Mouse Drawer System (MDS): An automated payload for supporting rodent research on the international space station

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Abstract

Mice represent one of the most important animal models for biomedical research. For the scientific community, the ability of flying mice under weightless conditions in space offers many valuable advantages respect to other rodents. These advantages includes the option of testing a wide range of transgenic animals, the ability to increase the number of animals that can be flown, and reduced demands on shuttle resources (food, water, mass and air refreshment) and crew time (for water refill). In this study, we report the biocompatibility tests of the first European automated rodent spaceflight payload, the Mouse Drawer System (MDS), which is compatible to the configuration of space shuttle middeck and space station freedom express rack. Six mice were housed individually inside the MDS engineering model for 20 and 100 days. All mice survived, lost weight after the 20-day test, but gained weight after the 100-day test. No abnormality was detected by haematological analysis, or by necropsy. These tests showed that the payload meets NIH guideline for temperature, humidity, food and water access, air quality, odor and waste management; mechanical and electronic components of MDS can support animal experiments for at least 100 days. The payload is now ready to take off in the upcoming MDS space mission.

Key Words: MDS, animal habitat, spaceflight payload, welfare evaluation, international space station

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Animals have been crucial to support human space exploration. Before human went into space, animals were sent up in rockets as surrogates to help us in understanding if a living being could withstand and survive a journey beyond the Earth's protective environment. Followed the success of sending the dog "Laika" into orbit by the Russian satellite "Sputnik II" in 1957, other animal species have travelled into space as experimental subjects for biomedical researches. A rapid progress was made from 1973 to 2003 [9]. Many of these experiments used rodent payloads that were transported into space in satellite or aboard the space shuttle. The model rodents that have been flown in space missions are rat and mouse, with the former being flown far more frequently [3, 12]. However, the last decade has seen an increased interest in flying mice. A scientific benefit of using mice is the feasibility of examining the role played by specific genes played in the physiological adaptation to spaceflight in a large collection of spontaneous and transgenic mutants (over-expressing or deleting a particular gene). Many of them serve as models for human congenital defects. With the completion of the mouse genome project and given the remarkable similarity between the mouse and human genome [6], the benefit of spaceflight research on wild-type and transgenic mice assumes even greater importance. Besides, their In addition, the small body size and the short life cycle makes mice a very practical biomedical specimen. At approximate 10% the size of rats, mice require less living space with reduced mission logistic mass (body weight, food, water, waste), oxygen consumption and carbon dioxide removal.

Up to now the Animal Enclosure Module (AEM) and the Research Animal Holding Facility (RAHF) are the only two space-proven payloads, with which rats or mice have been flown abroad the orbit since 1981 [3]. AEM fits inside a standard middeck locker, and is a self-contained habitat that provides up to six rats with living space, food, water, ventilation, lighting and waste management. However, AEM is tightly sealed so that animals are not accessible for manipulation or treatment and does not have active thermal control [2]. RAHF has all life-supporting characteristics of AEM, in addition, it provides environmental control and monitoring for 24 rats with in-flight access. However,

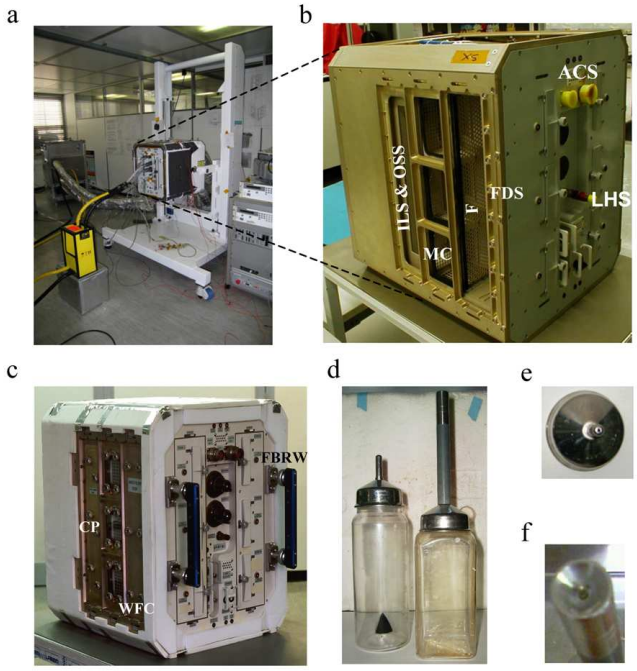


Fig. 1 *MDS payload. a) and b) photo of the MDS engineering model: ILS&OSS = ILlumination and Observation SubSystem, FDS = Food Delivery System, LHS = Liquid Handling Subsystem, ACS = Air Conditioning Subsystem, MC = Mice Chamber, F= Filters; c) photo of the MDS flight model. CP = Chamber Panel, FBRW = Food Bars Replacement Windows, WFC = Waste Filter Cover; d) photo of the drinking bottles: the normal bottle is on the left and the drinking training bottles connected to a stem activated valve on the right; e) the spout of a normal drinking bottle; f) the valve of a drinking training bottle.*

RAHF is designed to fly in the Spacelab module that is configured for the cargo bay. Due to the infrequent availability of Spacelab flights, rodent experiments rely heavily on the AEM [3]. With the decommission of the Spacelab components in 1998, no scientific experiments using RAHF was reported afterwards.

Other two rodent habitats were developed in the 1990s, but a report about their use in a space mission is not yet available. One habitat is the Advanced Animal Habitat (AAH), which was developed by NASA Ames Research Center to support rodent experiments in the Middeck, the Spacelab and the International Space Station (ISS). The AAH has many added features than AEM and RAHF, such as animal telemetry, on-orbit video and environmental recording within animal cages. Up to 12 adult rats or 30 adult mice for up to 30 days can be hosted by AAH [4]. The other one is the Animal Module for Autonomous Space Support (A-MASS), which was developed to enable 30-day spaceflight for mice on the first commercial experiment transporter mission. It meets NIH guidelines for animal welfare [2].

With the installation of the brand new ESA Columbus Module on the International Space Station (ISS) by the STS-122 crew in February 2008, the reality and perspective of conducting biological experiments in space are bouncing back. In line with the ISS development, the Italian Space Agency (ASI) contracted Thales Alenia Space Italia (Milan, Italy), the largest Italian aerospace industry, to design and build a spaceflight payload for rodent research on ISS, the Mouse Drawer System (MDS). To ensure MDS meet NIH guidelines for animal farewell, animal testing have been conducted with mice in every step during its development: a breadboard was first constructed to test single subsystems [7], then an engineering model was constructed for critical review acceptance by NASA, the flight experiment will employ the MDS flight model. In this study, we report the biocompatibility tests of the MDS engineering model for short and long durations. The MDS engineering model will serve as the animal habitat for conducting the ground control experiment for the upcoming MDS space mission that is currently scheduled to take off in 2009.

Materials and Methods

Animals

This study was approved by the Ethics Committee of the Animal Facility of the National Institute for Cancer Research (IST, Genova, Italy). The experimental protocols have been authorized by the Public Veterinary Health Department of the Italian Ministry of Health, and in accordance with the principles expressed in the “Guide for the care and use of laboratory animals" (Office of Science and Health Reports of the USA National Institutes of Health, Bethesda MD 20892).

Inbreed male mice (strains C57BL/J10) of 6-8 weeks old were purchased from Charles River Laboratory (Germany) and housed in the animal facility of IST. Under vivarium conditions (20-24°C ambient temperature, 40-60% relative humidity and a 12 hours light -12 hours dark regime), mice were hosted individually in a transparent plastic cage with a size of 267 x 207 x 140 mm (floor area 370 cm2) or 332 x 150 x 130 mm (floor area 335 cm2) (Tecniplast Gazzada srl, Milan, Italy) and fed with a laboratory animal diet “Mucedola” in pellet shape (Mucedola srl, Milan, Italy). Drinking water and food were provided ad libitum.

Animal habitat

The MDS engineering model (Figure 1a,b) was used in this study as the animal habitat. It was manufactured to test engineering features of the flight model (Figure 1c). MDS consists of one External Container

(dimensions 421 x 480 x 516 mm) in which the following subsystems are integrated: Mice Chamber (MC), Liquid Handling Subsystem (LHS), Food Delivery Subsystem (FDS), Air Conditioning Subsystem (ACS), Illumination Subsystem (ILS), Observation Subsystem (OSS). The MC is divided in 2 Habitats. Each Habitat is internally subdivided in 3 equal cages, each of a floor area of 116 x 98 mm and 85 mm height. Six mice can be kept individually inside a dedicated cage. Each cage is refurbished with a food bar dispenser, a drinking valve and a camera for observation. A cage has grids in all four walls, permitting olfactory but not physical contact between animals. The LHS includes a water tank of 0.5 litres that, once empty, can be re-filled in orbit. The LHS delivers water ad libitum to each cage independently through the relevant drinking valve. The FDS supplies each cage independently with a food bar of 149 x 73 x 7.5 mm and a mass of about 90 grams. The composition of a food bar can be decided by researchers; if necessary, additives can be incorporated. The FDS can mechanically deliver food in two modes: ad libitum or in rationed quantity/day. Once finished, the old food bars is to be replaced by new ones through six openings located in the MDS Front Panel. The ACS generates a continuous airflow of 0.1 m/s flowing through the cages to perform air renewal and remove waste products. About 5% of the total air is exchanged with ISS cabin in order to eject generated CO2 and inject consumed O2 by an open loop mechanism. HEPA In and Out filters are used to prevent possible microbiological contamination between the ISS cabin air and MDS. Removed waste products (urine, faeces, hairs, food debris etc.) are collected within Waste filters located below each Habitat. Air temperature inside the habitat is actively controlled, ranging from 25 to 26 °C, while air humidity is passively controlled by means of desiccant in the range from 40 % to 70 %. The ILS implements light/dark cycles that is programmable in steps of 5 min with a nominal status of 12 h light / 12 h dark. Light intensity is programmable from 0 to 40 lux in steps of 10 lux. Diffuse light (no bright spots) is provided during light periods, whereas infrared light source is available for mice observation during dark periods. The OSS permits the observation of mice through 6 video cameras (one per cage) around the clock. Video data are transmitted to ground to allow a near real-time verification of mice health status and behaviour. The CU permits the automatic execution of the tasks necessary to perform the experiment according to a command table previously loaded inside its internal memory.

Animal test protocol

In total two tests were performed, of which one lasted for 20 days and one for 100 days. A period of 20 days corresponds to the lifetime of a food bar. Each test employed three wild type C57BL/J10 male mice and three osf1 Tg male mice [7] that were housed individually in a single cage of the MDS engineering model. Test mice received a "drinking training" one week before the animal insertion under vivarium conditions by drinking water from "drinking training bottles". A drinking training bottle is a standard graduated water bottle connected to a commercial stem activated valve (model A160, Edstrom) (Figure 1d) at the bottle spout. When mice were inserted into the MC, the test began and was registered as day 1 (D1) of a test. During the test period, each mouse had water access ad libitum, and the water consumption was registered daily; each mouse was fed with "Mucedola" foodbar (Mucedola srl, Milan, Italy) twice a day delivered at 9:00 and 19:00 with a total amount of 5 g every 24 hours. For the 20 days lasting test, mouse body weight was measured weekly; for the 100-day test, mouse body weight was measured at the start and at the end of the experiment. The general health status was eye-viewed by a well-trained technician daily. At the end of the test, mice were sedated by injecting xilazine (1mg/50ml), whole blood was collected from the retro-orbital sinus and immediately placed into K2-EDTA-coated microhaematocrit or into Lithium-Heparin-coated tubes (Sarstedt, Numbrecht, Germany) for haematological analysis. Animals were then euthanized and necropsy was conducted by a veterinary to examine any macroscopic irregularities and to dissect organs. Two groups of mice were included in the control: both groups were housed in standard cages and under vivarium climate conditions, except that one group ate food ad libitum and drank water ad libitum from standard bottles, while the other group ate food rationed at 5 g / day and drank water ad libitum from the training bottles.

Haematological analysis

Blood samples were analyzed by using the Advia 120 haematology system (Bayer, Tarrytown, NY). Results reported here include the following: red blood cell (RBC), white blood cell (WBC) and platelet complete analysis, haemoglobin concentration (Hb), Urea nitrogen (BUN), creatinine, total protein, calcium, potassium, chloride, aspartate aminotransferase (AST), alanine aminotransferase (ALT). Blood parameters obtained from 10 age-matched wild type mice that were breed in the IST animal facility for 3 months were used as baseline values.

Odor test

To meet the environmental criteria of NASA for human space flight, 21-23 volunteers participated the odor test during the 100-day test by using a protocol approved by NASA. The participants were sophomore or 3rd year university students majored in biotechnology or biology; they were informed about the scientific objectives and the aim of the odor test;

Table 1. *Timeline and major events of the 100-day test*

D-14	D-4	D1	D19	D36	D59	D67	D75	D94	D99	D100	D101
19 Feb	29 Feb	3 Mar	21 Mar	9 Apr	30 Apr	8 May	16 May	4 June	9 June	10 June	11 June
Animal handling											
Drinking training		Insertion								Retrival	
Body weight measurement											
1st		2nd								3rd	
Odor test											
	1st			2nd		3rd			4th		
Filter change											
	1st		2nd	3rd		4th			5th		
Food bar change											
	1st		2nd	3rd	4th		5th	6th			
Tissue sharing program excise											
											Dissection & Shipment

they had no history of working on animal experiments, nor exposure to an animal facility; they were non-smokers; they were not against experimentation on animals. In total four sessions of odor test were conducted: at fours four days before the test (D-4), and at 36, 67 and 99 days during the test (D36), at, D67 and D99). The test procedure required one participant entered the room where the 100-day test was running, stayed alone for three minutes, then exit and gave an anonymous evaluation. The evaluation was scored as 0 (undetectable or barely detectable odor), 1 (detectable odor compatible with a long permanence in the room) and 2 (unbearable odor not compatible with a permanence in the room). The test would be considered successfully completed if the mean calculated score would be less or equal to 1.25.

Statistics

Data on body weight and drinking quantity were presented as mean value ± standard variation. Student-t test were performed using the SPSS software. The probability of statistical significance (ρ) was reported.

Results

Overall observations during the 100-day test

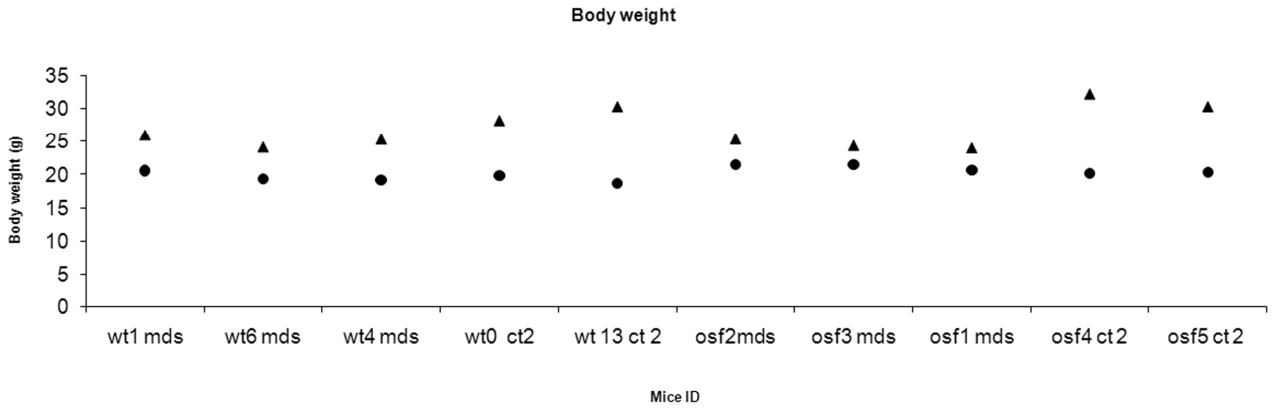
In total one 20-day test and one 100-day test were conducted to verify the biocompatibility of the MDS payload flight model. In IST animal facility mice normally drink from a standard graduated bottle connected to a spout, while the LHS of MDS employs a commercial stem activated valve. To prevent stress associated with the change of the drinking lixit, in the 20-day test and in the 100-day test a "drinking training" session was introduced before the insertion of animals into the MDS cages, by allowing mice to stay in the routine niche but to drink from a standard bottle connected with the commercial stem activated valve. This training obviously improved drinking adaptation in the 20-day test and in the 100-day test, in fact after the "training" mice drank water few hours after being inserted into the MDS cage.

During the test periods the animal welfare was checked twice a day through the OSS, no obvious abnormality in terms of routine behavior was observed. Subsystems of MDS functioned normally during all three tests. The food bars were changed about every 20 days; the LHS water tank was refilled every 4-5 days; waste filters were not changed during the 20-day tests. Since the waste filters were still in capacity after twice 20-day test, a 30-day interval was tested in the 100 day (Table 1). No leakage was observed at the bottom of the waste filters at the moment of its refreshment, showing that the lifetime of a waste filter can be extended from 20 days to 30 days.

Table 2. *Results of the odor test conducted during the 100-day test*

Session	Test day	Filter life	Score			Average score
			0	1	2	
1	-4d	baseline	22	0	0	0
2*	36d	1st filter + 20d	21	2	0	0.087
3*	67d	2nd filter + 30d	21	1	0	0.045
4*	99d	3rd filter + 30d	21	0	0	0
					Overall score	0.034

*NASA witness

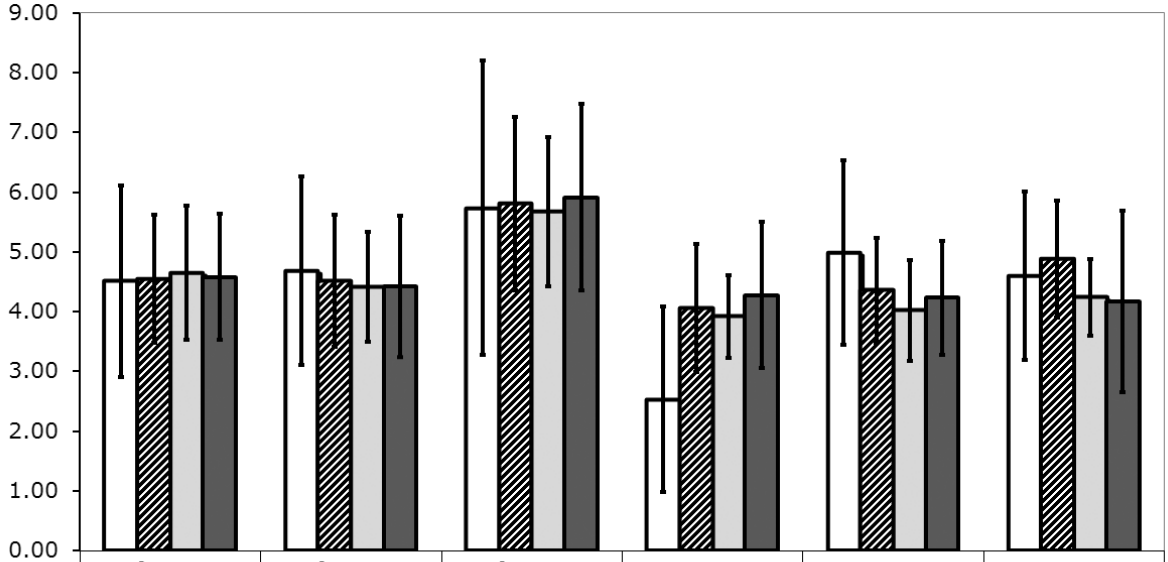


Test day	wt1 mds	wt6 mds	wt4 mds	wt0 ct2	wt13 ct2	osf2 mds	osf3 mds	osf1 mds	osf4 ct2	osf5 ct2
Δ D -14/0	-3.4	-1.2	1	0.8	1.5	-1.2	-2	-1.4	0.9	1.3
Δ D -14/+	5.36	4.74	6.14	8.4	11.6	3.9	2.9	3.3	12	10

Fig. 2 *Body weight profile of the 100-day test. End-point presentation of body weight measured at the beginning (black diamond) and the end (black dots) for 6 mice housed in the MDS payload (mds) and 4 control (ct) mice receiving 5g/d and water from the training bottles during the 100-day test. The change of body weight for each mouse during the drinking training period (ΔD-14/0) as well as during the experimental period (ΔD-14/+).*

Odor test

Using the protocol approved by NASA, four sessions of odor tests were conducted before (the first session) and during the test (the rest sessions). NASA personals witnessed three sessions. The first session was conducted 4 days before animals were inserted into the habitat (D-4), which served as the baseline odor level of the room where the 100-day test was performed. The 2nd, 3rd and 4th session was conducted respectively at D36, D67 and D99 during the test, all; these three sessions were conducted before the change of a waste filter. In all sessions, nearly every participant sensed no odor (Table 2). When participants that reported a slight odor was interviewed, nobody reported an odor



	osf1 MDS1	osf2 MDS2	osf3 MDS3	wt1 MDS4	wt2 MDS5	wt3 MDS6
Mar	4.51	4.68	5.73	2.53	4.99	4.60
Apr	4.55	4.51	5.81	4.06	4.36	4.88
May	4.65	4.41	5.67	3.91	4.02	4.23
Jun	4.58	4.42	5.91	4.28	4.23	4.17

Fig. 3 *Monthly water consumption profile of each mouse housed in the MDS for the 100-day test.*

Table 3. *Wild type mice blood analysis*

TEST	baseline values	MDS C57Bl/J10		CT 1 C57Bl/J10		CT 2 C57Bl/J10	
		mean	st dev	mean	st dev	mean	st dev
ALT (U/L)	26-120	291	239	204	26	230	23
AST (U/L)	69 - 191	446	245	312	83	435	78
BUN (mg/dL)	19 - 34	41	4	36	2	29	3
calcium (mg/dL)	9 - 12	7	0	8	0	7	0
chloride (mEq/L)	104 - 120	87	3	85	2	81	2
creatinine (mg/dL)	0.5 – 0.8	0.5	0.1	0.6	0.0	0.6	0.0
potassium (mEq/L)	5 - 9	8.8	0.7	8.7	0.6	8.3	0.1
total protein (g/dL)	4.3 - 6	6.1	0.6	6.8	0.7	6	0.4
RBC (10^6/ml)	5.0 – 9.5	10.4	0.5	9.9	0.6	11.3	0.0
Hb (g/dL)	10.9 – 16.3	16.0	0.6	15.3	0.9	16.3	0.0
Hct (%)	43.2	47.4	2.2	45.1	2.7	50.4	0.0
MCV (fL)	45.1	45.3	0.6	45.3	1.2	46.0	0.0
MCH (pg)	14.2	15.3	0.3	15.4	0.2	14.8	0.0
MCHC (g/dL)	33.2	33.7	0.7	33.9	0.5	32.3	0.0
RDW (%)		16.4	0.2	15.9	0.3	16.0	0.0
WBC (n/µl))	5.51 – 7.46	4.30	1.82	7.53	3.17	7.40	0.0
band neutr. (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
neutrophils (%)	6.53	34.00	8.72	18.00	5.00	38.00	0.0
basophils (%)	3.01	0.0	0.0	0.0	0.0	0.0	0,0
eosinophils (%)	3.89	0.67	1.15	1.17	0.76	2.00	0.0
lynphocytes (%)	84.6	65.3	9.9	79.3	7.0	60.0	0.0
monocytes (%)	1.41	0.50	0.00	1.67	2.02	0.50	0.0
Platelets (10^3/ml)	1084 - 1992	916.67	161.39	1116.00	580.66	1093.00	0.0
MPV (fL)	7.58	7.27	0.50	6.97	0.55	7.30	0.0

associated with animal smell, but rather a faint and unfamiliar smell to fresh air.

Body weight profile

The European Space Agency (ESA) is currently developing another automated rodent habitat, Mouse In Space (MIS), envisioned to fly in BION satellite. The cage and climate control units of MIS [10] have nearly identical features to that of the MDS. Since the MIS mice revealed no significant changes in myofibre size and type composition of skeletal muscle (type I vs. II) and quality of bone (3-D microarchitecture and mineralization of calvaria, spine and femur) determined by confocal and micro-computed tomography [8] indicating that the enclosure inside the MC does not affect the muscles and the skeleton of mice. The cage and climate control units of MIS [10] have nearly identical features to that of the MDS. Therefore, in the present study muscles and bones were not analyzed, and the body weight was used as the index to monitor animal well fare throughout all tests. In the 20-day test, body weight of mice was measured weekly. A fluctuated pattern of body weight change was observed (data not shown). At the end of this test, mice that were housed inside the MDS lost weight of 2.6 ± 1.9 g (n=6), while mice in the control group gained weight of 3.7 ± 1.6 g (n=6) .

In the 100-day test, body weight of mice was measured at 14 days before the "drinking training" started (D-14), at the insertion of animals into the MDS mice chamber (D1), at the end of the test (D100). During the drinking training period, some mice lost weight, while others gained weight. The loss or gain weight was observed both in the transgenic mice as well as in the wild type mice (Fig. 2). All mice gained weight after 100-day housing: mice that were housed inside the MDS gained 5.46 ± 1.87 g (n = 6), mice that were housed in the standard cages and received food at 5g/day and drank from the training bottles gained 9.38 ± 1.54 g (n = 4), mice that were housed in standard cages and received food ad libitum and drank water from normal bottles gained 8.82 ± 1.81 g (n = 6). The gain of body weight between the MDS group and the

Table 4. *Transgenic mice blood analysis*

TEST	baseline values	MDS OSF	C57Bl/J10	CT1 OSF/C57Bl/J10		CT2OSF/C57Bl/J10	
		mean	st dev	mean	st dev	mean	st dev
ALT (U/L)	26-120	101	66	246	133	208	125
AST (U/L)	69 - 191	341	79	423	303	508	180
BUN (mg/dL)	19 - 34	47	3	32	10	31	2
Calcium (mg/dL)	9 - 12	7	0	7	0	7	0.64
Chloride (mEq/L)	104 - 120	85	4	84	2	83	1.41
Creatinine (mg/dL)	0.5 – 0.8	0.5	0.1	0.4	0.1	0.6	0.00
Potassium (mEq/L)	5 - 9	8.5	1.0	8.6	0.6	9.2	0.07
Total protein (g/dL)	4.3 - 6	6.3	0.6	6.4	0.3	6.3	0.18
RBC (10^6/ml)	5.0 – 9.5	10.3	0.2	9.7	0.2	10.6	0.05
Hb (g/dL)	10.9 – 16.3	15.6	0.5	14.7	0.3	15.3	0.14
Hct (%)	4.,2	46.3	1.1	44.6	0.9	47.8	0.78
MCV (fL)	45.1	44.7	1.2	46.0	0.0	45.0	1.41
MCH (pg)	14.2	15.0	0.4	15.3	0.3	14.4	0.21
MCHC (g/dL)	33.2	33.6	0.4	33.1	0.4	32.0	0.14
RDW (%)		16.7	0.2	16.0	0.3	15.4	0.07
WBC (n/µl))	5.51 – 7.46	3.5	7.5	6.4	1.5	4.1	0.00
band neutr.(%)		0.0	0.0	0.0	0.0	0.0	0.00
neutrophils (%)	6.53	26.33	10.69	29.67	10.21	53.50	12.02
basophils (%)	3.01	0.0	0.0	0.0	0.0	0.0	0.00
eosinophils (%)	3.89	0.17	0.29	1.00	1.00	0.00	0.00
lynphocytes (%)	84.6	72.7	10.1	69.3	9.5	45.0	12.73
monocytes (%)	1.41	1.00	0.87	0.50	0.00	1.50	0.71
Platelets (10^3/ml)	1084 - 1992	1201	145	861	153	401	93
MPV (fL)	7.58	6.67	0.06	7.00	0.53	7.50	0.14

two control groups were statistically different (ρ = 0.009 and ρ = 0.01, respectively). The gain of body weight were similar between the two control groups (ρ =0.626)

Water consumption profile

During all tests water consumption was recorded daily for each mouse that was housed inside the MDS. Over the period of 20 days, a mouse drank 4.4 ± 1.1 ml per day (n = 6). In the 100-day test, a mouse drank 4.57 ± 0.72 ml per day. To understand whether season affects the drinking habit inside the habitat, the quantity of water consumption was plotted according to the month of a year. The water consumption rate by a mouse was very similarly distributed through March to June (Fig. 3), indicated differences suggesting a fully active constant climate control inside the habitat.

Blood analysis

Blood haematocrit counts and clinical chemistry parameters were used as indicators of mice wellbeing during the experiment period. Since vivarium conditions was shown to have a great influence on haematological parameters [11], the baseline values of the IST animal facility were obtained from 10 age-matched wild type mice that were bred in the facility for 3 months. When haematological values of the MDS mice and control mice were compared to the baseline values, majority of parameters fell inside the baseline range with high variability among individual animals (Tables 3 and 4).

Although for some of the measured parameters the values were unusually high and showed a high variability among individual animals. In all cases no major difference was observed between the experimental and the control groups.

Discussion

Engineering and scientific output of the 100-day test

The MDS engineering model has all characteristics of the flight model, and meets engineering requirements

Table 5. Comparison of MDS to other existing rodent flight payloads

Payload		Animal Enclosure Module (AEM)	Research Animal Holding Facility (RAHF)	Advanced Animal Habitat (AAT)	Animal Module for Autonomous Space Support (A-MASS)	Mice Drawer System (MDS)
Supporting modules		Shuttle middeck	Spacelab	Shuttle middeck, Spacelab, ISS	COMET-1 middeck, Space station freedom express rack	Shuttle middeck, Space station freedom express rack
Animal habitat	**Capacity**	6 rats* in 1 or 2 groups	24 rats* in-pair	12 rats or 30 mice in a gang-housed environment	2-6 mice	6 mice individually or 8 mice in-pair
	Cage dimension	24 x 36 x 22 cm (14 inches^2/mouse)	10.5 x 11 x 28 cm	65-97 cm^2	96.8 cm^2 /mouse	11.6 x 9.8 x 8.5 cm
	Air temperature	30 °C passive	23±1°C active	22-28°C active	22-26 °C active	25-26°C active
	Relative humidity	50±10%	30% - 70%	40% - 70%	70%	40-70%
	Light intensity	14lux illumination, 12:12light/dark cycle		0-40 lux It can raise to 100 lux to facilitate direct visual checks	25-40 lux	0-40 lux in steps od 10 lux
	Food	Food bars 1.8 x 1 x 8 inches		Food bar dispenser provides food for 11.5days 0-150 g	60g/day ad libitum (mice tipically consume 5g/d)	Food bar dispenser provides 90 g ad libitum or given quantity per day
	Water	2000 cc capacity	2 water lixit /cage 9.5l capacity	Via lixits fed from pressurized reservoirs, sufficient for 15 days 0-330 ml	60g/day ad libitum as NAPA nectar	500 cc capacity, refillable in orbit, ad libitum to animals
Monitoring		Daily observation by mission specialists through the transparent cover	Ability to manipulate animals	Video monitoring system and environmental control	Oxygen and humidity sensors, video system	6 vidoe camera, sensors for temperature, humidity, CO2, ammonium
Reference		[2, 5]	[2]	[6]	[7]	this study

for flight experiments of NASA. The life-supporting subsystems of the MDS flight model have different duration of autonomy. Whereas subsystems, such as OSS and CPU, have a guaranteed autonomy of 100 days, the LDS needs maintenance about every 5 days, one FDS is designed to last for 20 days, and the waste filters need to be replaced every 30 days. The upcoming MDS space mission has a nominal duration of 100 days, therefore, the 100-day test reported in this study not only tested the payload biocompatibility, but also tested the engineering performance of the payload. Results on animal welfare by using body weight and haematological analysis as quantitative indexes have showed that the MDS engineering model supported animal survival and well-being. Daily visual observation by OSS and autopsy at the end of the test did not find any irregularity on animal behaviour or internal organs. Subsystems of the MDS engineering model functioned normally throughout the test. The negative outcome of the odour test in all four sessions showed excellent performance of the waste management by waste filters and the Hepa filter. Mice have been flown in animal enclosure module (AEM) hardware only twice in Space Shuttle Transport system (STS)-90 and -108 for 13 days , whereas rats have been flown in the AEM more than 20 missions. This has been due, in part, to concerns that strong and annoying odors from mouse urine (vs. rat urine) will interfere with crew performance in the shuttle middeck.

Osteoporosis is a debilitating disease that afflicts millions of people worldwide. One of the physiological changes experienced by astronauts during space flight

is the accelerated loss of bone mass due to the lack of gravitational loading on the skeleton. This bone loss experienced by astronauts is similar to terrestrial osteoporosis in the elderly population and immobilized patients. A major objective of the MDS space mission is to investigate the effects of microgravity on transgenic or inbred mice as a tool to study genetic mechanisms underlying bone mass pathophysiology. To maximize the scientific output, the 100-day test was also designed as the science verification test by the principle investigators, in which a complex tissue sharing program was exercised.

Advantage and disadvantage of the MDS payload

The The MDS is a double middeck locker replacement payload, and can interface with space shuttle middeck, with the ISS express rack and with crew via portable glovebox. Compared to the two US payloads with flight history, AEM and RAHF, the major advantage of MDS is that it offers autonomy, interface with crews, and permanence on ISS. The major disadvantage is the limited number of rodents that can host. A major difference among these three payloads is that MDS can house rodents individually or in-pair, while other two payloads house rodents in group (Table 5). In the 100-day test, all mice gained weight, showing optimal performance of MDS to support the animal welfare. However, in the 20-day tests presented in this study as well as prominent difference between other reported studies fact, in conducted in AEM, MIS and A-MASS, all mice lost weight after a period of 20-30 days. This difference led us to hypothesize that mice need more than 20 days to adapt to a new habitat such as MDS.

MDS is the first European payload designed to perform rodent experiments in microgravity. The European Space Agency is currently developing another automated rodent habitat, MIS. Based on experience gained in MDS design, MIS will provide many improved engineering features. A common feature of these two habitats is that both offer single and paired housing of rodents. The difference between MDS and MIS is that the latter is designed for flying on biosatellite BION for duration of up to 30 days autonomy with on-board centrifuge [12]. With the realization of MIS, together with MDS Europe will broaden significantly the flight frequency and experiments capacity on rodents.

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