Preparing New World Monkeys for Laboratory Research

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Abstract

New World monkeys represent an important but often poorly understood research resource. The relatively small size and low zoonotic risk of these animals make them appealing as research subjects in a number of areas. However, historic portrayal of many of these species as difficult to manage and handle is one of the factors that has limited their use. Basic guidelines are provided on management and handling approaches for the New World monkeys most commonly used in research: marmosets, squirrel monkeys, owl monkeys, and titi monkeys. Topics include transport and acclimation to a new facility, location changes within a facility, diet changes, removal from and return to social groups, capture and restraint, handling for anesthesia, post-procedural monitoring, and staff training.

Key Words: acclimation; husbandry; management; marmoset; owl monkey; squirrel monkey; titi monkey; training

Introduction

The purpose of this article is to provide basic guidelines on management and handling approaches for the following four New World monkeys most commonly used in research: the common marmoset (*Callithrix jacchus*), the squirrel monkey (*Saimiri* sp), the owl monkey (*Aotus* sp.), and the titi monkey (*Callicebus cupreus*). Features both common to all species and unique to certain species are described. The text below begins with an overview of life history, reproduction, basic social structure, and response to novelty for each species.

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Overview of Species

Marmosets

Marmosets are members of the primate family Callitrichidae. The Callitrichidae is a species-rich family; however, only the common marmoset (*Callithrix jacchus*) is routinely used in laboratory research. Callitrichids are the smallest of the anthropoid primates; on average, common marmosets weigh approximately 350 g. They are primarily arboreal animals that have claws, allowing them to cling vertically. The animals use this form of locomotion and posture frequently. They have well-developed auditory and olfactory communication. With a fertile postpartum estrus and a typical litter size of two to three offspring, the callitrichid primates have the highest fertility rates of any primate. Readers are referred to Abbott et al. (2003) for a recent overview of marmoset biology and life history. Expert information is also available through the Marmoset Research Group of the Americas (MaRGA: www.marmosetresearchgroup.org) and the European Marmoset Research Group (www.emrg.org).

Marmosets are typically housed socially as mated pairs plus offspring. Such groups are easily formed and generally stable for many years (Layne and Power 2003). Their social system focuses around home range or group defense and cooperative rearing of infants. They are sexually monomorphic. Striking dominance relationships do not exist within their groups although breeding females often display dominance over access to a preferred food item (Tardif and Richter 1981). Marmosets begin puberty before 1 yr of age and are fully mature sexually and skeletally by 2 yr of age. However, due to physiological and behavioral suppression, offspring can remain with the breeding pair indefinitely and will generally not reproduce as long as the original mated pair remains healthy and intact. Multiple group members, including the dam, sire, and older offspring, all participate in the care of dependent infants. Care includes transporting the infants on their backs and providing them with solid food once weaning begins.

Social groups of unrelated adult common marmosets

may also be formed. These groups are particularly useful for mechanistic studies that capitalize on aspects of the dominance regulation of female reproduction such as hypothalamic amenorrhea (Abbott et al. 1997), hypocortisolemia (Saltzman et al. 1998), or maintenance of bone density in a hypoestrogenic environment (Colman et al. 1997). It is possible to maintain marmoset social groups that comprise two to four unrelated adult females and two to four unrelated adult males for many months to years (Abbott 1986, 1993). These groups are initially established in large observation rooms equipped with multiple nestboxes, tree branches, and cardboard boxes, which permit easy locomotion through a complex three-dimensional environment (Saltzman et al. 1998). Between 2 and 4 wk after initial formation, the intact groups may be moved safely to smaller, less complex cages.

Marmosets are both predators and prey in the wild, and their behavior in captivity reflects these roles—particularly that of prey. They are extremely vigilant and spend much of their time surveying their surroundings. They have well-developed alarm vocalizations, and alarm calling in one cage will frequently generate a chain reaction. Marmosets are also neophobic, although it is possible to quickly habituate the animals to new situations.

Squirrel Monkeys

Squirrel monkeys (genus *Saimiri*) are gregarious, socially living primates that adapt well to the captive environment. In captivity it is advisable to maintain the animals in species-typical social groupings as much as possible. Generally, social groups are maintained in captivity with only one or two males per group to avoid problems with aggression. Group size can range from a single pair to a maximum of 35 to 50 animals per group, based on available housing and the project for which the animals are being maintained.

Female squirrel monkeys reach maturity and begin breeding at approximately 2.5 to 3 yr of age. Males reach sub-adulthood by the time they are 2.5 to 3 yr old. In the wild, males of some species transfer from the natal group at that time. They may then join an all-male group of juveniles and sub-adults for a time until they become fully adult at about the age of 5 yr and are able to work their way into the male dominance hierarchy of an established group and to begin breeding.

Dominance hierarchies within squirrel monkey groups vary in form and degree among species. *Saimiri boliviensis boliviensis*, which show a high degree of sexual segregation within their social groups, demonstrate distinct linear dominance hierarchies among males. Dominance is associated with higher testosterone levels and, generally, copulatory frequency. A separate but less distinct dominance hierarchy is seen among females of this species (Mendoza et al. 1978). *Saimiri sciureus* spp., which tend toward sexual integration within a social group, demonstrate a linear dominance hierarchy that includes both sexes, with all males being dominant over all females (Mendoza et al. 1978; Mitchell et al. 1991).

Owl Monkeys

Owl monkeys (genus *Aotus*) are the only nocturnal simian primate. *Aotus* are arboreal (Kinzey 1997), although they have been reported to cross open savanna when moving between forest patches (Rathbun 1980). They become most active when the moon is bright, and they travel the same route each night, suggesting that they may memorize routes during bright moonlight travel (Wright 1989).

The basic owl monkey social unit is a breeding pair and their offspring. The young stay with their birth group until between 2.5 and 3.5 yr old, when both sexes then disperse (Kinzey 1997). The father is the main infant caregiver and gives the infant to the mother only to suckle (Jantschke et al. 1998). If the father dies when the infant is still young, other siblings may assume the caregiver role, but not the mother. Jantschke and colleagues (1998) reported that an infant reared without a father became independent at 12 wk versus 33 wk for an infant with a father.

Owl monkeys are very territorial. Territories are extremely small (usually $\leq$ 10 ha) for a primate of its size. Territories between neighboring groups overlap extensively (Wright 1978). Male-male aggression is common and is a factor in keeping groups apart (Moynihan 1964). Agonistic encounters involve back arching, stiff-legged jumping, piloerection, urination and defecation, as well as giving clicking/grunting alarm calls. Identical agonistic displays occur between conspecifics and other species (Wright 1978).

Titi Monkeys

Titi monkeys (*Callicebus cupreus*, previously classified as *Callicebus moloch*) are a somewhat less common laboratory primate than squirrel monkeys, owl monkeys, or callitrichids. Titi monkeys are small (~ 1 kg) New World primates.

Similar to owl monkeys, titi monkeys form small family groups that are centered around a male-female pair. Offspring of both sexes remain with the parents until adulthood. In the laboratory and in nature, young titi monkeys reach reproductive maturity at approximately 18 mo but do not breed when living with their natal group. The mated pairmates have a strong emotional bond (Mason and Mendoza 1998; Mendoza and Mason 1997a), which is demonstrated in the course of their normal behavior by huddling, tail-twining, and following closely behind the mate (Mason 1966, 1968, 1974, 1975). The selectivity of the bond clearly differentiates titi monkey relationships from those of non-monogamous primates such as squirrel monkeys (Cubiciotti and Mason 1978). Young titi monkeys form an attachment bond with their fathers and a weaker bond with their mothers (Hoffman et al. 1995a) (i.e., separation from the father, but not the mother, results in behavioral and physiological signs of distress). Parents do not form emotional bonds with their offspring and do not respond to separation from their offspring. Siblings do not participate in infant care and do not derive any future reproductive benefit from observing or

participating in rearing younger siblings (Mendoza, unpublished data).

Titi monkeys show a greatly heightened response to novelty (Hennessy et al. 1995). The animals have a relatively low baseline heart rate and a relatively greater increase of heart rate in response to administration of the cholinergic antagonist atropine (Mendoza and Mason 1997b). The negative feedback of their hypothalamic-pituitary-adrenal system is much stronger in titi monkeys than in squirrel monkeys (Mendoza and Moberg 1985). The implications of these physiological adaptations for well-being in captive colonies of titi monkeys have been fully considered and are reported elsewhere (Mendoza 1991).

Husbandry Issues Relative to Laboratory Research Use

Many excellent resources are available that provide overviews of husbandry, psychological well-being, and research management of various New World primates. These references are provided in Table 1. The ensuing discussion focuses specifically on the manner in which New World primates should be managed in the following situations that entail novelty or stressors: transport and acclimation to a new facility, location changes within a facility, diet changes, removal from and return to social groups, capture and restraint with and without anesthesia, handling for anesthesia, postprocedure monitoring, and a brief discussion of staff training.

Transport and Acclimation to a New Facility

Receiving animals. When receiving marmosets, owl monkeys, or titi monkeys from another facility, every attempt should be made to minimize stress. Transportation and sudden changes in housing, personnel, or feed are stressful for the common marmoset. It is advisable to supply food that is identical or similar to that of the source colony. New feed stuffs should be introduced slowly by mixing with source colony feed in increasing amounts over a period of time. It is also important to determine the type of water supply the animals were using previously —automatic sippers (ball-type or open) or water bottles—because some animals may not adjust to the new method quickly. After arrival, the animals should be monitored very closely to ensure that water and food are being consumed. A few extra pieces of fruit such as grapes or oranges may help ensure adequate hydration. Personnel traffic through the area should be limited, and animals that display clinical signs such as anorexia, lethargy, or abnormal behavior should be examined clinically.

Owl monkeys. Owl monkeys should be shipped as pairs or family groups whenever possible. These animals provide extensive paternal care to offspring, therefore isolating the dam and offspring for an extended period of time can lead to excess stress and infant abuse or abandonment. The shipping container should resemble the animal's nestbox. Including a perch will give the animals a place to get off the floor and help reduce contact ulcers. Small amounts of the animal's usual commercially prepared biscuits and fruit with a high-level water content should also be included.

New animals are best received during their light or sleeping period; the animals appear to be less reactive to the environment, and they typically move directly to their new nestboxes. The animals then awake at the onset of dark to a situation they have been able to observe quietly from the safety of their nestboxes. When receiving owl monkeys, it is important to be aware of and avert the following common medical problems: (1) *Aotus* show a tendency to cardiomyopathies, which the stress of shipment can worsen, and this condition can lead to sudden unexpected deaths. (2) Owl monkeys do not react well to quick changes in diet (see Diet Changes).

Squirrel monkeys. Shipping or receiving squirrel monkeys can be a relatively straightforward task. *Saimiri* are one of the first species that move into disturbed forests and appear to handle new environments with little stress. However, it is important to note that squirrel monkeys have a high metabolic rate and can slip into hypoglycemia if their food intake is not monitored. At all times during shipping, it is necessary to provide access to water and fresh fruit such as grapes, which have a high level of water and sugar content; however, apples should be avoided because they may cause digestion problems. It is also advisable to include a small amount of commercially prepared food so that the animals arrive with a familiar food. If the animals as being

Table 1 Overviews of management of various New World primate species

Species	Subject	Reference (see text)
Marmoset	Husbandry	Layne and Power 2003
	Clinical care	Ludlage and Mansfield 2003
	Research management	Schultz-Darken 2003
	Reproduction	Tardif et al. 2003
Titi monkey	Husbandry	Lorenz and Mason 1971
	Psychological well-being	Mendoza 1991
	Reproduction	Valeggia et al. 1999
Squirrel monkey	Husbandry	Williams and Glasgow 2000
	Clinical care	Abee 1985
	Reproduction	Williams et al. 2002
Owl monkey	Husbandry	Weller et al. 1985
	Clinical care	Baer 1994
	Reproduction	Merritt 1980

transported a long distance, we recommend providing the animals with extra fruit, biscuits, and water every 12 hr.

Shipping containers for squirrel monkeys should contain a perch or some way for the animals to get up off the floor. This design allows the animals to sit in a species-typical posture and decreases the chances of a tail or foot contact ulcer. It is important to know how the animals were housed before being shipped, including their previous food and watering schedule and the light/dark schedule. Shifting the animals to a new feeding schedule over a few days is acceptable as long as they are monitored for weight loss and dehydration.

Location Changes within a Facility

All of the genera can be trained to switch cages without being manually caught. A positive reinforcer can be used to induce the animals to transfer to a new cage rack. This technique provides a stress-free way to transport animals or to move them to special cages for experimental procedures (e.g., if the animals are used in an operant conditioning study in which the test cage can be moved around).

Chemical communication. All of the genera use chemical communication and scent-mark their environment. For this reason, frequent changes into a clean cage or a cage recently occupied by another animal can be stressful and result in excess locomotion and time spent marking their new environment. Marmosets, however, are routinely placed into cages with wood surfaces marked by other groups with no untoward effects on health, behavior, or reproduction.

Family unit separation. Marmosets can be removed from families and paired with an unrelated, opposite-sex cagemate; however, it is usually better to put newly formed paris in another room or well removed from their original family group. When a breeding adult in a family dies, it is possible, with care, to introduce an unrelated step-parent. Separation of the remaining juveniles into their nestbox during the introduction of the step-parent to the remaining parent will provide the juveniles with time to observe the adults from the nestbox and accept the new step-parent without the usual family mobbing that would otherwise occur.

Titi monkeys. A change from outdoor enclosures to indoor housing resulted in more successful reproduction in the California National Primate Research Center titi monkey colony (Mendoza and Mason, unpublished data). However, even relatively minor changes of location for titi monkeys should be considered carefully. It has been reported that a change in location, even one nearly identical to the homecage, results in elevated plasma cortisol level for approximately 48 hr, which could be important to the outcome of a study (Hennessy et al. 1995). The presence of the pair-mate reduces the sensitivity of the response (Hennessy et al. 1995).

Squirrel monkeys. If possible, squirrel monkeys should be housed with familiar cagemates initially. It may be possible to create new groups of unfamiliar females and young males; however, we advise against introducing unfamiliar males.

Diet Changes

All of the New World primates described here are omnivores, which eat fruits, leaves, and insects in the wild. In captivity, the animals are typically fed commercially available diets (pelleted commercially prepared biscuits, canned diet, or gelatin-based diet) formulated for New World Monkeys. An extremely wide variety of supplements (e.g., fresh vegetables, nuts, insects, yogurt, peanut butter) are generally provided, either daily or at a minimum of two to three times per week. Animals are typically fed each morning and again in the afternoon.

Generally, it is possible to change the diet of squirrel monkeys relatively easily. However, marmoset, owl, and titi monkeys respond poorly to abrupt dietary change. Any changes should occur gradually over several weeks to 1 month. New feed stuffs should be introduced slowly by mixing with source colony feed in increasing amounts over a period of time. Because of the very sensitive response to novelty demonstrated by titi monkeys (Hennessy et al. 1995) and the possibility that they will refuse to eat a novel food, it is advisable to present a relatively varied diet on a daily basis. We recommend a similar approach with many marmoset colonies, although care must be taken so that the animals do not self-select a diet that is poor in required nutrients. With an appropriate transition, marmosets can be maintained on a single diet if required for study purposes. Marmosets and titi monkeys are also susceptible to gastrointestinal diseases and diarrhea (Lorenz and Mason 1971; Ludlage and Mansfield 2003; Mendoza 1991), making it even more important that any diet changes are considered with the utmost care before implementation. However, once titi monkeys proceed past the initial reluctance to eat a novel food, they are relatively indiscriminate about what they will eat. Titi monkeys also demonstrate passive sharing of food between pair-mates. In addition, transfers of food between parents and offspring occurs in marmosets and titi monkeys (Fragaszy and Mason 1983), perhaps providing a means by which offspring learn to eat novel foods.

Research Use Issues

Removal from and Return to Social Groups

When marmosets are removed from social groups for short (< 20-min) procedures such as blood sampling (Hearn 1977, 1983), the individuals are gently herded into one of their home cage nestboxes for transport and return. When individual marmosets are removed from unrelated social groups for relatively lengthy (> 20-min) procedures, it is important to also remove the remaining same-sex animals and place

them in temporary single cage housing for the duration of the procedure. Failure to remove the remaining same-sex animals greatly increases the risk of group members attacking the removed individual upon its return to the social group at the end of lengthy procedures.

Short-term removal from a social group does not appear to affect squirrel or owl monkeys. Removal from 1 to 10 days for clinical or experimental treatment does not affect reintroduction into the social group. Colony management can include routine removal for up to 1 mo of new squirrel monkey mothers with the infants from their social group to a "maternity group" consisting of only dams and infants. In our combined experience with more than 700 such reintroductions, we have never observed dam-infant pair rejection.

An important consideration for *Aotus* is the fact that infants are carried most often by the father. New infants left with only their dam are in jeopardy of abuse and neglect. Owl monkeys assigned to protocols that require extensive removal of the male should not be placed in breeding groups.

Titi monkeys show sustained glucocorticoid elevations in response to separation from attachment figures (Mendoza et al. 2000). This response persists for long periods of time (at least 30 days) and decreases when the monkeys are returned to their respective attachment figures or upon formation of a new heterosexual pair (Mendoza et al. 2000). Although it is preferable to house titi monkeys in pairs or families, there is currently no evidence that prolonged individual housing is detrimental to the health of the animal (Mendoza 1991). However, offspring can be left in the natal cage with their parents well beyond the age of reproductive maturity without aggression, and female offspring at least can sometimes be returned to the social group after extended removal. In one study, adult daughters were removed from their natal cage, isolated for 1 mo, and then returned (Hoffman 1998; Hoffman et al. 1995b; Valeggia 1996). In seven of eight cases, the female was reintegrated into the group with minimal aggression; in one case, aggression was persistent enough that the female had to be removed.

A similar experiment in young adult males removed from their natal group has not been performed. However, it would probably be more difficult to return a male to his natal group after housing him separately for an extended length of time, even without exposing him to a female.

Capture and Restraint

"Box Training"

All of the genera can be trained to enter a box for transport. This box training requires shaping the animals' behavior to enter the box for reinforcement. First the box is shown to the animals, and they are allowed to move freely in and out. Then a positive reinforcer is used to induce the animal to stay in the transport box. Once the animal is trained to sit in the box for a period of time, the door of the box is slightly closed while the animal receives additional reinforcement. This procedure is continued until the animal will sit in the box with the door closed for extended periods. Subsequent use of the box provides a nonstressful way to transport these monkeys. Titi monkeys are considered slow to habituate to many behavioral tasks including learning and problem-solving, feeding, and behavior in a novel environment (Fragaszy and Mason 1983; Savastano et al. 2003). For this reason, extra time should be built into the habituation period of a study.

Laboratory Procedures Not Requiring Sedation

Detailed descriptions of a variety of capture and restraint methods for marmosets have been published recently (Layne and Power 2003; Schultz-Darken 2003). Straightforward training procedures can readily prepare common marmosets living in male-female pairs, families, and social groups (Abbott 1984; Baker et al. 1999; Saltzman et al. 1994) for a variety of laboratory procedures (Abbott et al. 1998; Barnett et al. 2005; Ferris et al. 2001; Hearn 1977; Schultz-Darken et al. 2004). With such training, marmosets can generally be handled for painless or minor procedures without sedation. The animals may be gently herded into their home cage nestbox. When a marmoset does not voluntarily enter its nestbox, it may be manually captured inside its homecage by a caretaker wearing leather gauntlets and placed into its nestbox. After the first 2 to 3 wk of regular capture, marmosets normally enter their nestboxes voluntarily when herded. Animals may be transported in a nestbox from their homecage to a nearby procedure room where they are manually removed from the nestbox and may be placed into a variety of restraint devices (see Layne and Power 2003 for details).

One commonly used marmoset restraint device is a short (~9.5 cm long × ~7 cm diameter) polyvinylchloride cylinder mounted approximately 60° from vertical on a customized cart with an adjustable working height (see Schultz-Darken 2003 for details). Once the blood sample is taken, the marmoset may be given a small food treat and then returned to its nestbox for transport back to its home cage. This entire procedure usually takes approximately 6 to 8 min per animal. Such repeated capture, restraint, sampling, and reward by the same personnel over approximately 4 wk results in acclimatization of marmosets to the procedure such that repeated blood sampling in the course of one morning does not elevate circulating levels of cortisol (Saltzman et al. 1994; Smith and French 1997). A key factor for such successful preparation of marmosets involves frequent and regular engagement of the animals to the same procedures using the same personnel. The following steps will further enhance the animal's ability to train for restraint:

- Strictly limit exposure to sights and sounds outside the training area (e.g., by use of a white noise machine);
- Use a "model/therapist" animal (i.e., an animal that is

already easily habituated to the restraint) during the training of difficult cases; and
- Move away from and slowly reapproach highly reactive animals, remembering that leaving a distressed animal may be just as reinforcing as a food reward.

Certainly, careful training results in marmosets that are better able to cope with laboratory procedures (Bassett et al. 2003).

Neuroscience Procedures

Marmosets can be trained for use in more manipulative procedures such as direct infusion of peptides into the brain (Barnett et al. 2005). For such procedures, marmosets are placed into a restraint tube with the head gently held manually to allow removal of a stylet, infusion of peptide, and insertion of a new stylet (Barnett et al. 2005). It has been reported (Barnett et al. 2005) that by completion of the first experiment in a three-experiment study, female marmosets voluntarily bowed their heads while in the restraint tube, thus enabling stylet removal and peptide infusion without head restraint.

For longer restraint during neuroendocrine experiments, marmosets can be adapted to sling-harness restraint at least 2 yr before a study (Schultz-Darken 2003; Schultz-Darken et al. 2004) by a series of trials of increasing duration. Marmosets are fitted with a sling-harness jacket and placed into the harness restraint apparatus that suspends the animals over a wire-mesh platform but permits a variety of species-typical postures including standing, squatting, or huddling (Schultz-Darken et al. 2004). During each restraint trial, the marmoset in the sling harness remains in full view of its cage mates that are situated within 0.3 m of the restrained animal in a small cage. Optimal adaptation training commences with 1 hr of restraint on day 1, 4 hr of restraint on day 2, 8 hr on day 3 or 4, and overnight (24-hr) restraint starting on day 7 or 8 (Schultz-Darken et al. 2004). During training, marmosets learn to eat ad libitum from the food tray and to drink fluids offered to them by syringe. From quantitative behavioral assessments, marmosets adapt to their sling-harness restraint by the 4-hr restraint trial (Schultz-Darken et al. 2004). Because circulating cortisol levels remain within the normal diurnal range during the entire push-pull perfusion and restraint procedure, and regular ovarian cycles are not disrupted in females with intact ovaries (Schultz-Darken et al. 2004), the careful training and preparatory techniques appear successful in acclimatizing marmosets to such neurobiological procedures. A similar training procedure is used for marmosets adapted to being awake in a horizontal restraint and head holder for functional magnetic resonance imaging experiments (Ferris et al. 2001).

Other Settings and Procedures

Capture and restraint of *Saimiri* and *Aotus* from a social setting are relatively easy as long as the animal to be captured is not an infant. In large cages (7 m × 2.5 m × 1.5 m), the capture is usually accomplished by suitably attired personnel (wearing protective leather gloves, standard latex gloves, and face mask) who move into the cages. When the animal runs past, it is caught manually. Two hands are used to control the upper and lower extremities of the animal. A similar procedure is used for *Aotus* and *Saimiri* housed in smaller cages, in which the technician reaches his or her upper torso or arms into the cage. Well-trained technicians can capture animals without harming the animals or being bitten (Bernacky et al. 2002; Fortman et al. 2002).

Adult female and male *Saimiri* will defend infants, and capture requires two individuals to enter the cage. As the individual with the infant moves out of the cage, the other technician stands between the infant and the rest of the social group. The use of nets or similar capture apparatus is discouraged, mainly because animals may get caught in the rope and suffer a bone fracture.

Restraint of nonsedated animals can be safely accomplished with both *Saimiri* and *Aotus*. Adults of either species rarely weigh more than 1.5 kg and can be handled by most individuals using two hands, one on the upper torso and the other holding the legs or pelvis area. It is possible to restrain animals for minor procedures using this technique.

Using manual restraint, two people can collect blood samples from both squirrel and owl monkeys. One person uses the leather capture gloves to manually restrain the torso of the supine subject, which is laid across a table. The person taking the sample can easily secure the legs of the subject with one hand and take a blood sample from the femoral vein.

Physical restraint methods can produce adverse effects, especially elevated cortisol levels (Butler et al. 1995; Cubicciotti et al. 1986). In addition, it has been reported that in squirrel monkeys, plasma glucagon and glucose levels increase in response to restraint procedures (Myers et al. 1988).

Titi monkeys are very timid and nonaggressive; however, when capturing and restraining them, it is advisable to wear elbow-length leather gloves. Titi monkeys can be trained to enter a transport box readily and then can be restrained manually for blood sampling. The person restraining the monkey should put one hand spanning the back with the thumb and two fingers under the armpits, with another two fingers immobilizing the head. The wrist should be flexed slightly so that the monkeys head is tilted backward. The other hand should hold the calves together directly under the knees, gently flexing the knees.

If it is necessary to remove a titi infant for experimental reasons, it is advisable to place the infant's carrier in the transport box. While one person lightly restrains the carrier at the shoulders and waist, the other person can remove the infant, which is usually on the carrier's back. As long as care is taken to disengage the infant's grip on the carrier's fur, the carrier is not disturbed by this procedure. If animals are well habituated, it is sometimes possible to peel an

infant off an adult's back gently without capturing or handling the adult.

Handling for Anesthesia

For all of the genera, isoflurane is the recommended anesthesia for extended periods (> 1 hr) and for anesthesia of compromised animals. Animals may be anesthetized easily using hand restraint, and induction usually requires less than 2 min. Use 4 to 5% isoflurane for healthy animals, and less for animals that are compromised. Once induced, animals may be maintained with 0.5 to 3% isoflurane. Marmosets can be intubated with an 8 French feeding tube cut to an appropriate size. This technique guarantees an airway in the case of emergency (Ludlage and Mansfield 2003).

For short-duration (20-min) anesthesia, ketamine alone is effective at doses of 20 to 40 mg/kg. For procedures that might be painful, xylazine should be given with ketamine at 20 mg/kg of ketamine plus 4 to 6 mg/kg of xylazine. Both ketamine and xylazine should be given subcutaneously or intramuscularly in the quadriceps.

Ketamine is a dissociative anesthetic. Its effects begin approximately 5 min after the injection and last for 20 to 30 min. When used with xylazine, it is possible to obtain good muscular relaxation (Bennett 2003; Butler et al. 1995; Clements et al. 1982; Ochsner 1977). Ketamine is known to elevate estradiol and luteinizing hormone levels in squirrel monkeys (Yeoman et al. 1988), to affect monoamine metabolites (Hamby et al. 2004), and to affect normal infant behavior for as long as 24 hr after administration (Pierre et al. 2003).

Another short-acting anesthesia frequently used is initial sedation with a very small dose of ketamine (1-3 mg/kg) followed by 0.01 to 0.03 mg/kg of medetomidine for quick procedures. This dosage is reversed with 0.05 to 0.15 mg/kg of atipamezole. The advantage is a quick recovery to normal function and avoidance of some of the dosing effects of ketamine (Ferris et al. 2004).

In titi monkeys, anesthesia is generally induced by ketamine at 10 to 20 mg/kg and maintained under isoflurane anesthesia at 1 to 2%. However, caution must be taken when sedating titi monkeys because these animals metabolize ketamine quickly. They also have a complicated lingual and hyoid apparatus that makes intubation difficult. The unusually high levels of parasympathetic activity in titi monkeys lead to lower overall heart rate (Mendoza and Mason 1997b) and much higher beat-to-beat variability. Because these characteristics, very low and irregular cardiac activity, might be considered pathological for a squirrel monkey or a macaque, it is important for clinicians accustomed to working with other species to realize that these characteristics are normal for a titi monkey.

Postprocedure Monitoring

Care of the surgical patient does not end when the procedure is competed. Controlling pain with preoperative and postoperative analgesics is a crucial component to surgical recovery (Ludlage and Mansfield 2003). For all species, it is best if the animal is recovered in a quiet area where it can be observed and then returned to its previous social environment. Due to the animals' small size but large relative surface area, preventing hypothermia is vital to postsurgical recovery. Methods to prevent hypothermia include heating pads, heating lamps, heating packs, and warm saline bags. It is important to prevent burns and avoid placing heating lamps extremely close to the animal. Very palatable food should be offered as soon as the animal is fully awake to prevent hypoglycemia.

Perhaps because of the unusual structure of the vocal cords or the tongue of titi monkeys, it is very difficult to force-feed them without liquid entering the lung and producing pneumonia. Force-feeding should be avoided postoperatively and at any other time unless there is absolutely no alternative.

Staff Training

New staff members should learn and understand normal behaviors of the species to provide the best care. Only then can the abnormal behavior of distressed or ill animals be recognized. Particular caveats related to the care and use of these animals in a research setting appear below, and we encourage the staff member to consult the additional information in the foregoing text.

Marmosets. It is important to understand cooperative breeding and extensive caretaking by older siblings and fathers and to expect twinning and triplet births. Caretakers should be made aware that the vast majority of births occur at night. Dystocia is usually noticed early in the morning, and clinical signs include lethargy, recumbency, straining, vaginal bleeding, abnormal posturing, and in some cases a partially expulsed fetus (Hill 1969).

Occasionally in group-housed marmosets, individual animals may be prevented from reaching the feeding platform due to one or more continuously aggressive cagemates. These animals may be seen frequently on the cage floor and be vocalizing. It may be necessary to remove one of these animals. It is preferable to remove the subordinate animal if there is more than one aggressor in the group or the aggression cannot be identified. However, if a single aggressor can be identified, that animal may be removed instead.

As with any primate, minimally aversive routine is essential. In the case of marmosets, proper handling and experimental procedures should minimize any disturbance in the animal area to avoid reproductive and/or health problems in the colony. Proposed solutions to such problems have been documented in association with animal facility construction (Johnson et al. 1991).

Squirrel monkeys. Squirrel monkeys appear to be more tolerant than owl monkeys, titi monkeys, or marmosets to noisy and light traffic environments, which can also

act as social enrichment. In contrast, staff should be quiet around marmosets and titi monkeys. Extraneous noise sometimes appears to result in an increase in "chain-reaction" calling. *Aotus,* a nocturnal monogamous species, is especially susceptible to environmental disturbance. As a consequence of stressful situations, these animals can develop persistent high blood pressure, which can lead to cardiovascular disease.

Titi monkeys. Unlike many Old World primates, titi monkeys do not display an aversion or negative reaction to eye gaze. Titi monkeys are normally very gentle and timid animals and are not normally aggressive toward humans unless they are being handled. However, attacks have happened in rare cases, primarily by nonhabituated monkeys, and staff should therefore be cautious particularly before monkeys are habituated. Titis become habituated to close proximity of humans but usually will avoid contact.

Conclusion

New World primate species represent a valuable biomedical research resource, both because of their unique features (e.g., small body size, relatively early maturity, and low zoonotic risks) and as a means to meet the resource demand created by limited availability of Old World species such as rhesus macaques. New World primate species can be used effectively in a broad array of studies as long as appropriate housing, husbandry, and diet are provided and the habituation needs of each species are understood. Many of these species are highly neophobic, therefore their effective management and use in research require both attention to consistency in their environment and behavioral shaping programs that encourage habituation to new situations. We urge individuals who will be managing or using any of these species for the first time to communicate with experienced investigators and veterinarians.

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