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Inducing Torpor in Non-Hibernating Mammals: A Systematic Review

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# Abstract

Crewed space travel is costly. The monetary cost of building and carrying the weight of astronauts, equipment, and resources is enormous. There is also the physical and mental cost on the astronauts, such as loss of bone density and increased agitation, that need to be mitigated in order to feasibly have crewed Mars missions. Their technological methods of mitigation are being examined by engineers such as simulating artificial gravity and developing radiation shielding methods. As biological researchers there are proposals to be made as an industry in this endeavour.

Hibernation is a biological phenomenon observed in a wide variety of animal species. It is charactised by depreciation of metabolism and body temperature leading to various ranging periods of dormancy. It is not unlike the scientific community to take note of existing processes and abilities to further its own advancement and studies into synthetic torpor could have various scientific implications.

Whilst temporary forms of induced torpor already exist in practice, a long-term option is required to be applied to spaceflight and the implications of such a methodology requires analysis of its feasibility and effectiveness. If successful it would reduce the volume of resources such as food, water, and medicine needed for the journey alone drastically. As well, it would reduce the mental and physical strain associated with months-long excursions in small spaces with a small contingency of people.

This systematic review scrutinises various methodologies proposed for inducing hibernation-like states in humans, ranging from pharmacological agents to environmental manipulation and genetic engineering. This is to assess the most viable method or combination of methods for torpor induction. Critical analysis of these approaches is conducted to assess their safety, efficacy, and potential translational applications in clinical settings.

A systematic review of currently available literature was carried out to examine experiments that pertain to or would be relevant in the development of a biological method to induce long-term hibernation or torpor in humans. A total of 592 papers from the database “Web Of Science” were assessed using a PRISMA Flowchart. 19 studies were retrieved using the prompt “synthetic torpor” and 573 were retrieved using the prompt “human hibernation”. After four treatment stages, a total of 10 studies were included in the final review, 3 from the “synthetic torpor” prompt and 7 from the “human hibernation” prompt.

Various studies examined hijacking the physiological processes that occur during hypothermia, in order to synthetically induce torpor. This includes the injection of GABA-A into the Raphe Pallidus region of the brainstem. An alternative approach employed was to inject adenosine 5’ monophosphate and maintaining a low ambient temperature to induce torpor. This combination ensures comprehensive coverage across methodological diversity, temperature reduction, and torpor duration.

# Introduction

Humanity's ambition to explore the cosmos remains tethered to Earth, constrained by the challenges of space travel. From ancient astronomers tracking celestial movements to the space race of the 20th century, our journey into the cosmos has been marked by curiosity, ambition, and technological innovation. In the modern era, private organisations like Blue Origin, SpaceX, and Virgin Galactic continue to push the boundaries launching missions and working with existing organisations such as NASA and the ESA to further space exploration (Weinzierl and Sarang, 2021). This raises the question of what barriers exist to manned exploration of even our local area to a planet such as Mars.

Many concepts in science fiction, to aid in space exploration, remain just that; however, this has not stopped life taking inspiration from the genre, and the various studies examining how to make spaceflight more feasible. One of the most popular tropes in movies is the concept of human hibernation (Nicholls, 1979). Putting a population to sleep for years of even centuries to keep them alive over vast amounts of time is a piece of science fiction that has been examined with sincerity over the last few years. From Arthur C. Clarke's "2001: A Space Odyssey" to Ridley Scott's "Alien", the idea of placing astronauts in a state of suspended animation has captured the imagination of audiences worldwide. While these portrayals often veer into the realm of fantasy, they have also inspired real-world scientific inquiry on a more realistic scale to our current technological development levels. Society is nowhere near being able to make centuries long hibernation a reality - however, a pragmatic start is underway in the research of inducing such hibernation for shorter manned missions, such as ones to Mars (Bartels, 2021).

Both hibernation and torpor are mechanisms used by some animal species to reduce metabolic needs, but they are distinct from each other. Hibernation is triggered by hormonal changes in anticipation of certain environmental conditions such a winter and is closely linked to the circadian rhythm as daylight shortens (Andrews et al., 1998). It typically lasts for a few months and is most common in mammals in arctic and sub-arctic climes but is found in some species living in temperate climes (Findlay‐Robinson et al., 2023). Torpor, on the other hand, is much shorter lasting typically a few hours or days, as an emergency when the same conditions that trigger hibernation suddenly occur unexpectedly for a short period of time (Melvin and Andrews, 2009). Small animals with extremely fast metabolism also use torpor periodically, particularly during situations such as migration, instead of hibernation due to an inability to store high enough body fat to meet their metabolic needs (Ambler, Hitrec and Pickering, 2022).

The notion of inducing a state of suspended animation in humans, akin to the hibernation observed in certain animal species, offers a wide range of possibilities for the future of space travel. By reducing metabolic demands and physiological functions, humans could endure the rigors of crewed missions to Mars, which in itself takes nine months, dealing with the issues of resource needs and extended close quarters contact with no break (Crawford, 2021). In recent years, research into human hibernation has gained momentum, driven by advancements in biotechnology and metabolic regulation (Yang et al., 2019). This paper examines the mechanisms underlying hibernation, we confront ethical considerations and practical challenges that accompany the realization of these ambitious endeavours. These insights not only have implications for space exploration but also hold promise for applications in medicine, such as organ preservation and trauma care.

This systematic review explores the current landscape of human hibernation research by synthesizing existing knowledge and shedding light on the complexities that lie ahead. From metabolic manipulation to ethical implications, our journey into human hibernation is as profound as it is promising. This review seeks to conflate and analyse the viability of various examined methods of synthetically induced torpor and postulate how that research could bring forth current and future biomedical methods of synthetic human hibernation.

# Method and materials

## 2.1 Review methods

This systematic review relied on the guidelines provided by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009) and was conducted by author J.A .

## 2.2 Literature search

The initial literature search was conducted using the electronic database Web of Science database using the prompts “Synthetic Torpor” (ST) and “Human Hibernation” (HH) on 12th February 2024. The database collates data “from 1900 to present” (Clarivate, 2023) ; however, all articles from this study range from 2014 to 2024. These searches yielded 19 and 573 articles respectively for a total number of 592 articles. This produced two Excel documents that extracted the following data from each publication: primary author’s name, publication title, publication type, publisher, overall topic, publication year, DOI number and affiliated institute. Only papers written in English were compiled from the database.

## 2.3 Eligibility criteria

To be eligible, articles had to meet these criteria: 1 – An experiment must have been conducted, therefore reviews, meta-analyses, white papers, and meeting notes were excluded; 2 – Torpor had to be synthetically induced so articles examining only natural hibernation without examining how to synthetically induce it were not suitable for review; 3 – Torpor had to be part of the experimental methods, even if it was not the focus of the article and was simply a means of experimentation. For each stage there was also eligibility criteria necessary for an article to move to the next one. The first stage where articles were actually excluded was for “title was not appropriate” or “abstract was not appropriate”. This would eliminate the vast majority of the articles and was important as a means to save time which may have led to over selection. Titles were deemed inappropriate if they did not include or relate to the terms “torpor”, “hibernation” or anything alluding to “space travel” as it was a simple method to whittle down the very large data pool for one person on a time constraint. Abstracts were deemed inappropriate on face value if the paper was presented as a conjugation of ideas on the concept of human hibernation, similar to a white paper, rather than a study that undertook some form of analysis and experiment.

# Results

## 3.1 Summary of Included Studies

A total of 592 articles were identified through the initial literature search using the electronic database Web of Science on February 12th, 2024. After screening for eligibility based on predefined criteria, 19 articles related to "Synthetic Torpor" (ST) and 573 articles related to "Human Hibernation" (HH) were included in the review. All included articles were published between 2014 and 2024.

## 3.2 PRISMA

Using the eligibility criteria, a PRISMA was conducted, and diagram produced (Figure 1). The screening stage is comprised of three parts and was independently conducted by researcher J.A. In the first part, articles were examined and excluded on two points; “title was not appropriate” or “abstract was not appropriate”. For the ST prompt, 8 articles were excluded for having inappropriate titles and 2 were excluded for having inappropriate abstracts, leaving 9 articles for further screening. For the HH prompt, 432 articles were excluded for having inappropriate titles and 110 were excluded for having inappropriate abstracts leaving 37 articles for further screening. Next, articles were screened to see if it was possible for the reviewer to retrieve the full text in order to complete screening. At this stage, 0 articles from the ST prompt and 1 from the HH prompt were removed, this was due to being behind a paywall making it inaccessible to the researcher, reducing the articles to 36. The final screening stage was assessing eligibility to be included in this systematic review. In order to be included in the final review, articles had to satisfy both “Being appropriate as a whole paper” and “An experiment was carried out” upon reading of the full text. Thusly, the ST prompt ruled out 3 articles for “No experiment carried out” and 3 articles for “Wider paper not appropriate”. The HH prompt ruled out 15 articles for “No experiment carried out” and 14 article for “Wider paper not appropriate”. Following reading of the full texts, 10 articles were identified and analysed for this systematic review.

***A diagram of a flowchart

Description automatically generated***

***Figure 1. Prisma flow diagram of data retrieved.***

## 3.3 Characteristics of full texts

Table 1 and Table 2 summarise the important characteristics of the full texts retrieved for analysis. Characteristics analysed were as follows: sample size, torpor mechanism, method, animal model, dosage, torpor durations, body temperature change/reached and ambient temperature. Table 1 demonstrates the 3 articles retrieved under the synthetic torpor (ST) prompt. Table 2 demonstrates the 7 articles retrieved under the human hibernation (HH) prompt.

Of the 10 papers analysed, 4 included rats as the animal model, 3 used mice, 1 used shrews, 1 used zebrafish and 1 used squirrels. In regard to the torpor methods used, 4 employed mechanical mechanisms (ultrasound (E10), temperature/humidity change (E6, E7), food access (E6) and light neural manipulation (E4)) and 6 used biological mechanisms (pyruvate (E5), GABA-A agonist (E1, E3), AMP (E2), melatonin (E9), AMPK-alpha (E8)).



E1

E2

E3

***Table 1. Synthetic torpor key characteristics***

***Table 2. Human Hibernation key characteristics***

E5

E4

E6

E7

E8

E9

E10

# Discussion

This study focussed on suggesting viable methods of human hibernation, by extrapolating and analysing existing synthetic torpor research using other species as models. Many previous reviews, including those excluded from this review, explored the theory of if human hibernation and associated evidence (Choukèr et al., 2018). This review in contrast takes it one step further and specifically examines how existing research is conducted and considering what can be translated for human experimentation. To do this, it is wise to begin by evaluating each article included in this review individually. One key component to understand is the relationship between body temperature and metabolism. It is not linear, as this would have impeded metabolic processes for people living at temperature extremes, however the general consensus is that for every 1°C drop in temperature there can be a 7-10% drop of metabolic rate (Moellering and Smith, 2012).

E1 (Morganti et al., 2019) and E3 (Luppi et al., 2020) are extremely similar, so for coherence they will be examined together. They were conducted by injecting GABA-A agonist into the Raphe Pallidus area of the hypothalamus section in the brain of Sprague-Dawley Rats. They both had the shortest torpor durations at 8 and 9 hours respectively however this is obfuscated by the fact that the experiment subjects were euthanised at this point so torpor may have lasted longer if not for this. Irrespective of this, both studies demonstrated a body temperature drop to roughly 20°C representing a roughly 45% drop. This can be inferred to have reduced the metabolism significantly, however if we used the human scale above it would drop to 0 so it cannot be used to infer the rat metabolism rate. The main point of contention is the method used to induce torpor. Drugs cannot be injected directly into the brain of humans for both ethical and practical reasons. The average thickness of a Sprague Dawley Rat skull is 0.5-1mm (O’Reilly, Muller and Kullervo Hynynen, 2011) whereas a human one is 8mm (Mahinda, H.A.M.;Murty, O.P, 2014), highlighting why this mechanism is not viable. Furthermore, even if the skull thickness was not an issue, injecting a drug into the brain that ordinarily could not pass the blood-brain barrier is dangerous as it would allow a pathway for pathogens to enter (Le Guennec et al., 2019). GABA-A agonist is already in use in healthcare; however, this is in a different capacity, reducing the pain and frequency of muscle contractions associated with seizures so it may not be appropriate in inducing torpor (Fukuda et al., 2013).

E2 used 5’ Adenosine Monophosphate (5’AMP) injected into the peritoneum (body cavity) of Sprague-Dawley rats (Puspitasari et al., 2022). This method resulted in the second longest torpor duration recorded, lasting 7 days. However, it is important to note that the specimens were euthanized at this point, suggesting that torpor could potentially have lasted longer. The methodology used is noteworthy; there is already precedent for both intraperitoneal injection and the use of 5’AMP in clinical settings. Currently, several drugs are administered via intraperitoneal injection for chemotherapy, such as mitomycin C and cisplatin, due to their efficacy in delivering high drug concentrations directly to the abdominal organs (Christou et al., 2021). This method allows for rapid drug uptake but carries the risk of misinjection and potential organ damage (Laferriere and Pang, 2020). Research into 5’AMP has shown its significant impact on metabolism. A study conducted in 2021 demonstrated that 5’AMP reduced both oxygen demand and mitochondrial metabolism in C57BL/6J mice, while also lowering body temperature to 32-34°C and protecting cells from hypoxia (Kondo et al., 2019). Another study by Zhang et al. (2020) highlighted the neuroprotective effects of 5’AMP under hypoxic conditions, reinforcing its potential for inducing hypometabolic states. These findings position 5’AMP as a promising candidate for inducing torpor in humans, which could have substantial implications for medical and space travel applications.

Moving on to entries from the human hibernation prompt, E4 employs the use of light to stimulate neurons in the hypothalamus of heterogenous mice lineages (Takahashi et al., 2022). The wavelength of light was 250 μW, giving the LED a weak blue colour. Each mice lineage had either Qrfp-iCre or Calb1-Cre knock in, meaning the sequences were added to the genome, and were all males of the C57BL/6J line. The Qrfp gene encodes for a neuropeptide of the same name, which plays various roles in the regulation of physiological processes such as food intake, energy metabolism, and stress responses. However, it's important to note that the specific functions and regulatory mechanisms of QRFP may vary across species (Jiang et al., 2003). The Calb1 gene codes for Calbindin 1, a calcium binding protein, which is found in specific populations of neurons in the brain, where it may play a role in modulating neuronal signaling and synaptic transmission. It has been suggested to influence neurotransmitter release and receptor function in some neurons (Gong et al., 2003). Mechanistically, blue light stimulation is known to activate light-sensitive proteins, such as channel rhodopsins, leading to depolarization and neuronal firing (Boyden et al., 2005). The use of blue light stimulation is relatively safe if well managed, however further research is needed to fully assess its long-term effects and translational potential for therapeutic applications in humans. A number of studies associate blue light with stimulating alertness and improving cognitive performance as opposed to inducing “sleepiness” or creating conditions congruent with torpor (Alkozei et al., 2016). Nonetheless, the use of blue light stimulation in conjunction with genetic manipulation techniques offers a powerful approach to dissecting the neural circuits underlying torpor induction.

E5 pertained to the use of saline diluted pyruvate injection of High Fat Diet male rats from the C57BL/6J line (Soto et al., 2018). Pyruvate is produced at the end of the glycolysis process and is a key intermediate in various metabolic pathways (Zangari et al., 2020). There is precedent of the use of pyruvate in human medical practice and in those situations, it speeds up the metabolism and aids in weight loss, particularly in obese people (Kalman et al., 1999). The rats in the study conducted were overfed, however pyruvate had the opposite effect, resulting in an apparent drop in metabolism as temperature decreased and torpor was induced. Furthermore, some studies suggest that dosing with pyruvate, outside of situations of being overweight or pyruvate deficiency, can have adverse effects on physical and cognitive functions (Mo et al., 2021). These factors together make it inappropriate for use in human hibernation as such treatment appears to have both opposite and adverse effects, especially considering their application need for hibernating astronauts who will need to be able to conduct important duties accurately.

E6 employed dropping the ambient temperature as a method to induce torpor (Horii et al., 2022). It specifically relied on Asian House Shrews (*Suncus murinus*) as their research demonstrated that this was a viable model for torpor synthesis. Asian House Shrews undergo torpor naturally for a few hours, especially when there is low food availability (ISHII et al., 2002), however the article did not specify if this was part of the experimental conditions. Its temperature drop is quite high at 10°C and had a middling torpor duration at 15 hours. The main issue with this method is that determining the difference between what the natural torpor would have been compared to this is difficult as no control of that kind was carried. Without a control such as this, assessing the viability of the simulation in inducing torpor is not possible. Reiterating a point, the simulation was of conditions that the shrew would usually be in to induce their torpor, humans however do not undergo torpor and the only equivalent, hypothermia induced shutdown, does not have the same level of protection. Overall this is an unfit method to translate to human torpor induction in the capacity required.

E7 (Tessier et al., 2014), similar to E6 (Horii et al., 2022) and E8 (Zhang et al., 2015), monitored and simulated condition similar to those naturally occurring for a hibernating mammal. The duration of torpor cycles in thirteen-lined ground squirrels can vary depending on factors such as environmental conditions, food availability, and individual physiological characteristics however typically episodes can last for several hours to a few days (Barnes, 1989). This once again causes contention as the experimental torpor duration was only 3 days, well within what could naturally occur in the conditions the article sought to simulate with a low ambient temperature of 5°C. Restating the focus, the simulation replicated conditions typical for inducing torpor in the squirrel. However, humans do not undergo torpor, and the mechanisms governing metabolic regulation differ significantly between the two species. Consequently, this approach proves inadequate for translating torpor induction to humans at the necessary level.

E8 (Zhang et al., 2015) examined how AMPK signalling could regulate the naturally occurring torpor of the Grey Mouse Lemur (*Microcebus murinus*). Instead of examining the whole organism, a PathScan ELISA was conducted on the liver, skeletal muscle, heart, brown adipose, white adipose and kidneys. The PathScan® ELISA (Enzyme-Linked Immunosorbent Assay) is a specific type of ELISA assay, using the sandwich method, developed by Cell Signaling Technology (CST). It is designed to detect and quantify specific signalling proteins or phosphorylated proteins within cell lysates (Cell Signaling Technology, n.d.). In this instance, this was the APMK protein being analysed in in each of these tissues and organs with its prevalence being linked directly with torpor induction (Giroud et al., 2010). The experimental approach undertaken in this study appears to have significant limitations. Despite replicating conditions conducive to torpor induction, similar to previous research efforts, the subsequent harvesting of organs prevented a thorough examination of the precise mechanisms underlying torpor initiation. Consequently, the study primarily reaffirmed the well-established association between AMPK and torpor induction in the Grey Mouse Lemur, offering little in terms of novel insights. Furthermore, the applicability of these findings to the induction of synthetic torpor in humans remains questionable, given the fundamental biological differences between humans and torpor-capable species. Thus, while contributing to our understanding of torpor physiology, the study's translational relevance to human applications appears limited, echoing similar concerns raised in preceding literature.

E9 (Cahill et al., 2021) differs by being the only non-mammalian entry in this review, instead using the Zebrafish. This article specifically considered its applications to human hibernation for Mars missions which, at least partially, overcomes reservations about how to translate its results from a non-mammalian to a human mammalian system. Its methodology was to dose the water the Zebrafish were housed in, a total of 24 fish with 4 per 6L tank, with 24 µM of melatonin per tank. The doses were administered daily for a duration of 10 days. Although the study did not report the exact temperature changes, thus limiting its broader applicability, torpor was effectively maintained throughout this period. The ambient temperature, while relatively low at 18.5C, fell within the natural range of temperatures experienced by Zebrafish in their natural habitats (Wakamatsu, Ogino and Hirata, 2019). Melatonin, commonly employed by humans as a sleep aid due to its ability to augment naturally occurring hormone levels and its lower incidence of adverse effects compared to pharmaceutical alternatives, presents a potential cross-mechanistic use (Costello et al., 2014). Moreover, the long-term use of melatonin raises questions about its impact on circadian rhythms and metabolic health. While short-term use as a sleep aid is generally considered safe, the effects of prolonged administration, especially at doses intended to induce torpor, are not well-characterized (Arendt, 2010). Potential applications of melatonin-induced torpor extend beyond space travel, such as in critical care settings where reducing metabolic demands could improve patient outcomes during extended surgeries or severe trauma. However, it is crucial to recognize that sleep and torpor are distinct physiological states. Therefore, further research, particularly involving mammalian models, is necessary to explore this potential, given the significant physiological differences between fish and mammals. Comparatively, the physiological responses to melatonin can vary significantly between fish and mammals. Previous studies have shown that melatonin can have diverse effects depending on the species and environmental conditions. For example, in Zebrafish, melatonin's influence on circadian rhythms might overlap with pathways involved in torpor induction, but this relationship needs to be explored in mammalian models to assess translatability (Feng and Bass, 2016).

E10 presents a notable approach by employing a non-invasive method to induce torpor, utilizing ultrasound instead of light for neural manipulation as seen in E4. This study used C57BL/6NCrl mice with UCP1 knockout and strain code 273 Wistar HenIGS rats. The observed temperature reduction was modest, at -3.26 ± 0.19 degrees Celsius, which is a promising finding for potential human hibernation applications. Specifically, the study employed ultrasound wavelengths of 2.3 MHz for the mice and 1.5 MHz for the rats, resulting in both groups maintaining torpor for over 24 hours. This outcome is exceptional and significantly enhances the potential utility of this method for inducing human torpor. Ultrasound is already widely used in medicine, primarily for diagnostic purposes such as detecting internal abnormalities (Evans, 2013). However, recent studies suggest that ultrasound can also facilitate the uptake of drugs targeting the brain, indicating a potential for combined methodologies (Mozaffari and Lee, 2017). This dual approach could balance torpor duration, temperature reduction, and overall viability, addressing multiple methodological challenges. For instance, the use of focused ultrasound has been shown to transiently open the blood-brain barrier, potentially enhancing the delivery of therapeutic agents (Aryal et al., 2014). The mechanism by which ultrasound might induce torpor involves its interaction with neural circuits. Ultrasound waves can stimulate specific brain regions, potentially mimicking the neural pathways that regulate natural torpor states (Kubanek et al., 2016). This method's non-invasive nature makes it a safer alternative compared to invasive procedures, which is crucial for ethical considerations in human applications (Ranjan et al., 2022). In comparing this method with other non-invasive techniques, such as pharmacological agents or environmental manipulations, ultrasound offers a unique advantage in precision and control over targeted brain regions. This precision reduces the risk of off-target effects and enhances the safety profile for potential human trials. Future research should focus on confirming these findings in larger mammalian models and exploring the long-term effects of repeated ultrasound exposure. Additionally, studies should investigate the combined use of ultrasound with pharmacological agents to optimize torpor induction protocols, ensuring both safety and efficacy (Lipsman et al., 2014).

# Conclusions

This systematic review set out to identify existing research into synthetic torpor and hypothesise using the evidence garnered what could be translated into possible human hibernation for future crewed Mars missions. Having examined 10 relevant articles a few methods stand out for possible use. For a solely drug based biological method, the use of adenosine 5’ monophosphate appears the most promising. This is due to it providing protection against hypoxia which could occur from depressed metabolic processes as well as providing a suitable drop in temperature whilst still maintaining torpor for a suitable duration. It already has precedent being used in humans however research is necessary for effects in long term use. On the mechanical mechanism front, neural manipulation with ultrasound or light both present viable options it is best to er on the side of using ultrasound as for the same torpor duration there is a lower temperature decrease that would be most suitable for human hibernation use. Furthermore, there is the possible opposition in the use of blue light for safety and research reasons as current understandings of its use in humans provides a conflicting result. The ultimate method would be a combination of the ultrasound method and adenosine 5’ monophosphate as a way find a middle ground and they could be complementary due to ultrasound aiding uptake of drugs in the brain as demonstrated by some studies highlighted above.

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