New Development of Space Utilization in the Life Sciences Field

TOSHIMASA OCHIAI[*1] HIROCHIKA MURASE[*2]
ATSUKO HOMMA[*2] JUNICHIRO GYOTOKU[*2]

Mitsubishi Heavy Industries, Ltd. (MHI) has conducted parabolic flight experiments to assess the potential of life science studies in space and has investigated the impact of microgravity environments on small animals and humans at the molecular level. The possibility of space environments contributing to the establishment of new central nervous system pathological models has been confirmed. Conducting experiments using small animals in space and creating opportunities for their utilization can be expected to improve our lives because the in-space scientific data thus provided may help us to understand pathological mechanisms that cannot be obtained on the ground. This report describes our research projects that will enable the performance of small animal experiments in space.

1. Introduction

Using space shuttles, Japan started conducting life science experiments in space in the early 1990s. Since then, MHI has been engaged in the development of experimental equipment. The need for in-space experiments on small animals, especially mice, is high in Japan, as well as in other countries. However, these experiments have previously been conducted exclusively by NASA and Russia. As in experiments performed on the ground, mice and rats are indispensable to the understanding of biological mechanisms. In the field of space medicine, test results, including in-space experiments, demonstrate that microgravity environments can affect vital reactions in the bones, muscles and cells of mammals. In fact, major pharmaceutical company in the U.S. have repeatedly invested money to conduct in-space experiments, but none of the results have been disclosed to the public. This, in itself, indicates the importance of conducting in-space experiments.

In response to this situation, MHI has focused on research in the drug discovery field with objectives such as the provision of laboratory animals that have been placed under microgravity environments, and the establishment of new pathological models. Our research projects have been conducted based on two different perspectives: 1) the assessment of the impact of microgravity environments on biological mechanisms, and 2) the establishment of technological backgrounds for a platform for in-space experiments. As far as the former is concerned, we performed the microgravity experiments on small animals by parabolic flight and verified the effectiveness of the experiments based on evaluations by universities and pharmaceutical companies. With regard to the latter, we conducted research to establish a recoverable capsule-type experiment system that can be piggybacked as a payload on the H-IIA rocket. The component technologies for the maintenance of an appropriate habitat for mice were examined. Tests related to the closed environment and environmental resistance were also performed using mice. Results of these studies have confirmed the feasibility of the tested technologies. In addition to the system mentioned above, research is being conducted to develop any possible system that can facilitate the realization of in-space experiments.

*1 Manager, Space Technology Application Department, Space Systems Division, Aerospace Systems
*2 Space Technology Application Department, Space Systems Division, Aerospace Systems

2. Impact of Microgravity Environments on Animals and Its Utilization

Previous studies on space medicine and in-space animal experiments have demonstrated that microgravity environments can affect, within a relatively short time, a variety of biological reactions in the mammalian body such as bones, muscles, the cardiocirculatory system, immune system, and central nervous system. However, the details of individual mechanisms are still unknown. MHI has repeatedly conducted different basic experiments, and considered the possibility of drug discovery studies related to the provision of laboratory animals that have been placed under the influence of microgravity environments, the establishment of new pathological models, etc. The following is a discussion of the results showing the effectiveness of microgravity environments created during the experiments on stress associated with gravity change, including those of parabolic flight experiments using aircraft.

2.1 Experimental hypothesis

The involvement of serotonin, a neurotransmitter, in the sophisticated control of emotions in animals (mammals, in particular) and in the development of mental diseases has been demonstrated. According to previous studies conducted by co-researchers, serotonin is associated with the occurrence of panic induced by sudden exposure to microgravity, which is an unknown environment to mammals. Corticosterone, a stress marker in mouse blood, is significantly elevated after the experience of eight successive parabolic flights, as compared to an onboard control group. This preliminary experiment, therefore, demonstrated that mice were stressed by eight successive gravity changes (including microgravity). Mice became free from stress three hours after the first parabolic flight, as they got over the influence of acute stress. To understand the details of this phenomenon associated with stress, we conducted gene expression analysis considering serotonin as the key molecule.

2.2 Gravity change-associated stress experimental system

A parabolic flight with MU-300 can create a microgravity field of approximately 10^{-2} g for 20 seconds at most. In a single flight, a parabolic flight can be repeated a maximum of 20 times. In this experiment of stress associated with gravity change, an aircraft was carefully maneuvered; the applied gravity during the control of aircraft orientation before/after each parabolic flight was maintained at (or below) 1.4g to avoid hyper gravity as much as possible. The duration of microgravity achieved in a single parabolic flight was approximately 15 seconds. Gradual change in aircraft orientation allowed this microgravity flight to be repeated eight times within the overall aircraft flight time of 1 hour. This flight method is hereafter referred to as the gravity change-associated stress experimental system (**Figure 1**).

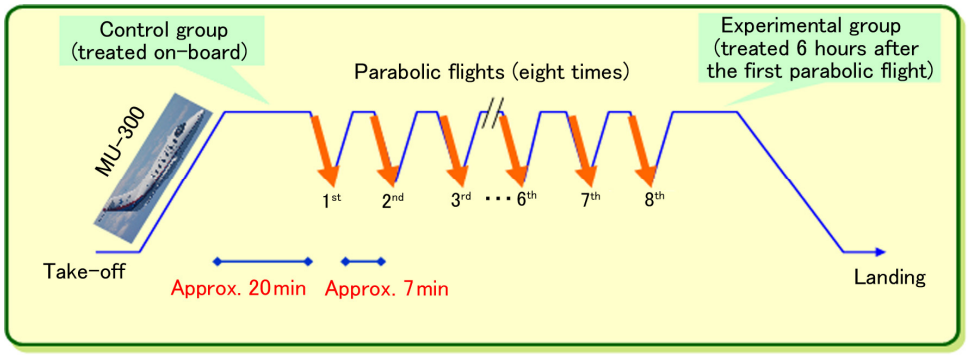

Figure 1 Image of an aircraft experiment

2.3 Experimental method

In this experimental system, based on the data obtained from previous basic experiments, the control group was defined as the on-board group treated immediately before the first parabolic flight. The experimental group was defined as the group treated six hours after the first parabolic flight (Figure 1). Features of this experimental system include the capability of conducting various on-board treatments, such as blood or tissue sample collection from the experimental animals before/after each parabolic flight, if necessary. In this experiment, mouse blood and brain/small intestine tissue samples were collected on-board to perform genetic analyses.

2.4 Results

Of serotonin-related genes, Tph1 is associated with tryptophan hydroxylase, which acts on the peripheral nervous system, such as the small intestine. Tph2 is related to another form of tryptophan hydroxylase that acts on the central nervous system such as the brain. In this experiment, only the Tph2 level was significantly elevated, exhibiting increased gene expression in the serotonin synthesis system (**Table 1**).

Table 1 Analysis results of stressors associated with gravity change and acrophobia

	Gene name	Functions	Gravity change*1	Acrophobia*1	Social situation
Tph1	Tryptophan hydroxylase	Serotonin synthesis system (peripheral nervous system: small intestine)	–	–	ND
Tph2	Tryptophan hydroxylase	Serotonin synthesis system (central nervous system: brain)	↑	–	–
Maoa	Monoamine oxidase A	Serotonin degrading system (substrate: serotonin)	–	–	ND
Maob	Monoamine oxidase B	Dopamine degrading system (substrate: dopamine)	↑	–	ND
Sert	Serotonin transporter	Serotonin transport (for recycling)	↑	↓	–
Slc7a5	Large neutral amino acid transporter 1	Tryptophan	↑	–	ND
Htr1a	Serotonin receptor	Serotonin receptor protein	–	↑	–
Th	Tyrosine hydroxylase	Noradrenaline, dopamine nervous system	⇑	–	ND
Gad1(67kDa)	Glutamic acid decarboxylase 1	GABA nervous system	–	–	ND
Gad2(67kDa)	Glutamic acid decarboxylase 2	GABA nervous system		–	ND

* 1 ↑ Red arrow: pertaining to the brain (with significant change)
↓ green arrow: pertaining to the small intestine (with significant change)
⇑ purple arrow: pertaining to the brain (tendency, without significant change)
– horizontal line: representing "no change" ND: representing "not done" (based on references)

Note : The RNA expression level was quantitatively estimated from real-time polymerase chain reaction (PCR) with the intercalator method, SYBR Premix Ex Taq II, where synthesized cDNA of the target and housekeeping genes was used as a template. HA067799 (18SrRNA) was selected as the housekeeping gene.

Conversely, because Tph2 expression is not accelerated by acrophobia or social stress, the serotonin synthesis system in the midbrain was stimulated by the stress caused by gravity change. Maoa is a gene related to a serotonin-degrading enzyme, while Maob is a gene associated with a degrading enzyme acting on another substrate, dopamine (also a neurotransmitter). The experimental results indicate that the stress associated with gravity change enhanced only Maob gene expression (dopamine-degrading enzyme), facilitating dopamine metabolism while selectively retaining serotonin levels. The expression of Sert, which is involved in the recycling pathway related to the effective use of synthesized serotonin, was enhanced by gravity change, but was lowered by acrophobia-induced stress. Slc7a5 functions as a large neutral amino acid transporter to take tryptophan into the cells. The increased gene expression of Slc7a5 also indicates the facilitation of the serotonin synthesis system. The increased expression of Htrla, a synthesized serotonin receptor, was seen only under acrophobia-induced stress.

Tyrosine is an amino acid from which other neurotransmitters such as adrenaline, noradrenaline, and dopamine are produced. Th is a gene associated with a tyrosine synthesis enzyme that is involved in the first stage of tyrosine synthesis. On the other hand, GABA is a neurotransmitter that inhibits brain excitation. Gad1 and Gad2 are the genes that are associated with GABA as glutamic acid decarboxylase. Of these three, Th showed a tendency for enhanced expression, which indicates that the stress caused in this experiment increased the expression of Th in the brain. Therefore, the tendency to activate the entire nervous system by facilitating the activation of dopamine, noradrenaline and adrenaline systems was observed. However, this tendency did not reach statistical significance.

2.5 Discussion

In the conventional methods for stress induction used in studies on mental diseases such as the elevated plus maze, the release of not only serotonin but also other amino acids including dopamine and GABA in the brain, is accelerated. Therefore, it was not possible to selectively facilitate the expression of serotonin synthesis genes. The results of the gene expression analysis in the midbrain or small intestine showed that the gravity change associated-stress experimental system was a new stress induction method by which the serotonin synthesis system can be selectively stimulated in response to stress. Use of this new experimental system in research may lead us to the discovery of new pathological mechanisms in the central nervous system.

3. New Experimental Platform

MHI has developed space experiments in the life science field, including the cell biology experiment facility (now in operation) on the International Space Station (ISS), which is in orbit. Based on this experience, MHI has conducted research related to a recoverable capsule-type experiment system as a means of realizing the utilization of space for drug discovery since 2004. This system is mounted on the H-IIA rocket as a piggyback payload and can offer an experimental platform for biological experiments from launch until recovery. In the development of the system, basic data in terms of metabolism and behavior of small animals, etc. were examined to determine the conditions for small animal habitat in space. To realize such conditions, component technologies were developed and their verifications were conducted. Thus, we have accumulated technologies that can serve as an infrastructure for small animal experiments.

If this system becomes available for practical use, space can be utilized as a new experimental site for small animal experiments. This may enable not only the discovery of biological findings from small animals used in in-space experiments but also the use of such a system as a platform for drug discovery.

3.1 Our original technology for small animal experiments

The biological experimental system using the H-IIA rocket offers an experimental environment starting from the launch of small animals (mice) into space until their return to the ground. The system consists of a satellite bus (transportation infrastructure) and an experiment unit (infrastructure for small animal experiments). The experiment unit is equipped with sub-systems for environmental control and life-support functions and other experimental functions (**Figure 2**).

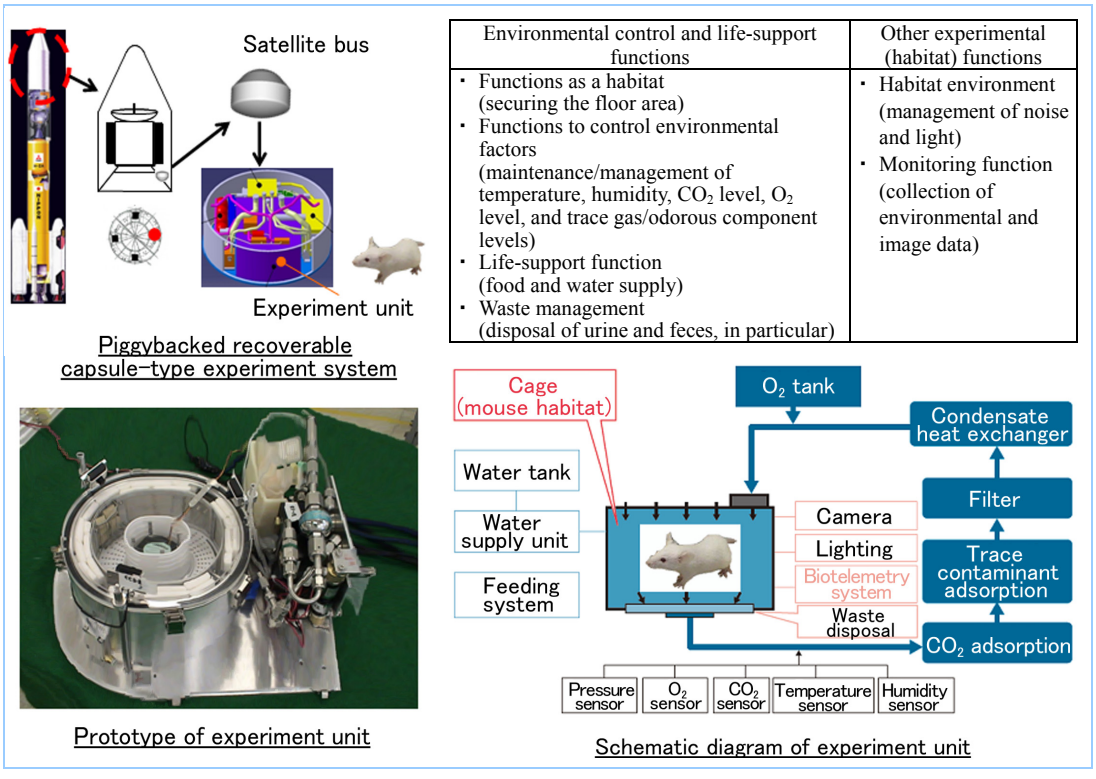

Figure 2 Schematic diagram of the piggybacked recoverable capsule-type experimental system

For realization of the above functions and conditions, it is important to overcome the technical challenges resulting from the distinctive features of in-space experiments, such as microgravity (space with no sense of direction), change in wettability of a liquid and environmental change during launch or re-entry (**Table 2**).

Table 2 The technical challenges and corresponding suggestions for technical solutions

Function	Major technical challenges peculiar to the environment in space	Suggestion for technical solution	Demonstration status
For habitation	Necessity of securing a suitable habitat size according to the number of mice aboard and shortening the length of air flow inside the habitat to prevent cross-contamination of floating waste to mice.	Adoption of a habitat type with a doughnut shape (a loop of corridor) to create the least restriction of activities among mice and to shorten the length of air flow, which has been realized (Figure 3).	・34-hour closed environment test, performed (Number of mice: 3) ・2-week closed environment test, performed (Number of mice: 5)
Waste disposal	Necessity of preventing the diffusion of urine onto the mouse body surface or the attachment of feces to mice.	Use of absorptive walls to immediately absorb liquid waste (urine, in particular) and adoption of air circulation to collect solid waste at the end of air flow, which has been realized (Figure 3).	・Waste-disposal component test, performed (Parabolic flight experiment with an aircraft)
Environmental control	Necessity of removing the influence due to the change of experimental environment (e.g., pressure change on launch, and change in air composition, temperature and humidity due to metabolism of astronauts, etc.) and preventing the contamination of experimental environment (especially, contamination of habitat environment by astronauts).	Adsorbent and valve-control technology to achieve a closed type of environment control with the external influence being shut out from the mouse habitat environment (removal of CO_2 and harmful gases, and control of O_2 supply), which has been realized. With regard to a type of habitat with an air vent connected to the outside (residential area of astronauts, in particular), adsorbent and filtering technology to keep the concentrations of trace gases and odorous components in the habitat at desirable levels for habitation, and to prevent their leakage into the outside, has been developed.	・34-hour closed environment test, performed (Number of mice: 3) ・2-week closed environment test, performed (Number of mice: 5) ・Deodorizing effect verification test, performed
Animal fixation	Necessity of receiving the least influence of disturbances such as hypergravity created before/after the microgravity experiment, because these parameters are considered uncertain in the assessment of results.	Automatic animal fixation technology using gas displacement and cooling, which has been realized. New experimental method by which samples are fixed before/after microgravity without the influence of disturbances, which has been established (Figure 3).	・Verification test for animal fixation characteristics/ quality of fixed tissue, performed

The functions for habitation and waste disposal are closely related to each other. From a habitation viewpoint, a sufficiently large space should be secured so as not to constrain the activities of small animals. On the other hand, a smaller space is preferable from a waste-disposal point of view, because small animal urine and feces can be removed before the waste, especially urine, spreads over the body surface (prevention of cross-contamination). In response to the functional requirements that contradict each other, we developed the component technologies to optimize factors such as the habitat shape, air flow inside the habitat, and waste disposal method. The closed environment test, in which the environment inside the habitat was controlled without the influences of external environment, and the adaptability test to microgravity environments by parabolic flights were conducted. Thus, the verification of each component technology has been performed (**Figure 3**).

In the waste disposal method screening test, mock mice with an imitation body surface were made. When the microgravity experiment started, the mock mice automatically started to excrete mock urine that was also created using a dyed liquid with viscosity and surface tension equivalent to the real liquid waste (urine) of mice. The behavior of mock urine in terms of spreading over the mouse body surface was examined. The results show that the passive adsorption method was effective for the disposal of liquid wastes from a short-term perspective, while the active adsorption method should be adopted for a long-lasting effectiveness.

To enable the evaluation of the impact of environmental change before/after the in-space experiment (gravity change, in particular) on small animals, MHI has also made the animal fixation technology available for practical applications. **Figure 4** shows an example of the results of the assessment test regarding the whole-body fixation of mice with our animal fixation device.

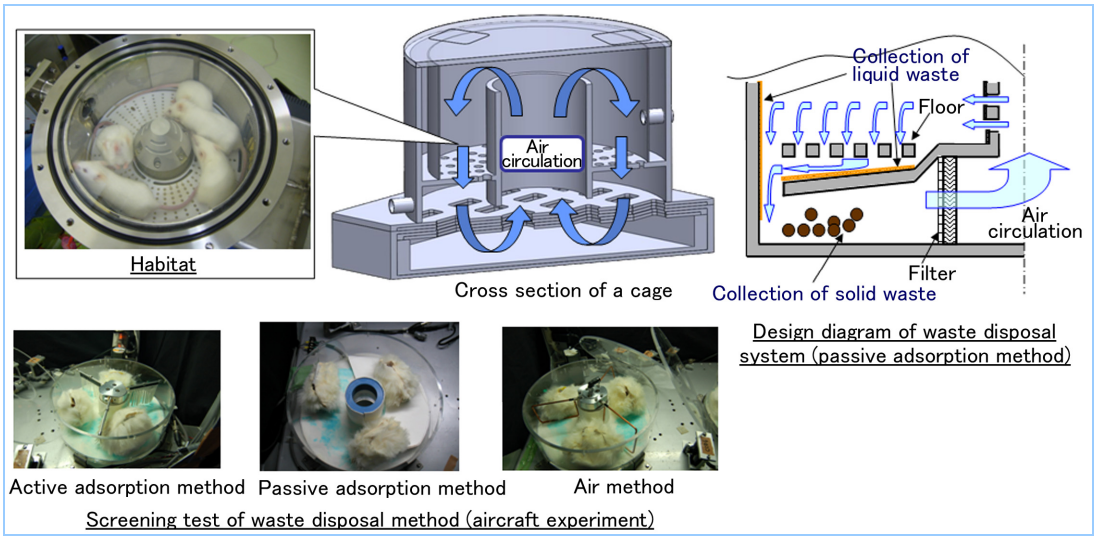

Figure 3 Functional verification tests for habitation and waste disposal

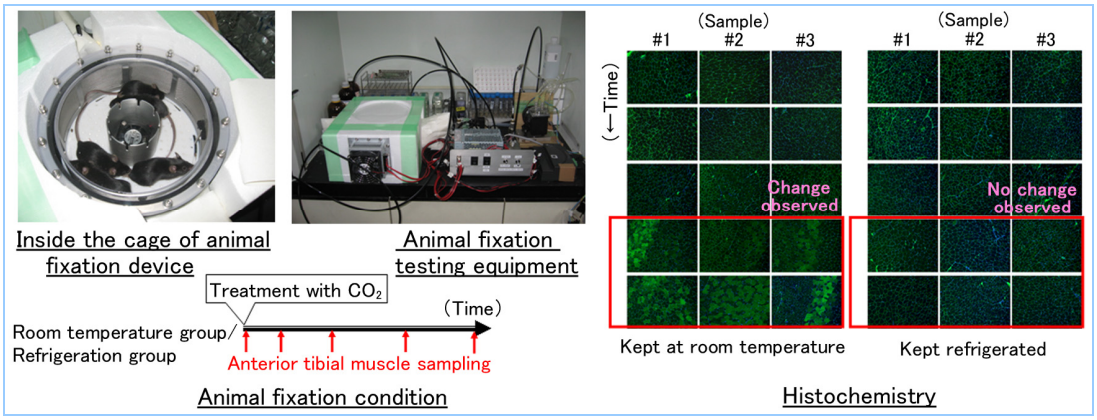

Figure 4 Animal fixation technology test

With this device, the air inside the mouse habitat can be displaced by carbon dioxide gas within a short time, enabling the treated mice to be kept refrigerated. In the assessment test, 10 week-old male mice were treated with carbon dioxide gas before being kept either refrigerated or at room temperature. Protein, RNA, and a transverse muscle slice samples were then removed from the mouse anterior tibial muscle to compare the fixed samples according to the preservation method. As a result, the presence of differentially-localized muscle fibers, as seen in the damaged muscles, was observed in the samples kept at room temperature. On the other hand, in the samples refrigerated after the animal fixation, the condition of fixed samples was preserved normally. The effectiveness of the device has been sufficiently demonstrated.

3.2 Toward more advanced small animal experimental devices

The microgravity environment created by a parabolic flight only lasts a number of seconds. However, the use of accumulated technical knowledge with regard to small animal experiments will enable experiments conducted by suborbital flights, in which microgravity duration reaches several minutes, or even the development of small animal experimental devices with which an experiment can be conducted on the ISS for several months.

Particularly when it comes to the devices used on the ISS, only minimum life-support functions (e.g., food and water supply, waste disposal, and ammonia removal) are required as basic specifications because the environment control functions equipped in the ISS can be utilized. Depending on the objective or needs of each small animal experiment, it is also possible to adopt a system in which the functional components can be replaced as cartridges, to achieve lowered CO_2 levels, and/or to be equipped with an animal monitoring system or animal fixation device. An operational scenario for the use of small-animal experimental devices on the ISS is shown in **Figure 5**. In this scenario, the restrictions on the timing for late access and the on-board weight, depending on the means of transportation, are considered. The device consists of three components: the main body, a cage in which mice are reared, and spare goods (cartridges). The device itself will

be on board the ISS until all experiments are finished. A cage with mice inside will be replaced according to the experiment that is to be conducted. Cartridges will be replaced regularly, based on the schedule of experiments. Thus, the continuous and effective performance of experiments with different objectives will be possible. Looking further ahead, we will develop an analysis device with which the fixed samples can be examined while they are still in orbit. The combined use of the analysis device and small animal experimental devices will shorten the time necessary for experimental assessment. We expect that we can expand the number of possible users of small animal experimental devices on the ISS.

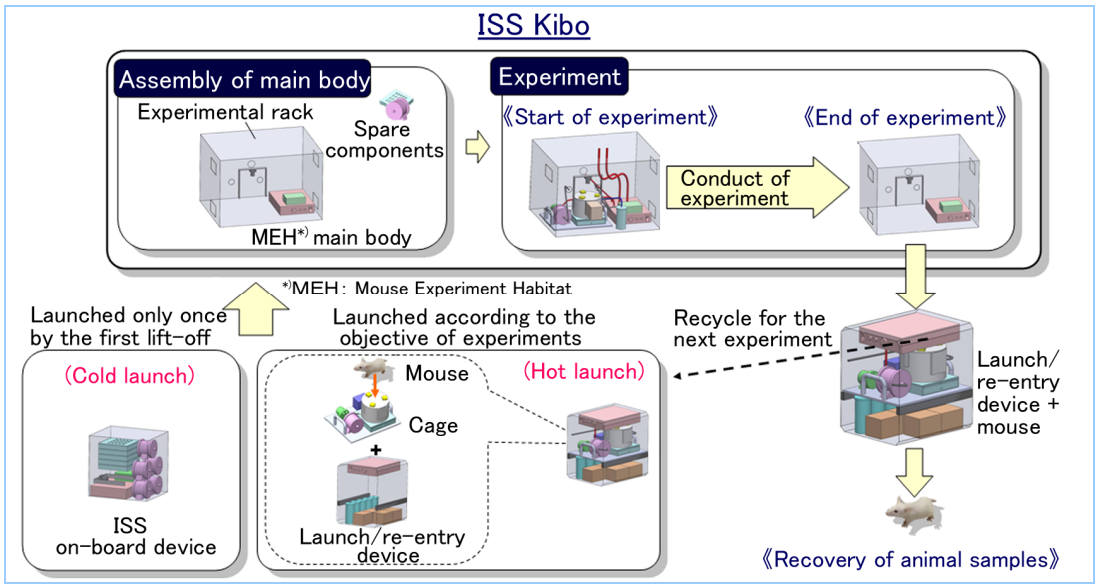

Figure 5 Scenario of in-orbit demonstration

4. Conclusion

Through the development of equipment for life science experiments, MHI has focused on microgravity impact on the body and has conducted research to utilize space as a platform creation for drug discovery. We will deepen our understanding through the verification of the effectiveness and assessment of microgravity impact on the body. With this in mind, to provide small animals that have been placed under the influence of microgravity environments, we will continue the research projects and establish the infrastructure for in-space experiments on the ISS or by suborbital flights. This will create potential new clients such as universities, research institutes and pharmaceutical companies, which may contribute to active space utilization.

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