated, requiring no or limited crew intervention and can be accomplished even in the absence of a sample return to Earth. If sample return is possible, additional "omics" type analyses (genomics, proteomics, metabolomics), histochemistry, and molecular biological and biochemical analyses will be conducted on the ground following in-flight sample freezing and/or fixation capabilities, complemented by additional histochemical assays. Finally, the return of live samples would permit detailed behavioral, organ function, and electrophysiological assays as well as recovery assessments.

In conclusion, the genetically tractable *Drosophila* model would provide invaluable insight into the molecular, genetic, cellular, and physiological pathways in both response and adaptation to space conditions, and the adaptation of an autonomous MVP system would allow complex investigation of organismal responses to the lunar environment with a minimal footprint on crew time and resources, as well as serving as the first step in applying it to investigate other model organisms and potentially even individualized human models. Besides, there is already a wealth of both ground and flight data; continued research would expand in important ways on this investment. Future research will leverage this information to further our understanding of the genetic, molecular, cellular, and organ system response to space, thereby aiding in countermeasure and biomarker development for deep-space habitation.

References

1 Lang, T. *et al. J. Bone Miner. Res.* 19, 1006–1012 (2004)
2 Perhonen, M. A. *et al. J. Appl. Physiol.* 91, 645–653 (2001)
3 Chylack, L. T. *et al. Radiat. Res.* 172, 10–20 (2009)
4 Kalb, R. *et al. Archives of Neurology* 64, 485–490 (2007)
5 Pierson, D. L. *et al. Brain. Behav. Immun.* 19, 235–242 (2005)
6 Reiter, L. T. *et al. Genome Res.* 11, 1114–1125 (2001)
7 OMIM Database
8 Gatto, C. L. *et al. Curr. Opin. Neurobiol.* 21, 834–841 (2011)
9 Şentürk, M. *et al. Curr. Opin. Neurobiol.* 50, 24–32 (2018)
10 Kim, M. *et al. Int. J. Mol. Sci.* 18, 1596 (2017)
11 Cammarato, A. *et al. Mol. Biol. Cell* 19, 553–562 (2008)
12 Cammarato, A. *et al. PLoS One* 6, e18497 (2011)
13 Ocorr, K. *et al. Trends Cardiovasc. Med.* 17, 177–182 (2007)
14 Ocorr, K. *et al. Methods* 68, 265–272 (2014)
15 Benguría, A. *et al. J. Biotechnol.* 47, 191–201 (1996)
16 Ikenaga, M. *et al. Biol. Sci. Sp.* 11, 346–350 (1997)
17 Miller, M. S. *et al. J. Gravit. Physiol.* 6, P99-100 (1999)
18 Dolling-Boreham, R. *et al. Life Sci. Sp. Res.(in review)*
19 Marcu, O. *et al. PLoS One* 6, e15361 (2011)
20 Taylor, K. *et al. PLoS One* 9, e86485 (2014)
21 Ogneva, I. V *et al. PLoS One* 11, e0166885 (2016)
22 Hateley, S. *et al. Genomics* 108, 158–167 (2016)
23 Hosamani, R. *et al. J. Proteome Res.* 15, 4165–4175 (2016)
24 Walls, S. M. *et al. SSRN Electron. J.* (2020)
25 Mhatre, S. D. *et al.(in preparation)*
26 Iyer, J. *et al.(in preparation)*