Preface

These guidelines were developed by the Japan Neuroscience Society in light of the recent increase in international collaboration in neuroscience research using nonhuman primates and the increasing frequency of collaborative experiments and sharing of experimental data. These researcher-initiated and -led guidelines are focused on the use of macaque monkeys and common marmosets in neuroscience and behavioral research. Different wording is used to indicate three strengths of recommendation in these guidelines. “Must” indicates duty or requirement that has to be followed. “Should” indicates that the procedures described are strongly recommended in principle, but alternative procedures are permitted depending on the situation only if the procedures are approved by the institutional animal care and use committee. “Advisable” indicates a suggestion to be considered. We chose to use the term “well-being” in the context of a better environment for animals. Some argue that it would be better to use other terms such as “tranquility” and “welfare,” while others say that these terms are more suited for use in the context of human society. We agree with the latter opinion and opted to use “well-being.” Another reason for this choice is that “well-being” is used in the Guide for the Care and Use of Laboratory Animals (8th ed.) issued by the National Research Council.

These guidelines also include two major proposals. The first is to promote the introduction of equipment (cages) that can accommodate multiple animals. We aim to achieve this goal by 2030. However, we have little knowledge or experience regarding the advantages and disadvantages of keeping Japanese macaques in pairs (knowledge in Europe and the U.S. is primarily derived from rhesus and cynomolgus macaques, and it is unclear whether it can be immediately applied to Japanese macaques). Therefore, we will conduct a survey of the actual status of this proposal and other necessary surveys in Japan in about 5 years to see whether reconsideration is necessary. The second proposal is to establish a collaborative relationship with veterinarians regarding veterinary management. We aim to achieve this goal by 2025.

We hope that these guidelines will contribute to the proper care of laboratory animals and the proper conduct of animal experiments in compliance with the “3Rs" principle.

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Guidelines for the Care and Use of Nonhuman Primates in Neuroscience Research

April 20, 2021

The Japan Neuroscience Society

Contents

Introduction

In Japan, policies on the handling of animals, including laboratory animals, have been developed based on several laws and guidelines, such as the Act on Welfare and Management of Animals (1973) and the Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals (2006). We believe it is advisable for researchers, who best understand the necessity of animal experiments, to take responsibility for regulating animal experiments on their own initiative, under administrative guidance rather than by law. At the same time, there are calls for a certain level of standards for the proper conduct of animal experiments and for guidelines that can be provided to foreign collaborators in light of the recent trend toward active collaboration in international research. The Ministry of Education, Culture, Sports, Science and Technology (MEXT), the Ministry of Health, Labour and Welfare (MHLW), and the Ministry of Agriculture, Forestry and Fisheries (MAFF) have issued Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions (2006), Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of the Ministry of Health, Labour and Welfare (2006), and Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of the Ministry of Agriculture, Forestry and Fisheries (2006), respectively. The Science Council of Japan published Guidelines for Proper Conduct of Animal Experiments (2006) at the request of the MEXT and MHLW. Each research institution carries out institutional management while establishing its own rules and regulations based on these laws and guidelines.

In light of the increasing internationalization of research and the accelerated pace of international collaboration, we decided to develop researcher-initiated and -led guidelines focused on the use of macaque monkeys (hereinafter, simply “macaques”) and common marmosets (hereinafter, simply “marmosets”) in neuroscience and behavioral research. We hope that these guidelines will assist research institutions in implementing more appropriate care of animals. All individuals involved in animal experiments must strive to conduct proper animal research based on scientific rationale.

Definition of terms

Terms used in these guidelines are defined as follows based on the definitions provided in the Guidelines for the Proper Conduct of Animal Experiments by the Science Council of Japan:

Animal experiments
Utilization of animals for education, testing, research, manufacture of biological products, or other scientific purposes.

Facilities
Facilities and equipment used to perform animal experiments.

Laboratory animals
Animals of mammalian, avian, or reptilian species used in animal experiments. In the present guidelines, this term is used synonymously with macaques or marmosets.

Institutions
Organization (university, institute, independent administrative body, company, etc.) where animal experiments are performed.

Director of the institution
The person with overall responsibility in the institution for proper and safe conduct of animal experiments (the dean, director of an institution, principal of a school, chairperson of the board of directors, president of a company, head of an institute, etc.).

Animal experiment protocol
A protocol drafted beforehand for the conduct of an animal experiment.

Researcher
A person who performs the animal experiment.

Principal investigator
The researcher who is in charge of all duties related to an animal experiment protocol.

Manager
The person in charge of the management of laboratory animals and facilities under the director of the institution (the head of the animal research facilities, department head, etc.).

Laboratory animal manager
The laboratory animal manager assists the manager and is in charge of the management of the laboratory animals.

Animal technician
Person(s) in charge of the care and management of laboratory animals under the laboratory animal manager or researcher

Manager and other personnel

Collectively refers to the Director of the institution, manager, laboratory animal manager, researchers, and animal technicians.

Policies
Refer to the fundamental guidelines and basic policies specified by government agencies and the

Guidelines for Proper Conduct of Animal Experiments issued by the Science Council of Japan.

Regulations
In-house regulations of research institutions specified for the proper conduct of animal experiments and the proper care and management of laboratory animals based on related laws, ordinances, and policies.

Chapter I. Guideline for Experiments Using Macaques

Section I: Basic policies

1. Introduction

The purpose of the "Guideline for Experiments Using Macaques" is to assist researchers and research institutions engaged in neuroscience and behavioral research in conducting experiments using macaques in a scientifically, humanely, and ethically appropriate manner. Macaques must be handled properly based on the knowledge and skills necessary to keep them in good health. All those who keep, manage, and breed macaques must be responsible for their behavioral, physical, and mental health. This Guideline has been developed to dispel the public's concerns while meeting their expectations and to ensure that animal experiments conducted in an appropriate manner will produce scientifically correct results. The instructions and recommendations in the Guideline are based on scientific evidence, expert opinions, and practical experience and should be referenced by research institutions for the management and care of macaques and the development of protocols when conducting neuroscience/behavioral research in Japan.

2. Scope and goal of the Guideline

This Guideline is applicable to neuroscience and behavioral research activities using macaques. Researchers in other areas should also refer to this Guideline when performing similar procedures with macaques.

The goal of this Guideline is to encourage the humane care and use of macaques for research. The guideline also aims to raise awareness of the profound link between the evidence-based care and management of laboratory animals and the quality of research, and to provide researchers as well as animal experiment committees, veterinarians, and other persons concerned with knowledge and techniques regarding medical and laboratory animal science based on sufficient experience. Of course, the information will be updated as scientific knowledge progresses. The content of this Guideline should also be updated based on the latest scientific evidence.

Each institution can refer to the instructions and recommendations set out in the Guideline while complying with institutional regulations on animal experiments established by each institution, and can use the Guideline to develop plans for the care and use of macaques while adding custom modifications. We also hope that this Guideline will contribute to the development of guidelines for the use of macaques in various other areas of research.

3. Evidence-based care and use of laboratory macaques

The care and use of laboratory macaques must be based on the concept of evidence-based well-being. This Guideline is also based on a diverse body of literature and specific values are included in the appendices. In some cases, however, sufficient data are not available for macaques. In such cases, data for related species or other laboratory animals are provided. Truly appropriate conditions for macaques need to be further explored, and the content of this Guideline must be updated based on such data.

4. "3Rs" principle

The "3Rs" principle was first proposed by W. M. S. Russel and R. L. Burch in 1959 as a set of strategies that researchers should consider when planning animal experiments, consisting of "replacement" (using alternative methods), "reduction" (reducing the number of animals used), and "refinement" (reducing pain/distress through refinement of techniques), and may be simply referred to as the "3Rs." In Japan, a provision on this principle was included in the Law for Partial Revision of the Act on Welfare and Management of Animals (Law No. 68 of 2005), which was promulgated in June 2005. The content of the provision is provided in Section V, Paragraph 5 of this Guideline. Among the 3Rs, "refinement" is often interpreted as "reduction of pain/distress," and there seems to be an overly narrow understanding, even among researchers, that "refinement" refers to the use of analgesics. In fact, "refinement" means reducing pain and distress in animals through refinement of care and experimental

techniques. It should also be kept in mind that unthinkingly repeating experiments that cause pain/distress and stress in the same animal (i.e., "reuse") should be avoided as a means to achieve "reduction." Careful consideration must be given to whether reuse is indeed appropriate. Planned euthanasia of animals and humane endpoints at the end of the experiment must be defined based on a proper assessment of the pain/distress and stress experienced by animals.

Section II. Housing environment and management

1. Introduction

The development of an appropriate housing environment for laboratory macaques is not only essential from animal welfare perspectives—that is, ensuring the growth, health maintenance, and well-being of laboratory animals—but also contributes to the collection of reliable research data and even to the health and safety of researchers and animal technicians. Many factors must be considered in maintaining the housing environment and sufficient understanding is required regarding specific recommended values to be observed for each factor. To improve the housing environment, it is important to maintain appropriate management of laboratory animals in the housing facilities by continually inspecting the current situation and making improvements as needed. Japanese macaques are designated as "specified animals" and rhesus and cynomolgus macaques as "invasive alien species." Each institution that keeps laboratory macaques must follow the necessary procedures in accordance with the relevant laws and regulations.

2. Animal research facilities

(1) Separation of animal research facilities from other areas

To ensure the proper care and management of laboratory macaques and the health and comfort of human staff, staff areas such as offices, conference rooms, and laboratories must be separated from animal research facilities (animal housing and research facilities). This prevents contamination of staff areas by animals and animal-derived biological samples, and at the same time prevents the transmission of pathogenic microorganisms from human staff to macaques. In addition, this separation also prevents vocalizations and sounds made by macaques and noise produced by animal care activities from affecting staff areas, and conversely prevents noise from staff areas from affecting the animal housing and research facilities.

(2) Composition of animal research facilities

Animal research facilities at each institution basically consist of two types of facilities: animal housing facilities where laboratory animals are continually housed, and animal research facilities where animal experiments are carried out. These components may vary depending on the purpose and size of the institution and other factors. It is advisable to have multiple facilities with necessary functions so that activities associated with a high risk of infection or other hazards that may affect the hygiene and health of researchers and animal technicians can be performed separately from activities less likely to have such risks. It is advisable to clearly distinguish work facilities and to pay sufficient attention to the flow (traffic lines) of people, animals, and equipment and materials within and between these facilities. If this is not feasible, necessary risk mitigation measures should be taken, such as fumigation and disinfection of a room after completion of activities associated with a high risk of infection or other hazards in order to eliminate such risks.

Animal research facilities for macaques should meet the requirements set out in Appendix 1-1.

3. Animal rooms

Animal rooms must be large enough to allow individual animals to perform their natural daily activities without interfering with achieving the purpose of the experiment, and the rooms must be structured to maintain temperature, humidity, ventilation, airflow, lighting, and noise/odor levels that do not cause undue stress in animals.

The recommended environmental conditions for macaque rooms, including temperature, humidity, ventilation, airflow, lighting, and noise/odor levels, are provided in Appendix 1-2.

(1) Temperature and humidity

Temperature and humidity are the most important elements of the physical environment in animal rooms and affect the metabolism and behavior of macaques. Special attention should be paid to temperature and humidity when housing macaques that have just been introduced and have not been acclimatized to the new environment. If animals are kept indoors, the temperature control capacity of the air-conditioning equipment must be checked regularly. Proper dehumidification and humidification should be used, with attention to excessive humidity during the rainy season and dryness resulting from air heating during the winter. Attention should be paid to the cage environment, as the temperature and humidity in the macaque room (macro-environment: secondary enclosure for the animals) may not necessarily reflect the temperature and humidity in the cage (micro-environment: primary enclosure for the animals).

(2) Noise

Vocalizations and sounds made by macaques and noise produced by animal care activities are unavoidable. Sound control should be considered when designing animal research facilities. Consideration for neighborhood residents is also important. For activities that may generate noise, the intensity, frequency and duration, potential for vibration, and audible range of the noise should be evaluated, and hearing protection gear or other necessary equipment should be provided to the animal technicians who perform such activities as needed. Whenever possible, it is advisable that such activities be carried out in a place away from macaque rooms to minimize stress in the animals. If a noisy activity must be carried out in a macaque room, consideration should be given to temporarily moving the animals to another macaque room.

4. Cages

(1) Cage structure

Cages must be constructed with the behavioral characteristics of macaques in mind.

Cages should be designed and constructed in accordance with the recommendations listed in Appendix 1-3.

(2) Housing space

In addition to making individual living spaces as large as possible based on scientific rationale, consideration should be given to increasing the potential for spatial utilization by taking into account the behavioral characteristics of the species. The minimum space required per macaque for paired or group (multiple animals) housing is provided in Appendix 1-4. Note that this is only the minimum space requirement and it is advisable to provide a larger space. Because macaques are highly social animals, care must be taken to ensure that they can adequately communicate with other macaques through visual, auditory, and tactile means. To ensure the maintenance of social relationships, which are very important behavioral and psychological traits of the species, an environment should be provided where multiple macaques can be kept (see Section IV). It should also be noted that group housing has the risk of causing undue stress if incompatible individuals are housed together. Individual housing may a better option if no suitable combination of macaques can be found. Group housing should be implemented after carefully observing the compatibility of the macaques with one another. Even when macaques need to be kept individually for experiments or veterinary care, the individual housing period should be made as short as possible. In the meantime, individual macaques should be allowed to have visual, auditory, and tactile communication with other macaques. At institutions that do not have facilities to accommodate group housing cages, they should still try to create an environment to allow group housing by connecting cages or by other strategies.

5. Feeding and water supply

(1) Feeding

Laboratory macaques must be fed appropriate feeds that are free of chemical and microbiological contaminants, with attention to nutrition and animals' preferences. Proper feeding is essential for the normal growth/development of macaques and maintenance of health. The basal metabolic rate per kilogram of body weight in macaques is shown in Appendix 1-5. Macaques should be fed primarily commercially available solid diets. Macaques are unable to biosynthesize vitamin C and therefore must be fed a diet containing vitamin C or given vitamin C supplementation. Feeding small amounts of food in multiple feedings is advisable. Supplemental feeding is also advisable for enrichment purposes. Some animals may develop acute bloat, a condition characterized by gas accumulation in the gastrointestinal tract and subsequent breathing difficulty. Acute bloat is thought to be caused by changes in food type and environment, abrupt change in feeding time, or intake of large amounts of solid feed and water at a time. Animals that routinely consume large amounts of solid feed and water at a time or that tend to have abdominal bloating must be given food and water at fixed times of the day, divided into small amounts, with due consideration to environmental changes. If it is necessary to restrict the amount of feeding (calories) or water supply for experimental purposes, attention must be given to the recommendations set out in Section V, Paragraph 6, Items 9 and 10.

(2) Water supply

Proper water supply, as well as proper feeding, is essential for the normal growth/development of macaques and maintenance of health. Routine water analysis must be conducted to ensure that macaques have access to water that is free of chemical and microbiological contaminants. Regular water changes must be performed by flushing and checks for blockages in water supply nozzles if an automatic water supply system is used; checks for leaks must be performed if water bottles are used. Consideration should be given to preventing acute bloat by not supplying large amounts of water at a time, especially for macaques that are subject to water restrictions. If there is an unavoidable need to restrict the amount of water supply for experimental purposes, attention must be given to the recommendations set out in Section V, Paragraph 6.

6. Methods and procedures for the management of specified animals and invasive alien species

Japanese macaques are specified animals. According to Article 26 of the Act on Welfare and Management of Animals, specified animals are defined as animals that may harm human life, health, or property. An ordinance of the Ministry of the Environment specifies that these animals must be kept with the permission of the governor of the relevant prefecture. For the structure and size of housing facilities for specified animals and the methods of keeping specified animals, proper procedures must be followed based on a full understanding of the relevant laws and regulations listed in Appendix 1-6.

Macaques such as rhesus and cynomolgus macaques fall under the category of invasive alien species, which is defined by the Act on the Prevention of Adverse Ecological Impacts Caused by Designated Invasive Alien Species (Ministry of the Environment Law No. 78, 2004) as species that harm or may harm local ecosystems, human life/health, and the agriculture, forestry, and fisheries industries. The rearing of invasive alien species must be permitted by the Minister of the Environment through submission of an Application for Permission for Raising Invasive Alien Species. The standards for the structure and size of housing facilities for invasive alien species are basically the same as those for specified animals, although permission must be obtained from the Minister of the Environment for invasive alien species, whereas it can be obtained from the prefectural or municipal governor for specified animals. In addition, housing facilities for imported monkeys must be designated in accordance with the provisions in the monkey section of the table in Paragraph 1, Article 1 of the Ministerial Ordinance Specifying Import-prohibited Areas, etc. (Agriculture and Health Ministries Ordinance No. 2 (1999)) set forth in Article 54-1 of the Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases. To do this, the applicant must submit one copy each of the application set forth in the Act to the Minister of Health, Labour and Welfare and the Minister of Agriculture, Forestry and Fisheries.

Being designated as specified animals or invasive alien species, macaques must be kept by knowledgeable and skilled personnel with a thorough understanding of their biology.
It is advisable for each institution or facility to establish special rules and regulations based on this Guideline.

7. Individual identification and recording

Individual identification of specified animals and invasive alien species must be done by tattooing or microchip implantation. It is also advisable to use cage labels with individual IDs for work purposes. The manager and principal investigator must prepare, fill out, and maintain animal cards to record the basic information of each animal, as shown in Appendix 1-7.

Section III: Veterinary management

1. Introduction

Management of macaques based on veterinary knowledge is essential not only for the health management of the animals but also for the safety of researchers and animal technicians, and it is important for obtaining reliable experimental results. Veterinary management must be carried out either directly by a veterinarian or by a laboratory animal manager/researcher/animal technician working with a veterinarian in close collaboration with the manager and principal investigator. For this reason, it is advisable that each institution employ or appoint a full- or part-time veterinarian. If this is not feasible, a collaborative system with external veterinarians must be established. It is advisable for collaborating external veterinarians to have experience in handling macaques. Veterinary management includes the activities listed in Appendix 1-8. Upon noticing or suspecting any abnormality in the health or behavior of an animal, researchers or animal technicians must promptly address it in collaboration with a veterinarian

It may be necessary to determine a humane endpoint based on the health status or suffering of the animals. It is advised that principal investigators familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals, and describe the anticipated pain/distress in the animal experiment protocol and the humane endpoint (see Section V, Paragraph 8).

2. Introduction of macaques

Each institution must legally introduce laboratory macaques with known birth and management status that have been bred for use in experiments and research. Prior to introduction, the institution must obtain the animals' identification information and quarantine certificate from the supplier to confirm that the animals are in good condition and legally raised. For transportation of laboratory macaques, the transportation plans, including the means and duration of transportation, the type of container to be used and feeding and water supply during transportation, must be checked in advance to minimize transportation-related stress on the animals. Because Japanese macaques are designated as specified animals and rhesus and cynomolgus macaques as invasive alien species, necessary procedures must be undertaken with the relevant ministries and municipalities to obtain permission for transportation of these animals. Immediately after introduction, macaques may have health problems due to environmental changes or excessive stress. Frequent observation should be performed to closely monitor each animal's health status.

3. Quarantine and acclimatization

Introduction quarantine is important for preventing disease transmission to laboratory animals already kept at an institution and for preventing zoonotic diseases. Macaques to be introduced must be quarantined by the supplier or isolated from existing macaques and other laboratory animals after introduction to the institution. Because it is possible that for latent pathogens to become active due to transportation-related stress, the health status of introduced macaques should be monitored during

quarantine, microbiological testing should be performed if necessary, and the animals should be transferred to a regular macaque room after they are determined to be in good condition. It is advisable for there to be a period of acclimatization to allow the introduced macaques to adapt physiologically and behaviorally to the new housing environment. It is advisable for researchers and animal technicians to establish a relationship of trust with the macaques, because this leads to less variable experimental data and allows for more reliable animal experiments.

4. Disease monitoring and control

All macaques must be observed on a daily basis by the laboratory animal manager, researchers, animal technicians, or veterinarians who are trained to identify signs of disease, injury, and abnormal behaviors. A macaque with a disease or injury should be immediately treated in cooperation with a veterinarian. It is advisable that animals showing any abnormality without obvious disease or injury be brought to a veterinarian's attention. Regular weighing should be performed, as should visual and palpation examinations of restrained animals.

Prevention of infectious diseases is important not only for the health management of laboratory animals, but also for the safety of personnel. To prevent the transmission of infectious diseases within the population of captive macaques of the same species, between macaques of different species, between humans and macaques, and between macaques and other animal species, necessary infection control measures must be implemented, such as restriction of human access to macaque rooms and control of traffic lines, use of appropriate personal protective equipment (PPE), and sanitary control by disinfection of animal housing facilities.

Because macaques are closely related to humans, special attention must be paid to zoonotic diseases. Considering the safety of personnel as the top priority, biological materials such as feces, blood, and tissues of macaques must be handled appropriately under the assumption that they might contain pathogens. Macaques, in particular, must be thoroughly examined for the possibility of carrying the B virus and other viruses that can cause fatal infections. At the same time, the possibility of human to macaque disease transmission must also be noted. A person with any symptom(s) suggestive of infection, such as fever, cough, and rash, must be restricted from entering animal research facilities. Any animal suspected of having an infectious disease must immediately be brought to the attention of the manager and other personnel as well as a veterinarian, and must be isolated from researchers, animal technicians and healthy laboratory animals, or handled appropriately by taking equivalent action. Precautions for disease control in macaques are summarized in Appendix 1-9.

5. Surgical procedures and postoperative management

Surgical procedures must be performed aseptically using appropriate anesthetic/analgesic methods, considering the nature of the procedure (technique, invasiveness, and time required) and must be based on preoperative health check-up results and a postoperative management plan. In general, major survival surgery that involves invasion and exposure of body cavities or results in physical or physiological damage must be performed by a trained researcher or under the guidance of a veterinarian, using equipment designed for such procedures. Postoperative management consists of observation of the operated animal until it is completely recovered from anesthesia, administration of necessary medications, such as analgesics and antibiotics, and fluid replacement. It is advisable that an environment be provided where temperature, humidity, and oxygen concentration can be controlled. There must also be an adequate recovery period, during which careful monitoring and management must be performed. Researchers must take necessary measures to minimize pain experienced by macaques throughout the preoperative, intraoperative, and postoperative periods. Researchers need to be up to date on the latest information and must be willing to constantly incorporate better surgical techniques and anesthesia and analgesia techniques. Appropriate procedures performed by adequately trained personnel lead to "refinement," an element of the "3Rs."

6. Anesthesia and analgesia

Appropriate use of anesthetics, sedatives, and analgesics to reduce pain and distress in animals is necessary from animal welfare and scientific perspectives and is an obligation of those who perform animal experiments. Anesthesia must be performed by researchers or veterinarians with knowledge and skill in the use of the anesthetics and anesthetic methods to be used. Before anesthesia, the animal's health status must be checked, except in an emergency. Macaques are prone to vomiting and associated aspiration. Since even small amounts of vomiting can cause fatal aspiration, it is advisable that macaques be fasted or premedicated with antiemetics before anesthesia to prevent vomiting. During anesthesia, the animal's vital signs, such as respiratory/circulatory parameters and body temperature, must be carefully monitored. Because sudden hypothermia and respiratory/circulatory depression may occur depending on the depth of anesthesia, it is advisable to take precautionary measures, such as keeping the body warm during anesthesia and being prepared for tracheal intubation during use of deep anesthesia and emergency drugs.

The precautions for anesthesia in macaques are summarized in Appendix 1-10, and selected anesthetics and analgesics that can be used in macaques are listed in Appendices 1-11 and 1-12, respectively, along with their usual doses. Some of these anesthetics (e.g., ketamine), sedatives, and analgesics are narcotics or psychotropics and must be maintained properly in accordance with the Narcotics and Psychotropics Control Act (Law No. 14 of March 17, 1953, amended as Law No. 69 of June 14, 2006). For pain management in macaques, a pain management protocol should be developed based on the estimated degree of pain, which is determined based on the degree of surgical insult and other factors. With the protocol in place, if the macaque is expected to be in pain, then its condition and behavior must be carefully observed to assess the degree of pain and appropriate analgesic treatment must be given. As anxiety may increase pain in macaques, it is also important to provide a safe environment for them, such as bedding and hiding places, if pain is anticipated. Regarding the use of analgesics, "preemptive analgesia," in which an analgesic is administered before a painful stimulus is applied, and "multimodal analgesia," in which a combination of multiple analgesics with different mechanisms of action, should be used. Anesthesia/analgesia in aged, diseased, or disease-model animals requires further caution and it is advisable that anesthesia/analgesia be performed in consultation with a veterinarian. Examples of actual anesthesia procedures are provided in Appendix 1-13.

7. Management of disease-model animals and genetically modified animals

In medicine and related fields, many small animal models of diseases, mainly rodents, have been developed and used to determine causes, elucidate pathogenesis, and develop treatments for intractable diseases in humans by using pharmacological methods, physical disorders, and even gene engineering methods. However, the extrapolation of animal data to human disease, especially psychiatric and neurological diseases, requires the creation of nonhuman primate models of disease that have similar neurological structures and functions to those of humans, or animals in which relevant genes are modified, as the physiological and metabolic functions of rodents do not necessarily reflect those of humans. Disease-model animals and genetically modified animals with psychiatric and neurological disorders or central nervous system disorder are likely to exhibit abnormalities and symptoms similar to those of human diseases, which may result in various behavioral limitations. Based on adequate estimation of the impact of disease and other abnormalities on the daily life activities of the animal, adequate preparations must be made, including making appropriate modifications to holding cages, strengthening of the animal monitoring system, feeding and watering assistance, and the development of criteria for determining humane endpoints.

8. Euthanasia

Planned euthanasia of macaques after completion of an experiment and euthanasia based on humane endpoints must be done by an appropriate method. Euthanasia is a procedure that causes loss of consciousness and irreversible death as rapidly as possible with minimal pain and stress. The circumstances under which euthanasia should be considered are summarized in Appendix 1-14. Methods of euthanasia are described below.

An internationally accepted method for euthanasia is to administer an overdose of anesthetics. Any barbiturate derivative may be used for intravenous administration. Intraperitoneal injection is also acceptable when intravenous infusion is difficult to perform, but may require higher doses. Sample collection, if required, must be performed after confirmation of respiratory arrest, cardiac arrest, and pupillary dilatation after sufficient time has elapsed. Suggested doses of barbiturates are shown in Appendix 1-15. When perfusion is to be performed, the same anesthesia procedure as for surgical procedures should be performed, followed by exsanguination and perfusion.

Section IV: Well-being

1. Introduction

When using laboratory animals for experiments and research, the manager and other personnel must be responsible for the well-being of the animals. The facts that animals do not just automatically respond to the environment and that they are able to indicate their intention via behavior have gradually been accepted as scientific knowledge. Therefore, especially when keeping and handling macaques, a nonhuman primate species, every effort must be made to ensure that the animals are in good physical as well as mental condition.

2. Considerations for behavior and psychological condition

Animal welfare refers to the state of animals being able to "cope" with the surrounding environment. To evaluate animal welfare, it is essential to have appropriate physiological, psychological, and behavioral markers, in addition to evaluating the presence or absence of illness, injury, or pain. The housing environment must also be improved to obtain better quality experimental data. It is important that the animal's innate physiological, biological, and behavioral patterns are preserved for the betterment of its psychological condition. Especially for macaques, which are highly social animals, social relationships among animals and between animals and humans have a significant impact on their psychological state. Therefore, care must be taken to ensure that laboratory animals do not become socially isolated and that relationships among laboratory animals housed together or nearby and relationships between laboratory animals and humans are kept in a good state. Users must not only comply with the Act on Welfare and Management of Animals and other relevant laws, but also conduct themselves with animal welfare always in mind. The housing environment and procedures must be adjusted so that experimental macaques can maximally express their natural behavioral patterns and do not exhibit abnormal behaviors related to stress caused by the housing environment and research use. Measures must be taken to improve the housing environment if stereotyped behaviors (i.e., repeating the same behavior), abnormal behaviors (e.g., plucking, excessive grooming, excessive marking, excessive threats, self-mutilation), or excessive obesity or emaciation is observed.

The specific goals are summarized in Appendix 1-16. To achieve these goals, it is advisable to respond flexibly to the current situation and actively improve the housing environment. It is also advisable to make maximal efforts to improve the environment for each animal while taking into account the objectives and practical possibilities of research and animal care and management.

3. Environmental enrichment

To improve the well-being of laboratory animals, it is advisable to actively introduce "environmental enrichment" that adds various functions to the housing environment. Improvement of the environment facilitates the innate habits and behaviors of animals. In addition, it also contributes to reduced stress and abnormal behaviors and increased opportunities for exercise and helps with the development and maintenance of various physical and social functions. It is advisable that modifications be made so that animals can control the environment according to their own independent choices. Where experimental or environmental constraints prevent satisfactory enrichment of all aspects of the environment, it is advisable to make the greatest possible effort to do so to the extent feasible. For example, if there is a

limited social environment, it is advisable to make modifications to enrich the eating and physical environments. However, introduction of various structures without sufficient planning may result in an inadequate effect or even increased injuries or conditions related to physiological stress, such as metabolic disorder. Thus, for effective environmental enrichment, enrichment should be applied to various aspects of environment concurrently while giving consideration to animal care and management. Specific strategies for environmental enrichment are provided below.

Introduction of novelty, variability, choice, and controllability

The housing environment for laboratory animals is generally monotonous, which is one of the major causes of various problems. It is thus advisable to introduce novelty and variability, as well as a system that allows laboratory animals to make choices and exercise control independently. For physical environmental enrichment, it is also effective to increase the potential for choices and control of the environment not only by introducing play equipment, but also by exchanging it with new equipment on a regular basis or introducing various types of play equipment that can be manipulated in various ways. Another effective strategy is to introduce partitions that allow animals to hide from people and other animals.

(1) Social environment

Because macaques are highly social animals, physical contact actions, such as grooming, and communicative actions via visual, auditory, and olfactory senses are important components of their daily life. They should be allowed to live in a social environment suited to these characteristics as much as possible. To form an effective group, it is important to facilitate the establishment of appropriate relationships among animals, while keeping in mind their social structure and the characteristics of their social behavior and considering intimacy and social hierarchy among them. It should be ensured that the established group is socially stable and that appropriate social interactions can be formed and maintained among the animals. When it is difficult to house multiple animals together, modifications can still be made such as connecting parts of the cages to allow physical contact and visual, auditory, and olfactory communication.

(2) Improvement of relationship with researchers and animal technicians

To reduce stress experienced by animals and increase the safety of researchers and animal technicians, it is advisable to establish a good relationship between these personnel and laboratory animals through routine activities. The establishment of such a relationship will help the personnel notice changes in the behavior of each animal and may even make animals cooperative to stressful procedures required for the purpose of research, such as restraint and blood collection. Moreover, if an adequate social environment with other animals cannot be provided for research reasons, good relationships with humans will compensate for the lack of social interactions to some extent.

To maximize the effects of various forms of environmental enrichment, attention must be paid to characteristics that differ among individual animals, such as sex, age, and origin. It is the responsibility of researchers and animal technicians to closely observe animals as part of routine animal care. In addition to implementing environmental enrichment, animals' behavior should be observed and recorded to the extent possible, and the effect of the enrichment should be evaluated. It is advisable that further environmental improvement be sought based on the evaluation results. It is also advisable that efforts be made on a routine basis to improve the environment even for animals that do not exhibit marked abnormal behavior. It is advisable to include the preparation of a good housing environment in the animal experiment protocol from the outset, based on the recognition that the research use of animals and animal welfare are not in conflict with each other, but rather go hand-in-hand.

Section V. Planning and conduct of experiments

1. Introduction

When using animals for research/education purposes, sufficient consideration must always be given not only to the proper care and management but also to the humane handling of animals. Therefore, appropriate animal handling and the development of a proper animal experiment protocol must be strived for based on a full understanding of the contents of the relevant laws and regulations and in accordance with the "3Rs" principle for animal experiments. The "3Rs" principle is an international norm for animal experiments and must always be considered. Consideration of "replacement," "reduction," and "refinement" is a prerequisite for all animal experiment protocols. Each institution must establish internal regulations based on laws and guidelines and must establish systems for animal facility management and protocol review by the institutional animal care and use committee.

2. Laws and principles

The laws/regulations, standards, and guidelines that must be followed in order to ensure proper animal care and experiments in Japan are listed in Appendix 1-17. An external review system should be implemented to ensure compliance with the related laws/regulations, standards, and guidelines of the ministries and agencies supervising research institutions.

The validity of an animal experiment is judged by a harm-benefit analysis of pain/distress experienced by animals and the significance (outcome) of the experiment. For this reason, there must be criteria for assessing the degree of pain/distress experienced by animals. Depending on the nature of the research being conducted, the degree of pain/distress experienced by animals is grouped into 5 categories as listed in Appendix 1-18. This pain classification is based on a classification table created by the Scientists Center for Animal Welfare (SCAW), a group of scientists in North America. Each institution may adapt this classification to macaques, taking into account the latest scientific trends and differences in the environment at each institution. When preparing an animal experiment protocol, the principal investigator must fully understand which pain category the protocol falls into. For protocols classified as Category E, the level of pain/distress and expected outcomes must be fully considered. On the other hand, protocols that are classified as Category A, but involve cell transplantation to other animal species or other equivalent procedures, must be reviewed for appropriateness, including ethical aspects. Protocols that only use cells must also be implemented with a full understanding of risks associated with the use of the cells. The use of hazardous substances or new chemicals, if any, must also be specified in the protocol.

3. Health and safety management of personnel involved in animal care and experiments

The conduct of experiments using macaques requires different knowledge and techniques depending on the degrees of contact with the animals and the invasiveness of experimental procedures. Personnel conducting these experiments must acquire the necessary knowledge and techniques in advance. Moreover, because many diseases in macaques are common to humans, having sufficient knowledge of these diseases is important in the dual sense of protecting against transmission of infections from researchers/animal technicians to macaques and from macaques to humans. Knowledge is also necessary of pathogenic microorganisms that require special attention in macaques, such as B virus and simian retrovirus, as well as those specified by the Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases and those for which notification is mandatory. Personnel expected to be involved in research activities using hazardous substances or equipment that are biological (e.g., pathogens), chemical (e.g., toxic chemicals), or physical (e.g., electromagnetic wave, radioactive materials) hazards must be trained in the handling of hazardous substances and equipment and, if applicable, obtain necessary qualifications.

Necessary health care must be provided to the personnel involved in animal care and experiments to

prevent them from contracting disease. Diseases associated with the use of laboratory animals include zoonotic diseases, animal allergies, and bites and scratches, as well as injuries and diseases caused by animal care and management activities and experimental procedures. The director and manager of the institution must identify risk factors that can affect occupational health and safety and ensure that all relevant personnel undergo necessary health check-ups in accordance with the Industrial Safety and Health Act. For disease prevention, the wearing of personal protective equipment (PPE) should be mandated, such as special clothing, masks, gloves, and various other types of protective gear, depending on the nature of the activities. In addition, the director and manager of the institution must ensure that necessary measures are in place in case of the occurrence of injuries or diseases in the relevant personnel, such as keeping emergency medical supplies on hand, maintaining manuals for first aid and emergency communication, and establishing a system for contacting medical institutions available in an emergency.

4. Planning for emergency response

Each institution must prepare an emergency response manual and emergency contact network in advance and make them known to the relevant personnel in case of natural disasters, fires, long-term power outages, and other emergencies. In an emergency, it is important to first ensure the safety of the personnel of the institution, keeping in mind that macaques are designated as either a specified animal or an invasive alien species, before seeking to complete the research project, ensure animal welfare, and preserve the surrounding environment. It is advisable to prepare an emergency response manual taking into account the points listed in Appendix 1-19. It is also advisable for each institution to refer to the "Guidelines for Preparing an Emergency Response Manual" set forth by the Japanese Association of Laboratory Animal Facilities of National University Corporations when preparing an emergency response manual. It is advisable that the emergency contact network be posted in corridors in front of laboratories and animal rooms to facilitate personnel's preparedness for an emergency response. Each institution should have a stockpile of feed/water and emergency power supply in case of a disaster.

5. Protocol review and the institutional animal care and use committee

Animal experiments must be scientifically, ethically, and humanely justified in terms of their purpose and content, and may be carried out only if the experiments are documented in an animal experiment protocol that has been reviewed by the institutional animal care and use committee and approved by the director of the institution. When conducting an animal experiment, the anticipated contribution of the research objectives to science and medicine must be fully considered, and the animal experiment protocol must be designed and reviewed in accordance with the "3Rs" principle of animal experiments, as described below.

(1) Replacement (using alternative methods)

Living macaques may be used in an experiment only when there is scientific justification for their use. When planning an experiment, other experimental options not involving animals or experiments using other species must first be considered; then, whether or not the purpose of the research can be achieved only by using macaques must be fully considered. Before conducting an experiment using living macaques, it is advisable to conduct a preliminary study using organs or cells derived from macaques whenever possible for the purpose of the experiment.

(2) Reduction (reducing the number of animals used)

The protocol should be designed to minimize the number of macaques to be used while improving the type, quantity, and quality of data, insofar as doing so does not compromise the objectives of the study. When planning an experiment, the protocol should be designed to allow for hypothesis testing with a small number of animals, with measures to minimize predictable individual differences in experimental results, such as analyzing changes over time before and after an experiment for each animal and conducting multiple evaluations for each animal, because macaques are not a genetically homogeneous population, in contrast to genetically defined mice and rats. For minimally invasive experiments that involve only medication, blood sampling and/or behavioral analysis, or less painful experiments such as

non-invasive bioimaging, it is advisable to use the same animal for another experiment after a certain rest period, in order to promote “reduction.” After an animal has been euthanized following completion of an experiment, it is advisable that organs and tissues not used in the experiment be used for other studies as much as possible without compromising the purposes of the studies. At the same time, repeating major survival surgeries (e.g., thoracotomy, craniotomy, laparotomy) on the same animal for the purpose of reducing the number of animals used is not recommended.

(3) Refinement (reducing pain/distress through refinement of experimental techniques)

Researchers conducting an animal experiment must make every effort to minimize the animal suffering such as pain, physical injury, anxiety, and fear. To this end, they must always strive to improve and refine their animal care and experimental techniques. The following fundamental principle must be followed: Procedures that are distressing for humans should also be considered distressing for monkeys, unless there is evidence to the contrary. Based on prior prediction of the physical and mental effects of the experiment on the animals, appropriate procedures and equipment for macaques, as well as appropriate anesthesia/analgesia and euthanasia procedures, must be planned. Researchers should be aware that poor techniques may result in increased pain/distress in animals, and therefore they must always strive to improve their technical skills. It is advisable that principal investigators familiarize themselves in advance with experimental procedures and the anticipated pain/distress in animals, and describe it in the animal experiment protocol when setting humane endpoints (see Chapter V, Section 8). Experimental designs involving multiple major, life-threatening surgeries on a single animal should be avoided unless the series of surgeries consists of a single experimental design and is judged to be essential based on scientific rationale or clinical (veterinary) necessity, or is deemed appropriate after due consideration of the degree of pain/distress in the animals and the significance (expected outcome) of the animal experiment.

Under the Act on Access to Information Held by Administrative Organs promulgated in 1999, animal experiment protocols and other related documents may be disclosed to outside parties as required, except for information whose disclosure may affect personal rights and benefits. Approval by the director of the institution assures outside parties that the relevant protocol has been reviewed and implemented in accordance with the Guideline described in this document. Principal investigators are thus required to prepare animal experiment protocols with due consideration of the purpose and significance of the experiments.

6. Conduct of experiments involving restrictions (including feeding and water restriction)

Restrictions on the intake of water, food, or selected nutrients, etc. are likely to adversely affect the development and health status of macaques and thus it is advisable that they be avoided as much as possible. For animals that exhibit human-like emotional responses, such as macaques, it is also advisable not to impose social restrictions, such as separating a developing juvenile animal from its mother, or other types of restriction, such as blockage or modification of sensory organs, because of their negative impact on the animal's development and health status. If these restrictions must be applied for the purpose of the experiment and there is no alternative experimental method available, the animal experiment protocol must include a detailed explanation of the need for such restrictions and their appropriateness must be fully discussed by the animal care and use committee. Even if approved, the frequency and duration of such restrictions must be set to a minimum, in conjunction with close monitoring of the development and health status of each animal through frequent observation and body weight measurement. Due to substantial differences in feed/water intake between animals, the baseline body weight and average feed/water intake of each animal should be measured to guide the adjustment of restriction levels and experimental schedules. Notes on feeding/watering restrictions are summarized in Appendix 1-20. Body weight and feed/water intake must be measured and recorded frequently during restrictions. Special care should be taken when using immature or aged animals in experiments involving restrictions.

Effects of feeding restriction on the health of animals, unlike those of water restriction, do not necessarily become apparent immediately. Because malnutrition may significantly affect the

development and health of animals, appropriate recovery periods must be scheduled depending on the duration and intensity of restriction. Feeding restriction must be carried out such that energy intake does not fall below the basal metabolic rate, which is determined as a function of species, developmental stage, and body weight (see Appendix 1-5), with the fasting period set as short as possible.

The health status of macaques during an experiment involving restrictions can be evaluated by, but not limited to, increases/decreases in body weight and feed/water intake, amount and condition of feces, skin and fur quality, and abnormal behavior. Changes in these parameters may be overlooked unless observed closely by an experienced observer. It is advisable that animals showing any abnormality be brought to a veterinarian's attention. If necessary, the experiment must be interrupted and effort must be made to restore the animal's health. The need for euthanasia as a humane endpoint must also be considered depending on the situation. Commonly used humane endpoints are provided in Appendix 1-14.

7. Education and training

The director and manager of the institution must provide the laboratory animal manager, animal technicians, and researchers with education and training necessary for proper animal care and experiments before they engage in any activity and on a regular basis thereafter. Only those who have acquired knowledge and skills in the biology and handling of macaques and zoonotic diseases should conduct experiments on macaques. In addition, invasive procedures on macaques should be performed by only those who have been adequately trained and mastered the necessary techniques under the supervision of a mentor. Furthermore, it is advisable that the personnel engaged in animal care and handling participate in internal and external training sessions, try to collect relevant information to acquire necessary knowledge and skills, and accumulate professional experience through their daily work.

8. Health management of animals during experiments and endpoints

Researchers and animal technicians must assume the primary responsibility for the health management of animals during experiments through careful observation of each animal, keeping in mind the experimental procedure performed. If any ill health or behavioral abnormality is observed in an animal, appropriate treatment should be given immediately to the animal in cooperation with a veterinarian. Animals used in experiments should also be provided the same appropriate veterinary management for disease prevention and treatment as other animals. It is advisable to provide animals with a suitable environment to promote their innate habits and behaviors and thereby improve their physical and mental health (well-being).

If an irreversible deterioration of health or unavoidable pain/distress occurs in an animal used in an experiment, the principal investigator must determine a humane endpoint in conjunction with a veterinarian. Principal investigators must familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals and describe the anticipated pain/distress in the animal experiment protocol. To enable objective determination of humane endpoints, the principal investigator must specify health parameters to be checked and assessments in the protocol and have them reviewed by the institutional animal care and use committee. It is advisable that researchers and animal technicians objectively assess the condition of each animal by observation, and a point when the animal's condition has deteriorated beyond a certain level should be defined as the humane endpoint (see Appendix 1-14).

9. Conduct of experiments using hazardous substances

Although not limited to experiments using macaques, when an animal experiment is to be conducted using hazardous substances that are biological (e.g., pathogens), chemical (e.g., deleterious/toxic substances, narcotics/psychotropic drugs, methamphetamine), or physical (e.g., electromagnetic waves, radiation) hazards, including the use of X-ray, PET, CT, MRI, and other imaging modalities, various types of equipment and systems must be in place to protect researchers, animal technicians, and laboratory animals from exposure to these substances and prevent environmental pollution. Dedicated

equipment must be used for experiments involving hazardous substances. These types of equipment must be installed in an area away from animal housing facilities and laboratories and appropriately indicated as a hazardous area. Personnel who use such equipment must obtain licenses required for the handling of the relevant hazardous substances and must be familiar with risk management procedures in case of accidents and other procedures. For the care of laboratory animals and storage, use, and management of animal waste, carcasses, and hazardous substances, clearly defined, safe operating procedures must be in place and sufficient education and training programs must be provided to protect personnel from hazards. All personnel must be well familiar with the properties of the hazardous substances to be handled and with required protective measures. Possibly contaminated equipment and waste must be disposed of appropriately at each institution to prevent the leakage of hazardous substances out of the hazardous area. Researchers who wish to conduct an experiment using biohazardous substances, such as microbes, or chemical hazardous substances must submit an animal experiment protocol to the relevant committees for approval. When an infection experiment is to be conducted, institutional codes must be established in accordance with the Safety Management Codes for Handling of Pathogens etc. of the National Institute of Infectious Diseases, and the experiment must be conducted in compliance with the relevant laws and regulations.

10. Conduct of animal experiments using genetically modified organisms

Although this is again not limited to experiments using macaques, the creation or transfer of genetically modified organisms and experiments using these organisms, such as genetically modified viruses, must be carried out properly in accordance with the “Ministerial Ordinance Providing Containment Measures to Be Taken in Type 2 Use of Living Modified Organisms for Research and Development,” which was issued based on the “Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms” (the Cartagena Protocol). When conducting an animal experiment using genetically modified organisms, the principal investigator must submit an application to the relevant authority or an application for the relevant minister’s permission, in accordance with the law, and obtain permission before starting the experiment. Animal experiments using genetically modified organisms must be conducted within a permitted research area.

Chapter II. Guideline for Experiments Using Marmosets

Section I. Basic policies

1. Introduction

The purpose of the "Guideline for Experiments Using Marmosets" is to assist researchers and research institutions engaged in neuroscience and behavioral research in conducting experiments using marmosets in a scientifically, humanely, and ethically appropriate manner. Marmosets must be handled properly based on the knowledge and skills necessary to keep them in good health. All those who keep, manage, and breed marmosets must be responsible for their behavioral, physical, and mental health. This Guideline has been developed to dispel the public's concerns while meeting their expectations and to ensure that animal experiments conducted in an appropriate manner will produce scientifically correct results. The instructions and recommendations in the Guideline are based on scientific evidence, expert opinions, and practical experience and should be referenced by research institutions for the management and care of marmosets and the development of protocols when conducting neuroscience/behavioral research in Japan.

2. Scope and goal of the Guideline

This Guideline is applicable to neuroscience and behavioral research activities using marmosets. Researchers in other areas should also refer to this Guideline when performing similar procedures with marmosets.

The goal of this Guideline is to encourage the humane care and use of marmosets for research. The Guideline also aims to raise awareness of the profound link between the evidence-based care and management of laboratory animals and the quality of research, and to provide researchers as well as animal experiment committees, veterinarians, and other persons concerned with knowledge and techniques regarding medical and laboratory animal science based on sufficient experience. Of course, the information will be updated as scientific knowledge progresses. The content of this Guideline should also be updated based on the latest scientific evidence.

Each institution can refer to the instructions and recommendations set out in the Guideline while complying with institutional regulations on animal experiments established by each institution, and can use the Guideline for developing plans for the care and use of marmosets while adding custom modifications. We also hope that this Guideline will contribute to the development of guidelines for the use of marmosets in various other areas of research.

3. Evidence-based care and use of laboratory marmosets

The care and use of laboratory marmosets must be based on the concept of evidence-based well-being. This Guideline is also based on a diverse body of literature and specific values are included in the appendices. In some cases, however, sufficient data are not available for marmosets. In such cases, data for related species or other laboratory animals are provided. Truly appropriate conditions for marmosets need to be further explored, and the content of this Guideline must be updated based on such data.

4. "3Rs" principle

The "3Rs" principle was first proposed by W. M. S. Russel and R. L. Burch in 1959 as a set of strategies that researchers should consider when planning animal experiments, consisting of "replacement" (using alternative methods), "reduction" (reducing the number of animals used), and "refinement" (reducing pain/distress through refinement of techniques), and may be simply referred to as the "3Rs." In Japan, a provision on this principle was included in the Law for Partial Revision of the Act on Welfare and Management of Animals (Law No. 68 of 2005), which was promulgated in June 2005. The content of the provision is provided in Section V, Paragraph 5 of this Guideline. Among the 3Rs, "refinement" is often interpreted as "reduction of pain/distress," and there seems to be an overly narrow understanding, even among researchers, that "refinement" refers to the use of analgesics. In fact, "refinement" means

reducing pain and distress in animals through refinement of care and experimental techniques. It should also be kept in mind that unthinkingly repeating experiments that cause pain/distress and stress in the same animal (i.e., "reuse") should be avoided as a means to achieve "reduction." Careful consideration must be given to whether reuse is indeed appropriate. Planned euthanasia of animals and humane endpoints at the end of the experiment must be defined based on a proper assessment of the pain/distress and stress experienced by animals.

Section II. Housing environment and management

1. Introduction

The development of an appropriate housing environment for laboratory macaques is not only essential from animal welfare perspectives—that is, ensuring the growth, health maintenance, and well-being of laboratory animals—but also contributes to the collection of reliable research data and even to the health and safety of researchers and animal technicians. Many factors must be considered in maintaining the housing environment and sufficient understanding is required regarding specific recommended values to be observed for each factor. To improve the housing environment, it is important to maintain appropriate management of laboratory animals in the housing facilities by continually inspecting the current situation and making improvements as needed. Each institution that keeps laboratory marmosets must follow the necessary procedures in accordance with the relevant laws and regulations.

2. Animal research facilities

(1) Separation of animal research facilities from other areas

To ensure the proper care and management of laboratory marmosets and the health and comfort of human staff, staff areas such as offices, conference rooms, and laboratories must be separated from animal research facilities (animal housing and research facilities). This prevents contamination of staff areas by animals and animal-derived biological samples, and at the same time prevents the transmission of pathogenic microorganisms from human staff to marmosets. In addition, this separation also prevents vocalizations and sounds made by marmosets and noise produced by animal care activities from affecting staff areas, and conversely prevents noise from staff areas from affecting the animal housing and research facilities.

(2) Composition of animal research facilities

Animal research facilities at each institution basically consist of two types of facilities: animal housing facilities where laboratory animals are continually housed, and animal research facilities where animal experiments are carried out. These components may vary depending on the purpose and size of the institution and other factors. It is advisable to have multiple facilities with necessary functions so that activities associated with a high risk of infection or other hazards that may affect the hygiene and health of researchers and animal technicians can be performed separately from activities less likely to have such risks. It is advisable to clearly distinguish work facilities and to pay sufficient attention to the flow (traffic lines) of people, animals, and equipment and materials within and between these facilities. If this is not feasible, necessary risk mitigation measures should be taken, such as fumigation and disinfection of a room after completion of activities associated with a high risk of infection or other hazards in order to eliminate such risks.

Animal research facilities for marmosets should meet the requirements set out in Appendix 2-1.

3. Animal rooms

Animal rooms must be large enough to allow individual animals to perform their natural daily activities without interfering with achieving the purpose of the experiment, and the rooms must be structured to maintain temperature, humidity, ventilation, airflow, lighting, and noise/odor levels that do not cause undue stress in animals.

The recommended environmental conditions for marmoset rooms, including temperature, humidity, ventilation, airflow, lighting, and noise/odor levels, are provided in Appendix 2-2.

(1) Temperature and humidity

Temperature and humidity are the most important elements of the physical environment in animal rooms and affect the metabolism and behavior of marmosets. Special attention should be paid to temperature and humidity when housing marmosets that have just been introduced and have not been acclimatized to the new environment. If animals are kept indoors, the temperature control capacity of the air-conditioning equipment must be checked regularly. Proper dehumidification and humidification should be used, with attention to excessive humidity during the rainy season and dryness resulting from air heating during the winter. Attention should be paid to the cage environment, as the temperature and humidity in the marmoset room (macro-environment: secondary enclosure for the animals) may not necessarily reflect the temperature and humidity in the cage (micro-environment: primary enclosure for the animals).

(2) Noise

Vocalizations and sounds made by marmosets and noise produced by animal care activities are unavoidable. Because marmosets are particularly sensitive to sound, special consideration must be given to noise control. Sound control should be considered when designing animal research facilities. Consideration for neighborhood residents is also important. For activities that may generate noise, the intensity, frequency and duration, potential for vibration, and audible range of the noise should be evaluated, and hearing protection gear or other necessary equipment should be provided to the animal technicians who perform such activities as needed. Whenever possible, it is advisable that such activities be carried out in a place away from marmoset rooms to minimize stress in the animals. If a noisy activity must be carried out in a marmoset room, consideration should be given to temporarily moving the animals to another marmoset room.

4. Cages

(1) Cage structure

Cages must be constructed with the behavioral characteristics of marmosets in mind.

Cages should be designed and constructed in accordance with the recommendations listed in Appendix 2-3.

(2) Housing space

In addition to making individual living spaces as large as possible based on scientific rationale, consideration should be given to increasing the potential for spatial utilization by taking into account the behavioral characteristics of the species. The minimum space required per marmoset for paired or group (multiple animals) housing is provided in Appendix 2-4. Note that this is only the minimum space requirement and it is advisable to provide a larger space. Since the ideal environment for marmosets allows for vertical movement, it is advisable that the housing space should have a sufficient height and be equipped with three-dimensional structures and play equipment. When installing these equipment and structures, sufficient consideration should also be given to cage position in each room (e.g., face-to-face positioning and the distance between each cage and the floor or ceiling of the room), depending on the characteristics of each housing environment. Because marmosets are highly social animals, care must be taken to ensure that they can adequately communicate with other marmosets through visual, auditory, and tactile means. To ensure the maintenance of social relationships, which are very important behavioral and psychological traits of the species, an environment where multiple marmosets can be kept should be provided (see Section IV). It should also be noted that group housing has the risk of causing undue stress if incompatible individuals are housed together. Individual housing may a better option if no suitable combination of marmosets can be found. Thus, group housing should be implemented after carefully observing the compatibility of the marmosets with one another. Even when marmosets need to be kept individually for experiments or veterinary care, the individual housing period should be made as short as possible. In the meantime, individual marmosets should be allowed to have visual, auditory, and tactile

communication with other marmosets. At institutions that do not have facilities to accommodate group housing cages, they should still try to create an environment to allow group housing by connecting cages or by other strategies.

5. Feeding and water supply

(1) Feeding

Laboratory marmosets must be fed appropriate feeds that are free of chemical and microbiological contaminants, with attention to nutrition and animals' preferences. Proper feeding is essential for the normal growth/development of marmosets and maintenance of health. The basal metabolic rate per kilogram of body weight in marmosets is shown in Appendix 2-5. Marmosets should be fed primarily commercially available solid diets. Because wild marmosets feed on resins (gum), fruits, insects, and bird eggs, it is advisable that natural and processed foods that satisfy their natural feeding behavior and preferences, such as gum arabic, fruits, mealworms, and boiled eggs, be provided as supplementary diet. The nutritional characteristics of captive marmosets include higher protein and vitamin D requirements compared with other species and inability to biosynthesize vitamin C, which must be provided through dietary supplements. It is advisable that animals be fed small amounts of food in multiple feedings, with due consideration to environmental changes. It should also be kept in mind that the nutritional requirements of marmosets have not been fully characterized and that current formulated diets do not always fully meet the nutritional requirements and promote the health of the animals. Because marmosets have a strong preference for sugary foods, care must be taken to manage their nutrition by ensuring that the animals do not consume only what they prefer. If it is necessary to restrict the amount of feeding (calories) or water supply for experimental purposes, attention must be given to the recommendations set out in Section V, Paragraph 6, Items 9 and 10.

(2) Water supply

Proper water supply, as well as proper feeding, is essential for the normal growth/development of marmosets and maintenance of health. Routine water analysis must be conducted to ensure that marmosets have access to water that is free of chemical and microbiological contaminants. Regular water changes by flushing and checks for blockages in water supply nozzles must be conducted if an automatic water supply system is used; checks for leaks must be conducted if water bottles are used. If there is an unavoidable need to restrict the amount of water supply for experimental purposes, attention must be given to the recommendations set out in Section V, Paragraph 6.

6. Care and breeding

Marmosets must be kept by knowledgeable and skilled personnel with a thorough understanding of their biology. Notes on the care and breeding of marmosets are provided in Appendix 2-6. It is advisable for each institution or facility to establish their own rules and regulations based on these notes.

7. Individual identification and recording

It is advisable that individual identification of marmosets be done by tattooing or microchip implantation. It is also advisable to use cage labels with individual IDs for work purposes. The manager and principal investigator must prepare, fill out, and maintain animal cards to record the basic information of each animal, as shown in Appendix 2-7.

Section III. Veterinary management

1. Introduction

Management of marmosets based on veterinary knowledge is essential not only for the health management of the animals but also for the safety of researchers and animal technicians, and it is

important for obtaining reliable experimental results. Veterinary management must be carried out either directly by a veterinarian or by a laboratory animal manager/researcher/animal technician working with a veterinarian in close collaboration with the manager and principal investigator. For this reason, it is advisable that each institution employ or appoint a full- or part-time veterinarian. If this is not feasible, a collaborative system with external veterinarians must be established. It is advisable for collaborating external veterinarians to have experience in handling marmosets. Veterinary management includes the activities listed in Appendix 2-8. Upon noticing or suspecting any abnormality in the health or behavior of an animal, researchers or animal technicians must promptly address it in collaboration with a veterinarian.

It may be necessary to determine a humane endpoint based on the health status or suffering of the animals. It is advisable that principal investigators familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals, and describe the anticipated pain/distress in the animal experiment protocol and the humane endpoint (see Section V, Paragraph 8).

2. Introduction of marmosets

Each institution must legally introduce laboratory marmosets with known birth and management status that have been bred for use in experiments and research. Prior to introduction, the institution must obtain the animals' identification information and quarantine certificate from the supplier to confirm that the animals are in good condition and legally raised. For transportation of laboratory marmosets, the transportation plans, including the means and duration of transportation, the type of container to be used and feeding and water supply during transportation, must be checked in advance to minimize transportation-related stress on the animals. Laboratory animals immediately after introduction may have health problems due to environmental changes or excessive stress. Therefore, frequent observation should be performed to closely monitor each animal's health status. Since marmosets are particularly susceptible to diarrhea, it is advisable to closely monitor for and take measures against diarrhea. Efforts must be made to closely monitor the condition of animals, especially immediately after their introduction, bearing in mind that due to their small size, sudden weight loss or deterioration of health can lead to death.

3. Quarantine and acclimatization

Introduction quarantine is important for preventing disease transmission to laboratory animals already kept at an institution and for preventing zoonotic diseases. Marmosets to be introduced must be quarantined by the supplier or isolated from existing marmosets and other laboratory animals after introduction to the institution. Because it is possible that latent pathogens become active due to transportation-related stress, the health status of introduced marmosets should be monitored during quarantine, microbiological testing should be performed if necessary, and the animals should be transferred to a regular marmoset room after they are determined to be in good condition. It is advisable for there to be a period of acclimatization to allow the introduced marmosets to adapt physiologically and behaviorally to the new housing environment. Immediately after introduction, marmosets may exhibit behavioral abnormalities, such as frequent movement and aggression toward humans, as well as abnormalities in their general condition, such as decreased appetite and diarrhea. It is advisable for researchers and animal technicians to establish a relationship of trust with the marmosets, because this leads to less variable experimental data and allows for more reliable animal experiments.

4. Disease monitoring and control

All marmosets must be observed on a daily basis by the laboratory animal manager, researchers, animal technicians, or veterinarians who are trained to identify signs of disease, injury, and abnormal behaviors. A marmoset with a disease or injury should be immediately treated in cooperation with a veterinarian. It is advisable that animals showing any abnormality without obvious disease or injury be brought to a veterinarian's attention. Regular weighing should be performed, as should visual and palpation examinations of restrained animals.

Prevention of infectious diseases is important not only for the health management of laboratory

animals, but also for the safety of personnel. To prevent the transmission of infectious diseases within the population of captive marmosets of the same species, between marmosets of different species, between humans and marmosets, and between marmosets and other animal species, necessary infection control measures must be implemented, such as restriction of human access to marmoset rooms and control of traffic lines, use of appropriate personal protective equipment (PPE), and sanitary control by disinfection of animal housing facilities.

Because marmosets are closely related to humans, special attention must be paid to zoonotic diseases. Considering the safety of personnel as the top priority, biological materials such as feces, blood, and tissues of marmosets must be handled appropriately under the assumption that they might contain pathogens. At the same time, the possibility of human to marmoset disease transmission must also be noted. A person with any symptom(s) suggestive of infection, such as fever, cough, and rash, must be restricted from entering animal research facilities. Any animal suspected of having an infectious disease must immediately be brought to the attention of the manager and other personnel as well as a veterinarian, and must be isolated from researchers, animal technicians and healthy laboratory animals, or handled appropriately by taking equivalent action. Precautions for disease control in marmosets are summarized in Appendix 2-9.

5. Surgical procedures and postoperative management

Surgical procedures must be performed aseptically using appropriate anesthetic/analgesic methods, considering the nature of the procedure (technique, invasiveness, and time required) and must be based on preoperative health check-up results and a postoperative management plan. In general, major survival surgery that involves invasion and exposure of body cavities or results in physical or physiological damage must be performed by a trained researcher or under the guidance of a veterinarian, using equipment designed for such procedures. Postoperative management consists of observation of the operated animal until it is completely recovered from anesthesia, administration of necessary medications, such as analgesics and antibiotics, and fluid replacement. It is advisable that an environment be provided where temperature, humidity, and oxygen concentration can be controlled. There must also be an adequate recovery period, during which careful monitoring and management must be performed. Researchers must take necessary measures to minimize pain experienced by marmosets throughout the preoperative, intraoperative, and postoperative periods. Researchers need to be up to date on the latest information and must be willing to constantly incorporate better surgical techniques and anesthesia and analgesia techniques. Appropriate procedures performed by adequately trained personnel lead to “refinement,” an element of the “3Rs.”

6. Anesthesia and analgesia

Appropriate use of anesthetics, sedatives, and analgesics to reduce pain and distress in animals is necessary from animal welfare and scientific perspectives and is an obligation of those who perform animal experiments. Anesthesia must be performed by researchers or veterinarians with knowledge and skill in the use of the anesthetics and anesthetic methods to be used. Before anesthesia, the animal's health status must be checked, except in an emergency. Marmosets are prone to vomiting and associated aspiration. Since even small amounts of vomiting can cause fatal aspiration, it is advisable that marmosets be fasted or premedicated with antiemetics before anesthesia to prevent vomiting. During anesthesia, the animal’s vital signs, such as respiratory/circulatory parameters and body temperature, must be carefully monitored. Because sudden hypothermia and respiratory/circulatory depression may occur depending on the depth of anesthesia, it is advisable to take precautionary measures, such as keeping the body warm during anesthesia and being prepared for tracheal intubation during use of deep anesthesia and emergency drugs. It is also advisable to use inhalation anesthesia because marmosets are known to be prone to decreased body oxygen levels resulting in hypoxemia during anesthesia. If this is not possible, measures should be taken such as administering oxygen. Due to their small body size and rapid metabolic rate, marmosets may develop sudden hypothermia and respiratory/circulatory depression depending on the depth of anesthesia. It is therefore advisable to take precautionary measures, such as keeping the body warm during anesthesia and being prepared for tracheal intubation during use of deep

anesthesia and emergency drugs.

The precautions for anesthesia in marmosets are summarized in Appendix 2-10, and selected anesthetics and analgesics that can be used in marmosets are listed in Appendices 2-11 and 2-12, respectively, along with their usual doses. Some of these anesthetics (e.g., ketamine), sedatives, and analgesics are narcotics or psychotropics and must be maintained properly in accordance with the Narcotics and Psychotropics Control Act (Law No. 14 of March 17, 1953, amended as Law No. 69 of June 14, 2006). For pain management in marmosets, a pain management protocol should be developed based on the estimated degree of pain, which is determined based on the degree of surgical insult and other factors. With the protocol in place, if the marmoset is expected to be in pain, then its condition and behavior must be carefully observed to assess the degree of pain and appropriate analgesic treatment must be given. As anxiety may increase pain in marmosets, it is also important to provide a safe environment for them, such as bedding and hiding places, if pain is anticipated. Regarding the use of analgesics, "preemptive analgesia," in which an analgesic is administered before a painful stimulus is applied, and "multimodal analgesia," in which a combination of multiple analgesics with different mechanisms of action, should be used. Anesthesia/analgesia in aged, diseased, or disease-model animals requires further caution and it is advisable that it be performed in consultation with a veterinarian. Examples of actual anesthesia procedures are provided in Appendix 2-13.

7. Management of disease-model animals and genetically modified animals

In medicine and related fields, many small animal models of diseases, mainly rodents, have been developed and used to determine causes, elucidate pathogenesis, and develop treatments for intractable diseases in humans by using pharmacological methods, physical disorders, and even gene engineering methods. However, the extrapolation of animal data to human disease, especially psychiatric and neurological diseases, requires the creation of nonhuman primate models of disease that have similar neurological structures and functions to those of humans, or animals in which relevant genes are modified, as the physiological and metabolic functions of rodents do not necessarily reflect those of humans. Disease-model animals and genetically modified animals with psychiatric and neurological disorders or central nervous system disorder are likely to exhibit abnormalities and symptoms similar to those of human diseases, which may result in various behavioral limitations. Based on adequate estimation of the impact of disease and other abnormalities on the daily life activities of the animal, adequate preparations must be made, including making appropriate modifications to holding cages, strengthening of the animal monitoring system, feeding and watering assistance, and the development of criteria for determining humane endpoints.

8. Euthanasia

Planned euthanasia of marmosets after completion of an experiment and euthanasia based on humane endpoints must be done by an appropriate method. Euthanasia is a procedure that causes loss of consciousness and irreversible death as rapidly as possible with minimal pain and stress. The circumstances under which euthanasia should be considered are summarized in Appendix 2-14. Methods of euthanasia are described below.

An internationally accepted method for euthanasia is to administer an overdose of anesthetics. Any barbiturate derivative may be used for intravenous administration. Intraperitoneal injection is also acceptable when intravenous infusion is difficult to perform, but may require higher doses. Sample collection, if required, must be performed after confirmation of respiratory arrest, cardiac arrest, and pupillary dilatation after sufficient time has elapsed. Suggested doses of barbiturates are shown in Appendix 2-15. When perfusion is to be performed, the same anesthesia procedure as for surgical procedures should be performed, followed by exsanguination and perfusion.

Section IV. Well-being

1. Introduction

When using laboratory animals for experiments and research, the manager and other personnel must be responsible for the well-being of the animals. The facts that animals do not just automatically respond to the environment and that they are able to indicate their intention via behavior have gradually been accepted as scientific knowledge. Therefore, especially when keeping and handling marmosets, a nonhuman primate species, every effort must be made to ensure that the animals are in good physical as well as mental condition.

2. Considerations for behavior and psychological condition

Animal welfare refers to the state of animals being able to "cope" with the surrounding environment. To evaluate animal welfare, it is essential to have appropriate physiological, psychological, and behavioral markers, in addition to evaluating the presence or absence of illness, injury, or pain. The housing environment must also be improved to obtain better quality experimental data. It is important that the animal's innate physiological, biological, and behavioral patterns are preserved for the betterment of its psychological condition. Especially for marmosets, which are highly social animals, social relationships among animals and between animals and humans have a significant impact on their psychological state. Therefore, care must be taken to ensure that laboratory animals do not become socially isolated and that relationships among laboratory animals housed together or nearby and relationships between laboratory animals and humans are kept in a good state. Users must not only comply with the Act on Welfare and Management of Animals and other relevant laws, but also conduct themselves with animal welfare always in mind. The housing environment and procedures must be adjusted so that experimental marmosets can maximally express their natural behavioral patterns and do not exhibit abnormal behaviors related to stress caused by the housing environment and research use. Measures must be taken to improve the housing environment if stereotyped behaviors (i.e., repeating the same behavior), abnormal behaviors (e.g., plucking, excessive grooming, excessive marking, excessive threats, self-mutilation), or excessive obesity or emaciation is observed.

The specific goals are summarized in Appendix 2-16. To achieve these goals, it is advisable to respond flexibly to the current situation and actively improve the housing environment. It is also advisable to make maximal efforts to improve the environment for each animal while taking into account the objectives and practical possibilities of research and animal care and management.

3. Environmental enrichment

To improve the well-being of laboratory animals, it is advisable to actively introduce "environmental enrichment" that adds various functions to the housing environment. Improvement of the environment facilitates the innate habits and behaviors of animals. In addition, it also contributes to reduced stress and abnormal behaviors and increased opportunities for exercise and helps with the development and maintenance of various physical and social functions. It is advisable that modifications be made so that animals can control the environment according to their own independent choices. Where experimental or environmental constraints prevent satisfactory enrichment of all aspects of the environment, it is advisable to make the greatest possible effort to do so to the extent feasible. For example, if there is a limited social environment, it is advisable to make modifications to enrich the eating and physical environments. However, introduction of various structures without sufficient planning may result in an inadequate effect or even increased injuries or conditions related to physiological stress, such as metabolic disorder. Thus, for effective environmental enrichment, enrichment should be applied to various aspects of environment concurrently while giving consideration to animal care and management. Specific strategies for environmental enrichment are provided below.

(1) Introduction of novelty, variability, choice, and controllability

The housing environment for laboratory animals is generally monotonous, which is one of the major causes of various problems. It is thus advisable to introduce novelty and variability, as well as a system that allows laboratory animals to make choices and exercise control independently. For physical

environmental enrichment, it is also effective to increase the potential for choices and control of the environment not only by introducing play equipment, but also by exchanging it with new equipment on a regular basis or introducing various types of play equipment that can be manipulated in various ways. Another effective strategy is to introduce partitions that allow animals to hide from people and other animals.

(2) Social environment

Because marmosets are highly social animals, physical contact actions, such as grooming, and communicative actions via visual, auditory, and olfactory senses are important components of their daily life. They should be allowed to live in a social environment suited to these characteristics as much as possible. To form an effective group, it is important to facilitate the establishment of appropriate relationships among animals, while keeping in mind their social structure and the characteristics of their social behavior and considering intimacy and social hierarchy among them. It should be ensured that the established group is socially stable and that appropriate social interactions can be formed and maintained among the animals. When it is difficult to house multiple animals together, modifications can still be made such as connecting parts of the cages to allow physical contact and visual, auditory, and olfactory communication.

(3) Improvement of relationship with researchers and animal technicians

To reduce stress experienced by animals and increase the safety of researchers and animal technicians, it is advisable to establish a good relationship between these personnel and laboratory animals through routine activities. The establishment of such a relationship will help the personnel notice changes in the behavior of each animal and may even make animals cooperative to stressful procedures required for the purpose of research, such as restraint and blood collection. Moreover, if an adequate social environment with other animals cannot be provided for research reasons, good relationships with humans will compensate for the lack of social interactions to some extent.

To maximize the effects of various forms of environmental enrichment, attention must be paid to characteristics that differ among individual animals, such as sex, age, and origin. It is the responsibility of researchers and animal technicians to closely observe animals as part of routine animal care. In addition to implementing environmental enrichment, animals' behavior should be observed and recorded to the extent possible, and the effect of the enrichment should be evaluated. It is advisable that further environmental improvement be sought based on the evaluation results. It is also advisable that efforts be made on a routine basis to improve the environment even for animals that do not exhibit marked abnormal behavior. It is advisable to include the preparation of a good housing environment in the animal experiment protocol from the outset, based on the recognition that the research use of animals and animal welfare are not in conflict with each other, but rather go hand-in-hand.

Section V. Planning and conduct of experiments

1. Introduction

When using animals for research/education purposes, sufficient consideration must always be given not only to the proper care and management but also to the humane handling of animals. Therefore, appropriate animal handling and the development of a proper animal experiment protocol must be strived for based on a full understanding of the contents of the relevant laws and regulations and in accordance with the "3Rs" principle for animal experiments. The "3Rs" principle is an international norm for animal experiments and must always be considered. Consideration of "replacement," "reduction," and "refinement" is a prerequisite for all animal experiment protocols. Each institution must establish internal regulations based on laws and guidelines and must establish systems for animal facility management and protocol review by the institutional animal care and use committee.

2. Laws and principles

The laws/regulations, standards, and guidelines that must be followed in order to ensure proper animal care and experiments in Japan are listed in Appendix 2-17. An external review system should be implemented to ensure compliance with the related laws/regulations, standards, and guidelines of the ministries and agencies supervising research institutions.

The validity of an animal experiment is judged by a harm-benefit analysis of pain/distress experienced by animals and the significance (outcome) of the experiment. For this reason, there must be criteria for assessing the degree of pain/distress experienced by animals. Depending on the nature of the research being conducted, the degree of pain/distress experienced by animals is grouped into 5 categories as listed in Appendix 2-18. This pain classification is based on a classification table created by the Scientists Center for Animal Welfare (SCAW), a group of scientists in North America. Each institution may adapt this classification to marmosets, taking into account the latest scientific trends and differences in the environment at each institution. When preparing an animal experiment protocol, the principal investigator must fully understand which pain category the protocol falls into. For protocols classified as Category E, the level of pain/distress and expected outcomes must be fully considered. On the other hand, protocols that are classified as Category A, but involve cell transplantation to other animal species or other equivalent procedures, must be reviewed for appropriateness, including ethical aspects. Protocols that only use cells must also be implemented with a full understanding of risks associated with the use of the cells. The use of hazardous substances or new chemicals, if any, must also be specified in the protocol.

3. Health and safety management of personnel involved in animal care and experiments

The conduct of experiments using marmosets requires different knowledge and techniques depending on the degrees of contact with the animals and the invasiveness of experimental procedures. Personnel conducting these experiments must acquire the necessary knowledge and techniques in advance. Moreover, because many diseases in marmosets are common to humans, having sufficient knowledge of these diseases is important in the dual sense of protecting against transmission of infections from researchers/animal technicians to marmosets, and from marmosets to humans. Personnel expected to be involved in research activities using hazardous substances or equipment that are biological (e.g., pathogens), chemical (e.g., toxic chemicals), or physical (e.g., electromagnetic wave, radioactive materials) hazards must be trained in the handling of hazardous substances and equipment and, if applicable, obtain necessary qualifications.

Necessary health care must be provided to the personnel involved in animal care and experiments to prevent them from contracting disease. Diseases associated with the use of laboratory animals include zoonotic diseases, animal allergies, and bites and scratches, as well as injuries and diseases caused by animal care and management activities and experimental procedures. The director and manager of the institution must identify risk factors that can affect occupational health and safety and ensure that all relevant personnel undergo necessary health check-ups in accordance with the Industrial Safety and Health Act. For disease prevention, the wearing of personal protective equipment (PPE), such as special clothing, masks, gloves, and various other types of protective gear should be mandated, depending on the nature of the activities. In addition, the director and manager of the institution must ensure that necessary measures are in place in case of the occurrence of injuries or diseases in the relevant personnel, such as keeping emergency medical supplies on hand, maintaining manuals for first aid and emergency communication, and establishing a system for contacting medical institutions available in an emergency.

4. Planning for emergency response

Each institution must prepare an emergency response manual and emergency contact network in advance and make them known to the relevant personnel in case of natural disasters, fires, long-term power outages, and other emergencies. In an emergency, it is important to first ensure the safety of the personnel of the institution before seeking to complete the research project, ensure animal welfare, and preserve the surrounding environment. It is advisable to prepare an emergency response manual taking into account the points listed in Appendix 2-19. It is also advisable for each institution to refer to the

“Guidelines for Preparing an Emergency Response Manual” set forth by the Japanese Association of Laboratory Animal Facilities of National University Corporations when preparing an emergency response manual. It is advisable that the emergency contact network be posted in corridors in front of laboratories and animal rooms to facilitate personnel’s preparedness for an emergency response. Each institution should have a stockpile of feed/water and emergency power supply in case of a disaster.

5. Protocol review and the institutional animal care and use committee

Animal experiments must be scientifically, ethically, and humanely justified in terms of their purpose and content, and may be carried out only if the experiments are documented in an animal experiment protocol that has been reviewed by the institutional animal care and use committee and approved by the director of the institution. When conducting an animal experiment, the anticipated contribution of the research objectives to science and medicine must be fully considered, and the animal experiment protocol must be designed and reviewed in accordance with the “3Rs” principle of animal experiments, as described below.

(1) Replacement (using alternative methods)

Living marmosets may be used in an experiment only when there is scientific justification for their use. When planning an experiment, other experimental options not involving animals or experiments using other species must first be considered; then, whether or not the purpose of the research can be achieved only by using marmosets must be fully considered. Before conducting an experiment using living marmosets, it is advisable to conduct a preliminary study using organs or cells derived from marmosets whenever possible for the purpose of the experiment.

(2) Reduction (reducing the number of animals used)

The protocol should be designed to minimize the number of marmosets to be used while improving the type, quantity, and quality of data, insofar as doing so does not compromise the objectives of the study. When planning an experiment, the protocol should be designed to allow for hypothesis testing with a small number of animals, with measures to minimize predictable individual differences in experimental results, such as analyzing changes over time before and after an experiment for each animal and conducting multiple evaluations for each animal, because marmosets are not a genetically homogeneous population, in contrast to genetically defined mice and rats. For minimally invasive experiments that involve only medication, blood sampling and/or behavioral analysis, or less painful experiments such as non-invasive bioimaging, it is advisable to use the same animal for another experiment after a certain rest period, in order to promote “reduction.” After an animal has been euthanized following completion of an experiment, it is advisable that organs and tissues not used in the experiment be used for other studies as much as possible without compromising the purposes of the studies. At the same time, repeating major survival surgeries (e.g., thoracotomy, craniotomy, laparotomy) on the same animal for the purpose of reducing the number of animals used is not recommended.

(3) Refinement (reducing pain/distress through refinement of experimental techniques)

Researchers conducting an animal experiment must make every effort to minimize the animal suffering such as pain, physical injury, anxiety, and fear. To this end, they must always strive to improve and refine their animal care and experimental techniques. The following fundamental principle must be followed: Procedures that are distressing for humans should also be considered distressing for monkeys, unless there is evidence to the contrary. Based on prior prediction of the physical and mental effects of the experiment on the animals, appropriate procedures and equipment for marmosets, as well as appropriate anesthesia/analgesia and euthanasia procedures, must be planned. Researchers should be aware that poor techniques may result in increased pain/distress in animals, and therefore they must always strive to improve their technical skills. It is advisable that principal investigators familiarize themselves in advance with experimental procedures and the anticipated pain/distress in animals, and describe it in the animal experiment protocol when setting humane endpoints (see Chapter V, Section 8). Experimental designs involving multiple major, life-threatening surgeries on a single animal should be avoided unless the series of surgeries consists of a single experimental design and is judged to be

essential based on scientific rationale or clinical (veterinary) necessity, or is deemed appropriate after due consideration of the degree of pain/distress in the animals and the significance (expected outcome) of the animal experiment.

Under the Act on Access to Information Held by Administrative Organs promulgated in 1999, animal experiment protocols and other related documents may be disclosed to outside parties as required, except for information whose disclosure may affect personal rights and benefits. Approval by the director of the institution assures outside parties that the relevant protocol has been reviewed and implemented in accordance with the Guideline described in this document. Principal investigators are thus required to prepare animal experiment protocols with due consideration of the purpose and significance of the experiments.

6. Conduct of experiments involving restrictions (including feeding and water restriction)

Restrictions on the intake of water, food, or selected nutrients, etc. are likely to adversely affect the development and health status of marmosets and thus it is advisable that they be avoided as much as possible. For animals that exhibit human-like emotional responses, such as marmosets, it is also advisable not to impose social restrictions, such as separating a developing juvenile animal from its mother, or other types of restriction, such as blockage or modification of sensory organs, because of their negative impact on the animal's development and health status. If these restrictions must be applied for the purpose of the experiment and there is no alternative experimental method available, the animal experiment protocol must include a detailed explanation of the need for such restrictions and their appropriateness must be fully discussed by the animal care and use committee. Even if approved, the frequency and duration of such restrictions must be set to a minimum, in conjunction with close monitoring of the development and health status of each animal through frequent observation and body weight measurement. Due to substantial differences in feed/water intake between animals, the baseline body weight and average feed/water intake of each animal should be measured to guide the adjustment of restriction levels and experimental schedules. Notes on feeding/watering restrictions are summarized in Appendix 2-20. Body weight and feed/water intake must be measured and recorded frequently during restrictions. Special care should be taken when using immature or aged animals in experiments involving restrictions.

(1) Effects of feeding restriction on the health of animals, unlike those of water restriction, do not necessarily become apparent immediately. Because malnutrition may significantly affect the development and health of animals, appropriate recovery periods must be scheduled depending on the duration and intensity of restriction. Feeding restriction must be carried out such that energy intake does not fall below the basal metabolic rate, which is determined as a function of species, developmental stage, and body weight (see Appendix 2-5), with the fasting period set as short as possible.

(2) The health status of marmosets during an experiment involving restrictions can be evaluated by, but not limited to, increases/decreases in body weight and feed/water intake, amount and condition of feces, skin and fur quality, and abnormal behavior. Changes in these parameters may be overlooked unless observed closely by an experienced observer. It is advisable that animals showing any abnormality be brought to a veterinarian’s attention. If necessary, the experiment must be interrupted and effort must be made to restore the animal’s health. The need for euthanasia as a humane endpoint must also be considered depending on the situation. Commonly used humane endpoints are provided in Appendix 2-14.

7. Education and training

The director and manager of the institution must provide the laboratory animal manager, animal technicians, and researchers with education and training necessary for proper animal care and experiments before they engage in any activity and on a regular basis thereafter. Only those who have acquired knowledge and skills in the biology and handling of marmosets and zoonotic diseases should conduct experiments on marmosets. In addition, invasive procedures on marmosets should be performed by only those who have been adequately trained and mastered the necessary techniques under the

supervision of a mentor. Furthermore, it is advisable that the personnel engaged in animal care and handling participate in internal and external training sessions, try to collect relevant information to acquire necessary knowledge and skills, and accumulate professional experience through their daily work.

8. Health management of animals during experiments and endpoints

Researchers and animal technicians must assume the primary responsibility for the health management of animals during experiments through careful observation of each animal, keeping in mind the experimental procedure performed. If any ill health or behavioral abnormality is observed in an animal, appropriate treatment should be given immediately to the animal in cooperation with a veterinarian. Animals used in experiments should also be provided the same appropriate veterinary management for disease prevention and treatment as other animals. It is advisable to provide animals with a suitable environment to promote their innate habits and behaviors and thereby improve their physical and mental health (well-being).

If an irreversible deterioration of health or unavoidable pain/distress occurs in an animal used in an experiment, the principal investigator must determine a humane endpoint in conjunction with a veterinarian. Principal investigators must familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals and describe the anticipated pain/distress in the animal experiment protocol. To enable objective determination of humane endpoints, the principal investigator must specify health parameters to be checked and assessments in the protocol and have them reviewed by the institutional animal care and use committee. It is advisable that researchers and animal technicians objectively assess the condition of each animal by observation, and a point when the animal's condition has deteriorated beyond a certain level should be defined as the humane endpoint (see Appendix 2-14).

9. Conduct of experiments using hazardous substances

Although not limited to experiments using marmosets, when an animal experiment is to be conducted using hazardous substances that are biological (e.g., pathogens), chemical (e.g., deleterious/toxic substances, narcotics/psychotropic drugs, methamphetamine), or physical (e.g., electromagnetic waves, radiation) hazards, including the use of X-ray, PET, CT, MRI, and other imaging modalities, various types of equipment and systems must be in place to protect researchers, animal technicians, and laboratory animals from exposure to these substances and prevent environmental pollution. Dedicated equipment must be used for experiments involving hazardous substances. These types of equipment must be installed in an area away from animal housing facilities and laboratories and appropriately indicated as a hazardous area. Personnel who use such equipment must obtain licenses required for the handling of the relevant hazardous substances and must be familiar with risk management procedures in case of accidents and other procedures. For the care of laboratory animals and storage, use, and management of animal waste, carcasses, and hazardous substances, clearly defined, safe operating procedures must be in place and sufficient education and training programs must be provided to protect personnel from hazards. All personnel must be well familiar with the properties of the hazardous substances to be handled and with required protective measures. Possibly contaminated equipment and waste must be disposed of appropriately at each institution to prevent the leakage of hazardous substances out of the hazardous area. Researchers who wish to conduct an experiment using biohazardous substances, such as microbes, or chemical hazardous substances must submit an animal experiment protocol to the relevant committees for approval. When an infection experiment is to be conducted, institutional codes must be established in accordance with the Safety Management Codes for Handling of Pathogens etc. of the National Institute of Infectious Diseases, and the experiment must be conducted in compliance with the relevant laws and regulations.

10. Conduct of animal experiments using genetically modified organisms

Although this is again not limited to experiments using marmosets, the creation or transfer of genetically modified organisms and experiments using these organisms, such as genetically modified viruses, must be carried out properly in accordance with the "Ministerial Ordinance Providing Containment Measures to Be Taken in Type 2 Use of Living Modified Organisms for Research and Development," which was issued based on the "Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms" (the Cartagena Protocol). When conducting an animal experiment using genetically modified organisms, the principal investigator must submit an application to the relevant authority or an application for the relevant minister's permission, in accordance with the law, and obtain permission before starting the experiment. Animal experiments using genetically modified organisms must be conducted within a permitted research area.

Appendix 1. Macaque-related materials (reference numbers are shown in brackets)

Appendix 1-1. Requirements for macaque research facilities

a. Sufficient consideration is given to the care, hygiene, and behavioral management of macaques
b. Quarantine rooms for infected animals or isolator cages equipped with a HEPA filter are available
c. The animal research facility is located adjacent or close to the animal housing facility
d. Dedicated operation/procedure rooms are available at the institution

Appendix 1-2. Environmental conditions for macaques

Environmental factor	Recommended value
Temperature	20-28°C
Humidity	30-70%
Ventilation rate	6-15 times/h [1]
Airflow velocity	13-18 cm/s (direct exposure of animals to drafts should be avoided)
Lighting	150-300 lx (40-85 cm above the floor) Usual cycle is 12-14 h light and 10-12 h dark
Noise	No more than 60 dB [2]-[4]
Odor	No more than 20 ppm ammonium

Appendix 1-3. Requirements for macaque cages [5]

a. Large enough to allow animals to move freely and maintain their natural posture, and designed to allow them to rest
b. Designed to allow animals easy access to feed and water
c. Designed to allow keepers to easily supply feed/water and to clean and exchange the equipment used for feed/water supply
d. Designed to permit easy handling of animals
e. Designed to prevent animals from escaping
f. Designed to prevent injury to animals
g. Designed to permit easy monitoring of the condition of animals

Appendix 1-4. Minimum housing space per macaque [1][6]-[11]

Body weight (kg)	Floor space/animal (m^2)	Height (cm)
0.0-1.5	0.20	76.2
1.5-3.0	0.28	76.2
3.0-10.0	0.40	76.2
10.0-15.0	0.56	81.3
15.0-20.0	0.74	91.4

* For individual housing, it is advisable that wider and taller cages be used. An environment that allows vertical movement is advisable for macaques.

Appendix 1-5. Basal metabolic rate of macaques [12]

Nonhuman primate species and developmental stage	Basal metabolic rate, metabolizable energy intake (kcal/kg body weight/day)
Rhesus macaque, adult	83.8 [13]
Rhesus macaque, adult	51.4 [14]
Rhesus macaque, adolescent	71.3 [15]
Japanese macaque, adult	55-63 [16]
Japanese macaque, adult	57-62 [17]
	Digestibility
Rhesus macaque, adult	87.0 [13]

Appendix 1-6. Laws and regulations pertaining to specified animals

a. Article 26-33 of the Act on Welfare and Management of Animals
b. Order for Enforcement of the Act on Welfare and Management of Animals
c. Article 13-22 and Supplementary Provisions of the Regulations for Enforcement of the Act on Welfare and Management of Animals
d. Details of the standards for the structure and size of specified animal holding facilities
e. Details of methods for care and management of specified animals

Appendix 1-7. Items to record for macaque management

a. Basic information about each animal: facility of birth, sex, date of birth, lineage, breeding information, physical characteristics, behavioral characteristics, etc.
b. Findings to be recorded during care and management: observation findings, body weight, test results, treatments, pathology results, etc.
c. Records of experimental use (procedures and manipulations)

Appendix 1-8. Veterinary management of macaques [18]

a. Daily observation of all animals for evaluation of their health status and housing conditions
b. Prevention, control, diagnosis, and treatment of disease and injury
c. Educating researchers and animal technicians on the proper handling, restraint, sample collection, operation, anesthesia, analgesia, postoperative management, and euthanasia of animals through training programs, and guiding or performing such tasks when necessary.

Appendix 1-9. Precautions for disease control in macaques [19]-[21]

Because macaques are highly susceptible to infection with *Mycobacterium tuberculosis*, it is advisable that infection control measures be taken to confirm that people entering the room are not infected with tuberculosis, such as chest X-ray and interferon-gamma release assays. It is also advisable to confirm that all people entering the room have a sufficient antibody titer against measles. (Positivity is usually determined by a titer of 1:16 by enzyme immunoassay, 1:256 by particle agglutination, or 1:8 by neutralization. In many cases, two doses of vaccine are required to gain access to the room.) The risk of transmission of infectious diseases between macaques and other monkey or animal species should also be noted, and it is advisable to divide animal care and management facilities and work traffic lines in animal research facilities. In particular, simian retrovirus (SRV), which is carried by cynomolgus and

rhesus macaques, can infect and cause severe illness in Japanese macaques, leading to death in many cases. Therefore, keeping these monkeys in the same room should be avoided. Given the possible transmission of unknown infectious diseases, keeping different species of monkeys in the same room should be avoided whenever possible. If animals are to be kept for more than several years, regular health examinations should be performed, including tuberculin, dysentery, and salmonella tests, as well as B virus and SRV tests.

Appendix 1-10. Precautions for anesthesia in macaques [22][23]

Neither muscle relaxants nor paralytic agents (e.g., succinylcholine and other agents with curariform action) are anesthetics. These agents should not be used alone in surgery, as they have no analgesic effect and do not cause loss of consciousness. Barbiturates should also not be used alone because of their weak analgesic effect and occasional risk of causing respiratory depression and delayed arousal at high doses. Inhalation anesthetics, such as isoflurane and sevoflurane, have a limited analgesic effect themselves and must be used in combination with sedatives, injectable anesthetics, analgesics, etc.

Appendix 1-11. Selected anesthetics that can be used in macaques [24]-[26]

Category	Agent name	Dosage	Note
Dissociative anesthetics	Ketamine	5-10 mg/kg IM	✓ For sedation and immobilization; should not be used alone in surgery ✓ Wide safety margin, but uncontrollable once administered ✓ Minimal effect on the respiratory system ✓ May cause increased blood pressure and increased heart rate ✓ Virtually no muscle-relaxing effect ✓ Strong analgesic effect ✓ Pre-medication with anticholinergics (atropine 0.02-0.05 mg/kg, IM) is recommended
Dissociative anesthetics	Ketamine (K) + medetomidine (M)	(K) 2.5 mg/kg + (M) 0.1 mg/kg, mixed and administered IM OR (K) 5 mg/kg + (M) 0.05 mg/kg, mixed and administered IM	✓ Antagonist: atipamezole (0.25-0.5 mg/kg IM) ✓ (M) can cause peripheral vasoconstriction, a transient increase followed by a drop in blood pressure, depressed heart rate, and sometimes arrhythmia ✓ Anesthesia time: 30-40 min

Dissociative anesthetics	Ketamine (K) + xylazine (X)	(K) 7 mg/kg + (X) 0.6 mg/kg, mixed and administered IM OR (K) 10 mg/kg + (X) 0.5 mg/kg, mixed and administered IM	✓ (K) + (M) is preferable ✓ Antagonist: atipamezole (or yohimbine at 1 mg/kg, IM) ✓ Pre-medication with anticholinergics (atropine 0.02-0.05 mg/kg, IM) is recommended ✓ Anesthesia time: 30-40 min
Barbiturates	Thiopental	25-40 mg/kg IV	✓ Given IV after immobilization is achieved with (K) or (K)+(M) ✓ Uncontrollable once administered ✓ Tachycardia, respiratory depression, decreased blood pressure, increased intracranial pressure ✓ Anesthesia time: 20-30 min ✓ For prolonged anesthesia, administer in divided doses through a venous catheter
Barbiturates	Propofol	5-10 mg/kg/h, IV Monitor the animal's general condition and adjust the dose accordingly	✓ Respiratory/circulatory depression, kidney/liver damage ✓ Anesthesia time: 30-60 min
Inhalation anesthetics	Isoflurane	1-3% (1 MAC = 1.28%)	✓ Rapid introduction/recovery and good controllability ✓ May cause respiratory depression ✓ May cause decreased blood pressure and increased heart rate
Inhalation anesthetics	Sevoflurane	2-4% (1 MAC = 2%)	✓ Faster introduction/recovery than isoflurane ✓ Good controllability ✓ May cause respiratory depression ✓ May cause decreased blood pressure and increased heart rate ✓ Nearly odorless

IM: intramuscularly, IV: intravenously; MAC: minimum alveolar concentration

Appendix 1-12. Selected analgesics that can be used in macaques [24]-[26]

Category	Agent name	Dosage	Duration of action	Administration route	Note
NSAIDs	Acetaminophen	10-15 mg/kg	6 h	PO	✓ May cause gastrointestinal disorder
NSAIDs	Aspirin	12-15 mg/kg	6 h	PO	✓ May cause gastrointestinal disorder
NSAIDs	Carprofen	4 mg/kg 1-2 mg/kg	12-24 h	IV SC PO	✓ May cause gastrointestinal and hepatic disorders
NSAIDs	Meloxicam	0.2 mg/kg	12-24 h	PO SC	✓ May cause gastrointestinal disorder/vomiting
Opioid	Buprenorphine	0.01-0.02 mg/kg	8-12 h	IM IV	✓ May cause mild respiratory depression ✓ Acts on μ-receptor
Opioid	Butorphanol	0.1-0.2 mg/kg	3-4 h	IM	✓ May cause respiratory depression ✓ Antagonizes μ-receptor and acts on κ-receptor (competes with buprenorphine and morphine)
Opioid	Morphine	1-2 mg/kg	4 h	IM IV SC	✓ Suppresses respiration and gastrointestinal motility ✓ Acts on μ-receptor

IM: intramuscularly; IV: intravenously; PO: per os; SC: subcutaneously

Appendix 1-13. Examples of anesthesia in macaques

Some examples of anesthesia procedures are provided below. These example procedures are only applicable to healthy animals to be anesthetized for research purposes. For anesthesia in aged, ill, or disease-model animals, further considerations must be given and it is advisable to seek veterinary advice.

Ketamine is commonly used for anesthesia in short and less invasive procedures. When ketamine is to be used alone, an anticholinergic agent (atropine or glycopyrrolate) should be pre-administered to prevent hypersalivation and bradycardia. Ketamine is commonly used with sedatives, such as xylazine and medetomidine, for reduced toxicity and better muscular relaxation. When medetomidine is used, its antagonist atipamezole should be administered at the end of the procedure. Medetomidine has a transient hypertensive effect (and a persistent hypotensive effect) and thus should not be used with anticholinergic agents. Due to its effect on hemodynamics, medetomidine should be given at a reduced dose or should not be used in aged or debilitated animals. When bradycardia or hypotension has been induced by medetomidine, atipamezole should be administered immediately. Both ketamine and medetomidine have an analgesic effect, although ketamine is considered less effective against visceral pain. Anesthetics commonly used in lengthy procedures include thiopental (a barbiturate), propofol (a GABA agonist),

and isoflurane (an inhalation anesthetic). All of these anesthetics have a minimal analgesic effect and should be used in combination with appropriate analgesics when used in invasive procedures. Bariturates such as thiopental should also not be used alone because of their weak analgesic effect and occasional risk of causing respiratory depression and delayed arousal at high doses, and they must be administered carefully by an experienced person.

a. Combination anesthesia with ketamine hydrochloride and inhalation anesthetics
    1) Anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or xylazine into the femoral or brachial muscle of the animal.
    2) Once the animal has been anesthetized and immobilized, transfer it to the operating table and administer an inhalation anesthetic (with isoflurane, 2-4% for induction and 1-2% for maintenance) through a mask or tracheal tube. During inhalation anesthesia, the vaporizer setting should be maintained carefully by a veterinarian or a researcher familiar with its operation. For the safety of the investigator, it must be ensured that the concentration of inhalation anesthetics in the surrounding environment does not exceed 2 ppm. If the operation is prolonged, additional ketamine or other analgesics should be administered as the analgesic effect of ketamine may wear off.

b. Combination anesthesia with ketamine hydrochloride and thiopental sodium
    1) Anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or xylazine into the femoral or brachial muscle of the animal.
    2) Once the animal has been anesthetized and immobilized, transfer it to the operating table and intravenously infuse thiopental sodium at 25-40 mg/kg. Keep in mind that rapid infusion of thiopental sodium may kill the animal. After about half of the total dose has been infused, check the respiratory rate and other physiological parameters and continue infusion at a lower rate thereafter. Since the effectiveness of anesthesia may vary from animal to animal, the depth of anesthesia should be evaluated by pain response or other means. If the operation is prolonged, additional ketamine or other analgesics should be administered as the analgesic effect of ketamine may wear off.

Appendix 1-14. Circumstances when euthanasia should be considered [27]-[29]

a. When it is determined that a humane endpoint has been reached due to difficulty in feed and water intake, self-injurious behavior, respiratory disorder, abnormal condition over a prolonged period (diarrhea, bleeding, etc.), rapid weight loss (20% or more over several days), or a marked increase in tumor size (10% or more of body weight).
b. When pain/distress persists for experimental or other reasons and cannot be relieved by analgesics or other drugs.
c. When an animal experiences severe pain/distress or stress in an infection experiment using a highly dangerous pathogen or a toxicity experiment, and is determined to have reached a humane endpoint.
d. When an animal experiences severe pain/distress or stress in a highly distressing experiment and is determined to have reached a humane endpoint.
e. The research purpose justifies killing the animal and there is no alternative method available at the moment (i.e., experimental killing).

For experiments that cause an intensity of pain that exceeds a certain level, the criteria for determining a humane endpoint should be established and reviewed by the institutional animal care and use committee. It is advisable that the criteria be determined through thorough discussion by the animal experiment committee, in conjunction with the laboratory animal manager, the principal investigator, researchers, and veterinarians. Principal investigators must familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals, prepare a scoring sheet that enables objective assessment of the condition of animals based on relevant parameters, and have it reviewed by the institutional animal care and use committee. It is advisable that researchers and animal technicians

objectively assess the condition of each animal using this score, and a point when the animal's condition has deteriorated beyond a certain level should be defined as the endpoint.

Appendix 1-15. Methods for euthanasia of macaques [30]-[32]

Euthanasia of macaques should be done by administering an overdose of a barbiturate anesthetic (≥100 mg/kg for thiopental sodium) and subsequent cardiac arrest, or by exsanguination after confirmation of loss of pain reflex under deep anesthesia (≥45 mg/kg for thiopental sodium). The 100 mg/kg dose of sodium thiopental may not result in cardiac arrest; if not, the dose must be increased as necessary.

The use of sodium thiopental has yet been well established in macaques and should be accompanied by adequate monitoring of the animal's condition.

Appendix 1-16. Goals for the well-being of macaques [5][18][33]-[35]

a. Animals should be housed in a residential environment that permits postural maintenance and movement.
b. Animals should be given opportunities to engage in behaviors such as eating, exploring, and manipulating objects, depending on species, age, sex, and individual condition.
c. Maintenance of social interactions.
d. Maintenance of appropriate relationships with humans.
e. Reduction of pain and stress.

Appendix 1-17: Laws, regulations, and guidelines related to animal experiments

Category	Laws/regulations/guidelines	Issuing authority	Year of issuance/ last revision
National law	Act on Welfare and Management of Animals	Legislation by the Diet Ministry of the Environment	1973/2012
Standards and guidelines based on the Act on Welfare and Management of Animals	Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals	Ministry of the Environment	2006/2013
	Standards Relating to the Methods of Destruction of Animals	Ministry of the Environment	1995/2007
National guidelines for animal experiments	Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions	Ministry of Education, Culture, Sports, Science and Technology	2006
	Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of the Ministry of Health, Labour and Welfare	Ministry of Health, Labour and Welfare	2006/2015
	Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of	Ministry of Agriculture,	2006

	the Ministry of Agriculture, Forestry and Fisheries	Forestry and Fisheries	
	Guidelines for Proper Conduct of Animal Experiments	Science Council of Japan	2006

Appendix 1-18:Categories of Biomedical Experiments Based on Increasing Ethical Concerns for Nonhuman Species [36]

Category	Representative procedures
A Experiments not using live animals	✓ Biochemical research ✓ Microbiological research ✓ Cell culture ✓ Research using autopsy-derived tissue samples
B Experiments considered to cause no or almost no discomfort to animals	✓ Restraining an animal for a very short period of time (≤1 min) ✓ Restraining an animal in an experiment cage for a short period (≤2-3 h) without immobilizing its limbs or head ✓ Administration of a minimally toxic substance or simple procedures, such as drawing a small amount of blood ✓ Experiments using animals made unconscious under sufficient depth of anesthesia and thus associated with no discomfort after completion of procedures ✓ Keeping animals away from feed and water for a short period, and killing animals using appropriate euthanasia procedures ✓ Non-fatal animal experiments (may involve infectious agents) that do not cause serious symptoms in animals
C Experiments expected to cause mild stress or pain (lasting for a short period)	✓ Restraining an animal on a monkey chair or other equipment for several hours ✓ Exposing a blood vessel under anesthesia or inserting a catheter for a long period ✓ Surgical procedures performed under anesthesia that cause mild post-procedural discomfort ✓ Painful but avoidable stimuli ✓ Non-fatal animal experiments (may involve infectious agents) that cause serious symptoms in animals
D Experiments expected to cause severe, unavoidable stress or pain (lasting for a long period)	✓ Intentionally causing stress in animals for behavioral observation ✓ Surgical procedures performed under anesthesia that cause significant, persistent post-procedural discomfort ✓ Experiments that expose animals to painful unavoidable stimuli ✓ Restraining an animal on a monkey chair or other equipment for more than several hours ✓ Experimental separation of a child from its mother, or applying sensory deprivation ✓ Causing an animal to exhibit aggressive behavior and injure itself or other animals of the same species ✓ Causing pain without using anesthesia ✓ Causing a nearly maximum level of pain that can be tolerated by animals (causing a facial expression of severe pain)

	✓ Fatal animal experiments (may involve infectious agents) that cause serious symptoms in animals
E Experiments that cause a level of pain close to or exceeding the maximum tolerable level in unanesthetized animals	✓ Carrying out surgery without anesthetics, but with a muscle relaxant or paralytic agent, such as succinylcholine and other agents with curariform action, only for immobilizing a monkey ✓ Causing severe burn or injury in an unanesthetized animal ✓ Causing severe, unavoidable stress in an animal or killing an animal by causing such pain ✓ Causing an animal to exhibit psychosis-like behavior by exposing it to nearly maximum tolerable pain or severe stress

Appendix 1-19. Items to consider in an emergency response manual for macaques

a. Protection against and prevention of escape by captive macaques and prevention of accidents. (handling of genetically modified and infectious animals)
b. Stockpiling (securing) feed and drinking water for animals
c. Response to power/gas outages (ensuring emergency power supply)
d. Appropriate management of research equipment and hazardous substances that could cause a secondary disaster

Appendix 1-20. Notes on feeding/watering restrictions for macaques [19][37]-[40]

In neuroscience and behavioral studies using macaques, feed/water intake restrictions are often used to make animal cooperative in performing cognitive tasks. Restrictions being too strict or too long may significantly affect the growth and health of macaques. The following points should be fully considered and reviewed by the animal care and use committee.

a. Not all experiments require feed/water intake restrictions. The availability of alternative methods should be considered. The use of highly palatable rewards can also be useful.
b. Prior to an experiment involving feed/water intake restrictions, an individualized protocol tailored to each monkey should be developed by examining each monkeys' preferences for feed and water and average feed/water intake.
c. In many cases, the monkey's behavior can be controlled by making them aware that they can get feed and water only within a limited time frame, rather than drastically reducing feed/water supply.
d. If the daily feed/water requirements of macaques cannot be met, supplementary food, such as fruits and vegetables, should be given to animals as needed to maintain their health.
e. Daily weighing and health checks should be performed throughout the experiment. A stable body weight is an important indicator of good health (growth-related weight gain should be seen in young animals).

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Appendix 2. Marmoset-related materials (reference numbers are shown in brackets)

Appendix 2-1. Requirements for animal experiment facilities using marmosets

a. Sufficient consideration is given to the care, hygiene, and behavioral management of animals
b. Quarantine rooms for infected animals or isolator cages equipped with a HEPA filter are available
c. The animal research facility is located adjacent or close to the animal holding facility
d. Dedicated operation/procedure rooms are available at the institution

Appendix 2-2. Environmental conditions for marmosets

Environmental factor	Recommended value
Temperature	27-30°C [1][2]
Humidity	30-70%
Ventilation rate	10-15 /h [3]
Airflow velocity	13-18 cm/s (direct exposure of animals to drafts should be avoided)
Lighting	150-300 lx (40-85 cm above the floor). Usual cycle is 12-14 h light and 10-12 h dark [4]-[6]
Noise	No more than 60 dB [7]-[9]
Odor	No more than 20 ppm ammonium

Appendix 2-3. Requirements for marmoset cages [6][10]-[12]

a. Large enough to allow animals to move freely and maintain their natural posture, and designed to allow them to rest
b. Designed to allow animals easy access to feed and water
c. Designed to allow keepers to easily supply feed/water and to clean and exchange the equipment used for feed/water supply
d. Designed to permit easy handling of animals
e. Designed to prevent animals from escaping
f. Designed to prevent injury to animals
g. Designed to permit easy monitoring of the condition of animals

Appendix 2-4. Minimum housing space per marmoset [1][10]-[12]

Floor space/animal*	Height
0.20 m^2	76.2 cm

* For individual housing, it is advisable that wider and taller cages be used. It is advisable that the ceiling of the cage be set higher than human eye level.

Appendix 2-5. Basal metabolic rate of marmosets [13]

	Basal metabolic rate, metabolizable energy intake (kcal/kg body weight/day)
Marmoset, 355 g	208 [2]
Marmoset, 300 g	142 [14]
	Digestibility
Marmoset, 355 g	75% [2]

Appendix 2-6. Notes for breeding and care of marmosets

a. Marmosets are diurnal animals and must be exposed to light at about 300 lx for 12 h a day in captivity. Keeping marmosets for a prolonged period in a poorly lit environment may result in reduced fertility and abnormal social behaviors [4]-[6][15][16].
b. It is advisable that animals be exposed to sunlight. If this is not feasible, vitamin D supplementation should be provided [17]-[27].
c. It is advisable to install a nesting box in which animals can hide at a position higher than human eye level. These boxes are also important as a sleeping place [12][18].
d. It is advisable to introduce wooden objects for gnawing and scenting behaviors [11].
e. It is advisable to avoid separating infants less than 1 year of age from their mothers as it may cause behavioral instability. For the same reason, it is advisable to avoid artificial nursing unless there is a specific reason [29]-[35].
f. Marmosets reach maturity in 2 years and can have conflict with their parents if housed together after reaching maturity. Therefore, it is advisable to separate juvenile marmosets from their parents before 1.5 years of age [36].
g. It is advisable that animals intended for future breeding be housed together with their parents up until about 1.5 years old. In particular, it is advisable to allow mothers to experience parenting of newborns before separating them from offspring [10][36]-[38].
h. Marmosets can become pregnant about 10 days postpartum. However, in order to avoid excessive maternal burden, it is advisable to closely monitor the health of postpartum mothers and consider pregnancy adjustment using PGF2α analogues or other agents, if necessary [39]-[41].

Appendix 2-7. Items to record for marmoset management

a. Basic information about each animal: facility of birth, sex, date of birth, lineage, breeding information, physical characteristics, behavioral characteristics, etc.
b. Findings to be recorded during care and management: observation findings, body weight, test results, treatments, pathology results, etc.
c. Records of experimental use (procedures and manipulations)

Appendix 2-8. Veterinary management of marmosets [36]

a. Daily observation of all animals for evaluation of their health status and housing conditions
b. Prevention, control, diagnosis and treatment of disease and injury
c. Educating researchers and animal technicians on the proper handling, restraint, sample collection, operation, anesthesia, analgesia, postoperative management, and euthanasia of animals through training programs, and guiding or performing such tasks when necessary.

Appendix 2-9. Precautions for disease control in marmosets [42]-[50]

Compared with macaques, marmosets have a lower risk of transmitting infections to humans because they do not carry B virus and are less susceptible to infection with *Mycobacterium tuberculosis*, and all marmosets bred and reared in Japan are kept indoors. However, given the possible transmission of unknown infectious diseases, sufficient care should be taken when handling marmosets and keeping them with different species of monkeys in the same room should be avoided whenever possible. It is advisable that infection control measures be taken to confirm that people entering the room are not infected with tuberculosis, such as chest X-ray and interferon-gamma release assays. It is also advisable to confirm that all people entering the room have a sufficient antibody titer against measles, given reported deaths of marmosets due to herd infection with measles or herpes simplex virus. (Positivity is usually determined by a titer of 1:16 by enzyme immunoassay, 1:256 by particle agglutination, or 1:8 by neutralization. In many cases, two doses of vaccine are required to gain access to the room.) The risk of transmission of infectious diseases between marmosets and other monkey or animal species should also be noted, and it is advisable to divide animal care and management facilities and work traffic lines in animal research facilities. If animals are to be kept for more than several years, regular health examinations should be performed, including tuberculin, dysentery, and salmonella tests.

Appendix 2-10. Precautions for anesthesia in marmosets [51][52]

Neither muscle relaxants nor paralytic agents (e.g., succinylcholine and other agents with curariform action) are anesthetics. These agents should not be used alone in surgery, as they have no analgesic effect and do not cause loss of consciousness. Barbiturates should also not be used alone because of their weak analgesic effect and occasional risk of causing respiratory depression and delayed arousal at high doses. Inhalation anesthetics, such as isoflurane and sevoflurane, have a limited analgesic effect themselves and must be used in combination with sedatives, injectable anesthetics, analgesics, etc.

Appendix 2-11. Selected anesthetics that can be used in marmosets [53]-[57]

Category	Agent name	Dosage	Note
Dissociative anesthetics	Ketamine	5-30 mg/kg, IM	✓ For sedation and immobilization; should not be used alone in surgery ✓ Wide safety margin, but uncontrollable once administered ✓ Minimal effect on the respiratory system ✓ May cause increased blood pressure and increased heart rate ✓ Virtually no muscle-relaxing effect ✓ Strong analgesic effect ✓ Pre-medication with anticholinergics (atropine 0.02-0.05 mg/kg, IM) is recommended

Dissociative anesthetics	Ketamine (K) + medetomidine (M)	(K) 1-3 mg/kg + (M) 0.05-0.15 mg/kg, mixed and administered IM	✓ Antagonist: atipamezole (0.25-0.75mg/kg IM) ✓ (M) can cause peripheral vasoconstriction, a transient increase followed by a drop in blood pressure, depressed heart rate, and sometimes arrhythmia ✓ Anesthesia time: short time
Dissociative anesthetics	Ketamine (K) + xylazine (X)	(K) 7-30 mg/kg + (X) 0.5-2.5 mg/kg, mixed and administered IM	✓ Antagonist: atipamezole (or yohimbine at 1 mg/kg, IM) ✓ Anesthesia time: 30-40 min ✓ Pre-medication with anticholinergics (atropine 0.02-0.05 mg/kg, IM) is recommended
Neurosteroidal anesthetics	Alfaxalone	5-12 mg/kg, IM	✓ Limited analgesic effect ✓ Minimal effect on the respiratory and cardiovascular systems ✓ Used for minor procedures and induction anesthesia ✓ Anesthesia time: short time
Neurosteroidal anesthetics	Alfaxalone (A) + medetomidine (M) + butorphanol (B)	(A) 4 mg/kg + (M) 0.05 mg/kg + (B) 0.03 mg/kg, mixed and administered IM	✓ Antagonist: atipamezole (0.25 mg/kg, IM) ✓ Used for brief procedures that require muscle relaxation and analgesia ✓ Anesthesia time: short time
Neurosteroidal anesthetics	Alfaxalone (A) + diazepam (D)	(A) 12 mg/kg, IM + (D) 3 mg/kg, IM	✓ (A) should be mixed with an anticholinergic (atropine 0.05 mg/kg) and administered IM ✓ (D) should be administered IM without mixing with other agents ✓ Used for induction anesthesia
Barbiturates	Thiopental	25-40 mg/kg, IV	✓ Limited analgesic effect ✓ Given IV 10 min after immobilization with (K) or (K)+(X) ✓ Uncontrollable once administered ✓ Tachycardia, respiratory depression, decreased blood pressure, increased intracranial pressure ✓ Anesthesia time: 20-30 min (for prolonged anesthesia, administer in divided doses through a

			venous catheter)
Sedative/analgesic combination	Medetomidine (M) + midazolam (M) + butorphanol (B)	(M) 0.04 mg/kg + (M) 0.4 mg/kg + (B) 0.4 mg/kg, mixed and administered IM	✓ Antagonist for medetomidine: atipamezole (0.2 mg/kg, IM) ✓ (Used for minor procedures and induction anesthesia) ✓ Anesthesia time: short time
Sedative/analgesic combination	Dexmedetomidine (D) + midazolam (M) + fentanyl (F)	(D) 0.1 mg/kg + (M) 1 mg/kg + (F) 0.001 mg/kg, mixed and administered IM	✓ Antagonist for medetomidine: atipamezole (0.2 mg/kg, IM) ✓ Used for minor procedures and induction anesthesia ✓ Anesthesia time: short time
Inhalation anesthetics	Isoflurane	1-3%	✓ Rapid introduction/recovery and good controllability ✓ May cause respiratory depression ✓ Decreased blood pressure
Inhalation anesthetics	Sevoflurane	2-4%	✓ Faster introduction/recovery than isoflurane ✓ Good controllability ✓ May cause respiratory depression ✓ Blood pressure decreased ✓ Nearly odorless

IM: intramuscularly, IV: intravenously

Appendix 2-12. Selected analgesics that can be used in marmosets [53]-[56][58][59]

Category	Agent name	Dosage	Duration of action	Administration route	Note
NSAIDs	Acetaminophen	5-10 mg/kg 2-4 times daily	6 h	PO	✓ May cause gastrointestinal disorder
NSAIDs	Aspirin	5-10 mg/kg Every 4-6 h	6 h	PO	✓ May cause gastrointestinal disorder
NSAIDs	Carprofen	2-4 mg/kg 1-2 times daily	12-24 h	SC PO	✓ May cause gastrointestinal and hepatic disorders
NSAIDs	Ketoprofen	2 mg/kg	12-24 h	IM, IV	

NSAIDs	Meloxicam	0.1-0.2 mg/kg, 1 time daily (0.2 mg/kg) 2 times daily (0.1 mg/kg)	12-24 h	PO, SC	✓ May cause gastrointestinal disorder/vomiting
Opioid	Buprenorphine	0.005-0.02 mg/kg 2-3 times daily	8-12 h	IM, SC	✓ May cause severe respiratory depression ✓ Acts on μ-receptor ✓ Antagonist: naloxone (0.1 mg/kg, IM) ✓ Start from a lower dose due to risk of hypersensitivity
Opioid	Butorphanol	0.01-0.02 mg/kg 2-4 times daily	3-4 h	IM, SC	✓ May cause severe respiratory depression ✓ Antagonizes μ-receptor and acts on κ-receptor (competes with buprenorphine and morphine)
Opioid	Remifentanil	0.1-0.25 μg/kg/min, IV Continuous infusion	Effect disappears 5-10 min after cessation of continuous infusion	IV	✓ May cause muscle rigidity, respiratory depression, decreased blood pressure, and bradycardia ✓ For muscle rigidity, muscle relaxants such as vecuronium bromide should be administered ✓ Concomitant use with benzodiazepines or barbiturates may enhance each other's effect and should be used with caution ✓ Acts on the μ-receptor
Opioid	Morphine	1-2 mg/kg 4 times daily	4 h	IM, SC	✓ Suppresses respiration and gastrointestinal motility ✓ Acts on the μ-receptor

IM: intramuscularly; IV: intravenously; PO: per os; SC: subcutaneously

Appendix 2-13. Examples of anesthesia in marmosets [56][60]

Some examples of anesthesia procedures are provided below. These example procedures are only applicable to healthy animals to be anesthetized for research purposes. For anesthesia in aged, ill, or disease-model animals, further considerations must be given and it is advisable to seek veterinary advice. Since procedures involving intramuscular injection may cause muscle damage due to a proportionally high dose volume for the body size of marmosets, it is advisable to use the thinnest possible needle and perform injections at multiple sites in the quadriceps, which is a large muscle.

Ketamine or alfaxalone is commonly used for anesthesia in short and less invasive procedures. When

ketamine is to be used alone, an anticholinergic agent (atropine or glycopyrrolate) should be pre-administered to prevent hypersalivation and bradycardia. Ketamine is commonly used with sedatives, such as xylazine, for reduced toxicity and better muscular relaxation. When xylazine is used, its antagonist, namely, atipamezole or yohimbine, should be administered at the end of the procedure. Combination anesthesia with alfaxalone, medetomidine, and butorphanol is another common option. In marmosets, unlike macaques, anesthesia can cause decreased body oxygen levels and resulting hypoxemia. It is thus advisable to induce anesthesia with the listed anesthetics, followed by maintenance with inhalation anesthetics, such as isoflurane and sevoflurane. Isoflurane and sevoflurane have a limited analgesic effect and it is advisable to use them in combination with the continuous administration of appropriate analgesics in invasive procedures. Bariturates such as thiopental should also not be used alone because of their weak analgesic effect and occasional risk of causing respiratory depression and delayed arousal at high doses, and they must be administered carefully by an experienced person.

Example anesthesia procedure

a. Anesthesia is induced by intramuscular injection of alfaxalone (4 mg/kg), medetomidine (0.05 mg/kg), and butorphanol (0.3 mg/kg) into the quadriceps of the animal.
b. Once the induction anesthesia takes effect and the animal has been immobilized, transfer it to the operating table and administer an inhalation anesthetic (with isoflurane, 2-4% for induction and 1-3% for maintenance) through a mask or tracheal tube. During inhalation anesthesia, the vaporizer setting should be maintained carefully by a veterinarian or a researcher familiar with its operation. For the safety of the investigator, it must be ensured that the concentration of inhalation anesthetics in the surrounding environment does not exceed 2 ppm. If the operation is prolonged, additional doses of the same or other analgesics should be administered as the effect of the analgesics may wear off.

Appendix 2-14. Circumstances when euthanasia should be considered [33][61][62]

a. When it is determined that a humane endpoint has been reached due to difficulty in feed and water intake, self-injurious behavior, respiratory disorder, abnormal condition over a prolonged period (diarrhea, bleeding, etc.), rapid weight loss (20% or more over several days), or a marked increase in tumor size (10% or more of body weight).
b. When pain/distress persists for experimental or other reasons and cannot be relieved by analgesics or other drugs.
c. When an animal experiences severe pain/distress or stress in an infection experiment using a highly dangerous pathogen or a toxicity experiment, and is determined to have reached a humane endpoint.
d. When an animal experiences severe pain/distress or stress in a highly distressing experiment and is determined to have reached a humane endpoint.
e. The research purpose justifies killing the animal and there is no alternative method available at the moment (i.e., experimental killing).

For experiments that cause an intensity of pain that exceeds a certain level, the criteria for determining a humane endpoint should be established and reviewed by the institutional animal care and use committee. It is advisable that the criteria be determined through thorough discussion by the animal experiment committee, in conjunction with the laboratory animal manager, the principal investigator, researchers, and veterinarians. Principal investigators must familiarize themselves in advance with experimental procedures and anticipated pain/distress in animals, prepare a scoring sheet that enables objective assessment of the condition of animals based on relevant parameters, and have it reviewed by the institutional animal care and use committee. It is advisable that researchers and animal technicians objectively assess the condition of each animal using this score, and a point when the animal's condition has deteriorated beyond a certain level should be defined as the endpoint

Appendix 2-15. Methods for euthanasia of marmosets [28][33][63]

Euthanasia of marmosets should be done by administering an overdose of a barbiturate anesthetic (≥100 mg/kg for thiopental sodium) and subsequent cardiac arrest, or by exsanguination after confirmation of loss of pain reflex under deep anesthesia (≥45 mg/kg for thiopental sodium).
The 100 mg/kg dose of sodium thiopental may not result in cardiac arrest and must be increased as necessary.

The use of sodium thiopental has yet been well established in marmosets and should be accompanied by adequate monitoring of the animal's condition.

Appendix 2-16. Goals for the well-being of marmosets [11][36][64]-[68]

a. Animals should be housed in a residential environment that permits postural maintenance and movement.
b. Animals should be given opportunities to engage in behaviors such as eating, exploring, and manipulating objects, depending on species, age, sex, and individual conditions.
c. Maintenance of social interactions.
d. Maintenance of appropriate relationships with humans.
e. Reduction of pain and stress.

Appendix 2-17: Laws, regulations, and guidelines related to animal experiments

Category	Laws/regulations/guidelines	Issuing authority	Year of issuance/ last revision
National law	Act on Welfare and Management of Animals	Legislation by the Diet Ministry of the Environment	1973/2012
Standards and guidelines based on the Act on Welfare and Management of Animals	Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals	Ministry of the Environment	2006/2013
	Standards Relating to the Methods of Destruction of Animals	Ministry of the Environment	1995/2007
National guidelines for animal experiments	Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions	Ministry of Education, Culture, Sports, Science and Technology	2006
	Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of the Ministry of Health, Labour and Welfare	Ministry of Health, Labour and Welfare	2006/2015
	Fundamental Guidelines for the Conduct of Animal Experiments in Affiliated Institutions of the Ministry of Agriculture, Forestry and Fisheries	Ministry of Agriculture, Forestry and Fisheries	2006

	Guidelines for Proper Conduct of Animal Experiments	Science Council of Japan	2006

Appendix 2-18: Pain categories [69]

Category	Representative procedures
A Experiments not using live animals	✓ Biochemical research ✓ Microbiological research ✓ Cell culture ✓ Research using autopsy-derived tissue samples
B Experiments considered to cause no or almost no discomfort to animals	✓ Restraining an animal for a very short period of time (≤1 min) ✓ Restraining an animal in an experimental cage for a short period (≤2-3 h) without immobilizing its limbs or head ✓ Administration of a minimally toxic substance or simple procedures, such as drawing a small amount of blood ✓ Experiments using animals made unconscious under sufficient depth of anesthesia and thus associated with no discomfort after completion of procedures ✓ Keeping animals away from feed and water for a short period, and killing animals using appropriate euthanasia procedures ✓ Non-fatal animal experiments (may involve infectious agents) that do not cause serious symptoms in animals
C Experiments expected to cause mild stress or pain (lasting for a short period)	✓ Restraining an animal on a monkey chair or other equipment for several hours ✓ Exposing a blood vessel under anesthesia or inserting a catheter for a long period ✓ Surgical procedures performed under anesthesia that cause mild post-procedural discomfort ✓ Painful but avoidable stimuli ✓ Non-fatal animal experiments (may involve infectious agents) that cause serious symptoms in animals
D Experiments expected to cause severe, unavoidable stress or pain (lasting for a long period)	✓ Intentionally causing stress in animals for behavioral observation ✓ Surgical procedures performed under anesthesia that cause significant, persistent post-procedural discomfort ✓ Experiments that expose animals to painful unavoidable stimuli ✓ Restraining an animal on a monkey chair or other equipment for more than several hours ✓ Experimental separation of a child from its mother, or applying sensory deprivation ✓ Causing an animal to exhibit aggressive behavior and injure itself or other animals of the same species ✓ Causing pain without using anesthesia ✓ Causing a nearly maximum level of pain that can be tolerated by animals (causing a facial expression of severe pain) ✓ Fatal animal experiments (may involve infectious agents) that cause serious symptoms in animals

E Experiments that cause a level of pain close to or exceeding the maximum tolerable level in unanesthetized animals	✓ Carrying out surgery without anesthetics, but with a muscle relaxant or paralytic agent, such as succinylcholine and other agents with curariform action, only for immobilizing a monkey ✓ Causing severe burn or injury in an unanesthetized animal ✓ Causing severe, unavoidable stress in an animal or killing an animal by causing such pain ✓ Causing an animal to exhibit psychosis-like behavior by exposing it to nearly maximum tolerable pain or severe stress

Appendix 2-19. Items to consider in an emergency response manual for marmosets

a. Protection against and prevention of escape by captive marmosets and prevention of accidents. (handling of genetically modified and infectious animals)
b. Stockpiling (securing) feed and drinking water for animals
c. Response to power/gas outages (ensuring emergency power supply)
d. Appropriate management of research equipment and hazardous substances that could cause a secondary disaster

Appendix 2-20. Notes on feeding/watering restrictions in marmosets [13][70]-[72]

In neuroscience and behavioral studies using marmosets, feed/water intake restrictions are often used to make animal cooperative in performing cognitive tasks. Restrictions being too strict or too long may significantly affect the growth and health of marmosets. The following points should be fully considered and reviewed by the animal care and use committee.

a. Not all experiments require feed/water intake restrictions. The availability of alternative methods should be considered. The use of highly palatable rewards can also be useful.
b. Prior to an experiment involving feed/water intake restrictions, an individualized protocol tailored to each marmoset should be developed by examining each animals' preferences for feed and water and average feed/water intake.
c. In many cases, the animal's behavior can be controlled by making them aware that they can get feed and water only within a limited time frame, rather than drastically reducing feed/water supply.
d. If the daily feed/water requirements of marmosets cannot be met, supplementary food, such as fruits and vegetables, should be given to animals as needed to maintain their health.
e. Daily weighing and health checks should be performed throughout the experiment. A stable body weight is an important indicator of good health (growth-related weight gain should be seen in young animals).
f. In marmosets, a small nonhuman primate species, feed/water intake restrictions can easily cause a rapid deterioration in physical condition. Experimental methods not involving such restrictions should be used as much as possible.

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Acknowledgments

In developing these guidelines, we received input from several experts whom we asked for opinions and many researchers in the research community.

We appreciate that we could have a constructive discussion based on their candid feedback. We would also like to thank the secretariat of the Japan Neuroscience Society for their tremendous support.

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