Environmental Enrichment in the ISS Rodent Habitat Hardware System

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ABSTRACT

Responses of animals exposed to microgravity during in-space experiments were observed via available video recording stored in the NASA Ames Life Sciences Data Archive. These documented observations of animal behavior, as well as the range and level of activities during spaceflight, demonstrate that weightlessness conditions and the extreme novelty of the surroundings may exert damaging psychological stresses on the inhabitants. In response to a recognized need for in-flight animals to improve their wellbeing we propose to reduce such stresses by shaping and interrelating structures and surroundings to satisfying vital physiological needs of inhabitants. A Rodent Habitat Hardware System (RHHS) based housing facility incorporating a tubing network system, to maintain and monitor rodent health environment with advanced accessories has been proposed. Placing mice in a tubing-configured environment creates more natural space-restricted nesting environment for rodents, thereby facilitating a more comfortable transition to living in microgravity. A sectional tubing structure of the RHHS environment will be more beneficial under microgravity conditions than the provision of a larger space area that is currently utilized.

The new tubing configuration was found suitable for further incorporation of innovative monitoring technology and accessories in the animal holding habitat unit which allow to monitor in real-time monitoring of valuable health related biological parameters under weightlessness environment of spaceflight.

INTRODUCTION

Animal behavior is a clear indicator into their wellbeing because it allows for objective observations to assess the animal-habitat interactions and ability to cope with their current situation (NHMRC, 2008). However, the microgravity environment makes monitoring indicators of an animal’s state of health problematic. Visual indicators, such as coat condition, as well as clinical signs, such as body temperature and heart rate (Burkholder, 2012), are therefore increasingly hard to see, making behavioral assessments even more important for the study of rodents in space.

Multiple stereotypies have been observed in rodents during spaceflight that would suggest that their wellbeing has been hindered by the microgravity environment or their housing. Some such behaviors include race tracking, which involves the mice using the habitat as they would a mouse wheel; traveling as fast as they can across the surface to expend energy (Rodent Research- 1). Additionally, a notable decrease in socialization is a common sign of stress seen in cages animals (Vessel and Russo, 2015) and is often observed among rodents used in space experiments. These documented observations of animal behavior, as well as the range and level of activities during spaceflight, clearly demonstrate that weightlessness conditions and the extreme novelty of the surroundings certainly exert damaging psychological stresses on the inhabitants. The result is an alteration in the animal's normal biological function as it attempts to adapt to the novel space environment.

Several notable behavioral activities have been observed during space experiments, including floating, hanging, and holding on to the cage bars (Cancedda et al., 2012). The mice adapted quickly to microgravity locomotion by using their paws and tails to grasp the cage's grid walls. Besides motion based behaviors, the mice interacted readily with additional stimuli, if present. The incorporation of stimuli and other pieces of environmental enrichment could greatly influence the wellbeing of these test rodents in space (Cancedda et al., 2012).

ISS RODENT HABITATS

Up until 2014 animal experiments aboard the ISS were generally performed utilizing the Animal Enclosure Module (AEM) and Animal Enclosure Module Extra (AEM-X), as seen in Figure 1. The AEM was a joint project between General Dynamics Company for the Student Shuttle Flight Program and the NASA Ames Research Center (ARC) in Moffett Field, California, and was designed to have enough food and water available for 30 days for 10-20 small rodents. The system has a habitable area enclosed with stainless steel mesh screens measuring approximately 24 X 36 X 22 cm. Unlike other ISS modules, the AEM is only cleaned upon reentry to earth due to the amount of rodent waste, which is partially filtered in the unit to allow for adequate air quality between cleanings (Borkowski et al., 1995).

The AEMs have recently been tested and modified to support future microgravity investigations with mice, leading to the current ISS habitat, the Rodent Habitat Hardware System. This hardware is closely modeled after the AEM and comprises of several different modules that allow for the storage, transport and testing of mice. This system was designed and built through a collaboration of NASA and the Center for the Advancement of Science in Space (CASIS) with the goal of providing a living space for rodents that could be used for space-based experiments (NASA). The data collected from these experiments is used to assess the impact of the novel space environment on living organisms, in addition to the study of general medical issues such as aging and bone density loss. However, in order to get quality data, the animals used in the experiments should be both physically and mentally healthy, with no signs of distress (Burkholder et al., 2012). This idea, paired with current observations of distress exhibited by mice in spaceflight, demonstrates that changes must be made to the current habitat hardware in order to produce significant experiment results.

The NASA/CASIS developed Rodent Habitat Hardware System underwent a series of validation studies performed by NASA's Rodent Research team. This research suggested there was no negative effects caused by their system, and concluded that it was adequate for rodent trials based on their comparison with previous habitat systems. To determine this, Choi et al. compared tissue samples whose weights are affected by stress (whole body and adrenal glands, liver, and others), as well as the amount of stress related enzymes. In addition, behavioral analysis was conducted both by ISS astronauts and trained ground crew with the help of video recordings from the habitat. The initial report from these observations suggested that the mice quickly acclimatized and performed regular eating, grooming and sleeping patterns compared to the control mice, with increased ease of travel through the habitat as the mission progressed. However, these finding fail to mention nesting, which is a key signal of wellbeing in mice (Burkholder et al., 2012).

In order to gauge whether or not the mice have low stress levels in spite of their open habitat, it would be necessary to provide shelter or nesting materials in order to compare to both the control data and data collected from previous experiments. It is possible that the lack of nesting is beneficial in a captive environment, but without comparing the data it is hard to know what is a behavioral sign of distress and what is a sign of acclimatization (Newberry 1995). However, different types of stress can add to the overall stress on the body, even if the original stressors show no visible signs of a change. A change in behavior is considered a stressor, so it is possible for an abnormal shift in behavior to compound with different stressors. Though incidents of stress may seem small, if they are added to an already stressed system they may have consequences that are larger than anticipated (Moberg and Mench, 2000). For example, it is possible for the inability to nest exacerbates the stress caused by handling and experimental procedures (Olsson and Dahlborn, 2011) or other effects of living in the novel space environment. Given that much of the research being done on the ISS is meant to understand the impact of microgravity and other factors, it is entirely possible that there are stressors we do not yet understand. As the duration of mice studies increases additional stressors are placed upon the mice, leaving the possibility for seemingly sudden behavioral and physical issues. With this in mind it is suggested that the active reduction of known stressors be a priority for rodent habitats used in these environments.

MODIFICATION DESIGN AND ASSESMENT

The goals of RHHS modifications are to improve the habitability of the microgravity environment and reduce its impact on animal physiology. This approach will increase the range of healthy activities the mice can choose from and add control over its social and spatial environment. As a group, rodents have certain behavioral characteristics that are helpful in understanding the most comfortable space flight living environment for them. They have evolved to utilize touch-sensitive whiskers and hairs to sense their environment, which incentivizes them to run close to walls in order to remain aware of their surroundings (CDC, 2006). Therefore, one of the key components of improvement of an environmental condition in spaceflight could be providing a divided narrowed environment. Figure 2 shows potential configurations of a metal mesh tubing system and tubing insertion. These models could be incorporated into the rodent habitat based on the needs of each study and mouse strain utilized. Tubes could be stacked (A) or twisted (B) to create different formations, and can be straight or incorporated into a ball of tubes.

Complexity and interconnectivity can be adjusted after review of rodent behavior inside habitats fitted with the suggested environmental enrichment. The implementation of a closed off tunnel system would allow mice to stay in contact with a wall and away from open spaces, making the mouse feel more secure and comfortable under the weightlessness conditions of spaceflight. The divided living space would also give the benefit of providing different areas for eating, sleeping and expelling waste, which is closer to the normal mouse habits in their natural environment. (Baumans, 2005). Rodents are more active when multiple pathways to food are provided, which would be a direct improvement offered by a sectional tubing system. The proposed tubing network could also be designed with combination of transparent and opaque parts providing the possibility for visual barriers or hiding places to minimize aggression (Stauffacher 1992). Such sectional tubing structuring will be more beneficial under microgravity environment than the larger space area currently present in the RHHS.

The proposed cylindrical design allows for more contact points in tubing system, making it applicable for space research in microgravity conditions, where mice may not have a preference which portion of the tube they traverse through. In addition, this design would create breaks in the light, providing the mice with areas where they can regulate their own light exposure during the preset habitat light cycle. While more complex, this design could reduce the amount of time needed for additional research and development compared to the grid implementation design, requiring only a new base shape.

As previously mentioned, current observed rodent locomotion styles in space utilize the mesh wall system, minimizing the time in contact with slick surfaces. Incorporating electrodes into grid system would therefore be an ideal scenario by utilizing surfaces that the rodents already voluntarily come in contact with. Implementation of a grid system decreases the impact of the design on the air ventilation, video monitoring and filtration systems, and reduce chances of waste buildup on the electrodes that might be observed in the other configurations. Given these benefits and design qualities the potential to cover more spaces within the habitat is also evident.

Figure 3 shows the preliminary tubing system prototype. It was constructed from a metal mesh with ¼ inch separation between the wires. Tube diameters measure ½ inches, with tube length measuring

7 ¾ inches. This design has two columns of 5 tubes which are interconnected with vertical pathways and have openings for access to food and water. A large variety of connections allow the mice to move freely regardless of attempted bullying or territoriality exhibited by captive mice. An almost infinite number of configurations could be constructed, but only thoroughly tested designs should be implemented, as some environmental enrichments could negatively impact test animals, despite the best efforts of their creators. Additional designs may have more or significantly less tubing systems than the provided prototype, depending on the needs of the species and experiment. The built tubing system prototype is designed to fit into the RHHS Rodent Habitat, which is sealed and stored in racks aboard the ISS (Figure 4).

IMPROVING/MEETING REGULATORY REQUIREMENTS

1. *Tubing Material*

The ideal tubing materials for this project are subject to the requirements of space travel, but also need to be rodent proof. We suggest that the same metal wiring/mesh used on the current RHHS cage be used at the tubing material. This mesh allows for paw and tail gripping, adequate air flow for ventilation, and has grid spaces small enough to prevent mice from crawling through the holes and becoming stuck. The metal has also been flight tested on many shuttle missions with the mice inside, reducing the amount of material testing required.

2. *Space/Volume/Surface requirements*

IACUC guidelines require that mice up to 25g need 12 in^2 of floor space per mouse, with 5 in of overhead clearance (National Research Council, 2011). The current design barely achieves that, with 710 cm2 floor space and 14750 cm3 habitable space (Borkowski et al., 1995). Although the addition of complete tubing system (Figures 2 and Figure 3) will decrease the habitable space, there will be an increase the usable floor space the enclosure by providing many more surfaces for the mice to traverse. Subsequent models with less tubing, would not reduce the habitable space but would increase surface area.

3. *Ventilation/Air flow*

   The ventilation system currently used on the RHHS adequately ventilates the enclosure, but the addition of internal modifications has the potential to alter the air flow. The suggested metal mesh tubing design should have minimal blockage of the air flow compared to full coverage tube designs.

4. *Feces removal/cleaning*

   All RHHS modules are cleaned on Earth, not by the ISS crew members, which reduces rodent feces contamination of the ISS. The proposed environmental enhancements could be sent back to Earth for the same treatment.

FUTURE MODIFICATIONS & TESTING

Non-invasive Health Monitoring Equipment

The use of rodents in space research is already being reduced due to the extended amount of time that handling the animals takes. Integration of telemetry equipment into the environment will allow for continuous active sensing which has the potential to reduce the busy work required by astronauts while actually increasing usefulness of the data. This data would not have the variable of astronauts handling the rodents prior to being measured, making the information obtained more characteristic of the average resting rate of the animals instead of the stressed rate they may exhibit after being transferred between modules for testing.

One such form of non-invasive technology which could be incorporated into the tubing designs proposed in this paper are non-invasive electrocardiogram equipment developed by Chu (Chu et al., 2001). This equipment uses electrodes on a surface which the mice touch with at least 3 paws, producing real time heart rate data without the use of surgery or implants (Figure 5A). Incorporating electrodes into the grid system would therefore be an ideal scenario by utilizing surfaces that the rodents already voluntarily come in contact with. The current demonstration design is a raised platform, which can accommodate one mouse at a time on the sensor. This technology could be redesigned to fit inside a modular tubing design in the form of a cylindrical configuration (Figure 5B), or could be incorporated into the grid system with which the tubes are formed (Figure 5C). The listed configurations could be used alone or in conjunction with each other in a more complex system, depending on the preferred inhabitation preferences determined through the design testing phase.

Radio Frequency Identification

Understanding how rodents spend their time within a habitat would shed a light on future developments and adjustments to benefit the test animals. A location monitoring system such as Radio Frequency Identification (RFID) is already employed in the monitoring of animals in many disciplines and could be implemented within current or future RHHS designs and layouts with the proposed tubing structure. Available videos (Rodent Research- 1) using analog video monitoring of rodents in their habitat aboard the ISS mention the presence of tail tags, which have proven difficult to see or read with currently implemented technology. An integrated system of low gain RFID antennas would allow researchers to place many units close to each other within the habitat, using newly developed “smartdust” chips (Hitachi, 2006) to tag individual mice within the enclosure. The movement patterns of the mice could then be assessed and taken into account during future design iterations with a focus on improving the usable space of habitat with further updates. Implementation of the smartdust tags, which are the size of a piece of sand or glitter, would require minimal effort to implement into the tagging process given their size.

Behavior Testing

Typical rodent stress tests like the open field test and elevated plus maze are measures of anxiety that will work during different scenarios, if at all (Ennaceur, 2014). These tests require placement into a specially designed test habitat, which would be a different environment than the one suggested here. This aspect will alter the data being generated, as the tests cannot be performed in the prototype habitats or current habitat, but must be performed in a specially designed structure not associated with the habitats. The inability to perform these tests inside the RHHS makes them inapplicable to any significant experiment that could be used to validate the environmental enrichments.

Although the aforementioned tests are not applicable to the suggested habitat design changes, it is still necessary to assess the reaction of rodents to the environmental enhancement in Earth gravity compared to the current RHHS design. By comparing animal behavior inside the modules, both with and without the enrichment, an assessment can be made of the legitimacy of the design. This assessment can be made by focusing on the amount of times specific behaviors are observed in the enclosure, including natural behaviors and gained stereotypies developed specifically during the experiment.

In this study the prototype of the modified configuration and original habitat unit have been compared for improving short-term welfare assessment by enabling video monitoring group of 6 mice for preferential occupation between these two habitat units during daylight and the dark phase. Rodents housed in this small groups were allowed to eat, sleep, drink, groom, and interact socially as normally used in laboratory research. The experimental design of this phenotyping tests relies on the animal being removed from home-cage environment of Animal Care Facility and placed in an unfamiliar apparatus giving them opportunity to choose the most preferable environment. As many tests measuring behaviors (for review see Crawley, 2007), the selected one may include elements of laborious and subjective variable influence of an experimenter (Wahlsten et al., 2003), which, however, applies equally to both the modified and original habitat units removing the presence of any possible experimenter bias, as well as any environmental perturbations.

Reproducibility and robustness of obtained data has been verified by using groups of the different mice in the same number in set of 3 experiments.

C57BL (6 weeks old) female mice were housed in home cages in groups of six mice per cage. Aggression in male mice is a reason why female mice are frequently preferred in space research as test subjects. The mice were kept under controlled light (light 8 a.m. to 7 p.m., dark 7 p.m. to 8 a.m.) at temperature 23°C. They had free access to water and were fed *ad libitum* on a commercial diet (SDS Rat and Mouse No.3). All procedures and animal studies were carried out in strict accordance with National Institute of Health guidelines (the Guide for the Care and Use of Laboratory Animals).

In the day of the recording sessions, the animals were transferred to the connected original and the enriched habitat units with fresh bedding, nesting material. Animal welfare checks were carried out visually twice daily. At the end of the recording period, mice were removed from the tested habitat units and returned to their home-cages.

The video system allows one to monitor a two habitat units of mice, and has been designed to fit within the adjacent rack space, houses an infrared camera, a computer and the appropriate power supplies.

The connected habitat units each containing three “numbered” mice was positioned on the baseplate such that the entire system could be visualized. A 20 min video of the mice was then acquired each two hours. The analysis was carried out using the preferential unit occupation of mice in sets of experiments with groups of different mice.

Figure 6 show result of the experiments as an occupation of the original and enriched habitat units with number of mice during daylight and dark phase in sets of experiments with group of different mice. Although C57BL/6J mice show difference in activity throughout the night and daylight periods their preferentially most of time occupy the enriched unit during all these time periods.

It seems that structural adjustments in the enriched habitat unit in comparison to the original unit benefit not only for social housing (e.g., perches, visual barriers, refuges), but also for the excess to important resources (e.g., food, water, and shelter) which provided a way that they cannot be monopolized by dominant animals. In addition, animals seems to appreciate the more adequate narrow bedding structures for resting and sleeping as well as the opportunities for species-typical behavior such as foraging, digging, burrowing, and nest building what is absent in the original unit. All of these enhances animal well-being by providing animals with sensory and motor stimulation, through enriched tubing structures that facilitate the expression of their natural behaviors.

Results of the behavior observation allow to expect that beneficial enrichment of the habitat environment in the proposed modification of the original space habitat unit tested in the "ground environment" will also be applicable under spaceflight environment where stress factor arising from long exposure to microgravity is substantially increased. The reduction of the open space and incorporation of narrow tubing environment into original mice habitat unit will allow animals to adopt most comfortable position avoiding floating in open space and grouping together as it has happened in the original habitat. We found that combination of transparent and not transparent parts of the tubing network which provide for animals opportunities for visual barriers or hiding places significantly minimize their aggression and allow the animals to control their environment. At the same time incorporation of barriers in the tubing network allow to position animals in specific places and restrict contacts for the selected animals if necessary. Additionally, Tubing network structure allows to place paper strips, commercial nesting fiber or wood wool to build nest-like place with these items while keeping them locally, what would not possible in open space of the original chamber.

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Legend to Figures:

Figure 1. The Animal Enclosure Module-Extra. The system was created by the Rodent Research Project at NASA Ames Research Center (NASA) – Image: NASA.

Figure 2. Potential configurations of a metal mesh tubing system and tubing insertion. These models could be incorporated into the rodent habitat based on the needs of each study and mouse strain utilized. Complexity and interconnectivity can be adjusted after review of rodent behavior inside habitats fitted with the suggested environmental enrichment. The tubing system is inserted into the RHHS Rodent Habitat, which is sealed and stored in racks aboard the ISS.

Figure 3. Proposed tubing system prototype. The prototype (blue) is shows alone and inside the ISS Rodent Habitat Hardware System which was designed and borrowed from NASA for use during this project. Tube inserts represent possible non-invasive technology implementation, or the inclusion of light shielding inserts that allow rodents to self-regulate their light exposure. The rodents will have access to water (green entrances) and food bars (orange entrances) from a variety of positions.

Figure 4. Artist's rendition of prototype with demonstration of pathways available for rodents. This tubing structure has increased surface area compared to an open cage model, and allows mice to travel many different ways within the habitat. One possible way is represented by the red line (A), with connections spanning horizontally and vertically within the structure. This structure slides in and out of the interior Rodent Habitat cage (B) which is then placed into the Rodent Habitat system when the lid is removed (C) – Image: NASA.

Figure 5. Electrode Configurations. Suggested configurations of the electrodes required for non-invasive ECG monitoring include (A) current models with horizontal electrodes (B) incorporation into a

cylindrical model for use in microgravity conditions and (C) implementation into the grid system already used in rodent cages for a seamless integration into the habitats. RFID antennae will allow researchers to track which mouse is coming into contact with the electrodes.

Figure 6. Occupation of the enriched (unit 1) and original (unit 2) habitat units with number of mice during daylight and dark phase in sets of three experiments with group of six mice.

Figure 1.

Figure 2

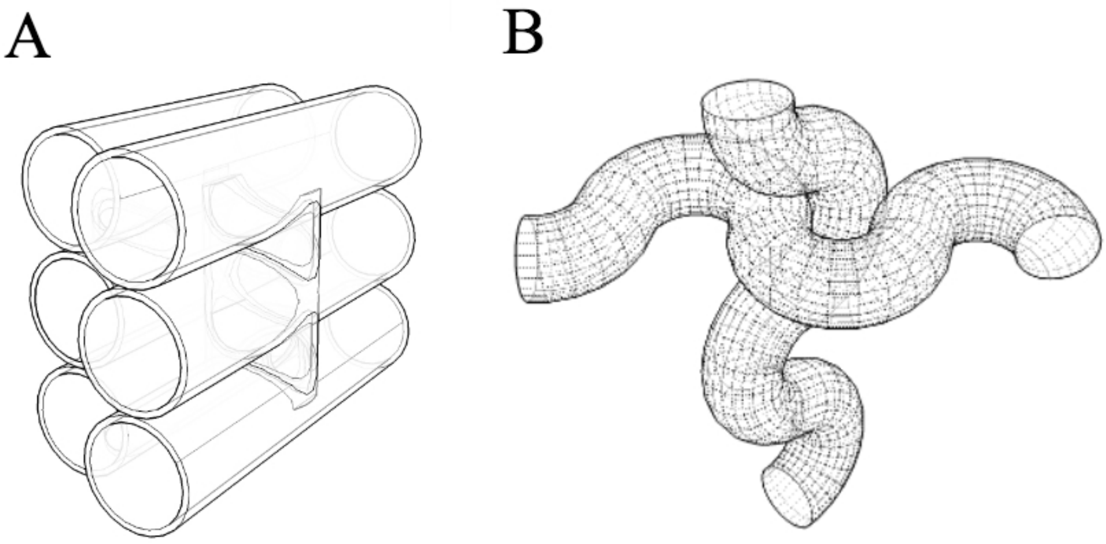


Figure 3

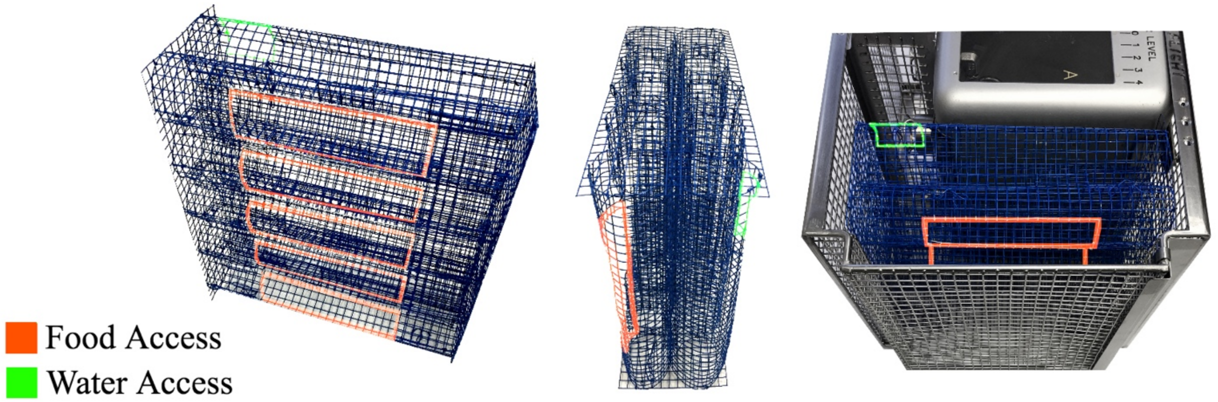


Figure 4

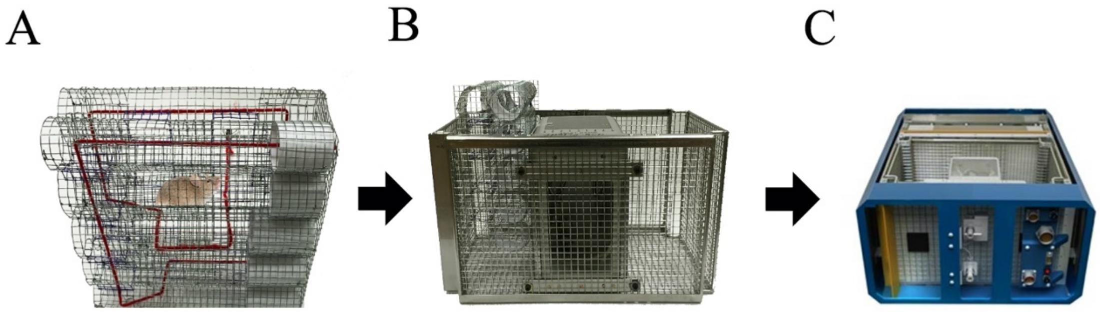


Figure 5

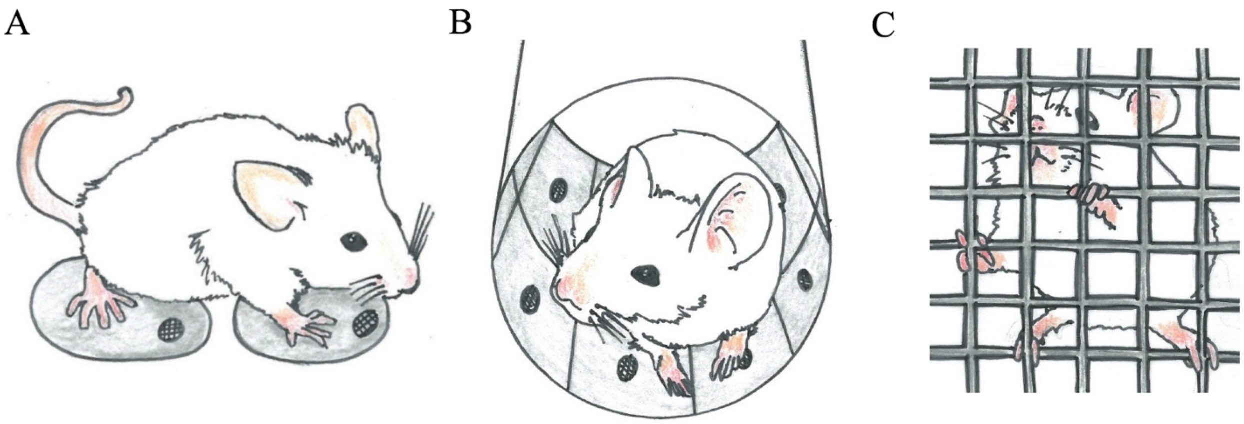
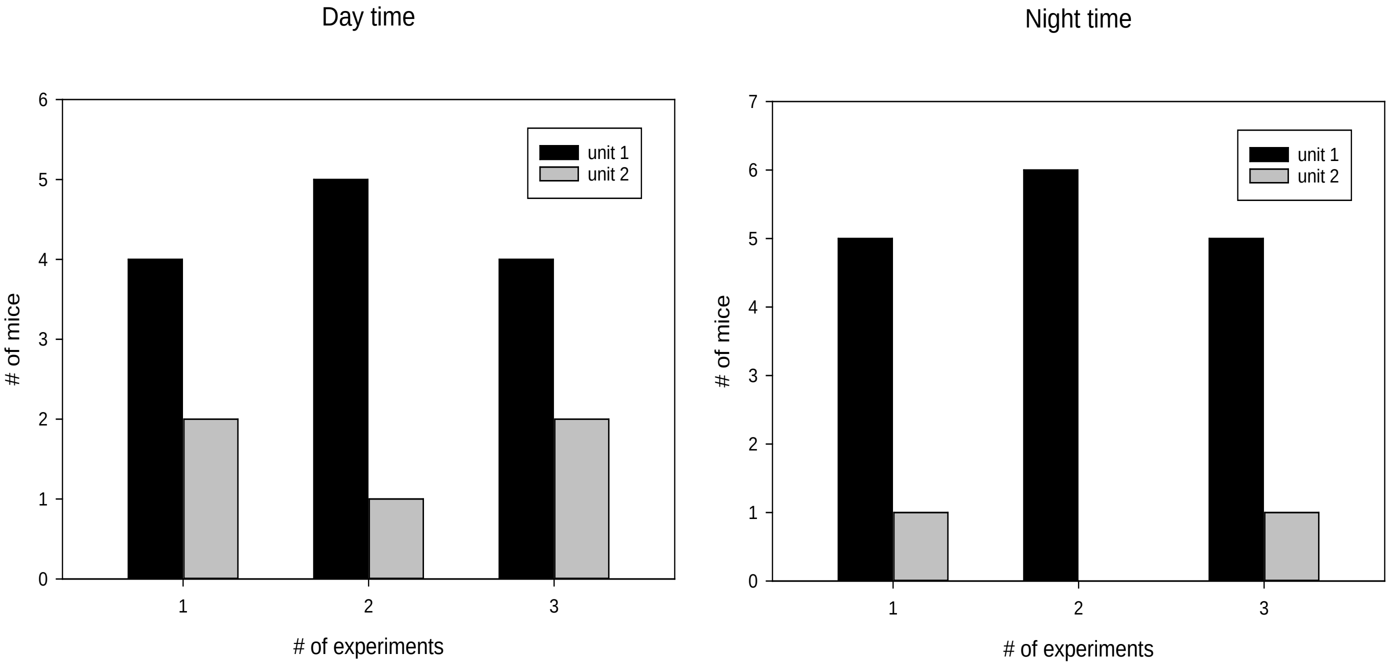


Figure 6



Day time
unit 1
unit 2
# of mice
0
1
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3
4
5
6
1
2
3
# of experiments
Night time
unit 1
unit 2
# of mice
0
1
2
3
4
5
6
7
1
2
3
# of experiments