Life Sciences in Space Research 16 (2018) 101–107

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

Review article

Synthetic torpor: A method for safely and practically transporting experimental animals aboard spaceflight missions to deep space

Yuri Griko[a,*], Matthew D. Regan[b]

[a] NASA Ames Research Center, Moffett Field, CA 94035, United States
[b] University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI 53706, United States

A B S T R A C T

Animal research aboard the Space Shuttle and International Space Station has provided vital information on the physiological, cellular, and molecular effects of spaceflight. The relevance of this information to human spaceflight is enhanced when it is coupled with information gleaned from human-based research. As NASA and other space agencies initiate plans for human exploration missions beyond low Earth orbit (LEO), incorporating animal research into these missions is vitally important to understanding the biological impacts of deep space. However, new technologies will be required to integrate experimental animals into spacecraft design and transport them beyond LEO in a safe and practical way. In this communication, we propose the use of metabolic control technologies to reversibly depress the metabolic rates of experimental animals while in transit aboard the spacecraft. Compared to holding experimental animals in active metabolic states, the advantages of artificially inducing regulated, depressed metabolic states (called synthetic torpor) include significantly reduced mass, volume, and power requirements within the spacecraft owing to reduced life support requirements, and mitigated radiation- and microgravity-induced negative health effects on the animals owing to intrinsic physiological properties of torpor. In addition to directly benefitting animal research, synthetic torpor-inducing systems will also serve as test beds for systems that may eventually hold human crewmembers in similar metabolic states on long-duration missions. The technologies for inducing synthetic torpor, which we discuss, are at relatively early stages of development, but there is ample evidence to show that this is a viable idea and one with very real benefits to spaceflight programs. The increasingly ambitious goals of world's many spaceflight programs will be most quickly and safely achieved with the help of animal research systems transported beyond LEO; synthetic torpor may enable this to be done as practically and inexpensively as possible.

1. The partnership between human- and animal-based researches

Efforts to understand the biological effects of spaceflight have typically combined careful observations of both humans and non-human animals. The combination of human- and animal-based research has served the interests of space biomedical research community effectively, addressing many important topic areas such as the effects of space on the musculoskeletal system, the neurovestibular system, the cardiovascular system, the vasculature of the eye, the hematopoetic system, the immune system, the reproductive system, and metabolism. Some of these experiments have been carried out on Earth, but the most powerful experiments—the ones that capture the full range of spaceflight's physiological effects—are those that are carried out on research animals in space. Certain characteristics of animal-based research such as relatively short generation times and the allowance of invasive experimental techniques have made it amenable to understanding the physiological, cellular, and molecular effects of spaceflight far beyond what would be offered by human-based research alone. When these animal-based findings are coupled with human-based findings, the result is a powerful partnership for understanding what happens when life is extended to the space environment.

2. The challenge of taking animal research beyond low Earth orbit

The facilities aboard the International Space Station (ISS) have made it possible to perform high quality animal research in space. While the results of this research have helped provide sufficient confidence to pursue longer duration human exploration missions beyond low Earth orbit (LEO), including asteroid, Mars, and long-duration lunar missions, there is still much we do not know. This includes the mechanisms of physiological plasticity that allow humans to acclimatize to the space environment, how these mechanisms are optimized to

* Corresponding author.
E-mail address: yuri.v.griko@nasa.gov (Y. Griko).

https://doi.org/10.1016/j.lssr.2018.01.002
Received 8 December 2017; Received in revised form 9 January 2018; Accepted 11 January 2018
2214-5524/ Published by Elsevier Ltd on behalf of The Committee on Space Research (COSPAR).

hasten acclimatization, and how they are impacted by long durations in deep space. Understanding these aspects of spaceflight is critically important to the success of future missions, and doing so will require a continued reliance on animal research aboard upcoming missions. But mass, volume, and power capacities of spacecraft design will present significant obstacles to this by constraining the required animal housing and life support systems.

The extent of these obstacles can be estimated using NASA's animal enclosure module (AEM) as an example. The AEM, which was designed for the Space Shuttle, is a self-contained rodent habitat that provides its occupants with living space, food, water, light, and ventilation. The system was designed to accommodate eight typical lab mice, and was used on 23 Shuttle missions between 2001 and 2011. We can use the capacities of this system to estimate mass, volume, and power requirements of keeping experimental mice alive and healthy on a long-duration mission beyond LEO. For mass, a mouse held within the AEM consumes approximately 4 g of food (in the form of NASA's nutrient-upgraded rodent food bar, NuRFB) and 4 mL of water per day (Moyer et al., 2016). For a 50-day mission with 24 mice on-board (a reasonable sample size for a single experiment) and a 25% safety margin, this would equate to 6 kg each of food and water. When the three 18.72 kg AEM units are factored in (mass does not include food and water load; NASA, 2017a), this would equate to 68.2 kg total mass. For volume, each AEM occupies 0.055 m^3 (NASA, 2017a), equating to 0.165 m^3 total volume. And for power, each AEM requires a minimum of 35.5 W (NASA, 2017a), equating to 106.5 W total power. Putting these mass, volume, and power values into context, they would respectively comprise 0.7%, 1.8% and 1% of total spacecraft capacities (based on Orion specifications; NASA, 2015). These values are sufficiently high to potentially exclude experimental mice, and subsequently animal research, from the mission.

Another major problem arises from the vast distances that separate Earth from even its neighboring planets, demanding long periods of travel time using current or foreseeable propulsion technology. For those aboard the spacecraft, including experimental animals, this will prolong exposure to the space environment and negatively impact health through continuous exposure to microgravity and space radiation, as well as increase the risk of life-support system failure. Solutions to these challenges must therefore remain effective for prolonged periods of time. The current suite of proposed solutions are directed mainly at the level of spacecraft design: thick shields incorporated into the walls of the spacecraft habitat can protect crewmembers from ionizing space radiation, and a rotating habitat design producing an artificial gravitational field can mitigate the tissue-atrophying effects of microgravity. While these ideas are theoretically effective, they are generally expensive, impractical, and currently at early stages of assessment and development. They correspondingly require a large, massive, and expensive spacecraft design, which is difficult to get off the ground both literally and figuratively.

All told, performing animal research beyond LEO will likely require new technologies to simultaneously limit the demands placed on spacecraft capacities and ensure the long-term health and survival of the animals. One technology, called synthetic torpor, has the potential to do this.

3. Synthetic torpor as a strategy for transporting experimental animals beyond LEO

Torpor is defined as a depression in metabolic rate below basal metabolic rate, the level of metabolism required to maintain an awake, resting, post-absorptive endothermic animal in its thermoneutral zone (Guppy and Withers, 1999). This reduced rate of energy consumption allows animals to prolong survival in environments that threaten the balance of cellular energy supply and demand. Typically, these are environments that lack (or are low in) some essential abiotic factor such as food, water, heat, or oxygen. Short-term torpor bouts (< 24 h) are called daily torpor bouts, while longer-term seasonal torpor bouts characterize hibernation (winter season) and aestivation (summer season). In the literature, 'torpor' is often used synonymously with 'daily torpor' and in a manner that excludes hibernation/aestivation. However, 'torpor' technically refers to a depressed metabolic rate, regardless of whether that rate is related to daily torpor or hibernation. We will hereafter use 'torpor' to refer to any state of metabolic depression, and where appropriate will clearly distinguish daily torpor-related torpor bouts from hibernation-related torpor bouts.

Many animal groups contain species that are capable of torpor [e.g., arthropods (Callier et al., 2015), gastropods (Adamson et al., 2017), fishes (Regan et al., 2017), amphibians (Donohoe and Boutilier, 1998), reptiles (Jackson, 1968), birds (Hainsworth and Wolf, 1970), mammals (Carey et al., 2003)]. Generally, endothermic animals induce torpor through an initial depression of metabolic rate, and then, crucially, maintain torpor through changes to their thermoregulatory set-points and thermogenic metabolism (Carey et al., 2003). This renders the mechanisms of torpor for endothermic animals inherently different from those of ectothermic animals (Richards, 2010). But while the mechanisms may differ, the physiological and biochemical effects are ultimately similar; namely, reduced rates of O_2 consumption, ventilation, heart beat, gene expression, protein synthesis, transmembrane ion pumping, and cell division (Guppy and Withers, 1999).

Recent advances in the metabolic control field have made it possible to artificially induce torpid states in animals that do not naturally do so, and then restore metabolic rate to routine levels with no apparent negative effects on physiological or neurological function (Blackstone, 2005; Cerri et al., 2013; Roth and Nystul, 2005). These techniques were originally carried out on relatively simple animals [e.g., yeast (Werner-Washburne et al., 1993), nematodes (Johnson et al., 1984)], but have recently been applied to more complex animals, particularly rodents (Cerri et al., 2013; Galicia et al., 2011; Blackstone, 2005; Tupone et al., 2013). A variety of terms have been used for this metabolic state, including metabolic flexibility, suspended animation and artificial hibernation. Here, we have decided to use 'synthetic torpor', a term first used by Cerri (2017) and defined as a reversible metabolic depression that is artificially induced. We have chosen this term because we feel it is the only one to sufficiently capture the key components of the phenomenon: depressed metabolism and non-natural, on-demand induction.

Synthetic torpor will offer two main benefits when transporting experimental animals aboard spacecraft on long-duration missions. First, it will reduce the mass, volume, and power required to keep the animals alive during the mission. Second, it will mitigate the negative health effects of spaceflight on the animals, which, depending on the proposed experiments, would avoid confounding results. These requirements could be addressed by using animals with natural metabolic flexibility for research beyond LEO (Griko et al., 2017), however a wider scope of studies is made possible with artificially induced metabolic depression in non-hibernating animals. This will place control in the hands of the crewmembers, and expand the repertoire of experimental animal species that can be taken aboard spaceflight missions.

We will elaborate on the advantages of synthetic torpor below, and then discuss current technologies for inducing synthetic torpor in mice. We focus our discussion on mice because of their widespread use in space biology research particularly aboard spaceflight missions (NASA, 2017a), and their current use in synthetic torpor research.

4. Advantages of synthetic torpor when transporting experimental animals beyond LEO

The mass, volume, and power savings that come with synthetic torpor are directly tied to the depth of metabolic depression. This depth will vary depending on the technique used to induce synthetic torpor (see next section), but in all cases, the savings are significant. A relatively shallow metabolic depression of 80% will theoretically reduce

food and water intake by the same amount, while a deeper metabolic depression will reduce food and water intake even more. Deeper depressions will also lessen the animal's ventilation requirements and negate the use of lighting, which will reduce the system's power requirements. The inactivity that results from torpor (natural or synthetic) will also reduce the required living space for the animal. Combined with diminished rates of food and water intake and a reduced need for bedding changes and habitat enrichment, transporting experimental animals in a state of synthetic torpor should reduce mass, volume, and power requirements by no less than 70%.

Torpor also mitigates the health problems that arise from prolonged exposure to space radiation and microgravity, two primary hazards of spaceflight. Space radiation is composed of the high-energy particles of galactic cosmic rays and solar particle events, including protons, helium nuclei, and HZE ions. This form of radiation is different than high-energy photon radiation such as x-rays and gamma rays, and correspondingly, it has distinct effects on biological molecules, cells, and tissues. These effects include direct damage to DNA and proteins, indirect damage to DNA and proteins resulting from the formation of reactive oxygen species, and an overall alteration of the cell's biochemistry that can negatively affect gene transcription and cell division, and ultimately, carcinogenesis (Cucinotta et al., 2017; Durante and Cucinotta, 2008; Zeitlin et al., 2013). Humans on Earth and in LEO are protected from space radiation by the Earth's magnetosphere, which deflects these high-energy particles. However, humans beyond LEO and outside the bounds of the Van Allen Belt have no such protection, and this is what results in radiation exposure during deep space missions.

Amazingly, torpid animals exhibit low levels of tissue damage when exposed to ionizing radiation. Though the exact mechanism is not known, recent data from NASA (Baird et al., 2011) along with other studies going back over 50 years demonstrate that metabolic depression per se reduces the damaging effects of ionizing radiation in mammals (Barr and Musacchia, 1972, 1969; Ignat'ev et al., 2006; Lisowska et al., 2014; Mraz and Praslicka, 1961; Musacchia et al., 1971; Smith and Grenan, 1951). Furthermore, the significantly reduced O_2 consumption rates of torpid animals suggest that their O_2 demands may be met under hypoxic conditions. Because much of the tissue-damaging effects of radiation arise from the reactive oxygen species produced from the interactions of high energy particles with di-oxygen molecules (Wilson and Hay, 2011), housing the animals in hypoxia so as to reduce tissue O_2 partial pressures may further reduce the harmful effects of radiation.

Most of the results described above were obtained from animals with natural abilities to induce torpor. The relevant question is therefore whether animals in synthetic torpor are similarly protected from radiation damage. The results of a recent study suggest this is the case. Mice were approximately four times more likely to survive total body radiation exposure when in a pharmacologically induced state of synthetic torpor (induced using AMP; see next section) than when in a normal metabolic state, showing ~80% survival at 30 days post-irradiation compared to ~20% survival for control mice (Ghosh et al., 2017). Pharmacologically induced synthetic torpor therefore appears to bestow similar radio-protection to the animal.

The microgravity environment of space has been shown to cause human crewmembers aboard Mir and the ISS to lose up to 2.3% of bone mineral density per month (Cavanagh et al., 2007; Lang et al., 2004; LeBlanc et al., 2000; LeBlanc et al., 2013; Vico et al., 2000), and similar results have been observed in non-torpid experimental mice exposed to weightlessness in ground-based experiments (Aguirre et al., 2006). The extent of these losses during long-term spaceflight beyond LEO is not known. However, it is known that animals in natural states of hibernation-related torpor, such as brown and black bears following six to eight months of hibernation, show no loss in bone mass and less muscle atrophy than would be expected over such a prolonged period of physical inactivity (Fedorov et al., 2012; Hershey et al., 2008; Ivakine and Cohn, 2014; Wojda et al., 2013). This suggests that animals in hibernation-related torpor possess natural mechanisms to prevent muscle and bone atrophy. It is not known what these underlying molecular mechanisms are, nor is it known whether they are inherent to torpor per se and therefore present during synthetic torpor as well. This is an important area of future research that might be done most effectively aboard spaceflight missions.

There are two ways that synthetic torpor could be incorporated into spaceflight missions. First, synthetic torpor could be used purely as a mechanism to transport experimental animals aboard the spacecraft as practically and safely as possible; practically, by minimizing the mass and volume requirements of the animals and their life support systems, and safely, by maximizing the health and survival of the animals themselves. The animals could then be aroused at the time they were needed for experimentation. Second, synthetic torpor could itself be incorporated into the experiments so as to explore how the space environment (e.g., microgravity, radiation) affects the biology of a synthetically torpid animal. Such experiments could serve as test beds for possible future projects investigating topics such as human-directed synthetic torpor (Cerri et al., 2016) and closed-loop bioregenerative life support systems (Niederwieser et al., 2018). In either case, the primary benefits of synthetic torpor—minimized mass and volume requirements, maximized animal health and survival—would be realized. This would allow additional supplies and/or objectives to be included in the mission, and enable certain experiments to be run that required the animals be in terrestrial-like physiological states at an advanced point in the mission and independent of the potentially distressing experience of launch and/or re-entry. The benefits of synthetic torpor are therefore clear and significant, but as the next section discusses, inducing synthetic torpor is not straightforward.

5. Metabolic control techniques for inducing synthetic torpor in mammals

The idea of placing spaceflight crewmembers in a metabolically depressed state has been discussed since the early days of Project Mercury (Hock, 1960). Since then, numerous biological compounds have been touted as torpor induction triggers, including iodothyronamines (Chiellini et al., 2007; Ju et al., 2011; Scanlan et al., 2004), 5′-AMP (Swoap et al., 2007; Zhang et al., 2006) and the aptly named hibernation induction trigger (Baldelli et al., 2006; Dawe and Spurrier, 1969). Efforts to translate these chemicals into synthetic torpor-inducing technologies have yielded mixed results that, for various reasons, are less than promising. Here, we will discuss three technologies that currently do hold promise. Two of these are pharmacological technologies, while the third is feed-related. The pharmacological techniques depress metabolic rate through mechanisms of action acting in one of two areas, respectively: the mitochondria and the central nervous system.

Mitochondria are obvious targets for synthetic torpor induction techniques because they supply the cellular energy (ATP, adenosine triphosphate) that ultimately powers the chemical reactions that comprise metabolism. Reducing mitochondrial activity would reduce the rate at which ATP is supplied, consequently reducing metabolic rate. Indeed, it has recently been shown that 13-lined ground squirrels appear to induce torpor through post-translational modifications to mitochondrial enzymes of the electron transport system (ETS) (Mathers et al., 2017). A method researchers have employed to do this synthetically uses H_2S to reversibly inhibit cytochrome c oxidase, the terminal enzyme in the ETS that transfers electrons to O_2. Inhibiting cytochrome c oxidase halts electron flux through the ETS, dissipating the inner mitochondrial membrane's electrochemical proton gradient and thereby arresting (or reducing) the rate at which ATP synthase can synthesize ATP. Using H_2S, Blackstone and colleagues (Blackstone, 2005) reduced the metabolic rate of mice by 90%, a depression that enhanced survival in what are usually lethal hypoxic conditions for mice (Blackstone and Roth, 2007). Despite cytochrome c

oxidase being widely conserved across eukaryotic species, attempts to induce synthetic torpor using H_2S in non-mouse mammals (particularly larger mammals) have failed (Drabek et al., 2011; Haouzi et al., 2008). However, crucially, these studies were able to independently induce synthetic torpor in mice (Volpato et al., 2008), verifying H_2S as a viable induction technique at least for this important experimental species.

A potential problem with inducing synthetic torpor at the mitochondrial level is that it depresses metabolic rate by reducing the supply of ATP. The long-term viability of this approach relies on a coordinated and concomitant downregulation of cellular ATP demand processes; in the absence of this, cellular ATP and ADP concentrations would respectively decrease and increase, leading to allosteric interactions with glycolytic enzymes that would increase glycolytic ATP production rates (Hochachka and Storey, 1975). Even if these glycolytic rates could supply enough ATP to meet cellular demand (which would be unlikely owing to the relative inefficiency of glycolysis), the combination of a finite glycolytic fuel store (glucose and glycogen) and the accumulation of glycolysis's deleterious end-products (lactate and protons) would render this balance short-lived and unsustainable. The ATP supply-demand balance would ultimately become mismatched, and the inevitable result would be the animal's death (Boutilier, 2001; Hochachka, 1986). Considerable debate exists around whether the cue for natural torpor induction resides on the side of ATP supply or ATP demand (see review by Guppy, 2004), with some studies presenting evidence to suggest the former (Bishop and Brand, 2000; Bishop et al., 2002; De Zwaan and Wijsman, 1976; Hochachka, 1985; 1982; Mathers et al., 2017; Plaxton and Storey, 1984; Rees and Hand, 1991) and other studies presenting evidence to suggest the latter (Caligiuri et al., 1981; Flanigan and Withers, 1991; Robin et al., 1979; Sick et al., 1982). Additional research is required to determine if mitochondrial-level synthetic torpor induction is ultimately stable in terms of ATP supply-demand balance, and if not, whether these induction techniques can be supplemented with techniques to reduce ATP demand processes.

Synthetic torpor can also be induced at the level of the central nervous system (CNS) by inhibiting thermoregulation in the hypothalamus. Different inhibitory mechanisms have been proposed (e.g., Cerri, 2017; Cerri et al., 2013; Madden and Morrison, 2009), but one that has proved effective and therefore received much recent attention involves the A_1 adenosine receptors (A_1AR) of the hypothalamus. These receptors mediate the central thermoregulatory control circuit in the hypothalamus (Nakamura and Morrison, 2008), which coordinates the activation of heat-generating tissues of the body including shivering thermogenesis, brown adipose tissue, and cutaneous vasomotion. By coupling a low ambient temperature with a centrally administered pharmacological A_1AR agonist called $_6$*N*-cyclohexyladenosine (CHA), researchers have been able to induce synthetic torpor in mice, rats, and arctic ground squirrels (Jinka et al., 2015, 2011; Muzzi et al., 2013; Tupone et al., 2013; Vicent et al., 2017). This technique can depress metabolic rate in rats by approximately 30% at an ambient temperature of 15 °C (Tupone et al., 2013) and presumably more so at lower ambient temperatures. The advantage of inducing synthetic torpor at the CNS level is that by resetting the thermoregulatory set point, whole body metabolic rate is thermodynamically downregulated to a new, stable, and homeostatically regulated state (a “shifted homeostasis”; see Tupone et al., 2016) that includes both ATP supply and demand processes.

A third technique that induces on-demand torpor in mice is non-pharmacological and involves caloric restriction coupled with a cool housing temperature of approximately 18 °C. Mice under these conditions will naturally exhibit daily torpor within 12 h of being withheld food (Sunagawa and Takahashi, 2016) and remain in this torpid state for up to half of each day (Hudson and Scott, 1979). The mechanistic site(s) of action are not fully resolved but may involve the CNS. Restricted (or eliminated) caloric intake is sensed by the hypothalamus via reduced leptin levels in the blood that result from β3-adrenergic receptor activation of white adipose tissue (Swoap et al., 2006). This alters thermoregulation by reducing the sensitivity of the mouse's thermoregulatory system (Sunagawa and Takahashi, 2016), enabling body temperature to remain low and stable for prolonged time periods. Metabolic rate during these times is depressed by approximately 70% (Sunagawa and Takahashi, 2016). While this depression could theoretically be deepened by housing the mice at lower ambient temperatures, the mice actually continue to regulate their body temperature during these states of torpor. Hibernators do this too, but while they typically regulate their body temperature between 3 °C and 10 °C during torpor (Carey et al., 2003), mice regulate their body temperature at somewhere between 12 and 16 °C depending on the study (see Hudson and Scott, 1979; Sunagawa and Takahashi, 2016). At temperatures below this threshold, the mouse's metabolic rate is elevated beyond what would be predicted so as to maintain a stable body temperature. The ideal housing temperature for inducing daily torpor in mice is therefore somewhere around 16 °C.

6. Challenges when applying synthetic torpor to spaceflight scenarios

While these technologies are promising, there are important considerations when applying them to spaceflight scenarios. First, natural multi-day torpor bouts such as those that characterize hibernation are punctuated with euthermic periods when metabolic rate is elevated to routine levels, returning body temperature to ~37 °C for a 12–24 h period before the animal falls back into torpor (Carey et al., 2003). These periods, called interbout arousals (IBAs), occur once every two to 21 days depending on the species and where in the hibernation cycle the animal is (higher frequencies at earlier and later points in the cycle; Carey et al., 2003). It is not known what signals these IBAs. Hypotheses range from electrolyte rebalancing (Jani et al., 2013), to metabolic fuel replenishment (Galster and Morrison, 1975) and/or waste clearance (Serkova et al., 2007), to the repayment of slow wave sleep debt (Daan et al., 1991). Whatever the reason, IBAs must be important to long-term torpor bouts because they account for up to 86% of the total energy consumed during the hibernation season (Karpovich et al., 2009). If IBAs are similarly important to long-term synthetic torpor bouts, they will need to be incorporated into the animal housing apparatuses. However, an alternative to inducing typical IBAs is informed by research on hibernating primates that reveals IBA use is temperature-dependent; hibernating lemurs, whose body temperatures closely track ambient temperatures, do not induce IBAs so long as the ambient temperature of their hibernacula periodically exceeds 30 °C (Dausmann et al., 2004; Dausmann and Warnecke, 2016). This minimizes the steep energetic cost of initiating IBAs while presumably providing the physiological benefits of IBA (von der Ohe et al., 2007). Consistent with this, bears, which reduce body temperature only to ~30 °C during hibernation, do not perform IBAs either (Tøien et al., 2011). Other hibernating species are affected by ambient temperatures differently, however. The golden-mantled ground squirrel (*Spermophilus saturatus*) displays shortened torpor bouts (i.e., more frequent arousals) as its ambient temperature increases (Geiser and Kenagy, 1988), and the Australian tawny frogmouth (*Podargus strigoides*) arouses from torpor when its ambient temperature rises above ~10 °C (Körtner et al., 2000). It is not known how animals in states of synthetic torpor will respond to ambient temperature. If they respond like the lemurs and bears, then periodically elevating the experimental animals’ housing temperature to ~30 °C may circumvent the incorporation of IBAs into the synthetic torpor cycle; if they respond like the ground squirrels and frogmouths, then elevating their housing temperature may be a relatively straightforward way to incorporate IBAs into the synthetic torpor cycles. In any case, further research is required.

Second, while it is known that CHA administration depresses metabolic rate (Jinka et al., 2011), it does not share all the hallmarks of naturally induced torpor (Vicent et al., 2017). This may be the case with other synthetic torpor-inducing techniques too. How this will impact

the potential benefits of these techniques to spaceflight scenarios is not currently know. So long as these techniques depress metabolic rate, the mass-, volume- and power-related benefits will likely persist. The radiation- and microgravity-related protective benefits, however, will require further research. Performing these experiments in space will probably yield the most conclusive data.

And third, synthetic torpor will optimally benefit spaceflight scenarios if it can be induced in the experimental animals over prolonged periods of time. It is not known how long the aforementioned techniques remain effective over, but, at least for CHA-induced synthetic torpor, longer-term synthetic torpor bouts (up to 24 h) require a continual administration of the compound (Jinka et al., 2015). Further research is needed to determine whether long-term synthetic torpor can be induced using a single dose, the scenario that would most benefit spaceflight applications. If this cannot be done, then compound administration systems would need to be incorporated into the spacecraft design, and this could offset some of synthetic torpor's mass-, volume- and power-saving benefits.

7. Conclusions

Current torpor-inducing technologies offer great potential to benefit animal research on spaceflight missions beyond LEO, but additional ground-based research programs are needed to optimize these technologies and their applications. Importantly, the value of this research goes far beyond the practical transport of experimental animals to deep space, which has been the focus of this communication. Other important and timely areas that stand to benefit from synthetic torpor include biomedicine, human spaceflight, and more generally, NASA's future role in space exploration. Biomedical benefits of synthetic torpor include the reduced tissue damage and increased response times that come with placing humans suffering from ailments such as stroke, sepsis, and cardiac arrest in metabolically depressed states, enhancing the probability of survival (see review by Cerri, 2017). Human spaceflight benefits of synthetic torpor are the same as those for experimental animals; namely, mitigated negative health effects of space travel and a reduced spacecraft size, mass, and cost. Furthermore, torpor-supporting spacecraft infrastructure for experimental animals would serve as test beds for human-directed torpor infrastructure. If NASA or any other agency or company is to pursue synthetic torpor for human crewmembers—and there are good reasons to do so (see review by Cerri et al., 2016)—this research is a necessary early step.

Finally, NASA's future role in space exploration stands to benefit from this research because more nations and commercial agencies are pursuing human spaceflight programs than ever before (e.g., Lele, 2013; Musk, 2017; NASA, 2017b). The ambitious goals of these programs will be achieved most quickly and safely by incorporating animal research models into their programs' development phases, and this may be done most practically and inexpensively with the help of synthetic torpor. The first agency to develop a synthetic torpor-inducing technology that is specific to spaceflight scenarios will create forward-demand in an age when the global appetite for space exploration is increasing, and when the technology to achieve these goals is developing more rapidly than ever before.

Acknowledgments

Supported in part by the NASA Human Research Program (HRP), San Jose State University Bridges Program, and NASA contract to the Carnegie Mellon University Silicon Valley # NNX08 –AB13A.

We thank Drs. David Loftus and Hannah Carey for their valuable comments and suggestions, and the anonymous reviewers for their insightful analysis of our manuscript.

References

Adamson, K.J., Wang, T., Rotgans, B.A., Kruangkum, T., Kuballa, A.V., Storey, K.B., Cummins, S.F., 2017. Genes and associated peptides involved with aestivation in a land snail. Gen. Comp. Endocrinol. 246, 88–98. http://dx.doi.org/10.1016/j.ygcen.2015.10.013.

Aguirre, J.I., Plotkin, L.I., Stewart, S.A., Weinstein, R.S., Parfitt, A.M., Manolagas, S.C., Bellido, T., 2006. Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. J. Bone Miner. Res. 21 (4), 605–615.

Baird, B.J., Dickey, J.S., Nakamura, A.J., Redon, C.E., Parekh, P., Griko, Y.V., Aziz, K., Georgakilas, A.G., Bonner, W.M., Martin, O.A., 2011. Hypothermia postpones DNA damage repair in irradiated cells and protects against cell killing. Mutat. Res. 711, 142–149. http://dx.doi.org/10.1016/j.mrfmmm.2010.12.006.

Baldelli, B., Vecchio, L., Bottone, M.G., Muzzonigro, G., Biggiogera, M., Malatesta, M., 2006. The effect of the enkephalin DADLE on transcription does not depend on opioid receptors. Histochem. Cell Biol. 126, 189–197. http://dx.doi.org/10.1007/s00418-006-0145-x.

Barr, R.E., Musacchia, X.J., 1972. Postirradiation hibernation and radiation response of ground squirrels: telemetry surveillance. Radiat. Res. 51, 631–637.

Barr, R.E., Musacchia, X.J., 1969. The effect of body temperature and postirradiation cold exposure on the radiation response of the hibernator Citellus tridecemlineatus. Radiat. Res. 38, 437–448.

Bishop, T., Brand, M.D., 2000. Processes contributing to metabolic depression in hepatopancreas cells from the snail Helix aspersa. J. Exp. Biol. 203, 3603–3612.

Bishop, T., St-Pierre, J., Brand, M.D., 2002. Primary causes of decreased mitochondrial oxygen consumption during metabolic depression in snail cells. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R372–R382. http://dx.doi.org/10.1152/ajpregu.00401.2001.

Blackstone, E., 2005. H2S induces a suspended animation-like state in mice. Science 308, 518. http://dx.doi.org/10.1126/science.1108581.

Blackstone, E., Roth, M.B., 2007. Suspended animation-like state protects mice from lethal hypoxia. Shock 27, 370–372. http://dx.doi.org/10.1097/SHK.0b013e31802e27a0.

Boutilier, R.G., 2001. Mechanisms of cell survival in hypoxia and hypothermia. J. Exp. Biol. 204, 3171–3181.

Caligiuri, M., Robin, E.D., Hance, A.J., Robin, D.A., 1981. Prolonged diving and recovery in the freshwater turtle, Pseudemys scripta—II magnitude of depression of O2 requirements and the relation to body O2 stores. Comp. Biochem. Physiol. Part A 70 (3), 365–369.

Callier, V., Hand, S.C., Campbell, J.B., Biddulph, T., Harrison, J.F., 2015. Developmental changes in hypoxic exposure and responses to anoxia in Drosophila melanogaster. J. Exp. Biol. 218, 2927–2934. http://dx.doi.org/10.1242/jeb.125849.

Carey, H.V., Andrews, M.T., Martin, S.L., 2003. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol. Rev. 83, 1153–1181. http://dx.doi.org/10.1152/physrev.00008.2003.

Cavanagh, P.R., Licata, A.A., Rice, A.J., 2007. Exercise and pharmacological countermeasures for bone loss during longduration space flight. Gravitational Space Res. 18 (2).

Cerri, M., 2017. The central control of energy expenditure: exploiting torpor for medical applications. Annu. Rev. Physiol. 79, 167–186. http://dx.doi.org/10.1146/annurev-physiol-022516-034133.

Cerri, M., Mastrotto, M., Tupone, D., Martelli, D., Luppi, M., Perez, E., Zamboni, G., Amici, R., 2013. The inhibition of neurons in the central nervous pathways for thermoregulatory cold defense induces a suspended animation state in the rat. J. Neurosci. 33, 2984–2993. http://dx.doi.org/10.1523/JNEUROSCI.3596-12.2013.

Cerri, M., Tinganelli, W., Negrini, M., Helm, A., Scifoni, E., Tommasino, F., Sioli, M., Zoccoli, A., Durante, M., 2016. Hibernation for space travel: Impact on radioprotection. Life Sci. Space Res. (Amst) 11, 1–9. http://dx.doi.org/10.1016/j.lssr.2016.09.001.

Chiellini, G., Frascarelli, S., Ghelardoni, S., Carnicelli, V., Tobias, S.C., DeBarber, A., Brogioni, S., Ronca-Testoni, S., Cerbai, E., Grandy, D.K., Scanlan, T.S., Zucchi, R., 2007. Cardiac effects of 3-iodothyronamine: a new aminergic system modulating cardiac function. FASEB J 21, 1597–1608. http://dx.doi.org/10.1096/fj.06-7474com.

Cucinotta, F.A., To, K., Cacao, E., 2017. Predictions of space radiation fatality risk for exploration missions. Life Sci. Space Res. (Amst.) 13, 1–11. http://dx.doi.org/10.1016/j.lssr.2017.01.005.

Daan, S., Barnes, B.M., Strijkstra, A.M., 1991. Warming up for sleep? Ground squirrels sleep during arousals from hibernation. Neurosci. Lett. 128, 265–268.

Dausmann, K.H., Glos, J., Ganzhorn, J.U., Heldmaier, G., 2004. Physiology: hibernation in a tropical primate. Nature 429 (6994), 825–826.

Dausmann, K.H., Warnecke, L., 2016. Primate torpor expression: ghost of the climatic past. Physiology 31 (6), 398–408.

Dawe, A.R., Spurrier, W.A., 1969. Hibernation induced in ground squirrels by blood transfusion. Science 163, 298–299.

De Zwaan, A., Wijsman, T.C., 1976. Anaerobic metabolism in Bivalvia (Mollusca). Characteristics of anaerobic metabolism. Comp. Biochem. Physiol., B 54, 313–324.

Donohoe, P.H., Boutilier, R.G., 1998. The protective effects of metabolic rate depression in hypoxic cold submerged frogs. Respiration Physiol. 111, 325–336. http://dx.doi.org/10.1016/S0034-5687(97)00125-4.

Drabek, T., Kochanek, P.M., Stezoski, J., Wu, X., Bayir, H., Morhard, R.C., Stezoski, S.W., Tisherman, S.A., 2011. Intravenous hydrogen sulfide does not induce hypothermia or improve survival from hemorrhagic shock in pigs. Shock 35, 67–73. http://dx.doi.org/10.1097/SHK.0b013e3181e86f49.

Durante, M., Cucinotta, F.A., 2008. Heavy ion carcinogenesis and human space

exploration. Nat. Rev. Cancer 8, 465–472. http://dx.doi.org/10.1038/nrc2391.
Fedorov, V.B., Goropashnaya, A.V., Tøien, Ø., Stewart, N.C., Chang, C., Wang, H., Yan, J., Showe, L.C., Showe, M.K., Donahue, S.W., Barnes, B.M., 2012. Preservation of bone mass and structure in hibernating black bears (Ursus americanus) through elevated expression of anabolic genes. Funct. Integr. Genomics 12, 357–365. http://dx.doi.org/10.1007/s10142-012-0266-3.
Flanigan, J.E., Withers, P.C., 1991. In vitro metabolic depression of tissues from the aestivating frog Neobatrachus pelobatoides. J. Exp. Biol. 161 (1), 273–283.
Galicia, E., Palma, E., Selch, F., Gomez, D., Grindeland, R., Griko, Y., 2011. Metabolic control as a strategy for payload cost reduction and mitigation of negative space environmental factors. Grav. Space Res. 25 (1).
Galster, W., Morrison, P.R., 1975. Gluconeogenesis in arctic ground squirrels between periods of hibernation. Am. J. Physiol. 228, 325–330.
Geiser, F., Kenagy, G.J., 1988. Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. Physiol. Zool. 61, 442–449.
Ghosh, S., Indracanti, N., Joshi, J., Ray, J., Indraganti, P.K., 2017. Pharmacologically induced reversible hypometabolic state mitigates radiation induced lethality in mice. Sci. Rep. 7, 14900.
Griko, Y.V., Rask, J.C., Raychev, R., 2017. Advantage of animal models with metabolic flexibility for space research beyond low Earth orbit. New Space 5, 110–120.
Guppy, M., 2004. The biochemistry of metabolic depression: a history of perceptions. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 139, 435–442. http://dx.doi.org/10.1016/j.cbpc.2004.02.019.
Guppy, M., Withers, P.C., 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol. Rev. Cambridge Philos. Soc. 74, 1–40.
Hainsworth, F.R., Wolf, L.L., 1970. Regulation of oxygen consumption and body temperature during torpor in a hummingbird, Eulampis jugularis. Science 168, 368–369.
Haouzi, P., Notet, V., Chenuel, B., Chalon, B., Sponne, I., Ogier, V., Bihain, B., 2008. H2S induced hypometabolism in mice is missing in sedated sheep. Respir. Physiol. Neurobiol. 160, 109–115. http://dx.doi.org/10.1016/j.resp.2007.09.001.
Hershey, J.D., Robbins, C.T., Nelson, O.L., Lin, D.C., 2008. Minimal seasonal alterations in the skeletal muscle of captive brown bears. Physiol. Biochem. Zool. 81, 138–147. http://dx.doi.org/10.1086/524391.
Hochachka, P.W., 1986. Defense strategies against hypoxia and hypothermia. Science 231, 234–241.
Hochachka, P.W., 1985. Assessing metabolic strategies for surviving O_2 lack: role of metabolic arrest coupled with channel arrest. Mol. Physiol. 8 (3), 331–350.
Hochachka, P.W., 1982. Metabolic arrest as a mechanism of protection against hypoxia. Protection of Tissues Against Hypoxia. Elsevier Press, Amsterdam, pp. 1–12.
Hochachka, P.W., Storey, K.B., 1975. Metabolic consequences of diving in animals and man. Science 187, 613–621.
Hock, R.J., 1960. The potential application of hibernation to space travel. Aerosp. Med. 31, 485–489.
Hudson, J.W., Scott., I.M., 1979. Daily torpor in the laboratory mouse, *Mus musculus* var. albino. Physiol. Zool. 52 (2), 205–218.
Ignat'ev, D.A., Fialkovskaia, L.A., Perepelkina, N.I., Markevich, L.N., Kraev, I.V., Kolomiĭtseva, I.K., 2006. The effect of hypothermia on the rat radioresistance. Radiats. Biol. Radioecol. 46, 706–712.
Ivakine, E.A., Cohn, R.D., 2014. Maintaining skeletal muscle mass: lessons learned from hibernation. Exp. Physiol. 99, 632–637. http://dx.doi.org/10.1113/expphysiol.2013.074344.
Jackson, D.C., 1968. Metabolic depression and oxygen depletion in the diving turtle. J. Appl. Physiol. 24, 503–509.
Jani, A., Martin, S.L., Jain, S., Keys, D., Edelstein, C.L., 2013. Renal adaptation during hibernation. Am. J. Physiol. Renal Physiol. 305, F1521–F1532. http://dx.doi.org/10.1152/ajprenal.00675.2012.
Jinka, T.R., Combs, V.M., Drew, K.L., 2015. Translating drug-induced hibernation to therapeutic hypothermia. ACS Chem. Neurosci. 6, 899–904. http://dx.doi.org/10.1021/acschemneuro.5b00056.
Jinka, T.R., Tøien, Ø., Drew, K.L., 2011. Season primes the brain in an arctic hibernator to facilitate entrance into torpor mediated by adenosine A(1) receptors. J. Neurosci. 31, 10752–10758. http://dx.doi.org/10.1523/JNEUROSCI.1240-11.2011.
Johnson, T.E., Mitchell, D.H., Kline, S., Kemal, R., Foy, J., 1984. Arresting development arrests aging in the nematode Caenorhabditis elegans. Mech. Ageing Dev. 28, 23–40.
Ju, H., So, H., Ha, K., Park, K., Lee, J.-W., Chung, C.-M., Choi, I., 2011. Sustained torpidity following multi-dose administration of 3-iodothyronamine in mice. J. Cell. Physiol. 226, 853–858. http://dx.doi.org/10.1002/jcp.22573.
Karpovich, S.A., Tøien, Ø., Buck, C.L., Barnes, B.M., 2009. Energetics of arousal episodes in hibernating arctic ground squirrels. J. Comp. Physiol. B 179, 691–700. http://dx.doi.org/10.1007/s00360-009-0350-8.
Körtner, G., Brigham, R.M., Geiser, F., 2000. Winter torpor in a large bird. Nature 407, 318.
Lang, T., LeBlanc, A., Evans, H., Lu, Y., Genant, H., Yu, A., 2004. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. J. Bone Miner. Res. 19, 1006–1012. http://dx.doi.org/10.1359/JBMR.040307.
LeBlanc, A., Schneider, V., Shackelford, L., West, S., Oganov, V., Bakulin, A., Voronin, L., 2000. Bone mineral and lean tissue loss after long duration space flight. J. Musculoskeletal Neuronal Interact. 1 (2), 157–160.
Leblanc, A., Matsumoto, T., Jones, J., Shapiro, J., Lang, T., Shackelford, L., Smith, S.M., Evans, H., Spector, E., Ploutz-Snyder, R., Sibonga, J., Keyak, J., Nakamura, T., Kohri, K., Ohshima, H., 2013. Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight. Osteoporos Int. 24, 2105–2114. http://dx.doi.org/10.1007/s00198-012-2243-z.
Lele, A., 2013. Asian Space Race: Rhetoric or Reality? Springer Publishing ISBN 978-8132207320.
Lisowska, H., Brehwens, K., Zölzer, F., Wegierek-Ciuk, A., Czub, J., Lankoff, A., Haghdoost, S., Wojcik, A., 2014. Effect of hypothermia on radiation-induced micronuclei and delay of cell cycle progression in TK6 cells. Int. J. Radiat. Biol. 90, 318–324. http://dx.doi.org/10.3109/09553002.2014.887233.
Madden, C.J., Morrison, S.F., 2009. Neurons in the paraventricular nucleus of the hypothalamus inhibit sympathetic outflow to brown adipose tissue. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R831–R843. http://dx.doi.org/10.1152/ajpregu.91007.2008.
Mathers, K.E., McFarlane, S.V., Zhao, L., Staples, J.F., 2017. Regulation of mitochondrial metabolism during hibernation by reversible suppression of electron transport system enzymes. J. Comp. Physiol. B 187, 227–234. http://dx.doi.org/10.1007/s00360-016-1022-0.
Moyer, E.L., Dumars, P.M., Sun, G.-S., Martin, K.J., Heathcote, D.G., Boyle, R.D., Skidmore, M.G., 2016. Evaluation of rodent spaceflight in the NASA animal enclosure module for an extended operational period (up to 35 days). NPJ Microgravity 2, 16002. http://dx.doi.org/10.1038/npjmgrav.2016.2.
Mraz, L., Praslicka, M., 1961. Influence of hypothermia on the survival of mice after large doses of x-radiation. Nature 189, 677–678.
Musacchia, X.J., Volkert, W.A., Barr, R.E., 1971. Radioresistance in hamsters during hypothermic depressed metabolism induced with helium and low temperatures. Radiat. Res. 46, 353–361.
Musk, E., 2017. Making humans a multi-planetary species. New Space 5 (2), 46–61.
Muzzi, M., Blasi, F., Masi, A., Coppi, E., Traini, C., Felici, R., Pittelli, M., Cavone, L., Pugliese, A.M., Moroni, F., Chiarugi, A., 2013. Neurological basis of AMP-dependent thermoregulation and its relevance to central and peripheral hyperthermia. J. Cereb. Blood Flow Metab. 33, 183–190. http://dx.doi.org/10.1038/jcbfm.2012.157.
Nakamura, K., Morrison, S.F., 2008. A thermosensory pathway that controls body temperature. Nat. Neurosci. 11, 62–71. http://dx.doi.org/10.1038/nn2027.
NASA (2017a). International Space Station: Animal Enclosure Module. https://www.nasa.gov/mission_pages/station/research/experiments/363.html .
NASA (2017b). Deep Space Gateway. https://www.nasa.gov/feature/deep-space-gateway-to-open-opportunities-for-distant-destinations.
NASA (2015). Orion Quick Facts. https://www.nasa.gov/sites/default/files/atoms/files/fs-2014-08-004-jsc-orion_quickfacts-web.pdf.
Niederwieser, T., Kociolek, P., Klaus, D., 2018. Spacecraft cabin environment effects on the growth and behavior of *Chlorella vulgaris* for life support applications. Life Sci. Space Res. 16, 8–17.
Plaxton, W.C., Storey, K.B., 1984. Phosphorylation in vivo of red-muscle pyruvate kinase from the channelled whelk, Busycotypus canaliculatum, in response to anoxic stress. Eur. J. Biochem. 143, 267–272.
Rees, B.B., Hand, S.C., 1991. Regulation of glycolysis in the land snail Oreohelix during estivation and artificial hypercapnia. J. Comp. Physiol. B.
Regan, M.D., Gill, I.S., Richards, J.G., 2017. Calorespirometry reveals that goldfish prioritize aerobic metabolism over metabolic rate depression in all but near-anoxic environments. J. Exp. Biol. 220, 564–572. http://dx.doi.org/10.1242/jeb.145169.
Richards, J.G., 2010. Metabolic rate suppression as a mechanism for surviving environmental challenge in fish. Prog. Mol. Subcell. Biol. 49, 113–139. http://dx.doi.org/10.1007/978-3-642-02421-4_6.
Robin, E.D., Lewiston, N., Newman, A., Simon, L.M., Theodore, J., 1979. Bioenergetic pattern of turtle brain and resistance to profound loss of mitochondrial ATP generation. Proc. Natl. Acad. Sci. U S A 76, 3922–3926.
Roth, M.B., Nystul, T., 2005. Buying time in suspended animation. Sci. Am. 292, 48–55.
Scanlan, T.S., Suchland, K.L., Hart, M.E., Chiellini, G., Huang, Y., Kruzich, P.J., Frascarelli, S., Crossley, D.A., Bunzow, J.R., Ronca-Testoni, S., Lin, E.T., Hatton, D., Zucchi, R., Grandy, D.K., 2004. 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat. Med. 10, 638–642. http://dx.doi.org/10.1038/nm1051.
Serkova, N.J., Rose, J.C., Epperson, L.E., Carey, H.V., Martin, S.L., 2007. Quantitative analysis of liver metabolites in three stages of the circannual hibernation cycle in 13-lined ground squirrels by NMR. Physiol. Genomics 31, 15–24. http://dx.doi.org/10.1152/physiolgenomics.00028.2007.
Sick, T.J., Rosenthal, M., LaManna, J.C., Lutz, P.L., 1982. Brain potassium ion homeostasis, anoxia, and metabolic inhibition in turtles and rats. Am. J. Physiol. 243, R281–R288.
Smith, F., Grenan, M.M., 1951. Effect of hibernation upon survival time following whole-body irradiation in the marmot (Marmota monax). Science 113, 686–688.
Sunagawa, G.A., Takahashi, M., 2016. Hypometabolism during Daily Torpor in Mice is Dominated by Reduction in the Sensitivity of the Thermoregulatory System. Sci. Rep. 6, 37011. http://dx.doi.org/10.1038/srep37011.
Swoap, S.J., Gutilla, M.J., Liles, L.C., Smith, R.O., Weinshenker, D., 2006. The full expression of fasting-induced torpor requires beta 3-adrenergic receptor signaling. J. Neurosci. 26, 241–245. http://dx.doi.org/10.1523/JNEUROSCI.3721-05.2006.
Swoap, S.J., Rathvon, M., Gutilla, M., 2007. AMP does not induce torpor. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R468–R473. http://dx.doi.org/10.1152/ajpregu.00888.2006.
Tøien, Ø, Blake, J., Edgar, D.M., Grahn, D.A., Heller, H.C., Barnes, B.M., 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. Science 331, 906–909.
Tupone, D., Cetas, J.S., Morrison, S.F., 2016. Hibernation, Hypothermia and a Possible Therapeutic "Shifted Homeostasis" Induced by Central Activation of A1 Adenosine Receptor (A1AR). Nihon Shinkei Seishin Yakurigaku Zasshi 36, 51–54.
Tupone, D., Madden, C.J., Morrison, S.F., 2013. Central activation of the A1 adenosine receptor (A1AR) induces a hypothermic, torpor-like state in the rat. J. Neurosci. 33, 14512–14525. http://dx.doi.org/10.1523/JNEUROSCI.1980-13.2013.
Vicent, M.A., Borre, E.D., Swoap, S.J., 2017. Central activation of the A1 adenosine receptor in fed mice recapitulates only some of the attributes of daily torpor. J. Comp.

Physiol. B 187, 835–845. http://dx.doi.org/10.1007/s00360-017-1084-7.
Vico, L., Collet, P., Guignandon, A., Lafage-Proust, M.H., Thomas, T., Rehaillia, M., Alexandre, C., 2000. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet 355, 1607–1611.
Volpato, G.P., Searles, R., Yu, B., Scherrer-Crosbie, M., Bloch, K.D., Ichinose, F., Zapol, W.M., 2008. Inhaled hydrogen sulfide: a rapidly reversible inhibitor of cardiac and metabolic function in the mouse. Anesthesiology 108, 659–668. http://dx.doi.org/10.1097/ALN.0b013e318167af0d.
von der Ohe, C.G., Garner, C.C., Darian-Smith, C., Heller, H.C., 2007. Synaptic protein dynamics in hibernation. J. Neurosci. 27, 84–92.
Werner-Washburne, M., Braun, E., Johnston, G.C., Singer, R.A., 1993. Stationary phase in the yeast Saccharomyces cerevisiae. Microbiol. Rev. 57, 383–401.
Wilson, W.R., Hay, M.P., 2011. Targeting hypoxia in cancer therapy. Nat. Rev. Cancer 11, 393–410. http://dx.doi.org/10.1038/nrc3064.
Wojda, S.J., Weyland, D.R., Gray, S.K., Mcgee-Lawrence, M.E., Drummer, T.D., Donahue, S.W., 2013. Black bears with longer disuse (hibernation) periods have lower femorial osteion population density and greater mineralization and intracortical porosity. Anat. Rec. 296, 1149–1153. http://dx.doi.org/10.1002/ar.22720.
Zeitlin, C., Hassler, D.M., Cucinotta, F.A., Ehresmann, B., Wimmer-Schweingruber, R.F., Brinza, D.E., Kang, S., Weigle, G., Böttcher, S., Böhm, E., Burmeister, S., Guo, J., Köhler, J., Martin, C., Posner, A., Rafkin, S., Reitz, G., 2013. Measurements of energetic particle radiation in transit to Mars on the Mars Science Laboratory. Science 340, 1080–1084. http://dx.doi.org/10.1126/science.1235989.
Zhang, J., Kaasik, K., Blackburn, M.R., Lee, C.C., 2006. Constant darkness is a circadian metabolic signal in mammals. Nature 439, 340–343. http://dx.doi.org/10.1038/nature04368.