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**—Review—**
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# Japanese Macaques as Laboratory Animals

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**Abstract:** The Japanese macaque (*Macaca fuscata*), along with rhesus and long-tailed macaques, is one of the macaca species. In Japan, it has been preferred for use as a laboratory animal, particularly in the field of neuroscience, because of its high level of intelligence and its gentle nature. In addition, the species has a relatively homogeneous genetic background and field researchers have accumulated abundant information on the social behavior of wild Japanese macaques. As future neuroscience research will undoubtedly be more focused on the higher cognitive functions of the brain, including social behavior among multiple individuals, the Japanese macaque can be expected to become even more valuable as a laboratory animal in the near future. The Ministry of Education, Culture, Sports, Science and Technology has launched a National BioResource Project (NBRP) to establish a stable breeding and supply system for Japanese macaques for laboratory use. The project is in progress and should lead to the establishment of a National Primate Center in Japan, which will support the supply of monkeys as well as social outreach and handling of animal welfare issues.

**Key words:** bio-resource, laboratory animal, *Macaca fuscata*, NBRP, neuroscience

## Introduction

Macaque monkeys have proved to be a useful animal model for a variety of reasons. First, they are phylogenetically closer to humans than other animals routinely used, e.g., mice and rats. Second, they have a similar body structure and physiological properties to humans, and consequently have proved of value for virology, neuroscience, and reproduction research, etc. Indeed, there are some types of research in which macaque monkeys cannot be replaced by other animal species, such as rodents, or by cell lines. Globally, rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*Macaca fascicularis*) are most commonly used primates as laboratory animals. In Japan, however, the Japanese macaque (*Macaca fuscata*) has been frequently employed, in part because Japanese macaques are native to Japan. Until recently, they were regarded in many rural areas as an agricultural pest, and were captured and legally transferred to researchers by local authorities. Now, for various reasons, the use of animals of wild origin for laboratory research is restricted. Nevertheless, Japanese macaques are still the preferred choice for many types of laboratory research, particularly in the field of neuroscience targeting the higher brain functions. Here, we describe the unique contribution of Japanese macaques to neuroscience research.

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**Table 1.** The research areas and the number of studies where macaca species were used

| | Micr | Neur | Bioc | Gene Cons | Cons Phar | HIV | Phar | HIV | Etho | Surg | Endo Phys | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. mulatta* | 102 | 251 | 36 | 27 | 13 | 76 | 106 | 35 | 35 | 46 | 39 | 766 |
| *M. fascicularis* | 38 | 132 | 26 | 11 | 6 | 51 | 16 | 13 | 36 | 18 | 12 | 359 |
| *M. fuscata* | 2 | 37 | 6 | 4 | 9 | 2 | 2 | 16 | 5 | 2 | 5 | 90 |
| Total | 142 | 420 | 68 | 42 | 28 | 129 | 124 | 64 | 76 | 66 | 56 | 1,215 |

Micr, microbiology. HIV, HIV/AIDS. Neur, neuroscience. Etho, ethology. Bioc, biochemistry. Surg, surgery/anatomy. Gene, genetics. Endo, endocrinology/reproduction. Cons, conservation/ecology/anthropology. Phys, physiology. Phar, pharmacology. (derived from Carlsson *et al*. 2004 [1])

## Macaque Monkeys in Life Science Research

*Global trend*

The number of articles published resulting from studies in which one of the macaque species was employed is shown in Table 1 (derived from Carlsson [1]). As is clear from the table, macaque species have been used in a wide range of life science studies, including microbiology, neuroscience, pharmacology, and HIV/AIDS. The overall proportion of studies involving Japanese macaques (*Macaca fuscata*) is small in a global context as they have mainly been used by Japanese scientists. In those studies in which they have been employed, Japanese macaques have been used most frequently in neuroscience and HIV/AIDS research.

## Why Japanese Macaques?

*Unique situation in Japan*

Initially, Japanese macaques were used in Japanese laboratories because they are a native species that was readily available from rural local authorities that trapped them as an agricultural pest. The number of Japanese macaques trapped has been reported to be about 10,000 per year [6]. The majority of these animals were killed, but in the past, some were transferred to researchers, mainly for use in medical studies. In neuroscience research, the number of articles published in the two leading journals, the Journal of Neurophysiology and the Journal of Neuroscience, with *Macaca fuscata* as the experimental model, dramatically increased after the mid 1980s (Fig. 1). Thus, *Macaca fuscata* has been one of the principal animal species used in this research field, especially among Japanese neuroscientists (Fig. 2B). However, in 2000, the Ministry of Environment in Japan decided to restrict the use of monkeys caught in the wild for research in response to concerns raised by primatologists regarding the possibly deleterious effect on Japanese macaque populations. Although the numbers of wild-derived animals used in laboratories was only a small portion of the total number of captured monkeys, it was argued that the existence of the path for such usage might encourage levels of capture that were inappropriate with regard to conservation of the species. The change in regulation reduced the use of wild-derived Japanese macaques, however, a demand for the species remains as many Japanese researchers regard the Japanese macaque as a valuable animal model, particularly in studies of higher brain functions. Japanese macaques have the reputation of being relatively tame and gentle compared to other macaque species, such as rhesus and long-tailed macaques. In some types of neuroscience research, macaques undergo behavioral testing in the laboratories after extensive training at particular tasks. The somewhat more gentle nature of Japanese macaques makes them more amenable to such test procedures. Moreover, although it is less widely appreciated, ethologists have accumulated a considerable amount of information on the behavior of Japanese macaques in the wild (see the next section). In neuroscience research, there is an increasing trend toward including studies of the neural basis of social behaviors. For such research, the possibility of integrating data collected in the field with that gathered in laboratory studies is attractive as it might provide valuable insights into many aspects of social behavior. To illustrate the potential benefits that might be gained from such studies, we briefly summarize the history of ethological research on Japanese macaques

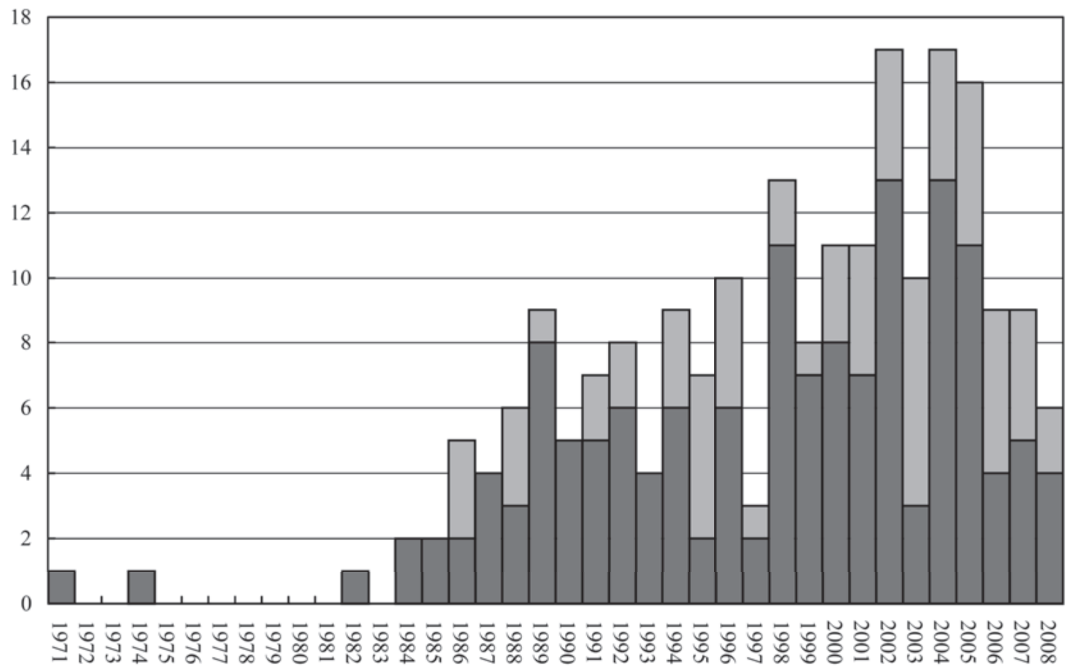


**Fig. 1.** The number of articles published in the Journal of Neurophysiology (■) and the Journal of Neuroscience (■) in which Japanese macaques were used. The abscissa (“x”) axis indicates the published year. The ordinate (“y”) axis indicates the numbers of articles.

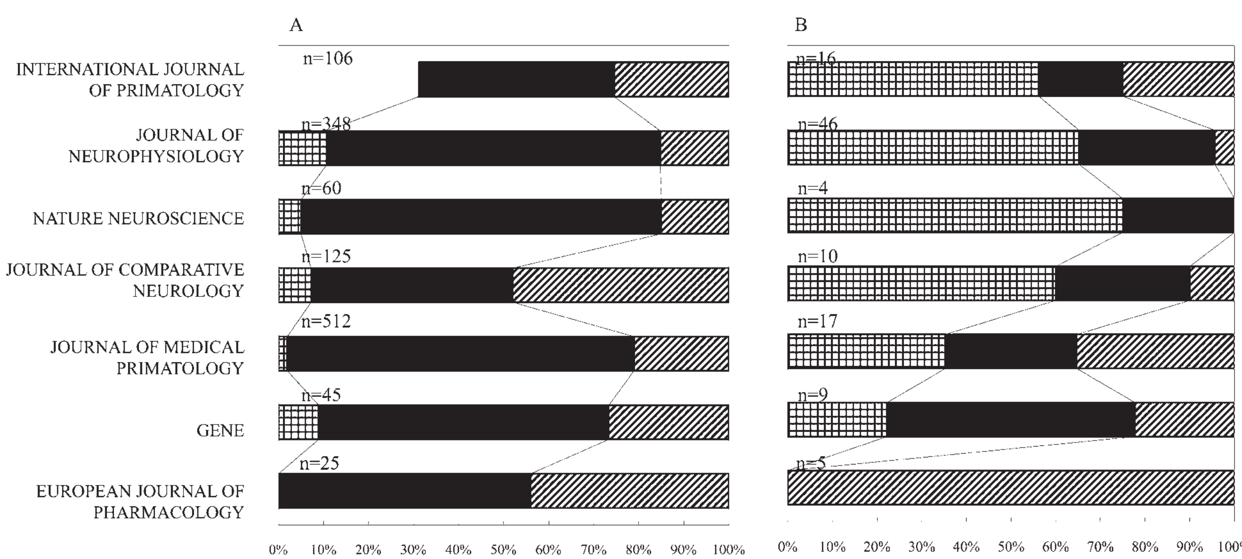


**Fig. 2.** The comparative rates of use of different macaque species in studies published in 2008. (A) Each bar indicates the percentage of each macaca species (▦ *M. fuscata*; ■ *M. mulatta*; ▨ *M. fascicularis*) per journal. The figure above each bar indicates the total number of articles. (B) The number of articles published by Japanese scientists using different macaque species.

in the next section. Another little known aspect of Japanese macaques is that they show considerably less genetic variance than other macaque species (Fig. 3). One possible explanation for this phenomenon is that only a small population of Japanese macaques initially colonized the islands of Japan during the Ice Age, when

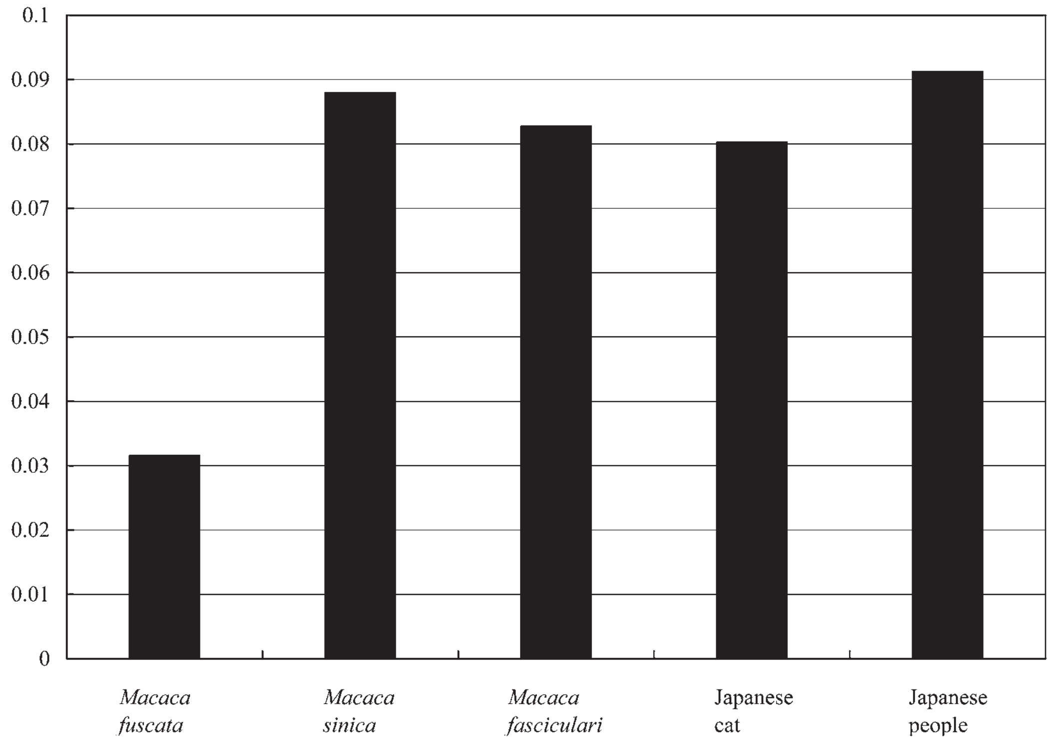


**Fig. 3.** Estimated genetic variability of various species. Each bar indicates the expected average heterozygosity calculated from the mean of gene frequencies in the subpopulations of each species (Nozawa *et al*. 1996 [10]). The ordinate ("y") axis indicates the value of heterozygosity.

these islands were connected to the mainland, and that the modern population is derived from this relatively restricted group of founders. This unique characteristic of Japanese macaques compared to the other macaca species (rhesus and long-tailed macaques) that are prevalent in south-east Asian countries is of considerable benefit for laboratory studies.

*Accumulation of ethological data*

From an ethological perspective, the Japanese macaque is one of the most well-studied primate species. The behavior of these monkeys has been under study for more than 50 years and there are continuous demographic records from various ecological settings.

In 1952, researchers from Kyoto University started provisioning and observing wild monkey troops at Koshima, Miyazaki and Takasakiyama, Oita, with a strong focus on evolution of sociality in their studies. In order to record details of social interactions, early Japanese primatologists adopted a unique observation method involving focal-animal data sampling based on individual identification. This approach led to the discovery of primate "pre-culture" (i.e., sweet potato washing [4]), which is often cited as the beginning of "cultural primatology". Furthermore, such "ethnography of monkeys" also attracted public attention in Japan. Six wild populations were designated as National Treasures, and as resources for tourism and education, monkey watching at "saru-yama" (zoo enclosures) and "wild monkey parks" (free-ranging, provisioned sites) became popular. Later, from the 1970s, researchers initiated attempts to habituate wild troops without provisioning to eliminate undesirable human influences on their behavior and ecology. As a result of these efforts over many years, an extensive understanding of the behavior and ecology of the Japanese macaque has been obtained.

An illustration of the extent of this data gathering comes from the Primate Factsheets at Primate Info Net, the information service site provided by the National Primate Research Center of the University of Wisconsin,


**Fig. 4.** Images of Japanese macaques displaying typical cognitive behaviors. (A) A juvenile monkey playing with a plastic film case lid. (B) An infant watching the behavior of its dominant kin behavior from its mother's back.

USA. There are 113 references to the Japanese macaque, the largest number of any primate species on the list, and more than twice as large as other well-studied macaque species *M. mulatta* (48 references) and *M. fascicularis* (45 references).

Chimpanzees and capuchins have been recorded as showing diverse tool-using behaviors in the wild. In comparison, Japanese macaques may not be tool-users in the wild, but in captivity or in provisioned settings, they are very adaptable and clever at making use of new, and often artificial, environments. In addition to the famous potato-washing behavior, monkeys at Koshima use seawater to separate wheat grains from sand; elsewhere, some make use of hot springs to ward off the cold, and others exploit fires built by humans. In many cases, young monkeys are the most curious about novel objects, are eager to play with them (see Fig. 4A), and eventually find a good use for their "favorite toys".

A vast amount of knowledge on social organization and communication has also been accumulated suggesting that the Japanese macaque has a highly developed social structure. Each troop consists of several matrilineal groups, and a stable dominance hierarchy among females is determined by their position in the matrilineal lineage. Young monkeys seem to learn appropriate social behaviors by watching their close kin (see Fig. 4B), and they assume a similar hierarchical status with the support of their kin group [5]. Intimate communication through vocalization, facial expression, and grooming plays an important role in reinforcing cohesion among different female kin groups. Males usually leave their natal groups before sexual maturity, join other troops, and acquire status in a male hierarchy according to their physical and social abilities. There is evidence that even alpha males often need the support of dominant females to retain dominance [9].

Japanese macaques seem to understand complex and dynamic relationships among their troop mates, and they restrain themselves from provoking pointless squabbles, which implies a high level of potential of cognition and decision making. On the basis of phylogeny, this capacity is probably also present in other macaque species, and of course, great apes are expected to have higher mental abilities. Nevertheless, the understanding that has been gained from more than 50 years of ethological research provides a strong foundation for neuroscience researchers who need to interpret their research outcome in the context of the social and ecological environments in which the Japanese macaques have evolved their cognitive abilities.

## Japanese Macaques in Neuroscience Research

During the 1990s and 2000s, Japanese researchers performed many studies on cognitive brain functions using Japanese macaques and a number of research articles were published in leading journals, including Nature and Science. These studies covered a range of different aspects of neuroscience:

(1) Neuronal activities in the inferior temporal cortex

related to storage and retrieval of memory of complex visual objects [7];

(2) Dynamic change in the response field properties of neurons in the parietal cortex during adaptation to tool use [3];

(3) Neuronal activity in the frontal cortex related to rule sets during solving maze tasks [8];

(4) Neuronal activities in the parietal cortex related to the spatial memory involved in route finding during navigation in virtual space [12].

Current trends in neuroscience research include analysis of the behavior of single subjects and also interactions between two or more animals. For example, Fujii and colleagues studied neuronal activities in the frontal and parietal cortices of two monkeys interacting with each other [2]. Studies such as this emphasize the utility of Japanese macaques for investigations of "social neuroscience".

The neuroscience studies listed above required the experimental animals to perform very complex and cognitively demanding tasks. Many Japanese neuroscientists believe that the intelligent and gentle nature of Japanese macaques in the laboratory confers a significant benefit to their studies.

Recent studies in Japan have identified genes that are uniquely expressed in the cerebral cortex of macaques, which are not present in rodents. Occ-1 is one such gene and is specifically expressed in the primary visual cortex of macaques [13]. There is no evidence of the expression of this gene in the cerebral cortex of rodents. Such species differences will be a very important topic in future research aiming to understand human-specific aspects of intelligence.

## Future Perspectives

*Current status of genomic analysis*

The complete genome sequence of the rhesus macaque was published in 2007. Moreover, microarray panels are now available for the rhesus macaque allowing systematic analyses of gene expressions in the macaque brain. As a result, data on the influence of genetic background on area specialization in the frontal lobe of the macaque cerebral cortex has been published [11]. Although a complete genome sequence of Japanese macaques is not yet available, BAC clones have been analyzed and these sequences are accessible in a database held at the RIKEN BioResource Center (http://www.brc.riken.jp/lab/dna/ja/macaque.html).

*National BioResource Project (NBRP) for breeding and supply*

As mentioned above, the use of Japanese macaques from wild populations has been strictly limited since 2001. Japanese neuroscience researchers have therefore worked to establish colonies of Japanese macaques for laboratory use. To this end, they have collaborated with both primatologists carrying out field research on wild monkeys and veterinarians specialized in the care of monkeys. The neuroscientists also succeeded in getting support from the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, which was initiated in 2002. NBRP aims to establish a system for the collection, preservation and supply of a variety of bio-resources for life science research (http://www.nbrp.jp/index.jsp). During 2001–2003, Japanese neuroscientists used approximately 270 Japanese macaques per year for their research. Based on this number, the breeding project sought to establish a system able to supply 200 Japanese macaques annually to researchers. The National Institute for Physiological Sciences (NIPS) became the core institute for this project and the Kyoto University Primate Research Institute (KUPRI) joined the program as a collaborative institution. NIPS initiated a breeding colony of Japanese macaques in collaboration with the private sector and now maintains a colony of 484 breeder monkeys and 239 purpose-bred monkeys. KUPRI currently maintains 233 breeder monkeys and 30 purpose-bred monkeys, but intends to increase the number of breeder monkeys to 350 by April 2010.

Each institution aims to be capable of providing 100 monkeys every year to researchers. The unique feature of NBRP is that a committee composed of All-Japan communities runs the system. The committee members include neuroscientists from many Japanese Universities and Research Institutes, primatologists, monkey veterinarians and a lawyer. Any scientist interested in using monkeys (currently limited to neuroscientists) can apply with a description of their research plan, information on their facilities for monkeys, and the formal approval of their institutional animal care and use committees. The initial supply system was run by KUPRI, which provided approximately 10 monkeys to researchers across Japan whose application had been approved by the NBRP committee. Subsequently, NBRP supplied 7 monkeys to 4 laboratories in fiscal year (FY) 2006 as test cases, then 56 monkeys to 14 labs in FY 2007, and 51 monkeys to 14 labs in FY 2008.

## Concluding Remarks

*Founding the National Primate Research Center*

NBRP was initiated in 2002 as a project that would be renewed every 5 years and now is in the third year of its second term. In FY 2009, long-term financing was promised to ensure the stability of the project. However, as described below, there are still reasons to believe that the current status remains unsatisfactory and that a more stable center of primate research is required. Such a center would include a breeding colony, a research facility and a public outreach section, and would be similar to the national primate research centers in the USA. Efforts are continuing to establish a national primate center for the supply of and research into Japanese macaques. Such a center might be expected to support not only the supply of monkeys but also to provide outreach to society and to address animal welfare issues.

*Research fields other than neuroscience*

Currently, the research use of Japanese macaques from NBRP is limited to the field of neuroscience, as this is the present policy of MEXT. In addition, use of these Japanese macaques is restricted to laboratories in Japan, which is a disadvantage in terms of this species becoming a universal standard for experimental use. Given the characteristics mentioned earlier of a calm and gentle nature, intelligence, and a relatively homogeneous genetic background, Japanese macaques have many advantages as experimental animals. We expect that scientists from non-neuroscience fields will pay more attention to Japanese macaques and efforts should be made to accommodate the needs of these scientists in the future.

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