

## Environmental enrichment techniques in non-human primates. The case of Callitrichids

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**Summary.** - In this paper, we discuss issues concerning the welfare of non-human primates used in laboratory research from an eco-ethological standpoint and suggest means of improving welfare. Following a brief review of the use of non-human primates in European countries and of the legislation that governs this use, we illustrate how a thorough eco-ethological knowledge of the species being studied can play a vital role in improving both its conditions and the quality of the experimental protocols, arguing that the animal's quality of life is closely linked to the quality of data. As a model for describing environmental enrichment techniques, we have used the common marmoset (*Callithrix jacchus*).

**Key words:** animal welfare, Callitrichids, environmental enrichment, European legislation, non-human primates.

**Riassunto** (*Tecniche di arricchimento ambientale in primati non umani: il caso delle Callitrichidae*). - Lo scopo di questo contributo è considerare alcuni temi rilevanti per il benessere dei primati non umani utilizzati nei laboratori di ricerca. Il caso preso in considerazione è quello della scimmia uistiti (*Callithrix jacchus*). Vengono suggeriti modi per migliorare lo stato di benessere di questi animali, applicando una prospettiva di tipo eco-etologico. Dopo una breve rassegna sulla legislazione e l'uso dei primati non umani nei paesi europei, viene suggerito che una conoscenza eco-etologica della specie studiata può giocare un ruolo cruciale nel migliorare le condizioni in cattività, la qualità del protocollo sperimentale e la qualità del dato sperimentale.

**Parole chiave:** benessere animale, Callitrichidae, arricchimento ambientale, legislazione europea, primati non umani.

### Introduction

Non-human primates are particularly useful in certain areas of biomedical research, in that they are the most phylogenetically similar animals to humans. For instance, *Homo sapiens* shares about 98% of its genome with the chimpanzee (*Pan troglodytes*) (for a review of the phylogenesis of primates [1, 2]). However, the use of non-human primates raises a series of ethical issues, including that of their treatment in captivity, not to mention the issue of whether or not animals whose cognitive abilities suggest that they possess sophisticated ways of experiencing pain and distress should be used at all for an ethical perspective ([3] and the suggested reading list before the References).

In the present work, we describe several means of enriching the lives of experimental non-human primates in captivity. As a model, we have chosen New World monkeys, in particular Callitrichids, a choice which was based on two main considerations: a) the popularity of these monkeys as laboratory animals has

increased significantly over the past 10 years; and b) we have firsthand experience in managing a colony of common marmosets (*Callithrix jacchus*) at the Istituto Superiore di Sanità.

### The use of non-human primates in biomedical research in Europe

Although the number of non-human primates used in biomedical research varies by species, country, and research area, according to the most recent data available from EU countries, non-human primates accounted for less than 0.1% of all laboratory animals used in 1999. A recent document issued by the European Commission indicates that the United Kingdom is the largest user of non-human primates as animal models in the EU, followed by France and Germany [5]. The most common research on non-human primates are biological studies considered to be of a fundamental nature, such as studies on neurology and physiology and on toxicology and other safety issues [6].

With regard to the specific species, the most commonly used Old World monkeys (Catarrhine) are macaques, in particular, the long-tailed macaque (*Macaca fascicularis*), whereas of the New World monkeys (Platyrrhine), the common marmoset (*Callithrix jacchus*), which belongs to the family of Callitrichids, has replaced the squirrel monkey (*Saimiri sciureus*) as the most commonly used primate in the past 10 years. Rylands reports that marmosets became acknowledged laboratory models in biomedical research in the early 1960s in the United States; before then, they were only used in anatomical and behavioural studies or for screening for yellow fever in the wild [7].

The advantages to using the common marmoset as a laboratory animal include its relatively small size (an adult male weighs approximately 250-300 g), which allows several marmosets to be housed in a smaller space. Furthermore, common marmosets are easily bred in captivity, giving birth to twins approximately every five months, so that colonies can be obtained with relatively few problems.

#### **Legislation on housing conditions for non-human primates**

In Europe, the use of animals in scientific and biomedical research is regulated by Directive 86/609/CEE (currently under revision). In Italy, this directive has been assimilated in Decree 116/92, which also includes non-mandatory recommendations on the housing conditions of non-human primates. In particular, Article 5 (Appendix II) states that, because primates greatly vary in size and characteristics, it is important that the cage meet the specific needs of a given primate species. In general, the cage should be high enough for the animals to stand erect and to swing from the top without their feet touching the ground, and it should be equipped with one or two perches so that the animals can make use of the upper cage (the Decree provides a table with the minimum dimensions of cages for non-human primates, in relation to their weight). With respect to social needs, the Article states that two individuals can be housed together as long as they get along. When this is not possible, the cages should be positioned so that the two individuals can see each other and, if necessary, avoid seeing each other as well. No further specifications are provided.

In any case, there is plenty of room for improvement for guaranteeing both the environmental and social needs of non-human primates. For example, the Italian Association of Primatology has published guidelines [4] which provide more detailed indications regarding, in particular, both feeding enrichment and environmental enrichment for non-human primates.

Although the guidelines do not provide a true species-by-species analysis, they do stress the importance of taking into account species-specific characteristics, which is of vital importance to the welfare of animals, not only primates. In fact, the recent document issued by the European Commission on the welfare of non-human primates used in research has the objective of revising the current European legislation, and it very clearly stresses the importance of species-specific characteristics [6].

#### **Three approaches to animal welfare**

In a book edited by Appleby and Hughes and published in 1997, Duncan and Fraser identify three approaches to studying animal welfare. In the first approach, it is proposed that the evaluation of an animal's welfare be primarily based on the subjective feelings of the individual animal. In the second approach, proper biological functioning is considered to be indicative of an acceptable level of welfare. The third approach promotes keeping animals in environments that allow them to express, to the greatest extent possible, the behaviours observed in their natural environment [8, 9]. In our opinion, common sense dictates that the three approaches be combined, so as to have a more comprehensive view of the welfare of the captive animal. However, as ethologists, we tend to place more importance on providing the animal with the possibility to express the behavioural repertoire observed in nature [10, 11]. Although it is impossible to recreate the African Savannah or a Brazilian Forest, a naturalistic laboratory environment should at least provide a suitable level of complexity and variability and allow the animal to have adequate control over this environment. Below we present suggestions for improving the welfare of Callitrichids, focussing on common marmosets, both in terms of social life and physical environment.

#### **Social wellbeing**

The majority of primate species live in very complex social systems, and the social dimension of their lives is vital for their wellbeing [12] and must thus be taken into account when housing them in captivity. With regard to the common marmoset, studies conducted both in the wild and in captivity have shown that this species, which lives in the Atlantic Forest of North-Eastern Brazil, lives in family groups composed of a minimum of three and a maximum of 15-16 individuals. Each family generally has only one pair of breeding adults, who are the

parents of a series of offspring present in the family. The breeding female gives birth to twins, and the gestation period lasts approximately five months. An important characteristic of this species' social behaviour is communal rearing, with the infants being cared for not only by the mother but also by the father and older siblings, who carry and feed them, offering them solid food, such as insects. These aspects have to be taken into account when housing a colony of marmosets in a laboratory. For example, it is good practice to house the breeding pairs with their offspring until the latter reach approximately 18-24 months of age and to allow the older siblings to care for the younger ones. In fact, experimental data have shown that adult marmosets will be better parents if, when young, they are provided with the opportunity to care for their younger siblings. To this end, the cage should be large enough to house from four to five individuals.

The colony of common marmosets at the Istituto Superiore di Sanità was founded in 1983 with four breeding pairs, and the number of animals has varied from a maximum of 27 to a minimum of 12. One of the reasons for this fluctuation has been what is referred to as "social expulsion", which consists of one family member suddenly and systematically attacking, chasing, biting, and fighting with another, with the situation potentially becoming quite serious after a couple of days, necessitating the removal of one of the animals. We have observed that this behaviour can vary in terms of both frequency and intensity; that both males and females have been perpetrators and targets; and that there is no apparent relationship with age. Moreover, the other family members neither interfere nor show interest in what is happening.

Because we have not fully understood this behaviour, it has been difficult to prevent it, and to the best of our knowledge there are no published in-depth studies on expulsions in common marmoset colonies, although there do exist data for related species, such as lion tamarins (*Leontopithecus rosalia*) [13, 14]. The general impression is that this behaviour mimics aggressive interactions in the wild which result in the dispersion of the expelled individual. Obviously, captivity has the effect of exacerbating such behaviour, where the attacked monkey cannot escape (violent expulsions have not been observed in the wild) [15].

In any case, what it is of interest is how to deal with social expulsion, which must be distinguished from day-to-day aggressive behaviour, and the promptness in doing so can prevent serious injuries from occurring. In families of common marmosets quarrels normally occur, yet they are usually temporary, the opponents reciprocate, and there are no evident consequences on the relationship between the two animals. By contrast, when social expulsion occurs, the victim rarely reacts to the attacks and continuously

tries to avoid the attacker; every time the victim sees the attacker, it emits vocalisations similar to those uttered by begging infants. This behaviour can continue on a daily basis, without interruption. At this point, one of the two individuals should be removed.

With regard to interactions between families, observations in the wild have shown that marmosets are quite territorial: when different families meet in the forest, the animals engage in a series of facial displays, body postures, and vocalisations aimed at scaring the other family away; actual physical aggression is rare. Thus, in captivity, where the animals cannot escape, different families must never have direct physical contact, and since even the constant view of different families can be a source of stress, the cages should be visually separated, for example using curtains. Nonetheless, acoustic communication among families can be beneficial. In the wild, some of the 30 sounds that comprise this species' repertoire are used to communicate with other groups, for example, the "phee-call", which is used by a family to communicate its position to other families. Acoustic communication is an essential aspect of the social life of common marmosets, which also engage in this behaviour when in captivity, with families housed in different rooms continuously exchanging these calls.

The potential of common marmosets to engage in aggressive behaviour should also be taken into account when creating new families in a captive colony. The following observations derive from our personal experience. It is good practice to initially allow individuals from different groups to familiarise themselves with one another through sessions of visual contact, the duration of which can be increased day to day. This can also serve as a means of checking for signs of aggressiveness. If the animals are calm, then a minimum of physical contact can be allowed, separating the monkeys with a wire mesh. The individual researcher, based on his/her knowledge of the temperament and personal history of the animals, can determine the number of sessions necessary before housing the monkeys in the same cage. Once housed together, the animals must obviously be observed very closely.

In practice, when pairing a male and female, relatively few problems occur. Signs of sexual behaviour are usually evident upon initial visual contact (e.g., tongue-flicking [16]), and although the female may not immediately accept the male's courtship behaviour, new breeding pairs can be easily formed. However, the procedure can be more difficult, at times failing, when three individuals are involved. For example, Yamamoto (personal communication), when pairing pairs of adult females with a new male, observed that the females promptly establish a dominance relationship, yet without obvious signs of physical aggressiveness. However, in some cases, one

of the females attempts to expel the potential sexual competitor. Thus the familiarisation procedure described above must be closely followed and carefully monitored.

Finally, it should again be stressed that in order to control the level of aggressiveness within a captive colony and allow the animals to live a relatively satisfying social life, the personnel involved with the animals, at any level, must have a knowledge of the temperament and personal history of all individuals under their care.

### Physical enrichment

As stated, the physical environment must be provide adequate complexity and variety and reflect to the greatest extent possible the natural environment. For example, in nature marmosets communicate their social status and the possession of a given territory by marking trunks and branches of trees with urine and gland secretions, which provides them with an important sense of identity. It is thus essential that the opportunity to express such behaviour be provided in captivity. To this end, wooden perches and branches of different sizes must be placed within the cages. A wooden nest is also necessary, in that it allows the family members to sleep in close contact with one another and makes them feel safe from possible predators (in captivity, monkeys retain the fear of predators, even if they have never actually encountered one). The wood of the nest becomes imbued with the smell of the members of that particular family, which every night confirms each individual's sense of belonging.

Another important aspect of the physical environment is the opportunity to develop and to exercise locomotory skills. In our colony, we have observed that infants spend a considerable portion of their time trying to balance on branches of different sizes and that they use twigs to practice their grasping skills. We have also observed that adults obtained from a breeding facility in which many marmosets were housed in small cages with no branches were unable to correctly balance themselves on horizontal branches and that when jumping from the top of the cage to a branch below they often missed the target and landed on the cage floor, whereas such locomotor difficulties were never observed in adults born in our colony. Thus marmosets should be provided with wooden perches of varied diameter and orientation, taking into account species-specific preferences [17]. Different degrees of firmness of the branches could help the marmosets to exercise their jumping skills. Finally, when furnishing the cage with such substrates, it should be considered that Callitrichids tend to avoid the lower parts of the

cage, which perhaps reflects a strategy against predator attacks. Unlike other New World species, such as *Cebus* spp., marmosets are not particularly manipulative; thus portable objects are not essential, although they can be a potential source of enrichment.

### Feeding enrichment

In nature, monkeys spend a good amount of time looking for and processing food [18], which also helps them to exercise their cognitive abilities [19]. Thus feeding enrichment in captivity is important, and it only requires a relatively small investment in terms of cost and time. For example, food can be scattered on the bottom of the cage, and experimental data have shown that, when given the choice, monkeys in captivity prefer working for their food rather than readily obtaining and consuming it (see, for example, [20]). Food can be made more difficult to obtain by using feeding devices or puzzle-feeders. McGrew *et al.* provided common marmosets, which are regular extractors of plant exudates, with a wooden cylinder drilled with holes filled with Arabic gum and reported that the animals readily learned to gnaw the device to extract the gum [21]. In another case, common marmosets were provided with a foraging tree, which consisted of a PVC pipe, connected with other T-shaped PVC pipes along its length; holes were drilled on the surface and food treats were inserted in both the pipes and the holes. When the foraging tree was not present, the marmosets spent 1-25% of their time foraging, whereas when provided with the tree they spent 80% of their time foraging, which more closely approximates the use of time in nature [22]. Puzzle-feeders have also been used with marmosets. In a study by DeRosa *et al.*, marmosets were given the choice of simply taking food from food dishes or working to extract it from plastic boards with a series of holes, hung at different heights. These puzzle-feeders were somewhat successful in increasing the time spent on feeding activities and thus contributed to more closely replicating the natural time budget. An important finding of this study was that families with younger individuals were generally more active and interested in using the puzzle-feeders than families that consisted only of adults [23], suggesting that a group's age composition can be important in determining the success of certain types of physical enrichment (for a brief review of different types of feeding enrichment [24]).

With respect to both physical and social enrichment, it is important to stress the need to provide monkeys with the opportunity to exercise some degree of control. As stated by Buchanan-Smith: "... many of the devices do not provide ecologically valid stimuli ... It is argued that rather than, or in addition to, providing

specific enrichment devices, the alternative is to design enclosures, and have routine housing, husbandry and feeding regimes which permit a degree of control. Variability within the environment alone permits an animal some control and exerts preferences, for instance by incorporating unpredictable environmental perturbations which require an adaptive response from the animal" [17, p. 48].

### Conclusions

Maintaining a satisfactory balance between welfare requirements and the need for reliable, high quality data is clearly not an easy task. However, we feel that in-depth ethological knowledge of the species being studied can play a vital role in optimising both the conditions of captivity and the carrying out of experiments, and that the animals' quality of life and the quality of the data collected will inevitably go hand in hand. Hopefully, the concerns raised by the use of non-human primates in biomedical sciences will pave the way for a more ethical treatment of other vertebrates as well. On this note, we wish to quote Marian Dawkins, one of the most attentive researchers in the field of animal welfare: "Let us not mince words: animal welfare involves the subjective feelings of animals. The growing concern for animals in laboratories, farms, and zoos is not just concern about their physical health, important though that is. Nor is it just to ensure that animals function properly, like well-maintained machines, desirable though that may be. Rather, it is a concern that some of the ways in which humans treat other animals cause mental suffering and that these animals may experience "pain", "boredom", "frustration", "hunger", and other unpleasant states perhaps not totally unlike those we experience." [25, p. 1].

For further reading on this subjects see:

- Cavalieri P, Singer P. *The great ape project*. London: Fourth Estate; 1993
- Davis H, Balfour D (Ed.). *The inevitable bond*. Cambridge: Cambridge University Press; 1992
- Segal EF (Ed.). *Housing, care and psychological wellbeing of captive and laboratory primates*. Park Ridge, New Jersey: Noyes Publications; 1989
- Wise SM. *Unlocking the cage. Science and the case for Animal Rights*. Oxford: The Perseus Press; 2002

or refer to the following Web sites and Discussion groups:

- Associazione Primatologica Italiana (Italian Association of Primatology): <http://www.unipv.it/webbio/api/api.htm>
- Mailing list: LAREF@yahoogroups.com. (discussion group on enrichment for laboratory animals)
- Animal Welfare Institute: [http://www.awionline.org/lab\\_animals/biblio/laball.htm](http://www.awionline.org/lab_animals/biblio/laball.htm). (bibliography on enrichment for laboratory animals)
- University Federation for Animal Welfare: <http://www.ufaw.org.uk>

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