LAREF discussions 2002-2023

The electronic Laboratory Animal Refinement & Enrichment Forum was founded 21 years ago. It allows the animal care community to exchange personal experiences and opinions about ways to develop and implement species-appropriate housing conditions and humane handling practices of animals assigned to research.

The present compilation is a collection of 324 discussions that have practical animal welfare relevance; they took place between September 2002 and September 2023. I am grateful to 237 LAREF members who participated in these discussions. Some comments were edited with the consent of the authors.

Mt. Shasta, California
October 2023

Viktor Reinhardt
viktor@cot.net

Table of Contents

Discussions on Macaques page 161

Basic Issues

< Does anybody know the approximate number of animals used in biomedical research? >

You are probably not surprised that the **annual usage of animals in biomedical research** is amazingly high. The estimated worldwide figure was over 192 million animals in 2015.

You start wondering if the gradual implementation of the three Rs really reflects a decline of the number of animals used and killed in laboratories, or if we are merely running in circles, blindly believing that we are reducing the suffering of animals kept in research labs.

One of the things one has to bear in mind is that in the United States—probably the primary user of animals in research—it is very difficult to get actual figures of the annual number of rodents used in research labs. This is not really surprising when you take into account that (a) rats and mice make up the great bulk of all rodents, and that (b) the U.S. is the only country that explicitly excludes rats and mice in its legal definition of the term animal; this implies that rats and mice don't count. They are not covered by the *Animal Welfare Act*, hence are not listed in the official annual reports of animals used in research. One also has to take into consideration that the number of genetically modified animals used in research has more than doubled in the last ten years and certainly contributed to the overall increase in the number of animals used in research.

I think the issues at hand are not specific distinctions of different animal categories but the huge number of living creatures killed for whatever research-related reason every year. A genetically modified animal is no less a sentient creature who clings to life than a traditional experimental animal.

< Why are **rats and mice excluded from regulations** and oversight in the United States? >

It's a purely economic decision. American research runs on rats and mice. In 2018, an estimated 111.5 million rats and mice, i.e., 99.3% of all mammals, were used in American laboratories (Carbone, 2021). Our laws are made by the most powerful lobbyists, not by the Congressmen themselves. In terms of animal suffering, we Americans are a lot less civilized than many of our coworkers in Europe.

I think it is important to add that although the Animal Welfare Regulations of the United States do not include mice and rats, the Public Health Service Policy on Humane Care and Use of Laboratory Animals

refers to all animals used in research, mice and rats included. This policy must be followed by any institution receiving federal funds. Additionally, many institutions—including private industry groups—are also AAALAC accredited, which uses the *Guide for the Care and Use of Laboratory Animals* as the current standard of care. So, while there are some facilities that do not fall into either of these categories (and there are a few, I will concede), the majority of animal research does follow a standard of care similar to that described in the animal welfare regulations promulgated by the United States Department of Agriculture.

I see here two problems:

1. "Animal facilities should (not *must*) be operated in accord with" the Guide and the PHS Policy (National Research Council, 1996, page 2).
2. The National Institutes of Health, who administers the federal funds, is not an enforcement agency; therefore, the public has no guarantee that the PHS Policy is actually followed by institutions receiving NIH grants.

< How do you **relate to the animals in your charge**? >

It is a custom in biomedical research to use the pronoun it rather than he or she when referring to an animal, even if the animal is assigned to a project in which gender-related phenomena—e.g., reproductive physiology/behavior—are studied. I want to question whether it is really appropriate to use the pronoun it for an intact animal.

I once referred to individual study animals as he/she. The principal investigator asked me to use *the animal* instead and lectured me that it is not scientifically appropriate to personalize an animal.

Perhaps you do personalize an animal, but this does not change the fact that using the **gender-appropriate pronoun** he or she is more accurate than using the pronoun it, as if the subject had no gender. Why would it not be scientifically appropriate to refer to intact animals with the proper pronouns he or she? I have always called animals, whether research subjects or not, he or she. To refer to an animal as *it* is to remove one's self from a living creature and regard and treat this animal like a thing. I think animals deserve some respect, and calling them he, she or who (rather than that) is the least we can do.

When I refer to an animal I'll use *he* or *she* or rephrase/reword to say *the mouse* if I don't know the animal's gender. That habit is left over from childhood grammar instruction: "Living beings are not it, that, or which."

I also refer to any animal as *she* or *he* and *who* rather than *it* and *that*. If I don't know the gender, I simply refer to *the animal* or the animal's species name, e.g., *the rat.* The pronoun it is reserved for inanimate objects/things.

Personalizing the animals provides them basic assurance that you are considerate of the fact, that they are living creatures who do feel pain, discomfort and distress in a similar manner as you do, and that their well-being is impaired when you expose them to discomfort, pain and distress. You will probably do your best to promote their well-being, which will also benefit scientific methodology. Not referring to an intact animal as *he* or *she* but as *it* is scientifically less appropriate than the reverse. After all, an intact female is not a neuter, and an intact male is also not a neuter. No scientist can, for example, study reproductive phenomena in an animal who is neither a she/female nor a he/male. Why pretend that animals have no reproductive organs and label them with the pronoun that we use for dead things, i.e., objects? We usually treat things differently than animals, because we know that they are not sentient, and hence do not suffer. Once we label an animal as a thing, the risk arises that we will treat the subject accordingly, for example, as a *standardized biological research tool* rather than a living creature.

The animals who serve us for experimental purposes should be treated with respect. They do deserve to be seen and treated accordingly as sentient beings who are, at the very least, referred to by their biological gender. At our facility we try to use the correct pronouns he or she for all our animals. Perhaps not surprisingly, the worst offenders for labeling an animal as "it" are our surgeons! We do discourage our personnel from using the pronoun it, since we do not want to encourage them to regard animals as mobile test tubes.

I am not convinced that using the correct pronouns will change the attitude of people who regard animals as sophisticated versions of *test tubes.* As a clinical veterinarian, I suspect that animals assigned to biomedical research have traditionally been labeled as quasi-objects in an attempt to protect the researcher from ethical concerns about the fact that he or she inflicts pain, distress, and probably also suffering on conscious creatures. The way we refer to animals in our language does reflect our attitude toward them, and the way we attend to their basic needs for well-being and safety. I guess the research laboratory is a place in which this kind of respect for life is not in high regard, because the research itself often implies the mutilation and killing of animals who are, after all, living beings just like scientists themselves.

It's sad when researchers are against referring to animals by their proper gender. I believe that some investigators need to distance themselves emotionally in order to feel okay with the research they are conducting with the animals. It's probably easier to inflict pain and suffering on a thing or on an object rather than on a real animal who wants to live and is endowed with feelings.

There are scientists who prefer to regard animals as objects because they believe that referring to them as *she* or *he* would hinder them from being objective while doing their research with these animals and interpreting the research data collected from them. Perhaps as a result of this objectification, scientists are often emotionally distant towards the animals they do research with and, therefore, care little about their well-being.

Calling animals by their gender (or names) is, in a subtle way, protecting them from being treated/used/disposed as if they were research objects, without consideration of their feelings and well-being.

As caregivers, we do not use the pronoun it when referring to an animal. An animal is not an object! We do not think that calling an animal *he* or *she* encourages anthropomorphism, but that it does acknowledge the fact that we are dealing with an individual sentient being who can feel discomfort, pain and distress in very similar ways as we do. Calling individual animals *he* or *she* helps us deal with something that deep down, we are not really comfortable with—namely the fact that these animals have no choice about deciding whether they want to be used in research and then killed.

< It seems that Refinement in the use of animals for research includes choosing **lower-order species** rather than higher-order species, presumably due to the assumption that the lower-order animals suffer less and that their use in experiments poses fewer ethical problems. Where do we draw the line? >

Personally, I have always had a problem with the terms higher and lower animals. I believe that it is our responsibility to reduce pain and distress in <u>all</u> species that we use, not just the ones that may be closer genetic cousins.

To me, non-human primates seem to be sufficiently different from other mammals—in having a sense of self and of the future—to deserve particular consideration, but what about dogs versus mice? There is a great cultural difference, in that humans tend to view dogs as beloved pets, and

mice as abominable pests. But does that mean that the mouse is of a lower order, and therefore suffers less from research than a dog?

As scientists, using animals for our research, we should be in the position to go beyond this weird idea of animals being of a lower or higher order. We are at a great risk of treating our research subjects not well when we consider them of so-called lower order, and by doing so, jeopardize the quality of our research methodology. When colleagues tell me that mice are lower mammals who cannot suffer from anything akin to human mental disorders, I ask them: “If mice are so different from us that they cannot suffer from mental disorders, then what is the point of developing drugs in mice to cure mental disorders of humans?”

For people who are using these terms, lower simply means less like humans and higher means more like humans. This terminology is tied in with the incorrect view of evolution as a ladder of progress toward especially evolved beings, such as humans. How would animals, used by humans for biomedical research classify the human species? Of a high order? Crown of creation? Very unlikely!

For me, it is very clear that so-called lower-order animals deserve as much animal welfare concern as higher-order animals do. People in general are biased in their perspectives. Sadly enough, some individuals still look at mice and rats and see *only rodents*, and do not look at them much more than that. Fortunately, there ARE also many other individuals—including yourself and probably all of us on this forum—who look at rodents and other so-called lower-order animals as amazing creatures who deserve our appreciation and, if needed, our unconditional compassion and care.

An animal species cannot be considered of a relatively higher or lower order on any scientific ground, because the idea of *lower* and *higher* is just a concept that does not reflect reality. We classify different animal species into a higher or lower order, depending on our personal, hence subjective relationship with these species. This view puts all animals commonly regarded as vermin or pests into the lowest order—e.g., mice and rats—and those animals who have a charismatic appeal, because we know them as companion animals—e.g., dogs, cats, rabbits, hamsters and guinea pigs—into a higher order. Finally, we put animals who look and behave in ways that are similar to humans—e.g., monkeys and apes—into the highest order. The fallacy in this categorization is that it does not help us determine whether one species suffers more during a certain experimental procedure (and hence deserves more of our concern) than another species.

Unfortunately, even professional animal care guides use these unscientific terms of lower versus higher-order animal species. I did a Google search on the exact wording "higher order species" and my first hit was the Canadian Council of Animal Care (1997), one of the most renowned resources on laboratory animal science. Here is the statement: "The creation of transgenic animals is resulting in a shift from the use of higher order species to lower order species, and is also affecting the numbers of animals used. .. An example of the replacement of higher species by lower species is the possibility to develop disease models in mice rather than using dogs or non-human primates."

This document does not elaborate on what scientific ground mice are categorized as a lower species that implicitly deserves less animal welfare concern than the so-called higher species of dogs or non-human primates. The fact, that rats and mice are commonly considered of lowest order, has probably allowed U.S. legislators to explicitly exclude rats and mice in the legal definition of the term animal, thereby negating the two most commonly used research animals legal protection of their basic welfare requirements. This begs the question: What is the point of having animal welfare legislation if it does not protect also the great majority of research animals, namely rats and mice?

I think all animals deserve the same consideration, whether they are a rat or mouse—of presumed low order—or a dog or monkey—of presumed higher order. It seems strange to me to simply categorize animals into different orders and then treat them accordingly; only the human mind can do that!

< Do those of you who work with different species in the research lab feel that the degree of discomfort and **distress** experienced in the artificial living quarters and during standard procedures differ significantly between **species of alleged higher versus lower order**? >

In my daily work with rabbits, rats, mice, hamsters or guinea pigs, I do not see species differences in the animals' reaction to discomfort and distress. When you ask, if it is less distressing for a mouse than for a dog or for a monkey to be killed, I think there is no difference. If there is a difference, it is probably due to the person who does the killing.

Working with quite a number of different species, I have found that the prey species—such as rodents and rabbits—tend to be more distressed during enforced handling and restraint than predator species—such as dogs and cats. All rodents are distressed when they are kept alone, perhaps not to the same degree as dogs or monkeys, but they are distressed nonetheless. To

this very day, I feel for every rat, mouse or guinea pig who had to live in our facility without contact with another companion. Frogs do not give the impression of being distressed in their living quarters, but they seem to be just as distressed as warm-blooded animals are when they are handled by people.

Many years ago, I worked with macaques and rats who were kept alone in barren cages. Both, the single-caged rat and the single-caged monkey were miserable—depressed and bored—and I must admit that I could not tell a difference in the degree of distress that they experienced. I have the feeling that even though we may categorize them as animals of lower order versus animals of higher order, rats and monkeys do not differ in their observable distress response to being permanently housed alone in boring living quarters. These animals were often restrained by humans for procedures. While the monkeys always resisted and gave the impression of being scared whenever they were restrained, the rats seemed to tolerate the procedure. The observer got the impression that being restrained was a much more distressing experience for monkeys than for rats. However, there is no reason to believe that this particular difference is somehow related to monkeys being more evolved than rats. The fact that rats do not show their distress during restraint is probably a biological trick that increases their chances of not being killed by a predator who has caught them. Being forcibly restrained is probably equally distressing for all animal species, but some show it while others don't for biologically sound reasons.

< How useful is the concept of **genetic relatedness** in terms of animal care and welfare? Does the genetic relatedness of animals with us, the human species, affects our concern for their well-being and our willingness to care for their welfare, while they are used for research and, when they are no longer used for research? >

To classify animals into those of higher versus lower order, or to classify animals according to their genetic relatedness to the human species may have theoretical value, but it would be unscientific to use these concepts to determine the relative importance of the respective animals' welfare needs.

It can be a little dangerous to suggest that a particular species deserves better care than another—for whatever conceptual reasons—because it implies that this species (for example chimpanzees) is more capable of suffering than another species (for example rats). This belief reinforces the misconceptions of those who might wish to protect non-human primates, cats and dogs, but not mice and rats. Genetic relatedness should have

nothing to do with our welfare concerns for animals. Suffering is a subjective experience; therefore it is impossible for us to know how another organism is suffering. It might be easier for us to appreciate that an animal is suffering in more genetically related species—e.g., monkeys—because they behave similarly to us, but it does not necessarily mean that a genetically less related species, such as rats, cannot suffer similarly as we do, or as monkeys do. We just don't know, and as long as this is the case, we must assume that suffering is a universal phenomenon that may vary from species to species and between individuals of the same species, but which is experienced as unpleasant by all animals independent of their genetic relatedness with the human species.

I do not believe we should be using something as vague as *genetic relatedness* to determine how an animal should be cared for. I care for all animals with the same concern for their well-being. Whether they are rats or primates, they all deserve optimal care. Humans share about 40 percent of their genome with bananas, and 85 percent with mice. If this is the case, do we give 98 percent of our welfare concerns to chimps with whom we share 98 percent of our genome, 85 percent of our welfare concerns to mice and 40 percent to bananas? Are we twice as worried about the welfare of mice as we are about bananas?

I have the uneasy feeling that genetic relatedness with the human species is just a pretext, while money is the actual cause for our relatively discriminating treatment of mice and rats. After all, it is less expensive to care for 100 mice than for 1 monkey, and it less expensive to replace 100 mice than 1 monkey.

< **Do animals have feelings**? >

The eyes of macaques are windows into their psyche. Because they can't speak English, or tell us in our own spoken words their life experiences and how they feel, many scientists think they have no feelings, at least nothing like humans have. But they can still effectively tell us through their expressions that they do have feelings.

Physical expression is the strongest form of communication, even stronger than speech. It is universally recognized by every culture, in every language in every corner of the world. Spoken words can be misunderstood or misleading, but there is little mistaking in what is spoken through expressive eyes and body language.

The expression of certain feelings—for instance anger—can be the same in different species, hence correctly understood by different species including humans.

I remember a female stump-tailed macaque whom I had trained to present her right leg through the partially opened cage door so that I can take blood samples. We had developed a strong bond based on mutual trust; I would often visit Kelly, talk to her, make her smile (left photo) or even laugh (right photo) by tickling her under the chin. Assuming that smiling and

laughing are expressions of enjoyment and happiness in humans, Kelly's response to being tickled suggests that she experiences the same feelings.

It is my experience that all animals I have worked with on a one-to-one basis have feelings that are expressed in simple movements, behaviors, reactions and responses, in sounds/vocalization and in the eyes. This does not only apply to non-human primates but also to rodents, ruminants, pigs, birds and frogs; maybe also to fish and insects? I would argue, the better you know a particular animal species, the better you are equipped to 'read' their feelings.

The fact so many scientists are reluctant, even unwilling to admit that their animals for research have any feelings may be related to their conditioning. Fathers have been conditioned by their fathers to be tough and not to have feelings, so they teach their sons also to be tough and have no feelings. Well, with such a mindset, you are probably unlikely to concede that the animals you do research with have feelings such as fear, anxiety, joy and frustration. Thanks to regulatory pressure, most scientists finally acknowledge that the animals they do research with have the capacity to experience pain; at least one first step in the right direction!

< Before entering an animal room, do you let the animals know that you are about to enter? Do you talk to them when you are in their room? >

When I started caring for macaques, I very quickly learned not to barge into animal rooms and have the macaques freak out but to calmly enter while **talking to them**. Once in the room, I would continue talking like, *Hi guys, I have to take a few blood samples; no big deal. When we are done, each one of you will get raisins and an apple.*

When entering an animal room I am also chatty. It may not always be the best approach for the dogs, but I can't help myself. We all get revved up together. The rats, hamsters, guinea pigs, rabbits, primates, pigs, goats, snakes and bats, they ALL definitely like to hear me enter. The mice, I am never sure if they really care.

I *Hoo* in the hallway outside to let all the animals in my charge be aware that I'm here and will enter their room. I talk incessantly to them, and usually in a fairly high soft pitch. I also sing songs to them—a lot—usually opera or folk songs. They probably identify me by my talking and singing: "Here she is … she's gonna sing … yup, there she goes!"

I have always talked to the animals I work with—heck, I even say *Hell!* to the zebrafish and *Xenopus* upon entry to the rooms. I use different intonations, and different types of speech, for different species, and always announce to the group what I have in mind to do, whether it be *I am really sorry, but we have to take blood samples today* or *Hey, guess what? It's goodie time!*

For rabbits, I tend to keep my words soft but, when I say "It's time to get out the hay," I will perk up. Also, I tend to sing (albeit somewhat poorly) bunny parody songs I've created like *Tiny bunnies, in the wine...* because it really does help them to get used to sounds from people.

With rodents, I'm straight up *Hey guys, how ya doing?* unless someone brings a sick/distressed rodent to my attention. Then, I get out my *poor little fella* calm voice. With swine, I always enter their room with a *Hey* or a *Yo, ladies, how are ya?*

With sheep, dogs and cats, I always say *Hello!* upon entering, and then assess their stance and see what's needed for that particular entry point. Fearful animals and fractious cats require completely different language and intonation than excited happy-to-see-you critters.

With monkeys, it was always a *Hey guys, what's up?* and we would go from there. I'd have individual conversations with each, once I learned their personalities, and even discovered with one group of Mauritius cynos that speaking French was the way to go. By entering with a sing-songy *Bonjour petits garçons,* they instantly knew it was me, and I would get some

hoots right back. I really miss my monkeys—they were some of my greatest confidants.

One thing I do quite often, that I have not seen many do, is ask permission of individual animals to handle them, provide treatment and take a sample. I had one research tech actually ask me last week what I would do if the rat looked at me and said *No!* This is something I'm going to have to deeply consider. And with that, I'm going to go ask a rat if I can have a look at her tumor.

< Is it unprofessional when you develop **affectionate relationships with animals in your charge**? >

Unprofessional? Absolutely never in my opinion. If you do not have an affectionate relationship with your animals, it is time to get out of the field.

Animal care personnel and researchers should be encouraged to develop affectionate relationships with their animals. We owe it to the animals who give their lives for our benefit. Having an affectionate relationship assures that you regard the animals as living beings, rather than biological test tubes. As such, you will be more careful and more patient. You will think more about what the experimental procedure implies to the animals. You will get more creative in refining procedures that are normally stressful or distressing to the animals. You will thus enhance their well-being and by doing so increase the scientific validity of research data obtained from them.

< How do you deal with **attachment to animals in your care**? >

A veterinary technician gave the following answer to this question: "It's hard because I am passionate about what I do and because our animals are long-term. It is important to be attached and there are certainly days when I am in tears, but I think if I ever felt unaffected by euthanizing our animals, it would be time for me to leave."

The American Association for Laboratory Animal Science (2001) notes that "The bond between people and animals in the laboratory, if understood and used consistently, can minimize certain variables related to stress in the animals." I agree wholeheartedly that developing a close bond with research animals can only be a good thing.

As a researcher, I do take the animals' welfare very seriously and get terribly upset if they suffer, even though I don't have an affectionate relation with them. It's perhaps not necessary to develop affectionate relationships, but kindness towards animals should be a professional prerequisite for any

person who is hired to care for animals in research labs. We owe this to the animals!

We also owe this to ourselves, because when we are not kind to animals, we are also not kind to ourselves and to other people. How can we ever expect to find happiness when we are not kind?

If you are not kind to your animals, make no attempt to enrich their boring, often depressing living quarters, and never show affection towards them, then I really don't think you should work in an animal research laboratory. Unfortunately, I did and still do find such people in animal quarters, so if anyone is offended, well, you just might be guilty!

The problem here is that nobody can make herself or himself develop affection and/or kindness towards another creature. Affection and kindness are spontaneous qualities over which we have no control. It's like love. You cannot make yourself love another person; true love is spontaneous. If love is pretended, the relationship with the other person will not work because your feelings are not genuine. The same applies for your relationship with animals. If you pretend to be kind and/or affectionate an animal will instinctively know that your feelings are not genuine and, hence will not feel at ease in your presence.

It's true, you cannot learn to be kind and/or affectionate, but you can follow professional ethical rules pertaining to your work with the animals in your charge.

< It is my experience that **mutual trust** is a key for safety when dealing with an animal, be it a laboratory animal, a wild animal or a pet. How do you know when an animal trusts you? And, how do you know when you can trust the animal? >

I was always taught when I worked in zoos that you should never fully trust animals who have not been domesticated. With enough love and mutual understanding and respect I can build a strong relationship with such animals, but I don't trust them 100%. After all, unlike domesticated animals, wild animals haven't been selectively bred to have a body-contact relationship with humans.

I absolutely agree, you should never 100% trust an undomesticated animal, however, I have come pretty close. One particular rhesus I used to work with would lie down in his cage, of course not close enough so that I could reach him. He was inviting me with his look to pet him, but I'd have to reach my entire arm through the feeder hole into the cage to do that. I was hesitant because I would not be able to pull my arm out quickly enough in

case he would have the intention to bite me. I wasn't yet quite sure if I could really trust him.

It's hard to explain what finally made me fully trust him, but one weekend after many visits, he just was lying there so still that I felt a strong connection with him; there was no separation between the two of us—just a deep feeling of oneness which is hard to put into words. From that day on I trusted him enough to reach my arm into his cage and give him belly rubs. He loved it, and I loved it; it became our afternoon thing. He was, and still is, my most favorite rhesus I ever worked with.

Trust and respect go together not only when dealing with wild animals but also with pets. I know without being able to explain how I know that I cannot trust a relationship when there is a trace of fear in me and/or when I feel that the animal has a trace of fear; just a little bit of fear makes the relationship unreliable, i.e., unpredictable, hence potentially dangerous. It is my experience that animals are very sensitive and read my emotional state spontaneously and correctly; there is no cheating possible!

When I am afraid that a male macaque could bite me, I will stay away from making attempts to handle the male in any way; he feels that I am afraid, and this will create anxiety and a readiness to defend himself. Such a situation can be very risky! It can also become risky when I am not fully present and/or when the animal is not fully present. When I trained potentially dangerous macaques with whom I had developed a mutual trust relationship, I always made sure that no environmental disturbances would distract us. Lunch breaks, weekends and holidays were always the best times for training sessions.

I worked with a particular Mauritius male cyno for a large chunk of my career. His name was Jordan; he was a beautiful boy and I used him as my training-monkey for new employees to learn on/from. He was perfectly pole/collar/chair trained (I had spent many years getting him there) and would almost put himself into the chair with no pole needed—he knew my every cue and we had a very special bond and high level of trust.

One day, when I was five months pregnant, I was chairing Jordan as I did so many million times before. He climbed into his chair, anxiously awaiting the treat I always gave him, and got settled. As I tightened his collar in place I noticed a tuft of his hair had gotten stuck in the track of the neck yolk; I reached down to adjust him, again, as I had done so many, many times before, and next thing I knew he snapped and there was a 2 inch canine sunk completely through my middle finger. My hand was pinned to

the yolk of the chair by his tooth; neither one of us could move/unhinge ourselves.

Long story short, a pregnant lady ended up with a very serious injury, a long hospital stay due to what became a blood borne infection, a central line placed, two+ months of IV antibiotics, several rounds of extremely painful debridement and hand surgery, a year of physical therapy to get my hand working again, and a permanently mangled finger with crushed bones and severed nerves along with loss of all sensation that will never work the same again.

I've been in this industry for 20+ years, and I have loved, bonded with and trusted more animals than I can ever count along the way—I still do, but that particular day taught me a very serious lesson about what I perceived as trust, and definitely brought me to a new level of combining trust with caution, and respect. Any little thing can spook even your best buddy animal, so please be careful!

I have been lucky enough to have many stories of mutual trust, but this one is my favorite: I adopted a small (spayed: LOL) female barn cat who was about 6 years old. Try as I might, I could not transition her to an indoor-only cat, although she was very friendly and was happy in the house. She was just too much of a hunter and would take every opportunity to escape. One day she was adamant that I follow her up the stairs into my son's room. She hopped into his dresser drawer and pulled up a giant kitten. Only one, but it was huge and all wet—she had just had him. She was showing him off to me. So I figured, after the kitten was weaned I would have her spayed. Well the kids let her out once again ...

One night watching TV she jumped onto my lap and was very lovely, dovey. Next thing I knew, my hand was all wet and she was starting to give birth. I could not believe she was doing this on my lap. She had three kittens and I could tell she appreciated my supportive belly rubs and such. Three healthy kittens and they all found homes the week they were weaned.

I operated a daycare at the time, and people were constantly coming and going and no one paid attention to the cat. So she escaped again and was missing for over a month.

When she came back, I could tell she was pregnant again, ugh. So again, one night watching TV, she jumped on my lap and wanted to have them on my lap again. She was talking to me and she would push the babies over to me while she had another delivery and cleaned up the new one; then she would have them nurse. It was amazing. I felt so blessed she chose to share this with me. So up to my bedroom she went with her babies and was

securely locked up until we got her to the vet for her spay. On one hand I was very glad we were not adding to the cat population problem. On the other hand she was the best momma I have ever seen and I know she missed having them and so did I.

< **Do you give names to the animals in your charge**? >

Naming the animals helps me realize that I am working with sentient beings who deserve my consideration of their well-being. It is probably more difficult to be callous toward a monkey who is called John than to a monkey referred to as 79045. As a clinical veterinarian, I observed that non-human primate caregivers became markedly more concerned for and interested in the animals in their charge when the ID number tags on the cages were replaced with name tags. I guess, we can all relate much better to names than to numbers, and we tend to treat named versus numbered animals accordingly. The naming of animals in research labs could serve as a safeguard for optimal animal care.

The employees looking after our macaques were discouraged by the principal investigator to name the animals of the research project. It was reasoned that the research team does not want the caretakers to be attached to animals who will eventually be sacrificed. The girls gave their animals names anyway.

At my last institution our monkeys were all numbered. I can't tell you how excited our animal techs were when I told them to name all of the monkeys. Many of them already had nick names from our care staff, but making it official was so much fun to watch! That became our husbandry staff's job whenever new animals came in. They loved it.

To deny or discourage attachment between technical staff and monkeys is a futile exercise, doomed to be unsuccessful. To be attached to experimental animals, and to feel ill at ease about their fate is just part of the responsibility we have to embrace when we decide to work with animals used for research purposes. And, as a matter of fact, this attachment has a positive effect on the level of care provided by the staff to those animals.

The former director of the Institute for Laboratory Animal Research shares his experience as it relates to naming monkeys. "I was encouraged not to assign names to the many rhesus monkeys in my charge. I was admonished that the animals are research subjects, not pets. The concern was that having names for the animals might blur this distinction between a research subject and a pet. ... It did not seem possible to remain distant—

emotionally isolated—from the animals. In fact, the inevitable closeness that resulted from those intimate interactions was precisely what made us capable of doing what we were asked to do. ... Eventually, we all came to know that F49 was Sam, A12 was Rosie, and Z13 was Curious. ... Such attachments are the results of compassionate people doing their job right" (Wolfle, 2002).

We have an investigator who is against the naming of rabbits assigned to her research protocol. The PI is afraid that, when bonding with her research subjects, we add a variable that is detrimental to performing research. Our staff feels that this is an antiquated mentality and we are all standing strong in our position of naming all rabbits in our charge!

I have run into that same mentality here, but ended up naming the animals anyway, using their ID numbers only for the records. I name our animals primarily because we have so many of them, and it helps our care staff and me keep better track of who is who. We have monkeys, cats, rats, rabbits and mice. All of them except the mice have their names. We have a high turnover of mice, and this makes the name-giving a bit of a challenge, but we name the mice who stay around for a while.

< **How do you know** for sure—and you must be sure for your own safety—**that an animal <u>wants</u> to be touched**, stroked or groomed by you? >

If a rat enjoys being groomed by me, she will respond with a relaxed stance and closed eyes, and then she will also start grooming my hand.

When a rhesus monkey approaches me and does a rump or chest present, I can tell the animal wants attention. Typically, a monkey will press his or her body up against the front of the cage, allowing me to gently tug at the fur as if I were grooming. I am sure the animals enjoy this as much as I do.

I have one rhesus girl, Meera, who loves to have her bum rubbed and her face groomed. She actually asks for it by presenting herself. This is a very clear signal that the animal is not afraid of you and wants you to come closer and start a grooming session.

We had another monk, Kiwi, who absolutely loved human contact. She would pretend to be asleep after a procedure, so that I would hold her longer before placing her in the recovery cage. I used to watch her squint her eyes slightly open to see what was going on, only to quickly close them if someone was looking at her!

The key signal that tells me that an animal likes to be touched is when she or he *presents*, i.e., entices me to do so. A chair-restrained rhesus

monkey, for example, will twist her body in an attempt to present her rear, thereby letting me know that she wants to be groomed. Under such a condition, the monkey will show no fear or aggressive-defense reactions but rather be relaxed and calm.

Cats are a bit tricky when it comes to trust. They can easily give you the wrong impression of enjoying being touched. Their time span for direct continuous social contact is usually very short when compared, for example, with dogs and monkeys. When you have reached this time limit, you may be in for a hiss or even a scratch. These critters can switch from *I am in bliss while you groom me!* to *Let me alone!* in a blink of an eye. I have had encounters with cats during which they allowed me to touch them, and then all at once, without any warning, turned around and gave me a swat. In some cases, the animal will solicit to be touched again right after swatting.

I have worked many years with pigs and can affirm that they do enjoy human touch very much, but it must be <u>their</u> idea, and they will of course let you know when it should end, usually by moving away or vocalizing if they feel trapped. I purchased a toilet brush for scratching the pigs in my charge. Most of them cannot resist, once they realize what it is for and how good it feels. They seem to like being scratched just about anywhere. When I need to obtain a rectal temperature, I scratch them around their tails quite a bit. They like this and stand still, allowing me to get their temperature without any ado.

< Working with animals for researchers can sometimes be very stressful, hectic and frustrating. Is it justified to **cry at work** once in a while? >

This is an important and difficult subject. I have been present at the final moment of some dogs who, I feared, would be frightened with strange people. It was hard and there was of course some crying, but I felt relief in the fact that I could offer these animals, who were so close to me, some comfort; it was worth the tears!

I have almost left my job a few times in my 24-year career while working with dogs in chronic studies. It can be very hard when an animal is euthanized with whom you have worked for quite some time and for whom you have developed affection and compassion. It helps me to share my feelings with others, who have similar experiences, and know that I am not alone but that it is okay to feel very sad and frustrated. I firmly believe that the feelings and emotions we carry for these little critters help us to make their lives as good as possible while they are in our care! It’s better when someone like me, who gets emotionally attached to the animals, is taking

care of them rather than someone who couldn't care less about the animals and is just doing the job for a paycheck.

Our caring for the critters can get the best of us emotionally but we should be able to express our feelings (within reason of course) and know that it is natural and normal to feel that way. I have shed many tears over monkeys, rabbits, rats and pigs, but the worst was two years ago the day before one of the greatest dogs in the world was scheduled to leave me. I had worked with countless dogs prior to her and it had never happened to me before, but she was MY dog, and knowing she was on a project which required sacrifice at the end was more than my heart could bear. The day before the procedure was due I sat with her and cried for probably about an hour while she licked my face. The PI had already told me I wouldn't have to be present for the surgery and that he would handle everything. It was a gift I didn't have to do the deed, but not being there for her was very hard.

If I cry due to work-related issues, I just remove myself from everyone. If anyone notices my emotions, it is labeled as unprofessional.

Are you joking? I cry at work all the time! More seriously, crying at work for the animals can mean that your empathic feelings are alert, rather than put to sleep by the routine of the lab work. So, to me, it is a healthy response to an emotionally upsetting situation. I would ask those who are uncomfortable with those of us who cry to just let us be, especially if it is not interfering with our work. Crying is an important safety valve that some of us need. I do the termination of my macaques, because I do want them to have the feeling that this day is not different from those when they are normally anesthetized. Some of these animals I have worked with weekly, if not daily, for up to five years. That loss deserves some tears! I believe in the research that is done with the animals, but this does not hinder me from offering them the best possible care, and I will cry when they are gone.

I have also cried at work on occasions when an animal suffered unnecessarily, or when I was involved in putting down an animal I had worked with for a long time. Expressing one's sadness is only unprofessional if it prevents me from doing a job in a way that is best for the animal. Otherwise it is simply an indication that I have compassion.

One of the most horrible times I ever had at work was when we traded out one dog for another, who would be used for a terminal study. We had received a group of dogs from a class B dealer, and a huge beautiful golden retriever pup who—we named Anton—was one of them. It was decided that, if we could find a trade with the dogs we already had, we would save Anton. When my supervisor brought the little female terrier mix over for the trade,

we just broke down. We used her because she would never be able to be adopted out due to the tick-borne disease research she had been used for. She was so sweet! I sat there for a good 30 minutes crying and talking to her and hoping someday she would forgive us. On the positive side, Anton is running on the prairie with a loving family!

Whenever an animal had to be put down, be it a mouse or a dog, my supervisor was very strict about respecting the animals' dignity. If anyone joked or kidded about it, she was like a cobra to correct them as to why it's no laughing matter. She is still my dear friend!

We do such a tough job, especially, since I think all of us are animal lovers. If we didn't cry, we would probably also not care, and wouldn't feel bad about what we are doing. If I didn't cry in the face of the animals' suffering, I wouldn't be in this profession. I, too, have hid in order to be alone, because crying is looked down upon here. I do support the research, but I am also sad that there isn't yet another way besides using animals to accomplish the goals of this research.

I would worry if I ever found that I wasn't disturbed by the thought of having to kill any creature, but it is much more difficult when you've become friends with an animal, and this happens to me quite often even though the animals in our lab are with us only for a short term. After 40 years in the business, I still find it difficult. Yet, I do get involved in the killing process because I strongly believe that it provides some comfort to the individual animal to have someone familiar present who maintains kind and gentle vocal contact up to the very end.

I am glad—as hard as it is on a daily basis—to work as an animal technician, because I feel that I can offer the animals a special gift. Every day, I do whatever I can to foster their well-being and make sure that, while they are here, they are getting the best care possible—and the best toys, of course!

After working with a group of beagles for several weeks, I was asked to assist in the euthanasia of my favorite one, whom we had nicknamed Cico; he was a porker but so cute! I did assist, but I cried like a baby. My co-workers' solution was that I should not be around for future euthanasia. But this was not the point. Even if I wasn't there for the euthanasia, I still would have been upset; it's hard not to be. I did and can do the euthanasia; that's part of my job. I have not had to say good-bye to any of our monkeys yet, and I honestly don't know how I'll deal with this situation.

Yes, I think it’s absolutely normal to be sad and cry sometimes with our line of work. To bottle up feelings of sadness, frustration or anger doesn't change the unacceptable situation but drains your energy and enthusiasm and makes you bitter. Walking through animals rooms, with row after row of cages in which lonely monkeys were sadly looking at me, made me often cry because of my limited power to change the situation. It's hard to be exposed to these realities. Crying is certainly a more healthy response than angrily arguing with investigators or administrators who are responsible for the situation. The first response gives you some relief, the second makes you even more frustrated, tense and angry—because you are usually talking to deaf ears.

People often make the mistake of assuming that we must not be animal lovers, because we work in biomedical facilities. I think it’s just the opposite. Most of the animal technicians and animal caregivers I know truly do love animals, and I think this is the main reason why we chose to work for the animals in research labs: We can make life easier for the animals in our charge. Yes, there are some days that are almost unbearable, but I know that I do make a difference for the animals, and this is what keeps me from running away. The animals need me!

A month ago, we had a young monkey experience a seizure after she was used for an experiment. After the seizure had stopped, she was paralyzed completely on her left side. She was awake, alert and hungry. Every time I tried to give her some food, she made an earnest attempt to sit up, but invariably would flop all over the place. It was heart breaking to witness this, and I couldn't help but cry. Here was this perfectly healthy animal, and we did this to her! Because I work with the girls on a daily basis, I can't help but become attached to them. I can't work any other way. I know what I'm in for, what they're here for and what will eventually happen to them. I'm fortunate to have an understanding boss. When the time comes to sacrifice an animal, I will inject the anesthetic and that's the last I see of the animal: a sleeping peaceful monkey. It is comforting to know that I am not the only one who gets upset with much of what we do and that there are other people with whom I can share my feelings.

< I am seeking input, feedback, and ideas about how to prevent or mitigate **compassion fatigue** and burnout when working with animals in research laboratories. What strategies or activities might offer support for the more difficult, emotional aspects of that work, for example euthanizing animals as part of your job or witnessing your animals suffering distress during certain research procedures? Some really good people leave science because they

simply cannot continue killing animals. Many of these individuals are the type of kind, sensitive, empathetic people we really want to be the ones working with the animals. Of course, some people who really do not want to do this work anymore should just not do it.

Perhaps some of you are willing to share what do you do personally to deal with compassion fatigue, and what approach do you take with your staff to address this issue? >

This is something that I continue to struggle with, despite being in the industry for 16 years. I find it emotionally very difficult to work in the lab on studies where I know the animals are going to be put down at the end of it all. Unfortunately, it doesn't ever get easier with time; you just learn how to deal with the given situation in your own way. Fortunately, other technicians at my facility are very understanding and sympathetic. We are sharing these sad experiences together.

How we cope with the unavoidable, emotionally very disturbing situations of our daily work is a personal thing, but talking about it certainly helps, knowing you are not alone. It has been my experience that it is beneficial to find colleagues who feel as I do, so we can share those feelings and find out about coping mechanisms that perhaps we never thought of. It is through forums like this that those connections can be made.

Compassion fatigue is an issue I have surely encountered in my 32 years of hanging around in the research field. I almost left a few times, especially when we used to have dogs. It's not that I think dogs are more deserving than rodents; it is just that they were here for longer periods of time and required more intensive nursing care in many situations. I spent more time with these dogs than my own one at home and of course would get very attached to them. It was hard to say good-bye when the time came, but I knew that it had to be and that it was for a greater good. This didn't stop the crying and sadness, but I looked at that as an intrinsic part of the whole process. I have been fortunate to never pass over the line of the natural grieving process and always bounced back again to continue serving the critters who have given their lives for the biomedical research endeavor. It was clear to me that I had made a difference in their lives by giving them the best care possible while they were with us.

I've definitely had to deal with this rather disturbing situation too. As a person who deeply cares and is passionate about animal welfare, I think it's very natural to feel some sort of sadness and/or compassion fatigue when animals are euthanized after they have served a scientific research purpose.

It is looked down upon in some facilities when you show signs of sadness when such animals are killed. You get hit with “Well, that’s part of the job” or “These are research animals, not pets” or even “Take your emotions out of it!” All these are extremely insensitive and disheartening statements and judgments of healthy humane feelings.

Unfortunately, I don’t have the solution to this problem. All I know is that it would be fair and reasonable if researchers and administrators of biomedical research facilities could at least make an attempt to be a little bit more sensitive and acknowledge the fact that the people who work day-in and day-out with and for their animals do have human feelings and humane concerns and emotions.

That is one of the hardest things in what we all do, isn’t it? Getting those in charge to STOP and THINK for a minute and try to see things from a different perspective.

I know colleagues who have quit because they could no longer deal with their sadness and helplessness when animals were put down with whom they had worked for an extended period of time. Maybe it would be indicated to establish support groups that can help us cope with these feelings.

Occasionally, when I can see it's all starting to get to people, I hold *group*. I put out a notice, say clearly that it's voluntary, and allow anyone who wants to come to share how they feel about a certain situation. I've had as little as one person show up, and during especially hard times—such as several weeks of terminal dog work—I've had a turn out of 15 animal care employees. I think it's good to help oneself and others get these emotional issues off the chest; it certainly makes the rest of our daily routines smoother.

I started a peer-based compassion fatigue support group at my facility. We meet once a month and allow people to express their feelings and help with self-care tips and ways to cope. We also work closely with the counseling department in our university and have a few counselors who are specifically earmarked for help with laboratory animal compassion fatigue.

Prior to euthanizing any animal, I speak with the caretakers, the researchers, the other vet staff and sometimes even the cage wash staff so they are aware of what will happen, and give them time to grieve the loss they may feel. I never remove an animal from a room for euthanasia (rodents included) without notifying the care staff. To unexpectedly find an empty cage or empty pen can be shocking and heart-breaking. We create

bonds with these animals and to lose one or several of them can hurt just as much as losing a family pet. I allow staff to get mad and speak their minds about the situation regardless of how negative the diatribe may become. I allow this in order for them to cope; it's a kind of release of the tension resulting from extreme frustration.

Emotional fatigue is a very important topic for all of us, and I agree that you should get out if it stops hurting. Finding ways to cope is an individual task, but knowing there is a group of others (supportive others!) going through similar experiences is a great relief. I love that you notify staff before euthanasia—nothing worse than going into a room and finding a buddy gone, not having had the chance to say good-bye. I try to convince myself that I won't get <u>that</u> close to another monkey (species I happen to work with), but then I fall in love all over again.

< The National Research Council claims that the goal of its Guide for the Care and Use of Laboratory Animals is to promote the "humane" care of animals used in research, but fails to explain what the term humane actually means. How would you **define the term humane** in the context of treatment of animals in research institutions? >

The term humane should be defined as "to treat an animal the same way you would treat a human being", that is to say, with respect and concern for her or his physiological and psychological well-being.

I am not sure I can tell you what the "humane" treatment of research animals means to me. Our chosen field of practice tends to skew our view of the plight of research animals. We amputate their toes for identification, we cut their tails for genetic analysis, we burn them to study healing, and we subject them to chemicals to see how these harm them. We have many, many ways of causing them harm to study abnormal occurrences in humans, and then we "sacrifice" them at the end of a study. This does not sound very "humane" to me, but who am I to judge.

< Do we really **sacrifice** animals and, how appropriate is it <u>not</u> to use the verb **kill** when we euthanize an animal at the termination of a study or because the animal is no longer of use for biomedical research? >

Many times, I got in trouble for writing in a protocol or report that the animals will be killed at the end of the study. In the interest of clarity and honesty, I always put "killed" in the first draft, but it is inevitably changed by someone higher up the chain to "euthanized" or "culled." It seems to me that it would be more honest to stick with the facts. When it comes to terminating an animal's life, **euphemism** is a cheap way of beating around

the bush. The word sacrifice implies that the act of killing is sacred (justified) and performed by a priest (the scientist), and that the subject is offered to a deity (science). I find this kind of euphemism to be a gross distortion of reality. Things are much more down to earth than this: we kill the animals!

The principal investigator who kills animals—or has others do the killing on his behalf—to achieve the goal of his scientific endeavor probably feels more at ease when he can hide behind the elegant phrase, "I have sacrificed animals for an important scientific project." This kind of wording is not scientific, because it has nothing to do with the reality as experienced by the animal who, de facto, is killed.

To me, the word killing paints a picture of violence, so I prefer to use the verb euthanize, because it makes me feel somehow better about the death and loss that I face daily. It can at times be overwhelming! When I have to euthanize an animal, I am not callous, but do it in the most humane way possible.

I agree, to euthanize seems to be the most appropriate verb, however, it may be misleading in regards to animals who are killed because they are surplus. When watching hundreds of surplus rats being killed or gassed in big tanks, because they are *spent*, have the *wrong sex*, have the *wrong phenotype*, or have reached the *end of res*earch, I don't feel the word euthanasia is appropriate. I am not so certain that rodents killed with gas as a way of "inducing death without pain" do not experience distress—either in theory or in practice. I believe the majority of them are in panic and feel considerable pain before they get unconscious, particularly in *bulk killing* settings.

This situation is certainly not a good example of the "humane care" of animals in research labs.

If we use the terms sacrificing or euthanizing with full awareness of what they actually imply to animals in research, we are honest to ourselves and we will do our very best to minimize the discomfort, pain and distress that the animals may experience during this life-terminating procedure. If, however, we use these polite terms to give the impression that what we are doing is justified and humane, we are dishonest to ourselves and to the lay public.

< I was wondering if I could get some feedback regarding **adoption of research-released animals**. We were able to get one dog adopted by an employee after the dog had chewed at an implanted probe. Our institution

had to get lawyers involved and go through a bunch of red tape, but the dog is now finally outside, living a normal life with a caring family. This ultimately positive experience made us ask ourselves, "Why can't we do this for more animals?" We currently have an investigator who would like to adopt one of his canine patient once the study is over. The dog could live a normal life. Can anybody share experiences on successful adoption programs? >

We had a strict no-adoption policy here but then we had an accidental beagle pregnancy. There was a lot of back and forth about the dam's future and if she would be allowed to go to term. Thankfully, she was too far along and too many staff members knew about the case—I may have helped with that. We quickly developed an adoption program and had the IACUC and a legal team draft the necessary protocol and contracts. We were then able to adopt the mom out and found a good home for her. We also got enough people lined up to take all of the puppies.

I was there when the mom went into labor and was able to see all the puppies being born! The runt had to be bottle fed by the third day; mom just could not supply enough milk. I decided to become the mom for one of them and brought her home; our little Belle. In fact, my son hooked up my computer to our TV last night and found the birthing video I took, so it's very fitting that this discussion came up today. Belle has been a great addition to the family!

We had a researcher who planned to continue a study with two cats who had lived at the facility already for one year. After no research was conducted with these animals in the course of the next five years, I approached the administration asking if I could take them home. My boss at the time convinced the researcher to let me adopt these cats who were already nine years old at that time.

They are currently 15 years old and living in the lap of luxury! They love lying in the sunshine, begging for food—not sure where they learned to do that!—and harassing my other two cats. Although they didn't die, they have definitely gone to heaven!

One good thing I got from my previous job is the joy that my three beagles Gabby, Dotty and Scrunchy bring me every day. I had named them at work, even though it was very much frowned upon. When they were at risk of being culled, I successfully pleaded with the management of the research facility to let me adopt them.

When I brought these three girls home, they were about 1.5 years old. They have adapted perfectly well to living outside of the research lab. They seem to be content and happy, and this makes me also happy, very happy.

This cute little female hairless rat ended up not being used on study, so she was slated for euthanasia. We have an adoption procedure here, so fortunately for both of us, I was able to bring her home. Her name is Tulip. The veterinarian checked her over and deemed her *ready to go home*.

I live in a drafty old farm house in Pennsylvania. Of course, Tulip was used to the wonderful climate-controlled atmosphere of her old home, and bringing her to my house was quite a change. One evening, while watching TV with her inside my sweater, I decided it was time for her to

have a sweater of her own. While she watched, I knitted a sweater out of some leftovers of lovely soft sock-yarn I had stashed away. She is very happy wearing this nice warm sweater that everyone refers to as her *tutu*.

In the Netherlands, there is an organization for re-homing animals, that is also specialized in re-homing dogs and cats who have been research subjects. This organization has contact with biomedical institutions, and I believe uses standard ownership transfer contracts. The dog or cat, who is no longer used for research, is placed with a foster family for an observation period. If the animal readjusts to normal life, bonds with the new family and is healthy, he or she can be adopted permanently in the new home.

We have an adoption policy that was drafted with the advice of our lawyers. We primarily adopt out cats, but occasionally also rats, frogs and rabbits. We have adopters sign a release/waiver of liability before the animal goes out. All potential adopters are screened as carefully as possible. The cats and rabbits are spayed or neutered and deemed healthy by the veterinarian before they leave our facility. We haven't—knock on wood—had any major problems with these adoption procedures. I think there's a good publicity potential in running adoption programs: (a) The facility shows people that it is concerned about the animals, and (b) gives evidence that there is research that doesn't harm the animals but leaves them fit enough to carry on a normal life as pets outside the laboratory.

For more than a decade, the University of Florida College of Veterinary Medicine has allowed investigators to arrange for the adoption of animals, who are no longer used in research. Di Gangi et al. (2006) surveyed 458 cats adopted over a period of six years and found that 91 percent of the animals were still in their original adoption homes, and 80 percent were highly valued family members.

At my institution pigs, sheep, chickens, ponies, dogs, cats, rats, mice, and guinea pigs have been successfully adopted out after completion of research projects. I have adopted several rats myself. They are very cute! Watching some of your favorite animals go to good homes after their hard work is quite rewarding. We have adoption forms that are almost identical to what one would fill out when adopting an animal from the Humane Society. I remember one instance where all 39 beagles of a study got homes after working for 2-3 years. It was a very, very positive experience for our entire staff!

I have brought home all kinds of animals in the past, from rodents and rabbits to livestock; my yard is full of chickens from my ocular research days. I will say it seems to be much easier to implement adoption programs in the academic setting versus contract research.

It's perhaps not as rare as one might think that researchers, like you, get so attached to their animals that they adopt them rather than have them killed after the termination of the study. When searching for photos for one of the LAREF books, I came across this photo with caption.

"Susie is an 8-yr-old pig who spent her first six months used in research studying lung ventilation. The researcher fell in love with her and didn't want to see her slaughtered. Hazel, Susie's companion, is a 6-yr-old pig rescued from a cruelty case where she was malnourished and infested with mange mites."

We have an adoption program at our facility and routinely adopt out rabbits, and occasionally mice, rats, ferrets and frogs. The ferrets and rabbits are always spayed or neutered before they move out, and we offer spay/neuter for rats and mice if the owner is interested. So it was very easy for me when I adopted a female NZW rabbit a number of years ago. She adjusted very well to her living environment; she was a little shy at first but soon came out of her shell and enjoyed the space and freedom that the small cage in the laboratory had not offered. It was great to watch her just being a quasi-free rabbit! I had never owned a rabbit before, and it wasn't until I had her that I realized how truly inadequate the standards are for rabbit caging in the research lab.

Our beagle and our cat were both adopted from a research facility that I used to work at. Both were babies when I brought them home, so they adjusted very well. They are a constant source of happiness for both me and my husband. When I worked at this facility, we had a fairly active adoption program, and from what I understand it is still going strong. I think it is high time that retirement and adoption become valid post-research end points. Simply because these animals were bred for research does not mean that their lives should end when their protocol is done; besides, what better way to say, "Thank you for your service to mankind!"

With concerted effort and good will it would probably be possible to re-home or retire the minority of animals released each year from research. The situation for rats and mice, the great majority of research-released animals, is bleak.

I would love to see rats and mice be re-homed but there must be sufficient suitable homes for them, and that may be the problem. I think when re-homing animals outside research facilities, it is easier to find homes for dogs and cats because they are more thought of as pets compared to mice and rats.

The large numbers of of mice and rats in research labs make it an unrealistic goal to find homes for all of them, unfortunately.

- We currently have more than 23,000 cages of mice and rats.
- Let's assume that 5% could be adopted in any given year. That's still more than 1000 cages, which for us is going to equate to roughly 2800 animals (based upon our average per cage census). And we're just one facility in the area.
- Now spread those numbers across all of our facilities and I think it's easy to see that this isn't a simple matter at all. I also think that's one of the reasons that facilities initiating adoption and retirement programs start with the larger, longer lived species like primates, dogs, and cats.
- It's not that rats and mice are less valued. It's simply that if I'm faced with adopting 5 dogs or 900 rats, which am I going to attempt first? Having a pet rat might be a whole lot cheaper, but it's still going to take me far longer to be able to adopt out all those 900 rodents. Whereas with 5 dogs, chances are good that I can adopt them out simply among the staff working at the facility.

< Re-homing of animals in research has been receiving increased attention both in and outside the laboratory. In the United States, a handful of states have passed laws encouraging it. If legislation is pursued, how do you think it can best benefit the animals? >

I worry that **mandating re-homing** for dogs will create pressure to euthanize dogs rather than deal with shelters or other organizations the biomedical companies may view as hostile or cumbersome. I would worry that the already overtaxed shelter systems would euthanize other animals to take these mandated dogs.

You are right, I also worry that mandating the retirement of research-released animals may have unintended consequences, especially within the current animal shelter systems that often struggle to find suitable homes for thousands of animals each year.

We should definitely encourage adoption and retirement of our lab animals and I'd be okay with legislation that encourages it as well. But having legislation that requires it? I think that would be the wrong answer to the problem. We would end up with facilities that don't/can't take the time to find suitable homes and so end up taking the first one that walks through the door, regardless of whether the new home is suitable or not. Not long ago, we struggled to find suitable homes for some rabbits. We had the luxury of being able to pursue several options and take the time to keep trying when some of the options fell through. But there are many facilities that won't have those luxuries and as a result our animals could end up worse off.

Legally mandating the retirement of research-released animals could easily be counterproductive, because many facilities would feel pressured and would wind up euthanizing rather than taking the time to retire the animals or creating a retirement program.

< Would animals living in minimum-sized cages benefit from **additional cage space**? >

Caged animals have little or no use for unstructured space beyond the space required for free postural adjustments and a few normal steps/hops.

The legal minimum space and exercise stipulations of the U.S. Animal Welfare Regulations do not make it clear that the prescribed space must be structured in a species-appropriate manner so that the confined subjects can make use of it.

Quite a number of articles have been published, showing that the legally prescribed minimum space is sufficient, and that the well-being of the caged subjects would not be enhanced by increasing the cage dimensions. The results and conclusions of these studies are questionable, if

not intentionally biased, because all of them were conducted with unstructured cages. I think it is quite obvious that an animal, and for that matter also a human, does not benefit from space by itself but from structures that make the space usable. Legal space requirement specifications are insufficient as long as they only prescribe **quantity of space**—usually based on body weight—and fail to define **quality of space**. For example, it should be mandatory that the space provision for a monkey's homecage is adequate for the proper placement of at least one sufficiently elevated resting surface.

Yes, this is a crucial point. To concentrate too much on minimum space distracts from the real question, which is: What can the animal do with the space in the enclosure? More space, if not structured, will not do much to the welfare of animals in captivity.

On the contrary, animals have the tendency to shun empty space but keep at the periphery close to the only structure available: the wall or the fence and corners. This intrinsic behavioral and emotional response to open space is termed wall-seeking or thigmotaxis (moving towards an object and keeping contact/touch with that object). The classic Open Field test for rodents actually demonstrates that empty space triggers fear because it doesn't offer any structural protection from potential predators or raptors.

It is hard to believe that animals don't benefit from additional space. I wish I had additional vertical space for my marmosets. I truly believe they would benefit from it.

They probably would, but only if you have placed branches or other structures in the additional vertical space. Those structures would be necessary to make the extra vertical space accessible for your marmosets; they cannot possibly perch in empty vertical space, they need some kind of structure to climb and sit on in the additional space.

< **Legal minimum cage space requirements** are usually based on body weight. How appropriate, from the caged subject's point of view, are such stipulations? >

Space requirements should be tailored in such a way that species-specific and species-adequate furniture can be placed in the enclosure without blocking part of the space that the occupant(s) need for free movement and free postural adjustments.

One factor that is important but is consistently overlooked is age. Very young animals need far more space than heavy animals!

Yes, juveniles need to have more space than adults, let alone adults who are overweight. Young animals are much more active and typically want to play; to do that, they need extra space.

In the revised Appendix A of the Council of Europe, minimum floor area is now not only based on body weight, but it also takes into account the need for young animals to play (Council of Europe, 2006). For example, for mice, the minimum floor area is 330 cm^2 per mouse, independent of the animal's body weight; this means that young and small, but relatively active mice grow more or less into their cage.

< When you check your animals, what **signs of impaired well-being** are you looking for? We often make use of these signs spontaneously, yet they seem to be very reliable. >

For most animals, the coat changes when they do not feel well. It may only be slightly off-color, dull and rough-looking with the hair clumping rather than lying sleek and glossy. Goats get a rounded face and a ridge along their backs due to the hair standing on end. Haven't observed any coat changes in sheep, but pigs will get a fluffy appearance when they are not okay.

Rodents, pigs, goats and sheep will take on a characteristic hunched look if they are in pain, with the back becoming arched and the abdomen tucked up towards the spine. I haven't observed this in rabbits. Sheep and goats will continually shift their weight from one leg to another when they are in pain, especially if the gut/abdomen is involved.

The guinea pigs, rats, rabbits, rhesus monkeys and dogs in my charge show typical positive responses when I enter the room and approach their cages. When one of them does not move but stays quietly in a shelter or in the back of the cage, I know for sure that this animal is not feeling well and needs to be checked more thoroughly. The response to my presence is probably the most reliable indicator of an animal's state of well-being, be it a dog, a monkey, a rat, a guinea pig, a chicken or any other animal who is in my charge. This leads us back to our discussions on the human-animal relationship. It would be impossible for me to take the subject's unusual response to my entering the room and approaching the cage as a sign of impaired well-being if the animal would not have a good relationship with me, but would be scared and always hide when I approach the cage. This scenario often happens with investigators who, therefore, are dependent on animal care personnel to check the health status of the animals assigned to their projects.

Rats are very good at concealing pain and health problems. However, if one of my guys is really ouchy he or she may show:

- decreased social behavior,
- decreased self-grooming when a rat is caged alone,
- skin twitching especially over the back area,
- spectacle effect, caused by haematoporphyrin-stained exudates around the eyes (Mason et al., 2004),
- decreased appetite notably for their treats,
- chewing bedding material,
- not moving around,
- not responding to external disturbances,
- hunched posture. Rats who do not feel well not only sit in a hunched position but they might even walk in a hunched posture, which make their legs look longer.

As for **monkeys**, I find the following signs useful indicators of an animal's impaired well-being:

- comes to front of enclosure but shows no interest in food treats (e.g., acute diarrhea, infection),
- does not come to front of cage when I approach (e.g., acute physical pain),
- does not look up when I talk encouragingly (serious health problem),
- crouches (e.g., fear, depression, physical pain),
- hides in far corner (e.g., fear),
- restless (e.g., boredom, anxiety),
- rough hair coat (e.g., chronic diarrhea),
- unkempt appearance (serious health problem).

When I worked with lots and lots of **mice**, I looked for hunched back, ruffled—or poorly groomed—hair coat as warning signals.

Aside from the obvious lack of food consumption or lack of urine/feces in a cage, I pay attention to the postures and movements of the mice. Typically their movements are a dead giveaway when they don't feel well. They might be sluggish, or hunched, or their gait might be a bit off.

I also check if they are grooming or interacting with their cage mates. It can take a bit of practice and patience but, once you know the normal behaviors of the animals in your care, you can usually pick up even subtle changes in those behaviors very quickly. Since mice—and rats—tend to be good about hiding their symptoms, this can mean the difference between an easy recovery or a rapid race to the euthanasia chamber.

Changes in posture in particular, but also piloerection, are reliable indicators that a mouse does not feel well.

Isolation from the group, hunched posture and starry coat—hair clumping or standing on end—are, in my experience, reliable signs that a mouse is going downhill. Such an animal *feels* light, compared to a healthy animal, long before you can actually see that the animal is losing weight. Yes, you need to know your charges very well in order to recognize such warning signals before it is too late.

Introducing a favorite food, such as a few sunflower seeds or some forage mix, is also a good well-being test: If a mouse doesn't try and grab her share, then you may well have a problem at hand.

When I was a young tech, listening to my animals was a good aid to detecting ill-health, as I could hear wheezing/sneezing; this was often the earliest indication of an outbreak of Chronic Respiratory Disease and appeared long before any visible signs of the problem.

We recently put Huntington's mice into enriched cages that contained a climbing rope and a beam. By monitoring the use of these enrichment structures, we noticed a decrease in usage by individual mice several days before any clinical symptoms of disease could be observed, and many days before they actually got sick.

We use the ability to run/walk on a beam or cling to a rotating rod as an indication of Prion disease development in mice. The inability to perform these tests and/or the time it takes a mouse to clear food out of a tube reliably shows that they have Prion disease well before any other signs are seen; this has enabled our researchers to refine the end point of the disease dramatically.

< What is the difference between **pain and suffering**? >

Pain is a physiological phenomenon. Based on my own experience, I would say that animals usually do not take a painful experience personally; it's not *their* pain, just pain that needs to be alleviated and avoided. They do not resist the pain, thereby making the sensation even more intense, but respond to it in the most appropriate way possible. Humans, however, have the tendency of identifying with their pain, thereby turning the perception of an objective phenomenon into a subjectively interpreted experience. The pain is now a personal problem, quasi an enemy that may trigger emotional reactions such as helplessness, self-pity, frustration, despair and worry. These emotional reactions transform pain into suffering. Pain is unavoidable for animals and humans alike, but suffering is a choice that humans make

probably much more often than animals. So it may then well be, that animals usually suffer less during painful situations than we do.

This is an interesting way of looking at pain. It seems to suggest that dwelling upon pain makes the pain even more painful. It could also suggest that captive animals, unlike wild animals, have nothing that could distract them from pain, so they are at a greater risk of dwelling upon the pain sensation thereby making it more intense. Gentle and Corr's (1995) study of chickens supports this hypothesis: When chickens were placed in pairs into pens containing a deep layer of wood shavings, they showed significantly less pain-related behavioral reactions to a joint inflammation than chickens placed alone in barren pens. When tested in the barren cage, the whole of a bird's attention was occupied in trying to reduce the pain as far as possible (one-legged standing, limping, sitting). In the more stimulating pen, the bird's attention was shifted from the pain to the social partner and the wood shavings, thereby reducing the intensity of pain that was actually experienced.

We cannot *objectively* measure the subjective experience of suffering, but this should be no hindrance for defining the term so that those who want to alleviate suffering can reason with those who inflict the suffering. Without such a definition, the animals are at the mercy of professional judgment, which is often influenced by egocentric interests.

Even if we cannot define the term suffering, we can agree, "If something is known to cause suffering in humans, it should be assumed to cause suffering in animals" (Organisation for Economic Co-Operation and Development, 2000). It seems reasonable to assume that a person who is forcefully restrained for a life-threatening procedure without being sedated is suffering, i.e., experiences extreme anxiety. I do believe that a monkey, a dog, a rabbit or a mouse is very likely to suffer in a similar manner during such a situation; anxiety-triggered diarrhea/urination, hyperventilation, struggling and significantly elevated stress hormones reflect the animal's state of suffering. I would also argue that a social animal who is kept in social isolation for an extended period of time is suffering, i.e., experiences extreme loneliness and boredom in a similar manner as a human prisoner does; the development of behavioral pathologies, for example self-mutilation, reflect the person's and the animal's distress, i.e., suffering.

I think the easiest way to define the term suffering would be "unrelieved pain." Of course, we must consider that there is not only physiological pain (e.g., injury of the body) but also emotional pain

(e.g., depression, frustration and boredom) that can lead to suffering if the pain is not relieved.

I checked the most recent published USDA report on animals used in research for the fiscal year 2010 and found that a total of 97,123 animals used in research experienced "more than slight or momentary pain or distress" that was not relieved with drugs. This figure, which does not include the most commonly used animals—rats and mice—makes it quite clear that many animals do suffer in research laboratories.

< The terms **stress and distress** are often used in the scientific literature but usually without a definition. If you use these terms, how do you define them? Are there signs that tell you that an animal is stressed or distressed? >

Stress and distress are physiological (stress) and emotional (distress) responses to events:

- An external situation (stressor) leads to stress, which implies an alteration of the subject's physiological and behavioral equilibrium (e.g., increased heart rate and fear). This kind of stress—*eustress* would probably be a more appropriate term—is not necessarily harmful, but it disturbs the subject's equilibrium, hence has the effect of a potentially data-biasing variable that needs to be accounted for in the research context. Being approached by unfamiliar personnel is a typical stress situation.
- If the subject cannot adapt to the stressor, i.e., return to physiological and behavioral equilibrium, stress turns into distress. Pathophysiological processes (e.g., chronic diseases, generalized alopecia), emotional disturbances (e.g., anxiety, frustration, depression) and/or maladaptive behaviors (e.g., self-injurious biting, hair pulling, stereotypical movements and gestures) often develop as a result of distress. Being permanently confined alone in a barren cage is a typical distress situation.

Although both "stress" and "distress" have negative connotations, distress is always bad, but stress can be both good or bad. A certain amount of stress is part of life and some mild stressors can make life a little more interesting. Introducing a new cage mate probably causes some stress for non-human primates—similar to how human primates might feel when going on a first date—but, assuming the companions are compatible, this is a good stress, as it breaks up the monotony and allows the animals to express their need for social contact and social interaction.

< Are **stereotypical behaviors** abnormal? >

Animals kept in legally minimum-sized, unstructured enclosures very often exhibit stereotypical behaviors. Traditionally, these repetitive movement patterns without obvious goals or functions are categorized as abnormal.

A healthy animal kept in a small, barren enclosure has little choice of expressing his or her biologically inherent drive to engage in species-typical behaviors, other than pacing back and forth, running in circles, somersaulting, rocking, self-biting, bar-biting, ear-pulling, hair-pulling, eye-poking and other bizarre activity patterns. There is nothing really abnormal, except the abnormally restrictive and abnormally boring housing conditions that induce the stereotyped expression of these activities. The majority of macaques who are kept in conventional, barren standard cages exhibit stereotypical activities. These behavioral patterns thus become **normative** under the given circumstance. In caged mice, barbering is another example of a stereotypy that has become a normative behavior within the context of inadequate living conditions.

When I worked with non-human primates, I often thought of things from their point of view. I would speculate how it would be to have nothing meaningful to do 24 hours a day, every day, for the rest of my life. I would envision my living space to be equivalent to the size of my kid's bathroom—not very big. There is water freely supplied to me and a means to go to the bathroom that suits me fine. I get the same kind of food every day for free, but what would I do with my time?! I am someone who is active and likes to exercise, so I would always imagine myself running back and forth from wall to wall, maybe running in place, doing sit-ups and push-ups over and over again to fill the utmost boring void, just to do something, trying not to go crazy. When I pondered in this way, I stopped looking at the monkeys' behaviors as abnormal but as a completely understandable **coping strategy** to an inadequate living environment.

When a human prisoner walks back and forth or walks in a circle for several hours each day, nobody would label his behavior as abnormal, as it is obvious that the restricted space of the cell leaves the person no other option to release the biologically inherent need to move/exercise the body. When a caged non-human primate or dog shows the very same repetitive movement patterns, we label the repetitive running in circles or the repetitive pacing back and forth as abnormal behaviors; why? It seems to me that the animal's repetitive behavior is not abnormal but the human-designed housing conditions that trigger it.

We tend to project abnormality onto animals rather than the people who create deficient living quarters for them. It would be fair to first focus on the husbandry conditions, study the environmental factors that lead to the development of stereotypical behaviors, and then correct these factors in order to prevent the development of stereotypical behaviors in the future.

The label abnormal would be more befitting for inadequate living quarters, rather the subject's frustrated attempt to adjust to them. When we say, "an animal shows abnormal behaviors," we make the animal quasi-responsible for behaving in an undesired manner; something is wrong with the animal, so we try to correct this behavior or make the animal stop showing it. When we do this, we are shifting the responsibility for the problem onto the animal, tacitly disregarding the fact that we—not the animal—created enforced, species-inadequate living quarters that make the animal behave in a strange manner. We—not the animal—created the problem, so it is up to us to fix it, i.e., refine the living quarters and living conditions in such a manner that the confined animal has no reason to develop stereotypical behavior patterns.

We know the cause(s) of most stereotypical behaviors. Why wait until an animal starts performing a stereotypical activity and then treat the symptoms?

It is important to work preemptively to avoid the development of stereotypical behaviors by meeting and maintaining the animals' behavioral needs from day 1 and throughout their life.

It is not unusual that animals and humans develop bizarre, repetitive behavior patterns when they are bored for a long time. Being confined in a more or less barren cage/room is probably such a situation in which parts of one's own body serve to provide some minimum stimulation for the mind. I would categorize such boredom-triggered behaviors not as abnormal but rather as normal attempts to cope with a biologically abnormal environment.

If animals develop stereotypical behaviors that cause physical (e.g., self-mutilation) or psychological harm (e.g., depression), then I would say that the animals cannot adapt to or cope with the human-created living conditions. I would classify such harmful activities as maladaptive behaviors or **behavioral pathologies** and argue that we have not only an ethical but also a scientific obligation to refine the animals' living quarters so that these behaviors stop completely and will not develop in any other animals raised and kept in such refined living quarters.

I think we could help laboratory animals more effectively by focusing our effort to prevent behavioral pathologies such as self-mutilation rather than engaging in the futile attempt to stop/eradicate normal adaptive behaviors, such as stereotypical pacing or running in circles.

< What does the term **environmental enrichment** imply? >

To my knowledge, the term environmental enrichment was originally introduced by the USDA in its Animal Welfare Final Rule (United States Department of Agriculture, 1991). These rules provide an **implicit definition** by stipulating under the heading *Environmental enrichment* on page 6500 that "The physical environment in the primary enclosures [of non-human primates] must [emphasis added] be enriched by providing means of expressing noninjurious species-typical activities." If facilities would follow this mandate to the letter, we wouldn't need to be too concerned about the behavioral well-being of non-human primates in research laboratories.

As a technician, I like the following definition, which I found on the title page of the Database on Environmental Enrichment and Refinement of Husbandry for Non-human Primates: "Environmental enrichment is the provision of stimuli that promote the expression of species-appropriate behavioral and mental activities in an under-stimulating environment."

As a veterinarian, I like the definition from the organization Shape of Enrichment: "Environmental enrichment is a process for improving or enhancing animal environments and care within the context of the inhabitants' biology and natural history. It is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out their species-appropriate behaviors and abilities, thus enhancing animal welfare."

In many circumstances environmental *enrichment* is not a suitable term. For example, when macaques are kept in barren single cages and are given puzzle feeders, that is not enriching their environment but it is making the animals' living quarters less poor.

If we provide animals in otherwise boring living quarters the opportunity to engage in behaviors that occupy a major portion of their lives in the natural setting, we do not *enrich* their unnaturally barren enclosures, but rather provide them with basic **necessities** required for the active expression of these behaviors also in the laboratory setting. I am sure the lay person has a different understanding of the nice term enrichment than most of us do.

To add biologically essential resources to a barren cage is over-egging it when calling it enrichment.

- Do we really "enrich" a monkey cage by installing a perch and adding a social partner?
- Do we really "enrich" a mouse cage by adding suitable nesting material?
- Do we really "enrich" a cage of a rat by adding a shelter and one or several other rats?
- Do we really "enrich" the animals' primary enclosures by allowing them to engage in foraging activities other than eating the freely available daily dry-food pellets or biscuits?

The answer is always No. Regardless of how we define the term environmental enrichment, it will always distract from the fact that we do not enrich the environment of captive animals, but provide them, at best, with opportunities to express very basic biologically programmed behavioral needs. That is the very minimum that the animals deserve.

It could well be that the term environmental ENRICHMENT was coined for the public, and not so much for insiders. We all know, for example, that providing nonhuman primates high resting structures so that they can access the "safe" arboreal dimension of the living quarters does not really "enrich" their enclosures; it only improves it, makes is more species adequate.

Environmental IMPROVEMENT would be a more honest and realistic term than environmental enrichment, but it would implicitly send the wrong, albeit correct message to the public.

I agree, environmental improvement sounds more honest and realistic but enrichment is much more pleasing to the ear and eyes :(

< Do **investigators** accept it when you enrich the living quarters of their animals? >

Investigators regularly object to the introduction of enrichment, whether it is food or toys because they fear for the comparability of their studies with previous work or with the work of others who do not provide enrichment. They insist on keeping their animals under standardized, albeit species-inadequate environments under the pretext that environmental variables need to be controlled in order to make their studies scientifically valid; yet they tacitly overlook basic variables such as the investigator himself, new caging design, cage location, cage illumination, new or

renovated animal holding facility, noise, etc. Using a double standard when it comes to extraneous variables may be convenient, but it is not at all scientific.

We have a form that all PIs sign, giving permission for enrichment. As more and more investigators become educated about the value of enrichment, some actually ask to provide more enrichment for their animals; this is so nice to see! However, our IACUC will give researchers permission to remove enrichment if they have a convincing reason for it.

At our facility, it is explained to the researcher that each animal cage has to contain certain enrichment objects or structures. An investigator has to have compelling reasons to keep his/her animals without such enrichment. When we get to know the techs working with the animals, we encourage them to distribute food treats, like cereals and also autoclaved hay, if this does not interfere with the research. I find that the techs are very willing to give these items to the animals in their charge. It makes all involved feel much better!

Before adding any kind of enrichment in the cages, we first seek the permission from the investigator to make sure that the enrichment does not affect the experiment. Our investigators have to inform us proactively if there are certain enrichments that must be withheld for study reasons.

In our facilities, we have begun the process of outlining in our IACUC protocol application forms what we consider to be standard practice, such as provision of nesting materials and shelters for mice, rats and hamsters. Researchers are asked to specify what enrichment provisions they want to be withheld and explain exactly how these enrichments would confound their experiments.

It is my impression that provision of basic environmental features is still not routine in the United States. The implementation of environmental enrichment in a facility seems to depend heavily on the motivation of key people.

I agree, environmental enrichment is often left up to the individual caretaker; there is no policy that has to be followed. The problem with this is that some animal care staff believe greatly in enrichment while others think it is unnecessary. This inconsistency is probably natural but it is very frustrating; without institutional standards, some animals will have the benefit of having their living quarters enriched by motivated personnel while others have to be content with barren cages because the attending personnel are not interested in providing environmental enrichment.

At our institution in the United Kingdom, environmental enrichment is the norm. If researchers want to opt out they must include scientific justification in their project license application so that it can be taken into account by both our internal ethical review process and the Home Office when they consider the license for approval.

Environmental enrichment is something that Brazilian researchers are not aware of at all. People usually do not give any environmental enrichment for rodents or rabbits, especially if it is a short-term experiment. Therefore, the question of obtaining permission to provide the animals with enrichment does not arise.

< How do you evaluate the **effectiveness of environmental enrichment**? >

The National Research Council (1998) makes it quite clear: "Enrichment methods that have not been subjected to empirical testing should be viewed simply as invalidated ideas, regardless of how well intended they might be. Without appropriate measurement and verification, we might do more harm than good in our efforts to improve animal conditions."

This sounds very reasonable, but it is a given, albeit sad reality that time, personnel, and budget are limiting factors that make it very difficult, if not impossible for us to evaluate all so-called enrichment items that we give our primates and rodents.

It may perhaps not be necessary to actually evaluate all enrichment items when we differentiate between biologically relevant enrichment—which should be mandatory—versus biologically irrelevant enrichment—which could be optional.

- **Biologically relevant environmental enrichment** triggers a response that has survival value for the subject, e.g., hiding in shelter, interacting with a compatible social partner, searching for and processing food and nesting material. The effect of this type of enrichment is predetermined by its intrinsic survival value and, hence, animals do not loose interest in it.

  Biologically relevant enrichment is by its very nature effective. For example: rodents do not get bored by a species-appropriate shelter; mice do not lose interest in species-appropriate nesting material; primates, dogs, cats and birds do not get bored by species-appropriate elevated resting surfaces; amphibians do not get bored by species-appropriate basking sites; animals in general do not lose interest in gadgets or other items that allow them to forage.

Biologically relevant enrichment is a necessity for captive animals; as such it should be a basic standard provision. Actually, the word enrichment is a bit misleading, making us think we are doing something as a luxury for the animals, when in fact it is often essential for the animals' physical and psychological well-being.

- **Biologically irrelevant environmental enrichment** triggers a response that has no survival value for the subject, e.g., pushing a ball, manipulating or gnawing a plastic toy, looking into a mirror or TV screen, listening to radio sound. The effect of this type of enrichment needs to be evaluated by means of behavioral observations, because the animals tend to get bored by it over time. It's effectiveness is dependent on it's novelty and, hence, requires regular exchange or rotation with new enrichment.

  Biologically irrelevant enrichment is usually not at all cost effective when managing large populations of animals. This is particularly true for toys or gadgets of which the animals quickly lose interest, hence several sets of such enrichment objects are then needed to rotate them—and sanitize them at that time—on a regular basis. Therefore, taking the cost benefit ratio into account, I feel it is prudent that we put our resources and manpower into enrichment options that are biologically relevant, producing long-term behavioral benefits to the animals.

< Does **music and radio talk** have any environmental enrichment value for animals in research labs, other than keeping the attending personnel in a good mood? >

For me it's all about attention and focus when I am in an animal room. This is one reason why I am not a fan of staff wearing headphones to listen to music or books on tape. It's so easy to let that capture your attention and your care just becomes rote. You have to be able to listen and pay attention to what's going on in the room, not just the cage right in front of you. When you are doing that it's much easier to think of them as little lives that need your attention rather than a collective mass of cages that need to be changed.

I have found in most primate research facilities I have worked and visited

- that radios or TVs are the main aspect of their enrichment plan, and
- that the animals are generally stressed by people.

Often the only contact they have is under stressful situations such as health checks, dosing and sample collection. Unless a great deal of effort has been

made to acclimate the animals to research procedures and human interaction, they are typically fearful and defensive-aggressive in our presence. Typically, the attending person listens to programs with people singing or talking. If the animals already find humans stressful, how enriching can it actually be for them when they are exposed for hours on end, against their will, to human voices from the radio?

It is questionable that non-human primates, and for that matter any animal species, like radio talk and music as their caretakers do. Being confined in a cage, not able to escape the source of loud and possibly disturbing music and human talk can probably be quite distressing for them. After all, what is music for the human ear is most likely noise for the animal ear, and if that's the case it is most likely a source of stress.

I've found that some technicians and care staff play the radios so loudly, you can hear them outside the animal rooms, sometimes even in adjoining rooms. I am lucky and can just leave when the noise gets too much on my nerves. The animals have no choice but listening to this cacophony; probably not a situation that is animal-welfare conducive.

We have had an ongoing problem with people playing radios in the animal rooms at excessive volumes, which could drive me—and probably also the caged monkeys—crazy! There was no way to get people change their habits voluntarily, so we had to make it a rule that no radios are allowed in the animal rooms and in the corridors. What a difference it made!

It is my personal experience that music in animal quarters does not necessarily make all humans who are exposed to it happy. Some people like loud rock music, others like soft background music, while others prefer silence. I would assume that animals also do not always share the musical taste of the attending care staff and, perhaps would chose silence if they could.

We used to have radios turned on in our marmoset rooms. After finding out that marmosets, when given the choice, prefer silence over any kind of music (McDermott & Hauser, 2007) we decided to turn all the radios off. There was some concern that the marmosets would be more nervous during any kind of disturbance, but this was not the case. When there was silence, the animals showed no conspicuous alarm reactions when people were talking in the hallways or someone entered their room. We now turn the radio on for short time periods only when we expect a sudden noise which might upset the animals, or when a technician is working in the room (to keep the technician happy).

That marmosets prefer silence to radio is not at all surprising when considering the fact that 30-minute exposure to playing radio is enough to double their salivary cortisol concentration (Pines et al., 2004); it's obviously stressful for them when they have to listen to the radio.

It is worth remembering that animals have very different hearing ranges than humans, for whom we have designed radios and speakers. What humans might find comfortable and pleasing, other species might experience as noisy and unpleasant.

I would argue that radio/TV music and talk should be allowed in rooms of research-assigned animals only if it has been documented that the music/talk does not disturb/stress the animals. Choice tests along with behavioral observations can easily clarify this question.

Non-human primates, being diurnal animals, may enjoy listening to certain types of music during the daylight hours, but rabbits and rodents are nocturnal animals who want to sleep during the day. Having to listen to music or radio talk is probably not beneficial for them. I don't have any experience with music in rabbit rooms here at work, but I do have a pet rabbit who very clearly prefers not to be in the same room in which loud or fast-tempo music is played. He will simply leave the room.

Krohn et al. (2011) exposed rats to different kinds of sound patterns, including radio, and found that the animals "showed a clear preference for silence to anything else, which may be taken as an indication that they feel disturbed by the sound from the speaker."

As long as the animals are not exposed to repeated noise—for example their room is located right beside the elevator machine room—the reason for the music is to keep us caretakers or animal technicians in a good mood. When the animals are alone, there is no need to keep a radio on. I am not sure if music per se is of any benefit to the animals.

< Are **running wheels** for rodents a necessity or enrichment? >

I see a running wheel as a necessity for caged hamsters and caged mice, because it allows them to release the biologically inherent drive for moving around; I am not an expert, but I would assume that in their natural habitat many rodents travel quite a distance within the area of their home territory in the course of a day. The running wheel is not a natural structure, but it promotes the expression of a behavioral drive that could otherwise find little release other than stereotypical movements. I am not sure what the situation would be for rats.

I wrote a review on wheel running and found that the distance run in 24 hours by animals in a running wheel can be as great as:

- 43 km for rats,
- 31 km for wild mice,
- 16 km for laboratory mice,
- 9 km for golden hamsters, and
- 8 km for Mongolian gerbils.

These findings suggests that rodents have a strong biological need to move their legs over considerable distances. In the artificial cage environment, a running wheel becomes a necessity for them because it helps them satisfy this need. Caged rodents don't lose their inherent drive for moving their legs, so as long as they are healthy they will not lose interest in their running wheels.

There was a research paper published a few years ago in which running wheels were placed out in nature and it was found that a variety of wild animals used them as well.

Your comment is so interesting that I checked the article; here is the reference with excerpts:

Meijer J.H. and Robberts Y. 2014 Wheel running in the wild. *Proceedings of the Royal Society B* 281: 2014.0210
https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.2014.0210

"Wheel running is often used in the laboratory for triggering enhanced activity levels, despite the common objection that this behaviour is an artifact of captivity and merely signifies neurosis or stereotypy. If wheel running is indeed caused by captive housing, wild mice are not expected to use a running wheel in nature.

Here, we show that when running wheels are placed in nature, they are frequently used by wild mice, also when no extrinsic reward is provided.

Some animals seem to use the wheel unintentionally, but mice and some shrews, rats and frogs were seen to leave the wheel and then enter it again within minutes in order to continue wheel running. This observation indicates that wheel running may well be intentional rather than unintentional for these animals.

Bout lengths of running wheel behaviour in the wild match those for captive mice. This finding falsifies one criterion for stereotypic behaviour, and suggests that running wheel activity is an elective behaviour.

Our findings may help alleviate the main concern regarding the use of running wheels in research on exercise."

< Is there any evidence that access to running wheels can prevent the development or decrease the incidence of behavioral disorders—such as barbering? >

Gebhardt-Henrich et al. (2005) found that single-caged hamsters show significantly less stereotypical bar-mouthing when they have access to running wheels. Similar findings have not been published for mice and rats.

< Do mice compete over access to one running wheel? >

The answer is definitively No. We have often seen several mice on one running wheel, and never witnessed any antagonism related to the wheel. It's not uncommon to have two mice running in the wheel and one or two, in addition, running on the top of the wheel. It is quite a sight!

< Do old rodents have any use for running wheels? >

Running wheels are great for young and adult animals who have the energy to exercise. Aged mice may sit in a wheel, but they are unlikely to run in it. I remember a study in which aged rats—24 months old—were tested on running wheels. These animals had hardly any use for the wheels. The researcher tested two separate groups of aged rats, and neither of them was interested in the wheels. For old rodents, an object for gnawing and manipulation is a better enrichment idea than a running wheel.

< What is the best strategy to **capture rodents who have escaped** from their cages? >

When rats or mice get out of their cages, we normally use a dustpan if the animals are scurrying on the floor—which they do most of the time. Most rodents, including guinea pigs, hamsters and gerbils, will run along the perimeter of a typical animal room offering no central shelter area. If you place the pan across the run, facing in the direction the critter is coming from, the escapee will run into it and happily sit there while you pick the pan up and safely and gently slide the animal back into the cage. This simple technique minimizes stress for the escaped rodent, eliminates the risk for the handler of being bitten, and it saves the elderly and arthritic amongst us having to get down on our hands and knees to awkwardly try to catch a swiftly moving, agile little animal.

If rats or mice have escaped overnight, we usually find them sitting in the food hopper of a neighbor's cage, finishing off the food they haven't managed to transport back to the homecage during the night. Sometimes their homecage gets so filled up with chow from neighbors, that they can't get back into it. This scenario typically implies that the neighbors have bitten

the tail and the feet of the scavenging escapee who, therefore, is relieved to be rescued by one of us.

I once had a rat escape and get inside an old radiator on the wall. Funny how dumb I was about it. I spent ages trying to reach in, stick things through the ventilation holes to get the critter and cut holes at various points. An hour later it was getting dark, the rat and I were both grimy and annoyed, and we were glaring at each other through the grill of the heater. Finally I stopped and thought: "What do rats like? Places that are familiar, dark and enclosed." I put the rat's homecage near the hole she had entered the radiator, and turned off the light. Thirty seconds later she was captured and returned back home.

< What are common **extraneous variables** that can affect the validity of research data collected from laboratory animals? >

handling practices

Handling procedures are a significant source of stress for animals in biomedical research facilities.

Unfortunately, inertia of tradition makes it difficult to change/refine stressful handling practices pretty much of all animals in biomedical research labs. This is very frustrating because in has been documented in the scientific and professional literature that animals typically can be trained or conditioned to cooperate rather than be afraid and resist during common husbandry and common research-related procedures, thereby reducing physiological stress reactions that can invalidate research data obtained from these animals. Investigators very often fail to describe where and how the test subjects were handled during data collection, even if stress-dependent parameters were measured (Reinhardt & Reinhardt 2000b).

witnessing stress reactions of another animal

< Do animals get stressed when they witness stress reactions of another animal? >

We have just finished a study on the effects on caged mice, when cage mates are removed and subjected to one-hour restraint stress in the animal room and then returned to the cage. We did this once daily for 14 days. The cage mice were not touched and had implanted telemetry transmitters to monitor heart rate and temperature. While cage mates were being restrained, the heart rates of the untouched mice in the homecage peaked at about 650/min, 15 minutes into the restraint period. Only by the end of the one hour restraint period, had heart rates of the untouched mice returned to

baseline. The untouched mice's stress response did not show signs of adaptation within the 14-day study period. A similar pattern of stress response to witnessing restraint stress of the cage mate was found in the rise of body temperature.

Several authors (e.g., Beynen, 1991; Crockett and Gough, 2002; Pines et al. 2004) documented a similar phenomenon in rats and cynomolgus macaques and marmosets who show physiological stress reactions when they witness how other conspecifics exhibit signs of stress during a handling procedure.

being removed from the homecage

< In your own experience, what can be done to avoid getting animals stressed when they witness another roommate being subjected to a distressing husbandry or research-related procedure? >

At our facility, only non-invasive procedures, such as weighing, can be performed in the animal rooms. Everything else has to be done in procedure rooms. This creates fewer disturbances for both animals and humans. I realize, there is some stress involved in temporarily moving individual animals away from their familiar quarters to procedure rooms, but I have the impression that this policy reduces the overall disturbance and stress that <u>all</u> animals of that particular room experience.

Whether we handle a research subject in the animal room—thereby disturbing many animals—or move the research subject to a procedure room—thereby causing additional stress prior to the actual handling, stress seems to be an unavoidable variable, unless we can train the research subject to voluntarily cooperate during the handling procedure in the familiar homecage.

I conducted a study with six single-caged adult rhesus males who actively cooperated during venipuncture in their homecage and who also actively cooperated during venipuncture in a mobile cage located outside in the hallways. The magnitude of cortisol increase was significant when the males were venipunctured away from their homecage but not when they were venipunctured in the homecage. My conclusion was that venipuncture per se was not a physiologically distressing event, but it became distressing when it was associated with a temporary removal from the home quarters (Animal Technology 1991, 42: 83-86).

When blood collection took place in the homecage, there were no signs that the other animals of the room got disturbed/stressed simply,

because the treated subject cooperated during the procedure and exhibited no behavioral or vocal signs of fear or alarm.

When doing injections for rhesus males in the homecage I never "snuck" the injection. I show the male the needle. This helps to keep him and his roommates calm. Often, the subject of my attention presents his thigh for the injection; that's a deposit in my trust bank-account.

I like your approach. Macaques are smart and spontaneously learn to cooperate with you rather than resist during a routine procedure such as capture, injection or blood collection when they know through experience that they can trust you. Too little use is made of the fact that macaques (probably also some other species) can easily be conditioned or formally trained by a kind person to work *with* that person during non-invasive procedures, thereby avoiding stress reactions. Yes, mutual trust is the key; but that's not a big deal.

noise in the hallways

< How do you minimize noise in the hallways of animal quarters? Have you come up with any great engineering solutions to minimize or even eliminate the sounds of caging or other equipment being moved around? >

In a word: Wheels ...

Make sure the wheels on all your movable equipment are in good order. In addition, one thing we've done that cuts noise is the elimination of most stainless steel lab carts. We now go with Rubbermaid carts; what a difference!

At our facility, we have also moved from metal carts to Rubbermaid carts.

We exchanged old, loud and squeaky casters and wheels of our carts with new ones that are rolling very smoothly and produce hardly any sound. It made a big difference!

At our laboratory, automatic swinging doors were installed at the two ends of hallways to reduce noise. The husbandry staff just pushes a button and the doors open to allow ease of movement of caging, equipment, etc., so there is no loud banging. The doors close automatically after a set period of time, and when they close (although still metal doors) it is very quiet.

Several years ago, we replaced heavy metal doors that divide corridors with plastic swinging ones. Pushing equipment through the plastic doors creates much less noise. Another thing I find very helpful is to move long equipment on carts with two people; that way both ends of the equipment can be controlled so there is less chance of scratching at walls.

I do like the idea of using two people to move large pieces. This not only is bound to cut down on noise but it is also much safer, ergonomically, for the staff.

ultrasonic sounds

< Evidence demonstrates that ultrasonic sounds of intensities common in animal facilities have deleterious effects on rodents physiology. What do you think are the biggest culprits for ultrasound in animal research facilities? >

Commonly used equipment that makes noise in the ultrasonic range are computers and monitors, ventilation systems (some are very bad!) and lights, even some red lights that are supposedly used for testing rodents during their active period.

One additional source of ultrasound that we noticed is running water. Taps seem to emit very loud ultrasound in the rodent audible range. We could clearly measure significant ultrasound noise from several feet away using an ultrasound microphone when the tap was running. Our technicians often run taps for several minutes to get the water cold before refilling bottles. When this happens, the rats get very alert and restless; obviously, the noise of the loud ultrasound disturbs them quite a bit.

Did you have metal or ceramic sinks? Was there anything you could do to eliminate this source of ultrasound? Especially when the water drips or runs in a stainless steel sink, that causes a lot of ultrasound.

All our sinks were stainless steel, and I'm sure you are correct that this would contribute to the noise.

Other sources of ultrasound noise that are not associated with equipment but with people are keys and sniffing. Dangling keys make a lot of noise. Human sniffing (because of a cold or allergies) is also making intense noise; it can startle rats and make them freeze.

We obtained a high frequency meter to detect sounds resulting from construction activities that might be problematic for our rodents. To our surprise, we discovered the noise associated with drilling and jack hammering was not within the animals' hearing frequency range, but jangling keys, water running into metal sinks, and glass water bottles clinking were significant sources of ultrasound that are likely to be perceived as very loud by the rodents.

construction noise

< Our city is going to build a tunnel running underneath our primate facility. I am concerned that our animals will be affected during the digging, drilling and dynamiting. How will our rodents and our macaques cope with the construction noise? >

We had a similar experience and noticed that our macaques were very disturbed in the beginning, but seemed to get used to the occasional bursts of extreme noise very quickly. It helps when the attending caretaker stays in the room, talks to the animals and offers favored food or other items that they find attractive.

I work in a monkey facility where the floors in the hallway right outside the primate rooms were recently jack-hammered and a tunnel built underneath the building. The flooring project took four months and the tunnel is still not completed. In my opinion, the noise is not the biggest issue with our monkeys, though I did notice an increase in locomotor stereotypies during the jack-hammering. What really distressed the animals was the fact that we had to keep moving them around the building, so that the workers could access the hallways to take the flooring up. That did upset the monkeys quite a bit, and it took them several days to settle down in their unfamiliar living quarters.

We recently had our cage wash outfitted with new tunnel washers. During that time, our non-human primates became extremely stressed and agitated. The monkeys alarm-called a lot during the day while construction was going on; many of them developed stereotypies that were not observed previously, like swaying back and forth and charging their own reflections. Others who had already displayed stereotypies—like hair-pulling—before the construction started, increased these behavioral disorders, and some even began self-biting after the construction got underway. We did our best to alleviate the animals' distress and occupy their time during the day by providing extra enrichment items like foraging boards and puzzle feeders.

The construction went on for several months during the summer, and I have to say that our primates did not adjust over time. Even after the construction was completed and the noise finally stopped, some monkeys continued with their new stereotypical behaviors.

We had a lot of noise going on over the last couple of months during construction of a new outdoor monkey area with a swimming pool, as well as a new indoor building for the monkeys. The new digs were built about 30 feet behind the existing facility, so the animals could see and hear everything that was going on.

The rhesus monkeys were stressed out during the first couple of weeks, but their stress levels decreased gradually as they became accustomed to the project. I knew they were stressed out at the first stage of construction, when I observed a lot of pacing, out of character screaming, fear grimacing, even leg-biting and arm-biting—behaviors that these

monkeys had never shown while they were in my care. So, during the first couple of weeks, I stayed with them in the indoor area for reassurance; I played some of their favorite movies with the volume up quite high to drown out the sound of construction. The only thing that remained an unwavering stressor was when the concrete trucks arrived. The trucks made a loud beeping noise as they were backing up; the monkeys never became used to that. So during concrete delivery we locked the animals indoors until the trucks were gone. I handed out treats and gave a lot of reassurance during that time. They could still hear the trucks and the beeping, but at least this extreme noise was somewhat muffled.

When construction was being done on campus within an acre of our facility, the barely audible noise and vibration threw all our rodent breeding programs out the window for quite some time.

We cut the foam mats that are used at the changing stations in the room into square pieces and place them under the wheels of static racks to buffer the vibrations to the cages on the rack. I have heard investigators say that they have noticed that the vibrations caused breeding issues in mice when no foam was in place, but that the mice maintained a regular breeding schedule when foam was placed under the cage racks.

While analyzing a rat adjuvance arthritis study, we found a significant and unexplained dip in body weight on one day, followed by immediate recovery over the next two days in all of our rats. When we asked the animal unit whether anything extraordinary had happened with our animals that day, they told us that the previous day, a large Positron Emission Tomography scan needed to be inserted in the building, and one side wall of the building had been opened. Obviously the noise created by this construction stressed our rats very much.

We have been in the middle of construction pretty much constantly for the past ten years. Most of the noise had little noticeable consequence, however, when a classroom building was built across the street from us, the ground was first tamped for three weeks and then pilings were driven for another three weeks. For six weeks the ground vibrated constantly for eight hours a day! We lost at least six months' worth of breeding of the transgenic mice; even the zebra fish stopped laying eggs. Unfortunately we did not have anywhere to move the animals within the facility to shield them from the commotion.

To my knowledge, the impact of construction noise on physiological parameters of laboratory animals has not been examined in any scientific publication. The implications of such studies' predictable findings are probably the reason why they have not been conducted, let alone published.

principal investigator

< It is my experience that many principal investigators show little or no interest in how their animals are housed and handled, and if they do handle their animals themselves, they often lack proper skills and patience. How do you train such individuals to realize that their attitude defeats sound scientific methodology? >

It is quite difficult to train researchers to do their work with the animals in a more considerate and compassionate manner. Unfortunately, investigators often see the results of the research as more important than the animals themselves. They are in a hurry to get results. Usually they do not take the time to get to know their animals, let alone work with them from time to time in a more relaxed, less stressful ambiance.

One thing that really bugs me about this business is, that a lot of the time investigators do not know how to treat the animals as sentient beings. A big problem is that first-hand experience with animals is often not a requirement for the researchers and their technicians to be hired, and/or to receive funding for their research proposals. I wish everyone involved in animal research was an animal person, but sadly, that is not the case.

Researchers don't need to be animal people, but they should be mature enough to respect animals (just like other humans) as sentient creatures whose physiological equilibrium will be compromised (extraneous variable stress) when they are not properly housed and when they are not properly treated/handled.

I think we (animal care institutions) need stricter regulations around what scientists/investigators/researches need to know and be proficient at before they start using animals in research projects.

I am working with investigators who do not know that the rats they are doing research with are nocturnal animals. I always love the statement, *They seem happy to me*. I actually hear that quite frequently from researchers. Usually they simply mean that the animal is moving about in the cage, but there is no comprehension about whether the movement is normal or indicative of stress or boredom or discomfort.

It all is so obvious for those who genuinely care for the animals and are concerned about valid scientific methodology, but it seems to be of little

or no relevance for those who see in the animal merely a means to get publishable data.

Our investigators usually have grad students doing the research-related procedures. It is not often that they will show up in the animal areas, while others I have yet to ever see. We had some grad students come in to work with mice, others to work with monkeys, but they had never actually worked with a mouse or a monkey before! Apparently, the principal investigators had failed to make sure that their students had received basic training and were actually qualified to work with the animals in an appropriate manner.

Time is a major factor when dealing with researchers and their attitude towards animals. Often, I have tried to help the researchers with a task involving animals, such as acclimatizing an animal to a restraint procedure, only to be told, "No, that would take too long."

There are many things that we could do to help alleviate stress, and improve the well-being of the animals, but these refined techniques may take a bit longer than the traditional, often quite brutal methods. The researchers usually give the impression of being in a hurry to get their data as quickly as possible and, therefore, that there is no extra time for the animals themselves.

Many investigators have no clue how "their" animals are housed and how they are handled during research-related procedures. Unfortunately, they make little or no attempt to communicate with personnel who work with and care for their animals; they could learn a lot from them if they were a bit more humble.

I find that 3 out of 4 times I communicate directly with a PI about their animals' behavioral needs, they are fascinated and very grateful. But there's always that one guy....

It is my experience that it is exceedingly rare to find researchers who get into the muck and have some appreciation of what it requires to provide decent housing and handling conditions for their animals. We still have quite a few who do not even want to walk through our dirty cage area side to drop off empty caging.

It always strikes me that many investigators hardly ever show up in our animal area. Some of them probably have never seen the animals assigned to their projects. Yes, they are familiar with the IDs and the subjects' history, but that is often all they know of the animals they are doing research with. A prestigious biomedical scientist puts it in a nutshell when

he concedes that, “Most investigators think only briefly about the care and handling of their animals and clearly have not made it an important consideration in their work” (Traystman, 1987).

weekends and holidays

< Do the animals in your charge behave differently on weekends and holidays than on workdays? >

Our rhesus and stump-tailed macaques, but also our guinea pigs, rats and chickens are less restless, less alert and apprehensive on weekends and holidays than during workdays, when personnel can enter their room any time, catch them and subject them to a painful procedure.

I agree that the animals—I refer to rhesus—seem to be calmer, more relaxed on weekends and holidays. This is perhaps not surprising, because the personnel who do the more invasive parts of the research are here during the week, not on weekends/holidays.

Hassler et al. (1989) and Schnell & Wood (1993) assessed cardiovascular stress parameters of rhesus macaques and marmosets and found, that values are significantly lower on the weekend than during workdays. Entry of technical staff into the colony could be clearly identified in the heart rate and activity recordings of the animals. Schreuder et al. (2007) made similar findings in rats: The animals’ heart rate, locomotor activity and blood pressure differed significantly on workdays versus weekends.

When I started training rhesus macaques to work with, rather than against me during husbandry and research procedures, I noticed very quickly that the animals get easily distracted by activities and noise in the hallway. Once distracted, they no longer held the focus on me; and this disrupted the training process. After I had learned the lesson, I scheduled all my training sessions at time periods when I could count on the fact that there is no noise and no human activity going on in the hallway: lunch breaks, Sundays, holidays and sometimes after work. Under these conditions, training the animals was easy, safe and very enjoyable,

multi-tier caging

< The cages of small and medium-size animals—such as rodents, rabbits, cats, monkeys, birds—are traditionally stacked on top of each other in a multi-tier caging arrangement to allow maximal usage of room space. Animals caged in lower rows live closer to the ground, in a less illuminated environment than animals caged in upper rows. These differences introduce extraneous variables that are usually <u>not</u> accounted for in scientific articles

(Reinhardt & Reinhardt, 2000b). Is this an issue we have to be concerned about? >

Ader et al. (1991) noticed in mice that animals caged on the top of a rack are more fearful than those caged on the middle or bottom shelf of the rack. Mice probably feel more secure and secluded in the relatively dark environment of lower shelves than on the top shelf that may expose them directly to bright light. Garner et al. (2004) found that barbering was significantly more severe in upper-row than in lower-row caged mice. Lagakos & Mosteller (1981) also studied mice and found that the incidence of certain tumors increases conspicuously from the bottom to the top shelf. Similar observations were made by Mantel (1980), Greenman et al. (1984) and Young (1987). These studies make it quite clear, that shelf level is an important extraneous variable that should be taken into consideration in scientific research with mice.

It is my experience with macaques that animal living in bottom-row cages show more behavioral stress responses—such as crouching in a back corner, alarm vocalization, hyperaggression—when an investigator, dressed up in protective garb and a surgical mask, enters the room than those living in upper-row cages. At the same time, lower-row caged animals tend to *escape* into a transfer box readily, while upper-row caged animals often stubbornly resist to leave their cage and exit into a transfer box. In a quantitative study I did on 20 pair-housed cynos, the animals spent 94 percent of their waking time in the upper part of the vertically arranged double cage. All food was given in the bottom section, yet the animals would bring the food to the upper part and consume it there. The monkeys' preference along the gradient of height was unequivocal!

illumination differences in cages

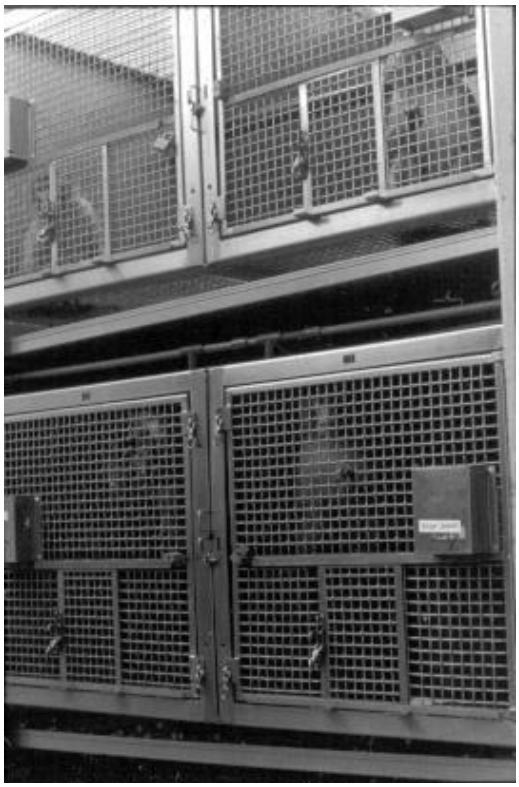
< Does anyone of you work in a facility that has successfully dealt with the illumination differences in cages arranged in multi-tier racks? >

I do not think the researchers over here have given it any thought. I do not even think that it crosses their mind, that the quantity and/or quality of light their mice are receiving could affect the findings of their studies with these animals.

The differences in illumination in upper- versus lower-tier cages are indisputable. Clough (1982) is probably not exaggerating when he states that light intensity in the cages is likely to be the most variable environmental factor in the average animal room.

The Animal Welfare Regulations pertaining to non-human primates of the U.S. admonish on page 6497 that, "Lighting must be

uniformly [emphasis added] diffused throughout animal facilities and provide sufficient illumination to aid in maintaining good housekeeping practices, adequate cleaning, adequate inspection of animals, and for the well-being of animals" (United States Department of Agriculture, 1991).



This legal mandate cannot be met when monkeys are kept in the traditional multi-tier caging systems. While animals in the upper row live in illuminated quarters those in lower rows often live in a semi-gloom environment, making it often necessary for care personnel to use flashlights in order to identify individual animals and assure the adequate cleaning of the cage. The striking difference of illumination between upper and lower cage rows belies the scientific principle that environmental variables must be controlled strictly to ensure the validity of collected research data.

The National Research Council (1996) advocates rotating cage position relative to the light source to account for the different housing environments of animals kept in upper-row versus lower-row cages. I very much question if this is an acceptable trick or if it simply rotates the problem without fixing it. If anything, rotating cage position is likely to make the methodological situation even worse, by introducing another source of variance.

In order to bring more light into the lower-row cages of macaques, I had all solid side panels replaced with mesh walls, allowing more light reflecting into the cages. This modification more than doubled the light intensity in the lower-row cages, but it did not eliminate the illumination difference between upper- and lower-row cages.

The differences in light could be addressed for rodents, by providing all animals with a species-appropriate shelter or nest. The animals will hide and rest in these dark places most of the time, thus being exposed to much more equal illumination.

individually ventilated caging

< Those of you who have first-hand experience with both the individually ventilated caging (IVC) system and the traditional caging system, which system is more animal welfare conducive? Economical factors should not influence your decision, please, only the standpoint of the caged animal. >

I believe that in terms of animal welfare, the development and uptake of individual ventilated cage systems is one of the worst *advances* in laboratory animal housing. I am regularly told that environmental enrichment is a threat to biomedical research and that more studies have first to be conducted before enrichment can be adopted by the research industry, but so many labs have gone over to one of the many, extremely various ventilated systems without raising this same objection. The lack of data on this subject boggles the mind, given the numerous variables that come along with this caging system, for example sound attenuation, smell attenuation, sensory deprivation, vibration, ultrasound, reduced handling, and movement to less preferred bedding types.

In our lab, IVCs are used only when researchers need frequent access to nudes and several strains of knockout mice who are immune compromised and, consequently, would not survive in open-top cages. We have not observed any detrimental effects of this caging system on the mice. However, IVCs and the associated equipment are relatively expensive and very labor intensive, so they are not something we would choose on purely economic grounds. There are two other drawbacks: The contact between animals and care personnel is reduced, and the complicated technology creates a comparatively high risk that something goes wrong. My biggest fear is a power outage, where the emergency back up does not kick in and whoever is on duty does not realize the implications and forgets to phone me.

restraint tubes for rodents

< Rodents are often restrained for blood collection and injection, by coaxing the animals into little restraint tubes. I wonder if this kind of enforced immobilization is not introducing stress as an uncontrolled variable into the data collected from such animals? >

We had a group of visiting scientists who used tube-restraint as classic stressor for experimental purposes in their research facility. When they saw us working with our rats, they could not believe their eyes: Our rats were quite happy to crawl into the tubes, go to sleep and show no apparent signs that they had become stressed by the procedure. It may well be, that our rats were particularly good-natured and laid back and/or were so well habituated to being 30-minute tube-restrained, that they calmly accepted the situation.

I also find that rats, mice and guinea pigs will enter restraint tubes quite happily, provided I am patient and gentle-and-firm the first couple of times when I prompt them to crawl into a tube. The initial experience associated with the tube is probably the determining factor of the restrained subject's response to subsequent restraint sessions. I encourage our researchers to handle their animals daily during the week prior to the actual studies. On these occasions they will also tube-restrain their animals without doing any other procedure that could possibly cause pain to the animals. This preliminary routine assures that the subjects not only will be familiar with the researcher, but they will be well acclimatized to the tube already at the beginning of the study. I think this provides a good condition for the animals to experience little or no restraint-related stress during the experiment.

Either we use ordinary transparent restraining tubes that we cover with a paper towel, or we use opaque tubes made of red Perspex so that they become a dark, safe hiding place. After all, the natural instinct of rodents is to seek a dark shelter in the event of danger, and being coaxed into a tube by a human hand must, indeed, be rather scary for them . When the tubes are transparent and uncovered, the animals will often wriggle about in what I presume is an attempt to get out. Since the scent of a stranger adhering to the tube is likely to induce a negative reaction in rodents, we thoroughly rinse the tubes between cages. We did notice that the animals are more reluctant to crawl into the tube and tend to be restless in the tube when we skipped the rinsing.

With a bit of training rodents do enter such tubes without appearing to be stressed, and as already pointed out, they will often fall asleep after a few minutes. They do give the impression of being relaxed, even though we take

tail-cuff blood pressure readings, or withdraw blood from previously implanted cannulae at various time points. When I take the animals out of the tube after a procedure, they are not agitated and usually resume their routine business, such as exploring the environment and grooming themselves. I really believe that gently habituated rodents do not experience undue stress, or any stress at all, while they are restrained in dark tubes during noninvasive procedures.

I should perhaps emphasize, it is very important to make sure that the animals do not get overheated while they are restrained in the tubes. They can get hyperthermic very easily, and this will certainly distress them; they will come out of the tubes in a state that I can only describe as *prostrated*—reluctant to move, panting, semi-conscious, damp or moist. This must be avoided, and it can be avoided by keeping the animals in the restrainer for only short periods at a time.

< Does anyone want to share tips, tricks, procedures, processes that have been implemented at your institution to **monitoring social housing**, maybe something that you found has really been a game changer for getting the program up and running, or something that might be unique to your institution? >

We have a card with multiple check boxes that gets placed on the cage of any singly housed animal, specifying why the animal is caged alone. The same card is used for mice and large animals and everything in between. That way, when IACUC, USDA, vets, or anyone else walks through the facility and sees a singly housed animal, there is a card on the cage indicating why the animal is singly housed (e.g., aggression, only animal left in the cohort, research-related reasons). This system pre-dated me here, so I had nothing to do with the idea, but I think it works really well.

At my previous institution, we had a spreadsheet for tracking the social-housing history of all of our monkeys. It was a handy way to see at a glance:

- which animals had been housed with whom,
- which animals are socially compatible,
- which animals are socially incompatible,
- which animals are singly housed, for what reason, and
- what are the plans to transfer singly housed animals to compatible social housing?

Our USDA VMO really liked this spreadsheet; it was pretty low tech, but effective.

That is a great idea. Too often animals are left singly housed because nobody is sure of the reason why the animal is living alone.

Sometimes we will hold off the weaning of mice for a day or two to ensure the pups can be weaned together with another litter of the same strain so that no mouse ends up singly housed. One of my colleagues used to put slips on the cages saying *Lonely female please give me some friends* when there were singly housed female mice.

The medical records database of our NHPs has housing status and cohort/partner information (social compatibility or incompatibility) as one of the fields. I can easily scan a room on the computer or run a report of singly housed animals. I use this information to keep a monthly report for the vets on the animals' housing status. The report helps me determine potential pairs in order to resolve single-housing status.

The pair-housing status and the reason(s) for single-housing of our macaques are entered in the animals' computerized records. Every month we document the reason(s) for single-housing and what attempts have been made to transfer single animals to compatible pair-housing arrangements.

< How do you go about it when you have an **environmental enrichment idea that you want to implement** at your facility? >

When I was involved in the animal enrichment program for my company we had an enrichment committee that would meet every month to investigate new enrichment devices and how to evaluate their usefulness for the animals. I personally would make full use every year of the resources and vendors at the Tri-branch Symposium. I would specifically hit all the enrichment vendors and obtain lots and lots of their environmental enrichment free samples to bring back to my company and distribute them to animals. When I saw animals showing interest in certain gadgets and interacting with them over several days, I put them on my list for our next committee meeting. We would order just a few of each of these samples, monitor their use by the different animal species on site over the course of several weeks and report back at our next meeting. We then made our request for funding to buy those enrichment items in bulk that had proven to trigger species-typical, non-injurious activities and behaviors in the animals over extended periods of time.

When I worked with macaques, a lot of preparatory work was needed before I could approach the administration with a proposal for the implementation of a new Refinement in our colony of several hundred

macaques. To start with, I had to get permission by the senior veterinarian to expose a few animals who were presently not assigned to any ongoing research to a new idea. Typically, I conducted a formal study to demonstrate if the new idea achieves the intended effect, creates no hygienic issues, causes no harm to the animals, causes no harm to personnel, is not labor intensive and does not cost much. Once this preliminary hurdle was passed, I could ask the administration for permission to go ahead with the implementation of the new idea.

This process did do the trick in most cases. I was able to implement quite a number of unconventional ideas: installing high perches in all cages, providing all animals with unsealed wood (gnawing sticks), transferring single-caged animals to compatible pair-housing, modifying the standard feeders into puzzle feeders, and providing each animal daily with a whole apple. There was one refinement idea that did pass the first hurdle, but I was not able to implement it on a large scale: training the animals to cooperate during injection and blood collection in the familiar homecage. In the course of a year, I successfully trained about 60 animals but finally gave up because of time constraints and lack of interest by the PIs.

My experience is much like yours. All new ideas must first be reviewed by the veterinarians. I will then submit a proposal to the director in which any published findings related to the new idea are discussed and permission requested for a preliminary testing of the idea with a small number of animals. When the findings of the pilot study demonstrate the usefulness of the new idea, I will draft an official guideline (Standard Operating Procedure), and once that's approved, the new enrichment/refinement idea is ready to be implemented. The whole process can take quite a while, especially when the new idea aims at refining traditional handling practices of non-human primates. For example, I have been working on implementing a positive reinforcement training program for our macaques for the last three years; I have just now submitted the final draft of the SOP.

Yes, although the process can be annoying and frustrating at times, the result can be very valuable and satisfying, especially when you have the assurance that the implementation of your idea is officially supported independent of you, the start-up person.

We almost always will ask for one or several samples of commercial enrichment items. We need to be certain that not only is it going to meet the enrichment need of the animals, but also that it can withstand the washing process. Will it go through our automated system without falling through the

disposal grate so that it can be retrieved? Does it have small grooves or crevices that will be difficult to clean? How many times can it be autoclaved before (if) it starts to show signs of stress?

After we have observed that the animals use it appropriately, we will have to ensure it doesn't create a nightmare for our cleaning or care staff.

I can't think of any item that we just took at face value, not because we were concerned the animals wouldn't use it, but because that piece of enrichment is going to become part of our entire process so we need to be certain we can truly incorporate it.

< Do you find it helpful **listening to in-house presentations of principal investigators/researchers** so that you can get a picture of the potential scientific benefits and the possible costs for the research subject(s) of an invasive study to be conducted or being conducted with animals in your daily charge? >

Our researchers are required to make presentations about projects before they can begin the studies. These informative presentations are very helpful. Unfortunately, I do not always find the time to attend them.

I wish we had those types of informative in-house presentations and discussions. Research protocols are available to us to read, but there is no time for us to review them thoroughly so that we can get an idea of their implications for the research subject's safety and well-being.

It has been my experience that animal caregivers are typically overloaded with work. Yes, we are invited to attend seminars that could inform us about planned and ongoing research in our units, but we are not given the time to do this.

As an animal technician—not a caregiver—I do not have the time built into my schedule to attend lab seminars. Very few of my colleagues who are actively involved in a study are given the time to attend these lectures. I barely have time to eat lunch some days!

I am also a tech. I have to make time to attend the programs I am responsible for, so that I can offer suggestions to refine studies that would otherwise stress my monks. For the most part, these important sessions are not built into my work schedule.

< As animal caretaker or animal technician, do you **communicate with the PI** about your ideas of avoiding or at least minimizing certain housing- and handling-related variables of which the investigator is not aware? >

I can discuss animal welfare concerns openly with several researchers without fear of them getting defensive or reading more into what I am saying

than necessary. There are others who are immediately defensive and can quickly become combative. They will take it as a personal offense when your suggestion implies that they may not be aware of everything. I'm sure there is a government program that requires research institutions to hire a certain quota of these difficult-to-like people.

We as animal techs have very little contact with the PIs. This can sometimes be frustrating especially when our views regarding animal welfare and species-appropriate housing and handling are kind of ignored by the PI who, typically, is not aware of his research subjects' fears, discomfort and distress.

Over the years I have realized that the varying ways people get into the research field and how the scientific research staff is reviewed and rewarded (grants and pay raises) makes many PIs unaware of the fact that the welfare of their animal subjects has a significant impact on the scientific validity of research data obtained from them. Animals are not a focus or even an interest of most investigators.

Having worked as a scientist with many scientists in the course of more than 30 years, I must say that the genuine scientific motivation of researchers is very often clouded by a fierce career-oriented competition that leaves little room for so-called sentimentalities such as compassion for animals and making sure that animals are properly housed and carefully handled during procedures.

I may not speak on behalf of all animal care techs, but it seems to me that we have chosen our profession because we are fascinated by animals and have compassion for them. Researchers on the other hand chose their work with animals not because they have feelings for them but because animals can help them promote their professional career in biomedical science. To find a consensus between these rather opposite goals and achieve a harmonious cooperation between both sides is a big challenge.

Husbandry staff is hired to provide basic care for the animals. They need to work on a schedule created by others, stay within a timeline as dictated by a time clock or supervisor, and do very repetitious assignments in order to keep their positions. Research staff is hired to conduct scientific projects; PIs need to write grants, publish papers and attend scientific meetings in order to keep their position and advance their career. It can be hard for the two groups to get on the same wavelength. And the techs, well, we have to float between husbandry staff and research staff who at times seem to speak completely different languages and are unable—or unwilling—to respect each other's view points and goals.

I tend to have a very good relationship with the PIs, their graduate students and staff. However, there are a few PIs who seem to believe that their academic education makes them superior. I have run into investigators who treated me as if I have *lower intelligence* simply due to the fact that I wear a blue coat and punch a clock. To communicate with such individuals not easy, sometimes impossible.

It gives me great pleasure to say that most of my institution's investigators are very open to animal welfare issues. In particular, I have an investigator who is amazingly concerned that his animals—dogs and swine—receive the best possible care. He's a physician by trade and is one of the most attentive and caring researchers I have ever worked with. He has actually come to me to inquire as to how certain animals should be handled in order to keep them *happy* prior to even submitting a new protocol. He ensures that his entire staff is well acquainted with the critters they are working with and how to treat and handle them, so that they experience the least possible stress during procedures. He is also open to having his animals adopted after they have been released from research. During the past three years, seven of his dogs and one swine found loving homes.

Thank you for sharing this exquisite example of a truly responsible and caring investigator. I do think that it requires some humbleness on the part of a PI to seriously listen to animal caretakers and technicians who, after all, have so much more first-hand experience with animals before, during and after research is done with them.

Discussions on Mice

< What kind of **bedding** do mice prefer? >

We use ¾ *dust free* autoclaved softwood sawdust as bedding substrate for our mice and have not encountered health-related problems any strain, including nude mice. We switched to that substrate after quite a number of our nude mice had developed conjunctivitis on fine sawdust bedding that had a relatively high dust content.

Anything that nudes receive should be dust/lint free, we have had eye issues with them in many years past because of bedding/nesting material that was dusty or contained lint.

Our mice prefer shredded paper and wood-wool over woodchips or sawdust as bedding probably because the paper and wood-wool not only serves as bedding but can also be used as nesting material.

We use corncob litter which absorbs urine pretty well. There is no indication that it irritates the skin of our mice, who use the corncob litter not only as a bedding, but also as a foraging substrate.

Mice probably prefer bedding that they can burrow in, something that doesn't quasi-flow but will form tunnels. Generally we use softwood beddings that are kiln dried and therefore highly absorbent; it is a relatively large particle-type bedding. Wood shavings are also suitable, but they can create problems with vacuum systems of bedding removal from dirty cages.

I wrote my dissertation about bedding preferences of group-housed female mice; I only tested wood product bedding, not nesting material. *My* mice clearly preferred wood shavings over wood chip bedding (Kirchner et al., 2012).

We presently use two types of bedding, Beta-chip (small hardwood chips) and Alpha-dri (small square chips of alpha cellulose). Beta-chip is very dusty; the cage wire lids and micro-isolator lids must be changed more frequently than usual. I need to wear a mask when handling this substrate a lot. Alpha-dri is more absorbent and not as dusty but it is harder to tell if a water bottle has a slow leak, because there is no change in color of the bedding when water is present. The cage looks dirtier than with Beta-chip because it is white, and you have to get over the urge to change it before the scheduled time.

As a side note, inbred mice who have not encountered a digging substrate for at least 20 generations will take just about 30 minutes to build a perfect burrow when they get access to a generous amount of peat. Mice are

highly motivated to burrow in suitable substrate; burrowing seems to be a behavioral need for them.

<What's your experience with the various shelter options for mice; do the animals have a preference for a special type of **shelter**? >

We have found that, if you are using **plastic shelters**, some strains need to quasi-grow up with them in order to use them when they are adults. When we first investigated plastic shelters, we noticed that adult mice rarely used them, but if introduced at the age of weaning, the young mice will accept them more readily and you will end up with almost all adult mice making use of the plastic shelters. From the mouse's point of view compressed-paper shelters would be more attractive, but these make the checking of the animals so difficult that we have phased them out in preference for transparent plastic shelters.

My experience with the plastic mouse house is not so favorable. I have noticed that, in a cage furnished both with the house and with paper tissues, mice will typically drag the tissues to a suitable location away from the house, build a nest and sleep in their own nest rather than in the house.

I have made a similar observation. Some of our females with pups just don't like this sturdy house. I would place a mother with her litter in a house, but she would soon move the whole litter out. I repeated this game several times, always with the same result. Some mothers simply refused to stay in these houses and preferred building their nests outside with paper tissues. It's not really surprising that some, perhaps most mice prefer to construct their own nests according to their mice-specific microclimatic needs, and sleep in them instead of a prefabricated structure.

We have a group of mice who, without apparent reason, showed a decline in breeding performance. After we placed plastic mouse igloos and Nestlets in their cages, these mice returned to their normal breeding performance. I have seen some of them take their Nestlet into the igloo, where they probably built their nest and keep their pups under the igloo. Possibly, the mice feel more secluded in the relatively small igloo, while the much bigger mouse house may feel too open for them. The igloo is also less heavy than the big mouse house, and the mice can push it around, adjusting the entrances/exits exactly the way they want them to be.

I gave mice a choice of a commercial **paper-based shelter** (Shepherd Shack) and a plastic shelter (Techniplast Mouse House) and noticed that all three strains of mice tested showed a significant preference for the paper shelter. The paper shelter was much lighter (20 g) than the plastic shelter

(95 g). This allowed the mice to move it around, manipulate it and change the position of the entrance within the cage. The plastic shelter was probably too heavy for such maneuvering and, hence, never changed its place. The mice also gnawed the paper shelter, occasionally nibbled an extra hole in the side, or shredded part of the walls, using the shreds to strengthen their nest. They could not do this with the plastic shelter. All mice slept inside the paper shelter but never in the plastic shelter. When they slept in the cage that contained the plastic shelter, they did so in the sawdust outside the shelter. When tissue paper was provided, the mice dragged the material into the paper shelter and built a nest, but they never combined this nesting material with the plastic shelter.

We recently began moving away from plastic igloos to commercial paper huts with several openings. Our staff report that the mice seem to prefer the huts. Different strains appear to customize their hut, adding a *sunroof* or a *canopy*, shredding it completely or using the hut simply as a shelter without damaging it in any manner.

I am not sure there actually is a best plastic shelter; we use several different types that the mice use unpredictably either as a nesting place, a hiding place or a toilet. What is most important for them, and what they use in a consistent manner is nesting material. Mice love to build nests. When we give our mice Nestlet and hay, they typically build a nice igloo-type shelter out of it even if they are also provided with a plastic shelter; I usually find them sleeping in *their* nest but not in the prefabricated plastic shelter.

I believe that nesting material is more important for mice than a prefabricated plastic shelter. If they have access to nesting material, healthy mice always build a nest and sleep in it. I believe a plastic shelter is useful when several mice are housed together, especially males. If an argument breaks out, the subordinate mouse has a place to hide from the aggressive individual—hopefully out of sight, out of mind. Nesting material doesn't give this kind of cover or protection. We offer both, a Nestlet and a shelter. We use mostly the plastic square houses with several openings. Some mice nest inside the shelter, but many use it to hide from us and from each other, and to climb on! I think both, the Nestlet and the plastic shelter, serve important functions for the mice, so both should be offered to them.

We use the commercial plastic mouse house in combination with cotton Nestlets. The mice use these shelters regularly. Some investigators noticed a better breeding performance when their mice had access to a mouse house plus a Nestlet. As a result of this, most of our mice have now a house along with a Nestlet.

When given a choice between a paper-based and a plastic nestbox, mice always choose the paper box. Usually they sleep inside this box and, when given nesting material, they drag it into the box and build a nest (Van Loo et al., 2005). When no extra nesting material is available, they will shred the paper box and use the shredded material to build their own nest and sleep in it.

We use hardpaper igloos. The mice climb on them, chew holes in the walls, and mark them with urine, thereby giving a personal touch to their homes. When we move these urine-impregnated igloos during the cage cleaning process to the new cage, the mice are much less restless and aggressive among each other. They probably feel at home, as it literally smells like home.

I think you are right. We give our mice paper-based nest boxes that we also move along with the animals into fresh cages. The repeated transfer of the soiled nest boxes and the scent marks adhering to them probably accounts for the fact, that we also see hardly any fighting in strains considered to be conspicuously aggressive. Over the six years that we have been using paper-based nest boxes, we have encountered no ill effects on the mice's health status.

Our mice get cardboard boxes brought in from home by animal care staff. We first autoclave these items before placing them into the cages. The animals seem to enjoy the boxes, and we like to think we are *being green* by not wasting paper. It often raises a smile to see a gang of mice using an empty cat food box as a house. Who said animal techs don't have a sense of humor! We also use egg cartons, which autoclave very well. The mice explore the little huts and quickly turn the cartons into shredded pieces that make a good bedding and can be turned into nests.

I use 10 cm deep Carefresh bedding, along with cardboard tubes and Nestlets, in regular mouse cages. The mice build amazing nests and dig tunnels in this paper-based substrate. It is quite a revelation to see laboratory mice burrow in substrate. I always have a broad grin on my face when watching mice dig so furiously that they flick the substrate out of the cages and all over the place—a technician's nightmare! The mice build the tunnels along the sides of the cage —touching the sides (thigmotaxis) seems to be reassuring to them—so you can see them running about and behaving in very different ways underground. It's fascinating to watch!

< It seems to be accepted language to use the term environmental enrichment for species-adequate **nesting material**. Is it really fair to speak of **enrichment** when the so-called enriching material is a **biological necessity**—rather than a generous luxury—for the subject's well-being? After all, a mouse has to build a nest in order to be protected. >

I couldn't agree more. We do need better terminology to separate behavioral requirements/needs from environmental enrichment/fun. Sometimes I can't get my head around that term enrichment and think it only applies to stuff given to an animal that is not really a necessity but a kind of entertaining toy.

I think the history of how we cage animals for research has made the barren cage the standard, validated by studies that are repeatable and have been documented over the past 50 years. To use the claim that nesting material is a necessity does not hold water with the older generation of administrators and scientists. The good news is those folks are retiring and the younger generation is already used to working with animals who live in enriched living quarters. For them, nesting material for mice is a standard supply for every mouse cage.

So, things ARE changing; it's just a matter of time!

< Mice are biologically programmed to build quite elaborate nests that take various behavioral and microclimatic conditions into account. What kind of nesting material is most suitable for mice to build nests in lab cages? >

Based on my experience, **soft paper**—not shredded paper—is the most appropriate nesting material for mice.

My facility uses mostly Nestlets, but I've found that the mice create much better nests with **Enviro-dri** (mix of shredded recycled paper and Kraft paper). This material allows the mice to build proper nests, but it makes it a bit difficult to properly check the animals—a minor trade off. **EnviroPaks** are USDA approved tea bag filled with approximately 6 grams of Enviro-dri; this amount probably does not provide sufficient thermal nesting material.

It is my experience that mice prefer the Enviro-dri over the Nestlet. With the Enviro-dri the mice build a fine nest that they usually keep so tidy that you can transfer the complete nest when changing the cage, without having to add extra Enviro-dri. This material has one minor disadvantage in that it makes the cages harder to check, especially with the mice who don't like coming out of their cozy nest for the daily health check.

< I'm curious about your implementation of the loose crinkle paper (Enviro-dri). My facility is currently evaluating enrichment materials and I'm getting a LOT of push back because the material gets everywhere! Plus there is concern about maintaining an 8 gram allocation per cage. Do you all have any tips or tricks that worked for allocating the loose material? It's so cheap and the mice love it. Do you have technicians or PIs complain about not seeing their pups or missing dead animals? >

We do have some complaints but I feel the benefits outweigh the risks. The most important thing to a mouse is a good nest. We may not see every mouse or litter and we explain to researchers that if date of birth is important then they should be checking their breeding boxes daily themselves. If we are checking boxes and notice a litter, we record it but otherwise it is up to the labs to check for litters. We do however look for issues within the box that may signal trouble. These would be a lone mouse away from the group, blood in the cage, a poorly built nest, too little/too much feces or urine, etc. If anything looks off we put the box in the transfer station and have a look. I have done this for many years and can say that I don't think I have missed a whole lot.

We add the Enviro-dri in the housing rooms instead of in cage wash. Essentially our staff have an autoclaved mouse cage with the loose crinkle paper in it and they simply portion it out into the cage under the transfer station. We measure out 8 grams into a small Ziploc bag that stays near the change station in the housing room as a guide for people to use because we found some people had no idea what 8 grams should look like and were essentially filling the cage with shred. The mice were not making nests in that and many were trying to push the crinkle paper out of the cage up through the wire bar feeder. We've been able to get people stay fairly close to the 8-gram-size, now that they can see what it looks like, but it did take some education, sometimes as a one-on-one conversation with a researcher.

Our care staff dispose the shred nest in the housing room rather than send it back to cage wash in the soiled cage. We use a vacuum waste system and those shred paper nests will completely block the line which can shut down our cage wash operations for a full day depending upon where it would get stuck. Initially we had a good bit of push back on that because people were concerned about the time it would take to do that during cage change rather than cage wash but it turned out it didn't add significant time delays.

< One more question: Do you also transfer the nest every other week to decrease aggression rates and increase breeding success? >

We do, for both males and females. This leads to most cages having a continual nest of 10-14 g. They're glorious! And hard to emergency check ... but, worth it!

Thanks for the comments everyone! I just hope it's enough to convince our program to increase their nesting provision!

This is difficult with regulators who expect visual check of every animal every day, especially when they're a USDA species. Even for one on the bandwagon, it's difficult to convince folks that it's OK not to see the mice every day, even if it's better for and the definite preference of the animals. The struggle is real!

You are absolutely right; we don't have the time or resources to look at every mouse. I would prefer to be able to see everyone of a cage everyday but for us that is not possible unless the time and staff were made available.

< For those who use Enviro-dri as nesting material for mice, approximately how much do you give per individual so that it is not an obstruction during **cage checks**? We have different sizes and strains of mice, so it's a challenge to provide sufficient quantities.

Did you have any problems with technicians being unable to do head counts because of the shredded paper? If so, how was this resolved? >

Our mice construct nice nests with Enviro-dri but it is very hard to see them when they don't want to be seen. We don't have the time to put every box in the transfer station and do health checks, but I try to look at other signs in the cage that something might be wrong. You don't want to see the mice when they have a big nest. Mice who are ill or in pain do not engage in nest building. Therefore you can use the nest as an indicator of how the mice are feeling that day. A mouse by herself outside the nest is usually sick or she could be bullied; the situation warrants a closer look. If a nest is not made, a thorough examination may be required and of course, if any signs of blood are noted there is most likely a problem that needs to be addressed immediately.

This is a tough discussion in some ways, good nest versus viewing the animals and going home feeling okay about not viewing them. I have been checking mice for about 37 years and must say I had a bit of a hard time letting go of not visualizing them. Years ago when we gave shavings and Nestlets for nesting material, it wasn't usually a problem to see the mice. When we moved to Enviro-dri, I used to go home thinking to myself, "What

if someone was in discomfort or distress and I didn't see it?" There were a few sleepless nights in the beginning but with more experience with Enviro-dri I was able to see first hand just how much happier the mice appear to be when they are able to build a cozy dome nest rather than sit on a mattress.

It may be time to start rethinking what it is we need to see during a cage-side exam. Perhaps not seeing the mice because they are all in a well-built nest can be taken as a sign that we have healthy animals.

I agree, a well-constructed nest with all the mice snuggled in it, tells me that the mice of this cage are most likely all healthy; a sick animal is typically kicked out of the nest and is easily detected during the health check.

We have started providing nesting material to a room of breeders and not laying eyes on them every day. I had to convince our husbandry staff not to 'earthquake' the cages so they could look at all the mice in the cage during sporadic health checks. It turns out that these mice seem to be arguably happier plus they are better breeders. I began tracking numbers of animals 'found dead' before switching to increased nesting material and can say that the rates have not gone up. Despite being 'out of sight' most of the time, these mice are thriving and do not seem to require any extra attention or special vet care.

A lot of our mice dig down to the bottom of their cage and build the nest up from there. When you remove the cage from the rack, you can see and count all their little bodies from the bottom of the cage without disturbing the nest.

I do that too, though I always feel slightly rude doing it! It's all feet and private parts, LOL!

I take care of about 1,500 ventilated cages. I do not find a large nest an added burden at all. If I need to observe the animals I just pull out the cage a bit and look from underneath. If I see movement then I assume all is well. The mice get a close health check during the cage change.

We currently use 15-17g of Enviro-dri per cage (which increases to even more, once you transfer a bit from the old cage), and have the same 14-day cage-change period. And, as you know, this makes daily emergency checking more of a challenge, but greatly enriches the mice in our care. Cage checks are now done with a small USB flashlight, which helps quite a bit in identifying many issues. It is no longer possible to visualize all mice in the cage every day, so we look for signs like uneaten food, undisturbed bedding, sick mice found outside the nest, and as many mice as we can visualize without disturbing them. If nothing looks amiss, we move on, if we suspect

something we have a closer look, or open the cage. Over 14 days of checks we expect that all of the mice will be visualized at multiple points. While we have seen a slight increase in deceased mice found on cage-change days, the amount of fighting injuries has greatly decreased.

There are some researchers who fill nearly half the cage with shredded, crinkle, nesting paper. The animals don't even bother trying to build a distinct nest because there's so much paper. They just sort of tunnel underneath/into the mass of paper. Combine that with a hut or platform, wire-bar lid or cotton Nestlet and there will be no way to see the mice nor even the water valve at the back of the cage. You have to try to look for other indications, such as food level dropping, evidence of urine or feces in the cage, movement of nesting material, no traces of blood, no obvious odors of dead animals. We try to avoid physically handling the cage just to do a visual inspection of the animals since that can be very disruptive to some of the sensitive (overly-sensitive) breeding moms. There have been only two instances out of nearly 24,000 cages where a health concern was missed. So at least for our facilities, we've found that daily visual inspection of each individual animal isn't a necessity. Obviously there are some instances— e.g., tumor monitoring, surgical recovery, wound monitoring— where that won't be the case. But for the standard breeding colonies, it doesn't appear to be the issue we all assumed it would be.

We also warn those researchers who overfill the cages with paper that we can't see the animals and thus early warning signs might be missed between cage changes.

Certain veterinarians and regulators insist that if you cannot see the mice you must open the cage and be sure they are OK. This attitude is unfortunate and shows a lack of understanding of what makes a less stressed mouse; it is based on the original regulations that were about husbandry and very basic care, at a time when "dead mouse in the cage" was a routine finding and hundreds mice a day were found dead. I question anyone working in a large facility who says that every animal is seen/checked every day. I know, in a room with 12 double-sided vent racks that is not true. We all have rodent cages that we overlook. It is human nature to sometimes spontaneously take short cuts and that means not every cage gets your attention every single day.

The argument from regulators about having to see the animals is also dated. NO ONE can check 20,000 cages a day individually regardless of how many staff you have. Good husbandry staff have always used the eyeball test to know which cage to look at individually. A sick mouse cannot

really be overlooked; being kicked out of the nest will make her very noticeable.

< **Nestlets** are 4.8 x 4.8 x 0.5 cm large compressed cotton fiber pads; they are commonly used to offer caged mice the opportunity to build a nest. The nests constructed from one or, even worse, a half Nestlet have never impressed me, so I am questioning if the provision of a Nestlet can really satisfy the biologically inherent need of a mouse to build a nest? >

We have tested cotton Nestlets in several strains and found that: MF1 nudes shred them and build nests, and ordinary MF1 and Balb/c mice seem to ignore them; the same is true for C3H mice. Some C57Bl/6 mice shred them or sit on them, while others also ignore them.

Many debilitated mice—transgenic, post-surgical, etc.—cannot use the compressed cotton squares—because they do not have the energy to shred this material and build a nest. I have actually seen such mice sitting on top of an unshredded Nestlet!

I have also seen some mice who don't know how to pull the cotton fibers apart and end up just sitting on top of the full sized squares. Sometimes, I'll rip the Nestlet and fluff the edges slightly to see if starting it for them helps, but then I find them just sitting amongst the pieces I tore.

With the mice who don't use the Nestlets, it's almost as if they don't recognize them as nesting material. It might help if you started them off, but it would be quite fiddly and labor intensive when you have several thousand cages to deal with. We have stopped using the Nestlets, as all strains of mice that we work with seem to be happy with shredded paper. The additional advantage of shredded paper is that it costs nothing.

< Do mice have **preferences for certain nesting locations**? >

Mice tend to prefer nesting in the rear, rather than in the center or front section of **traditional cages** probably because it’s darker, hence more secluded in the rear.

Mice are nocturnal animals, so it is a natural response to nest in the darkest area of the cage, which is normally the rear section of it.

Logically the location of the nest should be away from light and traffic.

Agreed, away from light and traffic, and also away from the water bottle and the food hopper.

I care for two **vent racks** with various strains of mice and have noticed that most mice build their nest away from the air flow vent.

This is also my observation; the mice avoid the air stream by choosing another resting place in the cage, or by piling up walls of sawdust around the nesting place or use provided nesting material or a movable shelter as a quasi-windshield.

In line with this is the observation by Scales & McDonald (2011) that "61% of mice housed in static caging preferred to nest in the rear of the cage, compared with 49% of mice in low ventilation (30 ACH) caging, and only 14% in moderate ventilation (70 ACH) caging." Obviously, mice don't like to be constantly exposed to a strong air stream, so they build their nests away from it, even if it implies away from the relative dark and undisturbed rear section of their cage.

< It is not uncommon that malfunctioning watering valves or leaks of water bottles result in **cage flooding**, a circumstance that can have serious implications for the animals trapped in such flooded living quarters. In your own experience, what can be done to deal with this problem? >

I rarely see this problem in rats, but relatively often in mice. It seems to be worse if the animals are nervous or have litters. Since we have moved all our breeding colonies to a separate unit, where the animals are disturbed very little and kept in cages that are provisioned with shelters and nesting material, the incidence of wet cages has become negligible. When we have an occasional problem cage, we reduce the amount of sawdust and use more shredded paper instead. In my experience, water leakage is primarily triggered when the animals build a nest up against a drinker spout but is rarely due to a malfunctioning of the spout.

We have a flow detection alarm on the water system and dedicated staff who respond to the alarm and find the offending cage at any hour.

It's my experience that the following measures keep the number of animals dying as a result of flooding very small:

- The drinking spouts have small metal gutters pointing downwards, away from the cage. Whenever a spout starts leaking, the water will drip on the floor, rather than into the cage. It is important to make sure that the nipple and gutter are placed correctly.
- Occasionally, the animals plug the spout with bedding material. When this happens, the cage will flood. To minimize this hazard, the whole watering system is cleaned by the manufacturer once a year. This is a bit costly, but worth the effort and money.

- One problem we occasionally encounter is that mice will push enrichment items against the nipple of the water bottle, thereby causing it to leak into the cage. We try to prevent this by fixing the enrichment objects to the cage/lid so that the animals cannot move them around.
- Animal caretakers make sure that no water tubes are left on top of cages; the mice would invariably gnaw on them thereby causing leakage.
- All cages are checked once a day, <u>including weekends and holidays</u>, so no cage is left uncontrolled for more than 24 hours.

We have moved away from automatic watering system so as to prevent flooding, but we still get leaky bottles. Mice typically build up a mountain of bedding near the water bottle—not sure why—and that readily causes flooding. There is disagreement among caretakers about the amount of bedding to use. Some reason that a very thin layer—hardly enough to cover the bottom of the cage—and a 250 ml water bottle will prevent the mice from pushing the bedding up into the sipper tubes, and if the cage does flood, *only* 250 ml of water will be contained in the cage. However, there are still incidences of animals, especially pups, dying as a result of water leakage. Other colleagues argue that 1.5 cm of bedding or more will help keep the mice alive, since the bedding will absorb all the water that leaks from one bottle. But pups are likely to die from this also, since they would be cold from sitting on wet bedding.

We hang plastic tubes and other resting surfaces off the top of the cage, functioning as life rafts so to speak. This doesn't do much for the pups, but at least we save the adults. We are hoping this will alleviate much of the drowning risk, since we did not see any better options at this point. In fact, we are currently working on SOPs that will make elevated furniture, such as tubes, a rule for all rodent cages.

I like the idea of tubes suspended on the side of the cages to keep adult mice dry and warm, but getting everyone else of the staff agree turns out to be very difficult. Some people have a hard time endorsing anything that looks like environmental enrichment, and tubes fall into that category. I have seen videos of mouse and rat mothers carrying their pups to new nest sites. If a cage was to incorporate an elevated dry refuge structure, I wouldn't be surprised if the mothers evacuated the young from damp substrate to this dry and safe site.

< Has anyone had experience with **mice building mounds with their bedding** near the sipper tubes? Do you know why they do this? Were you able to find a solution?

We recently changed from corn cob to aspen wood chip bedding for our mice, housed in Tecniplast individually ventilated cages with external water bottles. Initially, we used 1/2 inch depth of bedding instead of the typical 1/4 inch depth in the cage bottom.

Our pilot study using 1/2 inch depth of bedding showed that the interval between cage changes could be extended and that the mice seemed to enjoy burrowing in the bedding. Unfortunately when we made the transition to the full colony, many of the mice began making mounds in their cages near their sipper tubes, resulting in more than usual numbers of flooded cages. The mound building did not correlate with cage change days. We've decided to decrease the bedding depth back to 1/4 inch, which negated the benefits that we had been trying to achieve by using 1/2 inch depth. >

A reason that mice build mounds may be that there is draught in the cage. Years ago, when the first IVC racks came on the market, most had side-inlet of the air. This lead to serious draught and mice were building mounds to shelter from the draught. I also remember a discussion on CompMed where a researcher reported death of mice in IVC cages that had the air-inlet through holes situated around the drinking nipple. These animals dehydrated because they did not want to drink anymore. Nowadays most IVCs have their air inlet in the top of the cage, but draught may still be a problem. So my advice is to check how the air flow in your IVC is, and how strong the air flow is (maybe you can do an experiment with smoke in an empty cage). You may find that the mounds are built exactly at a spot where the mice can shelter from the draught.

< When you **change mouse cages**, do you transfer the nest? We change boxes twice a week, as we have static caging. I usually tell our animal care staff to transfer at least some of the nest but it is usually pretty soiled. Is it stressful for a mouse to rebuild a nest on a regular basis? >

I try not to make too much of a mess of the nest but, of course, because it is going into a clean cage, the mouse always has to arrange it somewhat. It seems that they usually start the fix-up shortly after the move, there is a short checking out the new environment phase, then repairs begin.

I actually don’t think it’s a problem if the nest needs tidying after it’s moved. If you watch mouse behavior in regard to nest building, they are

constantly re-building and making adjustments. This wouldn't be much different than in natural settings where ground shifts, weather, animal activity, etc. require the continual re-building and tidying of the nest. Plus, if we look at the purpose of enrichment to fill idle time, the more interaction that is elicited with the materials, the better.

If the nest material is really scattered and soiled such as with juvie mice, I only save a very small amount for the new cage. Mice are olfactory creatures and scent means a lot to them. I think the continuity of their scent in the new cage functions as a kind of stress buffer. Building a new nest in such a quasi-familiar environment is unlikely to stress the mice. I think the drive to build the nest is always there. If I keep adding nesting material, the mice will continue building and modifying their nest; they never seem to ever be done with it. Building a nest is probably an intrinsically satisfying activity of which mice don't easily get tired; they just start stuffing the material in the food hoppers and around the sipper tubes or in a corner. So I would say that building a new nest or rebuilding an old one is unlikely a stressful activity for mice; it just seems to give them a satisfying job to do. Did you ever notice how excited a room gets after a cleaning, with all the mice working on their new nests? I love spending a couple of minutes when I'm done just watching them get to work. I'll even rip the Nestlet apart if there are several mice so that they all get a little piece and can join in the nest building frenzy.

Always transfer the nest! I mean the proper nest, <u>not</u> the sawdust—as this might be soiled with urine. Odor cues of urine-soiled bedding and odor cues of old nesting material are not the same; they affect the mice differently. Mice show much more aggression in a completely clean cage or in a new cage with a handful of soiled sawdust—from the old cage—than in a clean cage in which the old paper nest has been transferred (Van Loo et al., 2000).

When changing mouse cages that contain breeding mice with offspring, I first move one parent to the clean cage, then the litter and only then the rest of the adults. The pups seem calmer when I do this, especially at the popcorn age. I like to think that the familiar smell of the parent in the new cage is the reason for this.

If you transfer singles it takes more time and the mice scurry all over the place; that's not good. We just try and scoop them all up at the same time. If a little soiled bedding goes along that's a good thing. The mice settle right in.

I agree; when you scoop up several mice at the same time and transfer them into a new cage, the animals are calmer than when they are transferred one by one. Also, if you scoop up a few adult mice, they are very unlikely to bite you whereas a single mouse will experience a lot of fear and, therefore, be more defensive and ready to bite.

The multiple-mouse idea is great but it gives me less control over the mice. I had the experience several times that some of them jumped on to the transfer station, then panicked and jumped down to the floor during the process. When this happens the whole group of mice has to be sacrificed. Researchers tend to get a little bent out of shape about this, especially if the mice are important or expensive. It bothers me because it really hurts the mice.

< It has been my experience that rodents are typically transferred into a new, clean cage after experimental surgery. If I were a mouse, I would feel pretty disturbed or even distressed in such a scenario, and I would think that my **recovery from surgery** would be quicker and less distressing if I would be surrounded by the familiar scents of my own nest/cage. Adding a little bit of the animals' familiar nest scent to the clean recovery cage is unlikely to jeopardize the research, but would it be too much to ask for in a busy work environment? >

No, I think it would be very little to ask for, and it seems like a sound idea.

There is no good reason for not transferring at least a little bit of the familiar nesting material—a piece of the Nestlet or a piece of the paper tissue—of the homecage to the new, clean cage to provide the recovering mice a familiar, comforting scent in an otherwise strange, presumably fear-inducing environment.

I believe, most people being uncertain exactly how contaminated old nesting material might be, simply omit transferring nesting to the new cage. I can't say I'm surprised, but it would be nice to have a better understanding of how contaminated old nesting might be as, if the risk is negligible, transferring relatively clean nesting would be an excellent practice due to the fact that most post-surgical mice are slow to build a new nest to aid in thermoregulation; if they could recover in their own already made nest, it would certainly speed their recovery. Would be a win-win!

A one-day-old nest will not be soiled, as rodents keep their nests clean and urinate and defecate somewhere else!

We clean cages one day before surgery. The clean cage in which the mouse regains consciousness after surgery is provided with some nesting

material of the one-day-old familiar nest of the homecage so that the mouse is surrounded by familiar odors while waking up.

We let our mice recover from surgery in their homecages whenever possible. This means that the animals recover in their home environment where they not only are surrounded by familiar scents but also receive the social, stress-buffering support of their familiar cage companions. To reduce the risk of suture biting by cage mates, we either use intradermal suture or metal suture clips.

< Male mice are often housed alone in toxicity studies. Typically such mice are relatively aggressive and not so easy to handle. Are there practical and safe options of **housing male mice** in pairs or in compatible groups during long-term studies? >

Your question was the subject of my thesis (Van Loo et al., 2001). My findings can be summed up in the following recommendations:

- Keep males in small stable groups of 3 mice. More individuals in a group increases the chance of aggression exponentially, fewer individuals (i.e., pairs) increases the chance of one animal being bullied without the opportunity of getting social support from another submissive animal.
- Transfer nesting material but NOT bedding material during **cage change**. Nesting material is kept clean from urine and contains aggression inhibiting pheromones from the plantar glands in the paws; urine, on the other hand, contains aggression-eliciting pheromones.
- Keep disturbances to a minimum. Each disturbance may elicit aggression between males, and once it has started in a cage, it is usually not easy to control. Although some disturbances may be unavoidable during a study, you may think of solutions such as combining handling procedures with cage cleaning.
- Use enrichment that can keep the mice busy (e.g., nesting material).

I have found that a happy number per cage is three males. They got Enviro-dri nesting material as well as two different types of shelters. It is hard to defend two shelters, so the dominate male would hang out in the one he deems the best, the other two mice will go in the second shelter.

Our male mice always get a new Nestlet in the clean cage. A nest building committee is quickly formed and aggression seems decreased because of the chore at hand. If the animals get into continuous fighting in the new cage, we don't wait long but check for the primary instigator and remove this individual from the group.

Group-housed male mice are relatively easy to handle and will let me pick them up by wrapping my hand around their body; no single-housed mouse has ever let me do this! Goes to show the importance of trying everything possible to keep them with a cage mate!

< Could you please share more about your experience of **removing the aggressor** in dysfunctional groups of male mice? >

It is not always easy to determine the aggressor right away; I must sit and wait, sometimes for quite a while. If I notice squabbling in a cage, I mark it and will then monitor the mice repeatedly on normal days when their cage is not changed. I mark the cage with a sticker, so the researcher or their techs will also be able to monitor the situation and let me know if they catch the problem mouse. The aggressor typically is the guy who is chasing and initiating an argument with the others. Patience may be required to identify him, but it will pay off. If the fighting has gone on for some time, then the guy with the least or no wounds is usually the culprit. Once we have identified the troublemaker, we take him out of the group.

When the rascal is removed, I like to keep the sticker on the back of the cage card so I can easily keep track of that group. Typically, another male will take the dominant position of the removed aggressor; I have never noticed that the new alpha male acted as aggressively as the old one, so I never had to remove one of them. It seems that the new dominant mouse is just less aggressive, so fights happen less often and they are usually no longer severe and a cause of concern.

The following quote from a published article (Emond et al., 2003) supports your observation that removing a particularly aggressive mouse can be very helpful to control aggression: “At our Center, two observation periods were set aside daily in order to identify, according to previously described behaviors, dominant mice and separate these when indicated. By reducing or eliminating the number of aggressive acts between group members in the same cage, our social conflict reduction program has led to a 57 percent reduction of mice being reported for clinical signs, death, and euthanasia.”

When I wrote this article at the time, we were using conventional cages—no filter tops. This made it quite easy to visually and audibly identify dominance behavior whenever it occurred at its early phase.

Since then we have been using filter top cages on ventilated racks. Under this new caging condition, we can barely hear the mice fighting because of the filter top barrier and the noise produced by the ventilators. Additionally, when we do spot an aggressive mouse, it takes a few more

steps to first remove the cage from the rack and then remove the filter top and the wire-bar lid from the cage in order to access the dominant mouse. By then, most of the time, we have lost visual contact with the perpetrator and it will take many more minutes of patiently waiting to see if the dominant mouse will act up again—or not—or until it is too late and we find injured animals the next working day.

So, identifying aggressive strains within a ventilated rack system isn't as easy as it is in the old open-cage system described in my article. We are now dealing with more casualties than before.

< I'm looking for suggestions of enrichment for mouse **metabolic caging**. We have the standard plastic caging with wire mesh. The mice will be housed in the cages four days per week for four weeks. The investigator will be **collecting urine**. >

Provide a hut so the animal can keep warm. Make sure that it is small and cozy inside, but that the mouse is unable to climb on it from the outside —and urinate on top of it.

When applying enrichment in metabolic cages, there are several aspects to consider both from the animal welfare point of view and quality of research: The mice are placed individually in surroundings that do not provide warmth, comfort or shelter. These are aspects that you wish to alleviate as much as possible. With regard to quality of research, on the other hand, you wish to collect urine samples as clean as possible, i.e., the least possible waste of urine left behind on inserts. This is especially important when collecting spiked (radiolabeled) urine. Inserting enrichment in those studies means that all inserts need to be rinsed and the rinsing water collected for analysis.

In studies where urine needs to be collected, my first questions are always:

- Does it need to be a 24-hour-sample? If not, then collection of urine can be achieved by putting the animals in a bucket just after they wake up and remove them as soon as they urinate. No metabolic caging necessary.
- If multiple hour-samples are necessary, can it be a pooled sample from several mice? If so, house them together in a metabolic cage.
- Can the time span of sampling be shortened, for example 6-8 hours rather than 24 hours in the metabolic cage per day?

< Based on your own experience, what is the most mouse-friendly and at the same time safe way of **picking up a mouse** in the research lab setting? >

Absolutely 100% tubing and cupping for handling of C57B6 mice. Compared to being picked up by the tail, mice are calmer when they are picked up in tubes or in cupped hands, and they are less likely to bite.

We moved to the tubing and cupping method in one unit first on a trial basis. After two weeks, everyone said that it was no more time consuming or difficult than picking the mice up by their tails. Cage change is now quicker because the mice are acclimated to being handled and can be swiftly scooped up.

We have a few years of experience with non-tail handling. Most animal care staff and researchers have switched, mostly using tunnels, but also huts, hands (cupping), gnawing sticks or ladders. Anything, as long as it is not the tail. Mice are much easier to handle and easier to train. It also affects their pups. I am sure that many of you who breed mice recognize the term 'popcorn-mice', when 2-3 weeks old mice jump around or even out of the cage as soon as a hand comes into the cage. I have heard from several breeders around Europe that this behaviour gets extinct when the mums are cup or tunnel handled. So I am a great advocate of non-tail handling.

Having said this, I would like to warn those of you who would like their facility to switch: You need a long breath and careful planning. The NC3Rs [National Center for the Replacement, Refinement and Reduction] gives good guidance for this. Apparently, it is very difficult to change people’s minds about this for two reasons:

1. They think it takes much more time, which is true in the beginning as is with everything you learn new. So give them time to adjust, for example by switching handling method one study/rack/room at the time.
2. Some really do not see what is wrong with tail handling. It may be difficult for them to realise that they have stressed the mice throughout their career; they do not want to accept that this is the case. So make sure they do not feel guilty, since none of us knew any better, but we do now.

For those of you who do not know the story about cup/tunnel handling: Jane Hurst, a UK researcher working with wild mice, performed a study with lab mice as control animals. She noticed that the lab mice were more stressed than the wild mice. The only difference was the way she handled the mice: She picked up the wild mice with a tunnel because she was unable to catch them by the tail. She then started to tunnel-handle the

lab mice as well and noticed a considerable difference. She told this story in 2010 at a rodent welfare meeting urging us all to try tunnel-handling rather then tail-handling of mice. She has been an advocate for this method ever since and finally, a few years ago, it has been picked up by NC3Rs where she got a podium to really spread the gospel.

I found the abstract of Jane's presentation:

Hurst, J.L. & West, R.S. (2010) Taming anxiety in laboratory mice. *Nature Methods* 7: 825-826.

"Handling experience (which includes routine maintenance) can have positive or negative effects on stress responses that influence experiments, depending on the animals experience during handling. The most common method used to capture and handle laboratory mice is to pick up and restrain the mouse by its tail. However, we show here that this induces a high level of anxiety and mice do not readily habituate to handling by this method. Even after multiple handling sessions, mice picked up by the tail avoided voluntary approach of a handler, showed a high level of urination and defecation during handling, and showed high levels of anxiety in an elevated plus maze test. By contrast, mice picked up either using a tunnel present in the homecage, or cupped on the open hands, quickly developed much higher levels of voluntary interaction with a handler, showed low levels of urination and defecation during handling, and low levels of anxiety in an elevated plus maze test. These responses generalised across different strains and sexes of mice, and across handlers with differing levels of experience. These notable differences in response may not have been recognised previously because picking up mice by the tail is so widely used in laboratories that the aversive and anxious response is perceived as normal. However, use of methods that do not induce strong anxiety responses will minimize confounding responses due to routine handling before and during experiments. In addition to providing more robust scientific outcomes, appropriate choice of handling method could enhance the welfare of the many millions of mice that are housed and handled in laboratories worldwide."

To be picked up by the tail must not only be a uncomfortable but also a very frightening experience for mice; after all the human who does the handling is a potential predator for them. This distressing situation is bound to affect the physiological homeostasis of the mice, thereby introducing uncontrolled variables which will affect the statistical validity of the research data collected from them. An additional number of mice will then be needed for the study to achieve statistical significance so that the research findings become publishable.

In my experience, mice still act crazy when you first start picking them up with a tube or cupped hands because they don't know what's happening. However, they quickly acclimate, and I think it's worth spending this little extra time. Even for myself, it is sometimes easy to forget and quickly grab a mouse by the tail. So, I think part of the value of handling via tube and cupped hands is that it forces you to slow down and be more aware that the little mouse is a sentient creature rather than a work tool. That attitude, I believe, plays its own role in decreasing handling stress. It increases, at the same time the scientific reliability of data that are collected from the mouse. A little bit of compassion along with some extra time for the mouse benefits not only the research endeavor but also the ethical well-being of the person who handles the mouse.

The Research Institutes of Sweden (RISE) produced very inspiring films on how they acclimatize their mice and rats to husbandry and research procedures. The video Housing, Handling and Training Mice at RISE shows how the tubing and cupping of mice works. Here is the link to the website with access to the 6-minute YooTube video:
https://www.ri.se/en/what-we-do/expertises/3r-focus-on-animal-welfare

I discuss tubing and cupping of mice in every Basic Mouse Handling class and teach it to new scientists and explain why it's superior to the traditional picking a mouse up by her tail.

The biggest hurdle to implement these refined handling techniques at our facility would probably be cage change since we have 60K mouse cages. I'm sure there would be a lot of concern regarding the additional acclimation time it would take to get staff used to these new ways of handling mice. The initial time increase would be tough to get people over when staff time is always an issue, but I would assume that after the initial acclimation period it would take the same or even less time to change cages.

People have concerns, but we found these were unfounded. The increase in time was only for one or two cage changes for us. Yes, there is a brief acclimation period when the handling of the mice takes a bit longer but then it becomes significantly less time consuming in our experience because the animals are much easier to catch for the cage change.

< Has anyone of you ever been **bitten by a mouse** while handling the animal before and/or during a procedure? >

Many times, usually at least 1 or 2 mice get me when **scruffing** agroup of mice for dosing; a couple of them are just faster than me. I usually place them on a bar lid so they can grab it. I have tried that tripod grip, but so far I haven't mastered it; my mice are not secure.

In my experience, the way a mouse is scruffed plays an important role in her stress response to manual restraint.

Many people scruff a rodent—for example, a mouse—by lifting a skin fold that is parallel to the animal's long axis. This will automatically put pressure on the throat, giving the animal the feeling of being suffocated, which of course intensifies fear dramatically.

A better way, in my view, is to scruff the mouse with three fingers, using a skin fold that goes at 90 degrees to her long axis. This doesn't put pressure on the throat and avoids the animal getting unduly stressed while being handled.

Most of our techs can't see a benefit in the 3-finger scruff. If some of you are seeing benefits for this scruffing technique please share your experience—our techs are open minded and I could perhaps persuade them to learn how to make use of this more mouse-friendly restraint technique.

I believe the 3-finger scruff was developed specifically for gavage. It gives you full control of the head but does not create tension pressure on the trachea/esophagus area.

Now…I will tell you; it took me a good 30 times of doing it before I felt confident enough, that I could do a gavage with that hold.

Thanks for mentioning the three-finger scruff technique. It wasn't developed just for gavage, but for any situation where it is necessary to immobilise the rodent without more stress than absolutely necessary (and to lessen the risk of getting bitten!).

We have collected some information here:

- https://norecopa.no/scruff
- and specifically the 2-minute film Three fingers better than two: A refinement of the scruffing technique: https://vimeo.com/290857433 (also available in Spanish)

If you look about 1:30 minutes into the film, you'll see that the secret is to make sure that the skin fold is rolled between your fingers until it's so high up on the dorsal neck so that the animal can't turn around and bite.

The confidence needed to make use of this technique can, to a large extent, be achieved using toy animals (and if necessary anaesthetised/dead animals) first for training purposes.

Hope this makes sense. I wouldn't dream of trying to immobilise the scruff of a rodent by any other method.

Thank you for your work Adrian!
Love,
180,000 *mice in Manhattan*

< What is the most mouse-friendly, low-stress way of giving a mouse an **IP injection**? >

There isn't a truly mouse-friendly way of doing this procedure; mice just don't like to be restrained, and they can't be blamed for that! I will say that scruffing them gently but securely the first time and then performing the injection quickly as well as correctly is very helpful. Have all that is necessary ready to go so that, once the mouse is restrained, the procedure can be completed swiftly. The less time the mouse is restrained the better.

< From the standpoint of the mouse, what is the least distressing **blood collection** technique? >

I have found that mice are less stressed when I take blood samples from the **submandibular vein** versus the saphenous vein. The mouse is subjected to less restraint, for a shorter time. The vein requires hardly any pressure afterwards to stop the bleeding; this again means relatively shorter restraint time. The vessel bleeds far better, especially in small mice, and a bigger sample can be collected if needed. I prefer to use a relatively large 21-gauge needle, because I think it is less stressful for the mouse to be punctured once with a larger needle than poked several times with a smaller needle to obtain a sufficient amount of blood.

I agree, the advantages of the mandibular method include:

- no need for a restraint device,
- no need to warm the mouse to induce vasodilatation, and
- the technique is relatively simple and easy to learn, and practical, especially when you have to collect numerous blood samples.

We use the **saphenous vein** approach with large mice who have a good sized vessel, but I also prefer the mandibular approach with small mice who have tiny saphenous veins.

With the saphenous collection technique you have to use a lot of caution not to hold the mouse too tightly; over-restraint can cause undue stress or even death. I have seen mice being held so tightly that it injured them while saphenous blood was drawn.

I also prefer the submandibular technique. It is fast, reliable and doesn't require holding off the vessel as does the saphenous technique. Restraint is one of the most stressful things for a mouse in my opinion. I assume this because they are usually vocal and almost always try to bite! The saphenous approach is okay, but sometimes the vessel must be held off for a relatively long time and the mice don't seem to like it all that much.

I would recommend the **tail incision** method for blood sampling of conscious mice. The mouse is placed on the cage lid, head and body covered with a tissue. The tail is bent upwards and with a sharp razor blade a perpendicular, small incision is made at the ventral side of the tail about 1.5 cm away from the tail base. Blood drops are then collected in a capillary tube. It is my experience that the mouse is very calm and hardly responds. When finished, a gauze pad is gently pressed on the incision for a moment before the mouse is returned to the homecage.

Having applied the tail-incision blood collection many times, I can testify that there is no indication that this technique leads to bone or cartilage damage. However, the incision is so small and superficial that it yields only very small amounts of blood.

We use the **tail nick** technique for serial blood sampling in one day. With the point of a scalpel we puncture the tail vein, then use a capillary tube to collect the sample. You do not need to re-nick at every time point as you can gently wipe the scab off with a tissue and collet the next sample. We collect up to 100 µl at a single time point, obviously dependent upon circulating blood volume. We often have 10 samples in a day and get this without problems from the tail vein. For samples over 20 µl we keep the mice in a warming cabinet for 10 minutes; for smaller samples we don’t warm the mice.

I have observed mouse behaviour with this technique and found that many of the mice don’t even move when the vein puncture is made and a blood sample collected. We use a modified restrainer that allows the mouse to move and not be restrained apart from having the tail held by the handling person.

I use a technique that is not universally approved, because it takes a fine hand. For serial microdosing, all day every 15 min or for a 1, 5, 15, 30, 60, 120 min type project, I like to do a **tail snip.** That name is inaccurate for what is done, if done correctly.

With the mouse in my left hand, I hold the end of the tail, find the very skin cap, JUST the skin not connective tissue and, using sterile scissors with my right hand remove just the skin cap; it's like shaving. Then, with a microcapillary tube I gently “milk” the tail and collect blood from the tip of the tail; this is followed by holding the vein off with sterile gauze. For subsequent bleeds I first immerse the tail in warm sterile water, pluck off scab and gently “milk” again. With the mouse standing on the lab bench, I allow her to walk along while I am holding the tail. Typically that keeps a mouse distracted without being tempted to turn around to bite me. I find a restrainer unnecessary in most cases.

The cavate of this technique is, you must have a person who compassionately cares for the individual mouse and has a fine touch. This is not a technique for most PIs or others whose view is not animal centric.

I am going out on a limb and state that I prefer the **retro-orbital technique** of conscious mice for the following reasons:

- done by a trained individual, this technique takes less than five seconds to complete;
- you can use a microhemocrit tube;
- if you need to take several samples at different times, the movement of the microhemocrit tube into the canthus is enough to break the clot and allow blood to flow;
- done correctly this technique does not damage the eye.

Performed by well-trained hands the submandibular, saphenous and retro-orbital blood collection techniques are equal in my opinion. Therefore, if you are the best at retro-orbital, you should use this technique; I certainly do.

As you say, you are going out on a limb! How do you measure lack of pain in the mouse when you have to scruff her, holding her so tight that she cannot move? I think the retro-orbital technique should only be done with an anesthetic to alleviate the animal’s distress and pain associated with this procedure. This is not a technique for the average vet tech and investigator; it requires extremely skilled and sensitive hands to protect the mouse from serious injury, pain and distress.

The eye is a particularly vulnerable organ, so just imagine being subjected to a retro-orbital blood collection gives us goose bumps. This is

probably the reason why we kind of instinctively infer that a mouse subjected to retro-orbital blood collection must suffer particularly much. It is my experience that this can be the case when the technician or investigator is poorly trained or, even worse, untrained, uses the wrong size tube, tries to perforate the conjunctiva and is neither careful nor skillful. I have taken many retro-orbital blood samples from awake mice and never encountered a serious problem. When you know what you do, and how to do it correctly, you can swiftly collect a sample from a conscious mouse without causing damage to the animal's eye and without causing undue restraint stress to the animal.

The first time I saw a retro-orbital bleeding was about five years ago. We needed a sample to test for MHV (mouse hepatitis virus). I called our vet and asked if he could teach us newbies how to get a blood sample from a mouse. He discussed various methods and then told us that he always does retro-orbital bleeding on mice. He then proceeded to do the deed, without any anaesthesia. It took maybe 4 to 5 seconds! I do not mind saying that I went completely weak in the knees and if I had not been standing next to a wall, I might even have gone down! We checked the mouse several times that day and he seemed fine, better than me in fact.

The retro-orbital bleeding technique does have important advantages:

- The technique is quick,
- easy in skilled hands,
- yields a relatively large sample.
- The mouse recovers quickly as reflected in corticosterone, catecholamine and behavioral responses (Van Herck et al., 1994).

These practical advantages are outweighed by serious ethical concers:

- The procedure is painful and, therefore, should never be done without proper anaesthesia.
- There is a risk of complications, especially forward protrusion of the eyeball, caused by continuous bleeding from the retro-orbital venous plexus. This leads to a gradual drying out and a constant itching of the cornea, as eyelids are no longer able to close properly. The mouse will react with excessive scratching, and by doing so will ruin the cornea. Within a short while you will find the animal with a blind eye.
- The procedure is esthetically unpleasant.

It's true, retro-orbital blood collection appears to be somewhat gruesome, but if you have a good teacher and enough practice—this is the

most important part of the puzzle—it isn’t a bad method. It is quick, provides a good amount of clean sample and, in my opinion, requires little to no anesthetic, depending on how much your mice resist. Now, I will admit that errors can occur during these bleeds, and I myself have made a few that have ended up in a way that definitely did not sit with me well at all.

Personally, I see the risk and trauma to the animal during retro-orbital bleeds without anesthesia as unacceptable. I perform several retro-orbitals monthly yet, I still prefer the submandibular. Done correctly on an adult mouse, it is faster and less traumatic than a saphenous, and less risky than a retro-orbital.

Topical anesthesia for retro-orbital blood withdrawal would not be enough. That would be only sufficient for anesthetizing the cornea. The pain comes from penetration of the conjunctiva and the deeper tissue layers. To anesthetize these you need to set a retrobulbar anesthesia, which is a rather painful procedure for humans, and probably also for mice. The best option, in my opinion, is a short isoflurane anesthesia for retro-orbital bleeding.

At the department where I am now working, retro-orbital puncture is routinely performed under light anesthesia. The technicians are very skilled and, therefore, the procedure causes no problems such as hematoma formation after blood collection.

I've seen seasoned techs who are so fast and precise with the retro-orbital bleed (the mouse is under anesthesia) that for those mice it seemed to be a fairly benign procedure. I'm not as well practiced and feel that I cause much more morbidity. I personally prefer the saphenous bleed, especially if only a small volume of blood is needed. The mandibular works well—if you can prick once successfully—but I've seen several mice develop large hematomas afterwards, so I think one needs to hold off for some time after the collection, which might be difficult in an awake mouse.

Based on my own experience as a veterinarian and based on what I have seen in research laboratories, I would argue that the degree of discomfort (physical restraint) and the intensity of pain (tissue poking, hematoma) and stress (duration of handling) experienced by an animal during blood collection is determined not so much by the technique applied but by the sensitivity and expertise of the person who performs the procedure. If you know how to do a procedure correctly, you probably inflict less discomfort, pain and stress on a mouse during retro-orbital blood collection than when you restrain a mouse incorrectly and have to poke the saphenous vein several times before you can collect a blood sample.

< For me, developing close relationships with the monkeys in my charge is always a spontaneous process. I know that I could develop this sort of rapport with other species, but based on my experience with mice I do wonder whether there is a size limit. >

It is entirely possible to establish a **close relationship with mice**, involving trust, petting and lots of physical contact. This is done with rats all the time, and the two species are not that different. The problem with mice is that most of the ones we're likely to come into contact with are wild. I have live-trapped hundreds of deer mice in my house and have never been able to turn them or their offspring into pets. You just have too many generations of skittishness bred into them. On the other hand, one of the best pets I ever had was a store-bought mouse. He was pure black and his name was Juarez. He lived in a small cage with a wheel, and he loved to come out every evening for some cuddle time and hand feeding. He was as tame as a dog—very responsive in every way.

Yes, you can develop a close, affectionate relationship with a mouse regardless of the fact that she/he is so small. Once, I was treating a mouse for a long-term eye problem. He was not fond of the cleaning with saline at first but with lots of time, patience and gentle handling I gained his trust and he finally decided to just sit in my hand and let me clean his eye without any restraint; he would even tilt his head toward the Q-tip. Getting his eye cleaned by me may have caused some relief of discomfort. It probably felt good, so why fight it? I think he had learned through experience that I was not going to hurt him but did something that actually was good for him, so he felt assured and at ease while I treated his eye.

I had a very similar experience. About 20 years ago we had a C57/BL female mouse; she had a very sticky eye and the clinical veterinarian asked me to put cream on the eye three times a day for 7 days. After 2 days, the mouse would stand on my hand and tilt her head sideways towards me so I could easily put the cream in her eye without restraining her. I was stunned that a mouse would do this; at first I thought it was by chance, but she did it every time. She made a full recovery. I did visit that little mouse pretty much every day while she was with us. Definitively, my relationship with her was affectionate; I will probably never forget her.

Mice are very charismatic when you deal directly with them, rather than with the idea of them. My students often say things like, “Oh, they're actually rather cute” when finally coming face to face with these animals.

I used to believe that mice do not like to be held, touched or handled. However, during the past year, I have found that it may all depend on who is working with the mice and how they are being handled. Granted, I would say that 90 percent of the mice at my institution would rather be left in their cages 24/7 without disturbance by human hands, but we have a lab here that has truly happy mice who literally buzz when you hold them. I wouldn't have believed that mice could be that way until I witnessed it with my own eyes. I was so amazed the first time I saw this that the person in charge of the mice placed one of them in my hands so I could feel the buzzing. The little mouse then closed her eyes as I gently rubbed behind her head. She even leaned into the direction of the rubbing so I could get behind her ears. I have since referred to this individual as the *mouse whisperer;* he has taught me that there is much more to the mouse than meets the eye.

< We have been trying to **breed wild forest mice** for almost a year now, and no litters have been born. The animals are pair-housed in standard cages; they do have nesting mater
ial and shelters. Any suggestions as to what we can do to get these animals reproduce? >

Wild mice usually breed in lab conditions reasonably well. I would suggest to breed your mice in larger than standard-size cages. As a general rule, wild mice breed better the larger their cage. Trios—two females and one male—tend to reproduce better than pairs, probably because a wild

female mouse likes to share a nest with another female. They need more nesting and bedding material than usual, as they like to bury their young. Try not to let them get too fat, and breed as young as possible. The breeding success can be enhanced when you try adjusting day light length and light intensity to mimic a biologically natural light cycle; this will fool your mice —just as ours—into believing that it's time to mate.

At our facility, we keep colonies of wild mice in 2 m x 3 m large enclosures with 100 cm high sheet metal walls. The floor is wood and covered with a layer of shavings and/or hay. The enclosure is provisioned with cardboard boxes, bricks and other objects behind which and in which the mice can hide. Overcrowding can quickly become a problem!

< What are good **feeding enrichment** options for mice? >

At every cage change I scatter a small amount of irradiated **sunflower seeds** on the bedding of our mice. I also distribute part of the daily standard food ration on the bedding; I have done this for years in many studies including GLP studies. The amount of the extra treats is so small that it does not affect calorie intake and body mass composition. However, I always make sure that the treats for foraging are mentioned in the study protocol and signed off by the study director.

We keep jars of sunflower seeds on the counters in the animal rooms, so that attending personnel can distribute them to their mice whenever they are inclined to do so. These regular visits enriched the daily routine not only of the animals but also of the personnel. At the same time, they foster a positive human-animal relationship.

Scattering seeds or other small food items on the bedding works great for mice; they love it. The problem is that these treats may interfere with experiments where bodyweight is an important parameter. We became aware of this some years ago when everybody was happy—especially the mice—until the researcher told me that he was surprised that the bodyweight was yo-yoing so much. We found out that the animal caretaker had given the seeds always at 4 p.m. before he went home. In just a few days the mice noticed this predictable routine. They liked the seeds more than their standard food, so they waited till four o’clock in the afternoon and then started to forage and eat the seeds, and continued eating pellets after they had finished all the seeds. We had to prevent this—for the sake of the study—and asked the animal staff to give the mice the seeds in small portions at different time points throughout the day. Animals always surprise you!

Corncob bedding provides a great feeding enrichment substrate, because it has invariably small pieces of corn hidden in it. Every time we change the cages, the mice scurry around searching for the corn.

I am involved in a project in which we are examining various enrichments for mice. One of these is **coconut shells** that the mice seem to enjoy immensely. They climb on them, use them as olfactory look-outs — rear on their hind legs and sniff the air—use them as shelters, and chew, chew, chew, chew on them! Often, the mice chew the coconuts from the inside, so when we pick the shell up a week later, it is paper-thin!

< Based on your own observations, why do you think that some mice engage in **excessive food grinding**? According to your own experience, what is your recommendation for addressing this problem, or perhaps you don't regard it as a problem? >

The only real problem this behavior presents is having to change the cage more often. At our facility, we typically see this in CD-1s/ICRs although, on occasion, mice of pretty much any strain will engage in this behavior. We have tried several methods to deal with it to no avail: increased bedding, increased nesting, chew toys, bamboo tunnels, running wheels, different food, HydroGel, and increased foraging opportunities.

We give excessively grinding mice extra food and change their cages twice as often as usual. Who knows, maybe they want a fresh clean apartment sooner and this is their way of forcing our hand.

Our mice show increased food crumbling behavior during high air humidity conditions. They are kept in a relatively old building that does not allow us to regulate air humidity with precision. It could be that our mice engage in more food grinding when their food gets relatively soft, hence easier to process. Maybe it's an innate response to gather all the food that is available; you never know, there may be a harsh winter to follow.

We don't give our mice extra enrichment with the hope of controlling the excessive food grinding but change the cage more often; once the air humidity lowers this behavior disappears.

At my last facility we had mice excessively grinding their food in a seasonal pattern; we tried pretty much every environmental enrichment option on the market. Nothing we tried helped with any of our strains! We were finally able to control the behavior by autoclaving the food for the rooms of the worst food grinding offenders. That pretty much stopped it in these rooms.

Quite a number of our mice also engage in excessive food grinding. It's not humidity for us, as humidity is very strictly controlled in the animal rooms. Our food pellets are hard and pre autoclaved. The pellet crumbles are causing a lot of problems with dust in our IVC cages. The mice just sit up by the bottom of the food hoppers and chew away! I do think, maybe it really is a stereotypical behavior.

< From what you have observed, do you get the impression that **barbering** is an animal welfare issue? >

Although it is often said that barbering is performed by the dominant animal, I dare dispute this. I often observed that not only subordinate but also dominant mice actively offer themselves to get barbered. They would lie down and relax while their cagemate is grooming and barbering them. When the barber stops too soon, he is nudged to resume the barbering. Of course these are only anecdotal observations, but it would not surprise me if being barbered would activate some endogenous opioid system and, hence, can become addictive. If this is true, the behavior itself may actually be stress-reducing and less of a welfare problem than we think.

I am also not convinced that the mice on the receiving end are in pain or that the act of barbering is motivated by aggression. I have often seen the animals sit quietly while the barber completes his task. Some of them seem to actively invite the barber to pull their hair. Over time, barbered mice develop bald patches, but this does not seem to affect their physiological and behavioral well-being. We do identify barbering as an unwanted behavior but we don't regard it as an animal welfare issue.

Rather than focusing on the barbered mouse, we should probably be more concerned about the mouse who does the barbering. After all, barbering is <u>not</u> a normal behavior as it is performed in a stereotypical, almost compulsive manner. Barbering may be a sign that a mouse is no longer able to cope with species-inappropriate living condition; if this assumption is correct the barbering mouse would experience distress.

Barbering mice show histological changes in the prefrontal cortex (Sarna et al., 2000) that are seen also in humans suffering from compulsive hair-pulling, a behavior classified as a mental disorder that can cause clinically significant distress (American Psychiatric Association, 1987). The hair-pulling mouse is perhaps also in a state of distress; if that's the case, barbering would certainly be a welfare concern with possible implications for scientific data obtained from affected animals.

Discussions on Rats

< Is it true that rats are **docile animals**? >

That rats get along with one another so well is one of the reasons I like working with them. I have never noticed any signs of aggression among rats. I have worked with rats in neuropathic pain research. The animals were always very friendly to each other, even when they were experiencing pain.

I have worked with rats for several years but never witnessed that they were aggressive with each other. Yes, they can be aggressive with humans; I have been bitten on several occasions.

The only time I've ever seen aggression among rats was when someone inadvertently put ex-breeder males together; predictably, this resulted in a major scrap. Unfortunately, this means that once a stud male has been used for breeding, he has to be housed singly from thereon unless he lives in a permanent pair or harem.

Diabetic rats can be aggressive towards each other if their insulin is out of control, but that is only temporary and, once insulin is back under control, they become their usual sunny selves.

During my undergrad training, I worked in a pet store for several years. We would routinely put together rats of various ages and genders. I don't recall that we encountered any aggression-related problems when the animals met each other for the first time. To make it even more interesting, we would often use nursing rat moms to foster pups from other rodents, such as hamsters and gerbils; we simply put the new pups in the pile, and the rat mom would take care of them.

I have always found rats to be the most accommodating of the lab critters: well-mannered, well-groomed, social, affectionate, and intelligent. When it comes to their relations towards each other, I've only ever seen one squabble in 13 years. This occurred when an older female's roommate died and I had a singly housed girl, who I thought would make a nice friend for her. When I placed them together, the older female did a little song and dance number with a bit of a hiss; but I think that was just to explain that she was Queen Bee. The next day I found them sleeping in their little snuggle patch, as rats do, and they continued to have a happy relationship. I love the creatures and would probably still be working with them exclusively had I not developed such a strong allergy to them in grad school. But that's something else I can thank rats for: if it weren't for the allergies, I would have never discovered monkeys.

< Rats like it when you pet them. Is there a body area where they prefer to be petted? >

I used to be a teaching assistant for a rat lab in college, and ended up *pardoning* a couple rats who became my pets. They always seemed to be soothed by me **petting** them, very gently and calmly, right at the base of their neck, especially while they were falling asleep. Prior to working with them, I didn't appreciate how very affectionate they could be!

I would say that, yes, rats do like it when they are petted. They seem to enjoy having their heads gently scratched behind the ears, and some will lie down for their abdomens to be scratched. If in free mode having an out-of-cage wander on the bench, rats will come up to where someone is working and get in the way until you give in and groom the critter. I find that all of our rats—not just pets—appear to welcome a gentle scratch whenever there is an opportunity. I always encourage investigators to spend some acclimatization time with their rats before they start experimenting with them. This should make them appreciate how amazing their research subjects are and, hopefully, re-evaluate the implications of their planned experiments on them.

Yes, having the investigators gently handle and groom their rats before starting an experiment is a great idea. An investigator, who has experienced the natural affection of rats and their spontaneous trust in humans, will be very careful not to cause them any avoidable discomfort or harm when conducting an experiment with them.

My rats have always loved being scratched/rubbed behind their ears and along the top of the head. Also, a few of them enjoy having their chins and/or upper backs rubbed as well. Then, of course, some will roll over for a good belly rub.

I had one girl who would grab my nose with both of her front paws and just lick away. Others liked grooming my eyelashes. Many of them preferred to lick my front teeth—I know, this is a bit gross, but for some reason it didn't bother me, probably because they were my pets. Some licked like crazy, some also nibbled a little bit with their teeth as they would when grooming their own fur. These were very special experiences for me.

We had two nude rats arrive at our facility this week, and I was assisting the research group getting used to the proper handling of these new animals. Both of the little guys were more than glad to curl up in my hands for a scratch behind the ear and then wiggled themselves into a position for a belly rub.

Rats are highly developed social creatures. That they typically establish such affectionate relationships with their human caregivers makes it even less acceptable that U.S. animal welfare regulations do not recognize rats as animals.

I completely agree. I once worked at a zoo facility that bred rats but kept the animals under very poor husbandry conditions. I fought to get better litter for them—they were kept on pine shavings, which are toxic to rats—and suggested that they need bigger cages. When USDA officers would come and do site inspections, they completely ignored the rat room! How can these wonderful, intelligent little creatures be so disregarded and treated as if they were disposable?

If rats have been properly socialized, they will often solicit attention. They seem to love it when you gently scratch them behind the ears, on the head between the ears and when you give them a mini-massage on the neck. I've had some rats who liked the tops of their front legs massaged too. I love those little guys! They are really underrated creatures—extremely affectionate and playful, and great learners.

< Can you **train rats to cooperate** with you during certain procedures? >

In one of our smaller facilities that usually only has a few cages of rats we were able to train Long-Evans and Sprague-Dawley rats to **self cage-change**: The tech puts the clean cage adjacent to the dirty cage; then, when the rats approach the side with the clean cage, the tech gently lifts the rats' rears so they can hop over. The animals quickly learn to jump over on their own; our smaller rats get a lift for a bit longer but only because they aren't long enough to make it up and over. We don't reward the rats with treats until the cage change is complete and all are over. The cages are changed 2-3 times a week (static shoebox style cages); usually within two weeks at the longest, the rats are changing themselves. The time required for self-cage change and manual cage change is about the same.

I did something similar during grad school when I had a colony of really old, crusty cranky rats who had been frequently handled when they were young. The cages needed to be changed 2-3 times a week, and I had gotten sick of being bitten when picking the rats up manually in order to transfer them into the clean cage. It turned out to be quite easy to get them to cooperate during cage change: Move the clean cage next to the dirty cage and then gently help the rats to get over, and reward them after everyone has made it into the clean cage. I did have to place a little box close to the adjacent sides of the two cages to assist some of the rats who couldn't climb up in order to hop over. They all learned to move from the dirty to the clean

cage. Some days, cage change took a while; it didn't really matter, but it made life less stressful not only for the rats but also for me.

One is tempted to wonder why this simple training exercise of rats is not a standard procedure.

When working with rats it became very quickly clear for me that they are very attentive and smart animals who can be trained—using basic and gentle skills along with a food reward—to cooperate with me. For example, rats on a gavage study learned to literally open their mouths and allow me to insert the feeding tube without struggling. I remember a study in which rats were receiving a subcutaneous **injection** once a day over a period of one year. Since quite a number of animals developed lesions at the site of injection as a result of the immunosuppression, the principal investigator told me that he usually adds 12 extra replacement rats for his study. I suggested that we would give the rats a food reward after each injection so that they could learn to associate the daily injections with a pleasant experience, hence accept them without fear. We would only need one extra potential replacement rat, just in case. The PI was very excited to hear this because it meant treating 11 rats less for 365 days, hence saving money and resources. He did ask me if I was sure that it works. Well, it worked just fine. I chose dog food as the reward for each injection. The rats loved it and readily learned to cooperate with the injections. We had zero lesions and all animals in the study survived the year! So yes, rats can be quasi-trained to overcome injection-associated stress.

The rats I am working with like it when I tickle them. Their response suggests that being tickled by my fingers puts them in a playful mood. They are not at all afraid when I approach them with my hand and tickle them. My hand becomes a playmate whom they will run after in a rough-and-tumble play fashion to entice more tickling. This makes it possible for me to pick them up, turn them over on my hand and give them IP injections. My rats show no stress reactions during this procedure after which I resume the tickling, serving now as a reward for holding still during the injection.

Touching is a great way to get rats used to being handled. Decades ago I had a group of hooded rats who couldn't get food rewards. I spent about two weeks with each rat, starting at 10 minutes and working up to 20 minutes per day, just holding them on a towel in my lap and finally petting them. They got so relaxed, we could eventually **gavage** them with no restraint. They would just lay back in our hands. Back then rat-tickling wasn't a thing!

Rats are smart animals who have also been trained with success to cooperate during blood collection (Shyu et al., 1987), saliva collection (Guhad & Hau, 1996), and oral dosing (Huang-Brown & Guhad, 2002; Rourke & Pemberton, 2007).

< What kind of **bedding, shelter and nesting material** work best for rats? >

Having tested different types of bedding for rats, I think there will be little debate when I say that woodchip bedding is the worst. Corncob and smaller wood flake bedding is not too bad, but I like the compressed paper chip the best because:

- the cage dries out better—better for the animal,
- the cage requires less changing—better for time management,
- there is less dust—better for the animals, workers, and air handling system.

We provide our rats with paper-based nest-building material, aspen wood chew-sticks and tunnels, all of which are used by the animals, although the paper strips tend to be laid one strip on top of the other to finally create a rather compact little heap rather than a nest as mice and hamsters would do.

In contrast to mice, rats have a strong preference for solid shelters. They have little use for nesting material unless it comes with a secluded shelter in which the nest can be built. Both female and male rats will move suitable substrate, such as straw, into a shelter and build well-formed nests.

Rats will rest in a shelter during the light period, and climb on it and spend much of the time resting in that elevated position during the light period. Almost any type of solid shelter will do for them, but they seem to have a particular preference for opaque boxes with two or more small entrance holes (Patterson-Kane, 2003).

It is not the general view at the facility I work, but I personally think that an appropriate shelter should be considered basic cage furniture. The majority of our breeder rats prefer rectangular PVC tubes over round pipes, probably because the pipes are not stable enough for quiet resting or sleeping, but easily roll over when the animals play on them and when the cage is moved. Pipes are accepted under the condition that they are firmly attached to a side of the cage.

One rectangular plastic shelter (Rat Retreats from Bio-Serv) is a standard furniture of every rat cage at our facility. We have a special waterproof cards (from Lithgow) that gets placed on any rat cage that cannot

have the shelter due to protocol exception. The cards have a spot to mark what enrichment is provided instead, e.g., shred paper.

I also consider a shelter a must for rats and would concur that the animals like small openings that the occupants can plug with their rumps. We use cardboard boxes or recycle old polypropylene mouse cages. Both are well accepted by the animals. I have never seen competition or aggression between rats over access to the shelter, although I am always careful to make it big enough for everyone to fit. They usually huddle together in it and very rarely sleep outside, even when they live in relatively large groups.

We provide more than one shelter whenever possible—one on the floor and one suspended with cable ties. The breeding mums use the suspended tubes to escape from the pups when they need a break.

We place stainless shelves in all of our rat cages. These give the rats not only a place to hide but adds floor space and allows the female to have a break from the pups.

The shelves are a great addition but still do not compensate fully for a loft area or another cage where the dam can "escape" the pups. I have seen pups hanging/swinging/climbing from their mom's tail while she is resting—or trying to rest—on the shelf. It is very funny to watch the pups but when you think about the mom, this is not a good situation.

Rats will use about anything that can cover them, even if it's not really suitable. I have videotaped a rat who tried hard to dig into and burrow under a handful of wood wool to become invisible to my presence. It is very important to realize that the domestication of rats has not eliminated their inheritance of being a prey animal. Their sense of security is very much dependent on being able to disappear from sight quickly, either by seeking cover or ducking under in a burrow.

< What are good options of providing **feeding enrichment** for rats? >

We buy cracked corn and wild birdseed mix, add popcorn, a few sunflower seeds in the shell, and occasionally some dry cereal or fruit-flavored bird treats. Toward the end of the day, I scatter a small scoop of the mix around the cages of our rats, and then add a little portion on top of the pellets in the hopper so that a few treats will trickle down here and there when the animals retrieve the chow. The daily provision of this mix keeps the rats busy for quite a while, and they really seem to enjoy it.

I throw daily a handful of their standard food pellets onto the cage bedding rather than into the feeder. The rats seem to like this; they get hold of pellets and run around with them before actually eating them.

We keep jars of whole peanuts, cereals and dried fruits—especially apples—on the counters in the animal rooms, so that attending personnel can distribute treats to their rats whenever they are inclined to do so. These regular visits enriched the daily routine not only of the rats but also of the personnel. At the same time, they foster a positive human-animal relationship.

< What are useful **inanimate enrichment** options for rats? >

I often observe some of my techs push strips of soft paper towels through the cage fronts to our rats, who then enthusiastically chase whoever is in possession of the strip and try to grab bits of it. The one who has the strip tries to sit on it or wrap it around her body, and you finally end up with a pile of rats and lots of smaller pieces of paper. Once the paper is torn up, the game is over. We have not seen any injuries during these games nor any signs of overt aggression. I am not sure who enjoys it more—humans or rats!

Rats like Nylabones. At cage-changing time it is useful to take the Nylabones out and put them all in one container so that the cage-wash person has them all in one place—less chance they will be thrown out by accident when the cage it dumped.

Our rats get Nylabones, gnawing blocks and manzanita sticks; we rotate those items. The Nylabones last the longest, but judging by the look of the sticks and blocks after one month of usage, the rats obviously like those gnawing sticks the best.

It's also my experience that rats clearly prefer manzanita sticks over Nylabones.

Our rats destroy aspen blocks within two weeks. Some rats really enjoy chewing, and their aspen blocks turn into little balls within three days. Some strains use several blocks per week per cage. The blocks simply vanish and no shredded leftover material needs to be discarded.

We use aspen sticks for all our rodents and rabbits. They are soft enough for a good bite. These sticks are used heavily, which suggests that the animals like them. The sticks can be sterilized for use behind barriers. Normally, they are changed every two or more weeks, depending how soiled they are and how much is left of them. From my experience, gnawing sticks do not lose their attractiveness over time, probably because they allow rodents to fulfill their inherent drive to engage in gnawing.

Perhaps the most important enrichment we can offer our rats is a **cage companion**. Often overlooked, a compatible cage mate plays a crucial role in enriching the lives of single-caged rats and, therefore, should always be present unless there is some very well-justified reason—an assumption or a belief is not enough—for depriving a rat of at least one cage mate.

Discussion on Chinchillas

< We have an investigator who will do research with chinchillas. I have never worked with these rodents before. It would be great if some of you who have direct experience with chinchillas can share practical suggestions on the species-adequate **housing and care** of these animals. >

In their native habitats at high elevation in the South American Andes, chinchillas live in social groups in burrows or rock crevices. They are slightly larger than ground squirrels, very active and agile jumpers who can jump up to 6 feet. They clean their dense, velvet-like fur by regularly taking fine-pumice dust baths. In captivity, they need extensive exercise to keep healthy and since they lack the ability to sweat, ambient temperature needs to be carefully controlled.

The provision of a **dust bath** is a MUST for chinchillas. Get some dust or very fine, dry sand; put it into a mouse box, place that into their cage and let them go bonko for about 30-45 minutes. It's a blast to watch!

I LOVE chinchillas! I worked with them for quite a while; they are so much fun! They can seem a little skittish upon arrival, but once they settle in, they're darling little things. You're going to love them. I've kept them in standard cat cages, large cat cages and standard rabbit cages. Chinchillas hop

(if they don't have enough room to jump), so they need relatively high living quarters. Grated flooring is not suitable for them as their feet can easily get caught in the wires. Thus, you need to provide them with a solid floor surface such as a cage liner.

Chinchillas like to take dust baths and they like to chew. We provide them with the dust (fine volcanic ash or eroded pumice rock) in a small cat litter box every day so that they can keep their fur nice and clean rather than oily. They also get wood blocks so that they can chew as much as they want, and we give them fresh hay on a regular basis and a hut in which they can hide and rest.

Chinchillas adore wooden blocks and other **chewy things** designed for smaller pets. Also, I purchased a couple of those large clear exercise balls so we could let the little guys go bananas about once a week for exercise—they're very active little critters!

As for food enrichment, small pieces of fruits and veggies, yogurt drops, raisins, and other small items are always a hit. Just don't overdo the sugar or you're going to have little beggars who won't eat their chow. Finally, hay is essential for chinchillas and should be provided daily as part of their standard diet. I've found they enjoy the timothy best.

Chinchillas **overheat very quickly**, so it is important to keep the room temperature a bit lower than the usual optimal temperature range for the other rodents. They need a **hiding/nesting box**, preferably one that is relatively narrow. A shoe box with a hole cut out or a long pipette box with the front torn off are well accepted by the animals; Carefresh or hay provide optimal bedding in such shelters.

When we housed our chinchillas in standard caging we used rabbit racks that we furnished with a structure for hiding (PVC tube or elbow or a plastic hut serving also as a perch) and an item for gnawing (pumice stone or wood block). The animals had weekly access to dust baths and daily access to loose hay. They got treats whenever they were handled by lab staff.

Our chinchillas are housed as compatible pairs (adults) and compatible trios (juveniles). We currently have to keep them in IVCs. Initially we put in plastic huts, but the animals were pushing them against the Lixit and flooding their cages. They still receive regular access to hay, gnawing devices, and weekly dust baths.

Discussions on Guinea Pigs

< What are practical, species-adequate ways of **housing guinea pigs**? >

We keep our group-housed animals in **recycled rabbit cages** with perforated floors. Each cage is furnished with a Macrolon Type IV rodent cage that has sawdust bedding with a generous layer—about 8 cm thick—of **hay**.[0121] The animals tunnel, hide and sleep in the hay. On top of that, hay is a favored foraging substrate for them. I do not find that hay creates a mess.

Guinea pigs like to have a clean sleeping area. They jump out of the Macrolone cage and defecate and urinate in a corner of the rabbit cage, in which they also find water and food pellets.

We have worked with this cage design many years, and I think the guinea pigs are no less satisfied with it than we are.

Our large breeding groups live in **floor pens.** For enrichment, hay is placed in plastic barrels that have holes in the bottom. Since guinea pigs love to go under anything that covers them, we mount the barrels on approximately 20 cm high iron legs, allowing the animals to run under the barrel. This arrangement also provides feeding enrichment, and the animals skillfully pull strands of fresh hay through the holes in the bottom of the barrel. The only occasional problem we have had with hay was when guinea pigs were tethered and long blades of hay would wrap around the cannula. We now prevent this by simply chopping the hay for cannulated animals into short (about 15 cm) blades.

Like hamsters, guinea pigs have a strong need to hide from the human predator. Their feeling of security depends on access to a covered refuge. PVC pipe sections provide great **shelters**. Group- and single-housed animals hide in them, run through or jump over them. I am sure they would prefer cardboard boxes, which they could gnaw and which would not roll over, but many of our researchers are concerned that the animals might ingest some of this easy-to-gnaw material, which then could exert an effect in nutritionally sensitive protocols. We have found no evidence that the animals gnaw the PVC pipes.

We use old polypropylene mouse cages with a hole cut out of one wall. They can be removed easily or flipped over when you need to get hold of an animal. Our guinea pigs use these shelters often, especially when people enter the rooms. I do recommend shelters for guinea pigs, because I see the animals making use of them so much, not only for taking refuge and sleeping in them, but also for sitting on top of them to get a better view of the room.

All our guinea pigs have access to little plastic huts. For group-housed animals, we provide enough huts so that they all can find a safe place in one of them. They definitely retreat into the shelter when they are spooked, especially the young ones, who are more skittish; I do think guinea pigs truly benefit from having a secluded place where they can take refuge. I like that the huts are open on both ends so that the animals can escape from each end if needed. Walters et al. (2012) found that pair-housed male guinea pigs with a hut had significantly lower fecal cortisol concentration than pair-housed animals without a hut. This indicates that a refuge can serve as a stress buffer for guinea pigs.

I think a hiding place for guinea pigs is a must. These are usually nervous animals and whether they are single- or group-housed, they do require access to a shelter.

Guinea pigs do not manipulate their food, but pick it up directly from the ground with their teeth. This suggests that any **toy-like enrichment gadgets** that may be useful for rats, mice, hamsters or rabbits serve no purpose for guinea pigs, especially those who are kept in single-cages. My pet guinea pig, whom I adopted after he was released from research, does

not care to play with any toy-like enrichment gadgets, but loves to chase my hand then run away from it. I wish, I would have the time to do this also with the animals in the lab!

< It is my experience with group-housed guinea pigs that the animals like **hay** more than any other substrate. Is it practicable on a regular basis to provide single-caged or pair-caged guinea pigs with an amount of hay that is sufficient for them to hide in/under? >

Loose hay autoclaved at 220º F for 5 minutes is the best enrichment for guinea pigs. I have used it successfully for a decade with our animals. They nest and hide in the hay, and they eat it. They will trill when you bring them new hay. Their excitement shows you that hay is a species-appropriate enrichment for them.

I believe the benefit to the animals outweigh all potential practical issues, such as extra time investment for cage cleaning and hay distribution. Hay is not costly, and it takes just a few minutes a day to give each animal a handful. When changing the bin, I have to dispose of the dirty shavings anyway, so some hay along with that doesn't take up any more time.

Hay is indeed the best so-called enrichment you can offer a guinea pig; it can be used for hiding, playing, nibbling and foraging. Unfortunately, some institutes will not allow hay behind the barrier without autoclaving, which makes it brittle and more sharp, hence potentially dangerous for the animals' eyes.

We also consider hay as an essential element of guinea pig housing and care. Our guinea pigs get a large slice from the hay bale on a daily basis; this is sufficient for them both to forage in and to hide under. To distribute the hay takes only a couple of minutes. We used to autoclave all our hay but now purchase it irradiated because it's easier to store. We have not experienced any problems with eyes irritated by hay, but straw can be a problem unless wheat straw is used—barley straw has awns that can penetrate soft skin and eyes.

All our guinea pigs also have access to solid shelters, but they clearly prefer the hay as hiding place.

If a restricted-nutrient study does not allow for the provision of hay, we give our animals wheat straw instead.

< Do you offer your guinea pigs **fresh produce** as a kind of feeding enrichment? >

Yes. Currently, I have our guinea pigs on a rotation of fresh vegetables four days a week as follows: Mondays - carrots, Tuesdays -

broccoli, Wednesdays - kale and Thursdays - lettuce; Fridays we suspend NutraBlocks. The guinea pigs relish all of it!

Our guinea pigs get green beans, broccoli, lettuce, apples, carrots and/or sweet potatoes on a daily basis.

Our guinea pigs get a variety of fresh and dried produce. We have not found anything they do not like, which even includes citrus fruit such as grapefruit.

We provide fresh fruit and veggies to our guinea pigs three times a week; this includes cucumber, lettuce, broccoli, kale and apples.

Unfortunately our facility just won't go for it, when it is so much easier and less costly to simply supply pellet food rather than green stuff that has to be washed and prepared as needed.

< We would like to transfer our pair-housed male guinea pigs to pen-housed group settings with more opportunity for exercise so that we can keep their body weight at around 500 g and **prevent obesity**. What would you consider to be the optimal housing environment for these animals? >

Keeping male guinea pigs in groups may cause you some headache. In the wild, guinea pigs live in large harem groups; they do not form bachelor groups, as many other species do, so keeping a group of males permanently together in the same enclosure can be problematic because victims of aggression have no way to leave the territory. Even numerous shelters will not be a guarantee that a group of confined guinea pigs will not become incompatible because more dominant males will bully, often quite mercilessly, subordinate males.

I agree, I would also have concerns with housing males together in groups; this may not go so well.

Rather than trying to give male guinea pigs more exercise in a group setting so that they don’t gain too much weight, it should be possible to prevent obesity by changing their diet. Guinea pigs love hay and vegetables, especially the green stuff. We usually feed guinea pigs in laboratories hay and greens as a supplement to their commercial pellet diet. Why not reverse it, and make the hay, greens and vegetables the staple diet and supplement it with pellets and vitamin C as needed?

I think that would be a reasonable approach to prevent guinea pigs from getting too heavy. Unfortunately, it would be hard, if not impossible, to get administrators and investigators realize that feeding caged guinea pigs veggies/greens/hay in the morning and pellets in the afternoon would be an optimal regimen to control the animals’ body weight and provide them with a diet that is much more species-adequate than dry pellets only.

Discussions on Rabbits

< Can anybody please share experiences on safe **pair formation and pair housing of female rabbits**? >

When attempting rabbit pairing in the past, I found a children's plastic swimming pool very helpful. The floor area is much larger than the cage, and items such as toys and vegetables can be placed on it to give the two animals some distractions if they aren't very comfortable with one another in the beginning. Also, should the two rabbits decide that they don't care for one another's company, the pool provides much more space to thump and charge than the cage. It also has sufficient space for me if I have to grab and separate them. The rabbits I have paired in the past were mature NZW (New Zealand White) females; they are larger, slower and more docile than Dutch Belted rabbits who can move very fast and tend to be quite high strung.

It has been our experience that female rabbits—including Dutch Belts—pair rather successfully when partners are introduced in the same cage as soon as they arrive at our facility, even if they are unfamiliar with each other. I think the transportation stress provides a bit of motivation to stick together with another conspecific. Most of our pairs are created in this way. It is not uncommon for paired does to groom each other; this suggests that they do enjoy the company of each other.

When pairing NZW rabbits who haven't lived together before, we give them a sedative, just enough to make them sleep. We then put the two partners side-by-side, touching each other, in a double cage. The important part of this pairing process is that the animals physically contact each other, thereby spreading recognizable smells, while they recover from sedation.

The front of the cage is covered with something to darken the interior—we use paper or black rubbish sacks so that we can easily lift a corner to observe without disturbing the occupants. We do not witness any overt aggression when we let them sleep the sedative off and slowly wake up together. The new pair’s cage is provisioned with an old mouse cage turned upside down that the two does can use as a lookout or refuge where they can quickly get out of each other’s way in case things suddenly turn nasty. We also provide plenty of hay, which probably also fosters partner compatibility by distracting the animals a bit.

Our female rabbits are routinely housed in pairs upon arrival. We first **mark them with urine from bucks** and then move them straight from their boxes directly into a cage with a partner, typically with their buddy who was

shipped in the same box with dividing panel. We tried the route of having the vendor pair the animals prior to shipping but honestly that turned out to be more of a hassle than it was worth, especially since we've had such good luck with pairing them regardless of their history prior to shipping. We've paired dozens of does this way with a very high success rate.

Can you please describe the urine marking procedure.

We use Annie Valuska's method:
http://pqdtopen.proquest.com/doc/1112868360.html?FMT=ABS
We collect urine from the bucks we have in house, dip a piece of gauze into the urine and rub it on the does' foreheads between the eyes/ears. Sometimes we 'dose' them again or add urine marks on multiple locations on their bodies if we have a pair that doesn't seem to be meshing right away—scuffling more than normal but not injurious aggression. Marking them again with buck urine calms those fractious females down.

A few years ago my charges were single-caged female rabbits on a 5-year-study. When I started working with these animals they had been there already for about 2½ years. They lived in banks and were developing hock sores and other foot issues.

Another tech and I developed a floor-housing system. We were in our own building, so we had plenty of space and were able to transfer all 50 rabbits to the floors of three rooms. The new housing consisted of collapsible dog pens with connected pet-shade on the tops for escape artists.

I arranged the does based on personalities and placed two of them as neighbors when I had the feeling that they would like each other. They could contact each other through the widely spaced bars of the pens. When they would lie next to each other consistently, were often grooming each other peacefully, and none of them showed any signs of depression, I paired them by removing the divider.

We were able to match up all 50 does into 25 pairs. There was only one pair formation attempt that resulted in a serious injury.

After my experience with those girls, I firmly believe that single-caging of does should be abandoned and replaced by pair-housing in floor-pens.

Your belief is supported by two studies showing (1) that does have a strong preference to spend time with another doe rather than alone in another cage (Brooks et al., 1993), and (2) that does are willing to push through weighted doors in order to gain access to another female companion (Chu et al., 2002).

We have watched our mature female NZW rabbits housed in pairs or groups go from doing really great together for one week to several months to finally being all separated and again single-housed. Serious fighting resulting in dangerous injuries, especially genital wounding forced us to stop the social-housing attempts for now until we come up with a new, hopefully better strategy for housing does in a social setting.

The NZW female bunnies that we receive from the vendor come as compatible pairs; they are approximately 70 days old. We keep them pair-housed in double cages that are provisioned in each cage section with a tunnel-like platform, serving as an elevated resting surface and hiding area for each of the two rabbits. There is a partial divider that is open in the front half and solid in the back half of the cage; it offers the two rabbits optional visual seclusion in the back of each of the two cage sections. Cage companions stay together through maturity. Within the past year, we had 36 adult female pairs; only 3 of these pairs had to be permanently separated due to injurious fighting.

< Can anyone recommend good **social-housing arrangements for female rabbits**? >

Our rabbits have been housed in **floor pens** for quite some time. Based on their size, we keep 2-3 does in each pen. The pens have a 42 x 42-inch floor space and a height of 30 inches. It was an animal victory to get the rabbits out of smaller, more sterile cages into these relatively spacious, bedded pens. We keep a pair of **guinea pigs** in the floor pens. They do a great job buffering aggression among the bunnies. I'm not sure if it is the constant movement of the guinea pigs that keeps everyone from setting up a territory, or what; but there certainly is more peace among the rabbits.

In our facility we use dog runs for group-housed, adult female rabbits. We remove the partition between the runs to expand the area for more room to move around. Betachip bedding is used to cover the floor in the runs and large cardboard boxes serve as shelters.

Our rabbit groups are also kept in dog exercise pens. We furnish the pens with plastic buckets lying on their sides (sheltered retreats for resting), plastic buckets without bottoms (tunnels to run through), milk crates placed upright (look-outs), one food bowl per rabbit and several Lixits. The floor is covered with wood shavings.

Our group-housed rabbits regularly use an old metal rabbit cage—with the door removed—as **s**helter. This cage is placed in the bedded area of the floor pen; the rabbits often sit inside or on top of it. They use a separate

area for a latrine, so the shelter is rarely soiled, but if necessary, it goes through the cagewash and autoclave.

We make use of the large barrels we receive our cleaning chemicals in. Once they are empty, they are thoroughly cleaned and then cut vertically and/or horizontally. We cut an entrance/exit either on the end or on the sides. Our bunnies use these shelters all the time.

Our group-housed female rabbits have access to Sonotubes and handmade **plywood shelters**, which they use as hiding/resting places and as perches. The plywood is sealed with four coats of polyurethane and hosed down when the pens are cleaned. The USDA inspector has not had a problem with them.

VERY interesting! However, don't they chew/scratch on the plywood? My concern is, if they created enough damage to the structure, then the sealant would no longer be valid, and the plywood would absorb water and/or any soap being used to sanitize the material. So, how do you deal with this? Or do you simply have ultra-chill bunnies?

The rabbits do nibble a little on the lower arch and top cutouts. But it takes the entire academic year to get to the point where the sealing coat starts to show cracks; a once-a- year paint job does not seem to be too much work for us.

What are **Sonotubes**? What material are they made off? Are they inexpensive?

They are the cardboard tubes used for holding cement in a cylinder, for instance to manufacture posts for a deck to be placed on. They come

from 8 to 24 inches in diameter. They are chewed by the bunnies and last about a month before they are either too dirty or too chewed up. Then we throw them out and replace them with new ones. A tube costs around 10 dollars. You can get them at any hardware store.

< We do a lot of repro-tox work with rabbits who are all kept in stainless steel cages. One of our techs and I are bothered by the fact that these rabbits don't have a suitable place to build their **bunny nest**; they are agitated and seem to be frustrated to not find a spot that could serve them as nest. So, we would like to add a solid floor space (like a low tray or a floor insert) to give them a place where they can build their nest with suitable nesting material. I imagine that this would decrease their distress toward the end of the study when they approach parturition; we desperately need data to quasi-sell the study directors our concern and refinement idea. >

We used the (very expensive) nesting box from Otto Environmental—a 10 x 10 x 20 in long [25 x 25 x 50 cm] stainless steel construction with removable polycarbonate floor—to breed some of the first transgenic rabbits. As long as this cage addition was specified in the study outline, our PIs had no problem with it.

Pregnant rabbits exhibit nesting behaviors as they near their parturition date, so providing them with a nest box and nesting material is a great way to support this natural behavior. We use the same box that you describe and we fill it with pine shavings. The box is given a few days before their due date, and of course the mothers add their own fur to the shavings and make nice bunny nests.

Our nest box version is also made from stainless steel and has a removable floor, but it has not only a front entrance but also a flip-top door so mom has privacy but we can peek into the box to check on her and the babies.

We house our rabbits in stainless steel cages with plastic perforated flooring. In each cage, we insert a tray filled with sawdust for all rabbits and extra nesting material for pregnant females. It works very well. You can buy different types of nesting material that is certified, so this should not be a problem for GLP repro-tox studies.

At our facility, shortly before the expected birthing date, a HDPE (high-density polyethylene) hut is placed in the cage along with wood shavings, and the doe takes care of the rest. The HDPE plastic is nice, as it absorbs warmth and the rabbits don't bother to chew this hard material.

< Is it safe to **pair-house adult male rabbits**? >

We do pair-house adult males under the condition that they are siblings. We have had success maintaining such pairs past sexual maturity without significant wounding. Daily monitoring for behaviors that tend to precede aggression allows us to avoid serious fighting. A quick spray from a water bottle is a great first step as it will give the two males just enough distraction to retreat to their own sides and groom away the water.

If your two males start fighting, your instinct will tell you to separate them immediately, yet it would be optimal for them to work out their dominance hierarchy on their own in order to establish a stable social relationship. While it may look and sound vicious, if there are no wounds on the genitals or on their eyes and if there are no profusely bleeding lesions, the two rabbits can usually be maintained with appropriate enrichment and veterinary monitoring. They re-establish their dominance relationship daily with chasing/mounting, so this is a natural behavior that needs to be allowed even though it may look aggressive at times.

We have an average census of ~400 male rabbits per year and have had only one interaction that led to a rabbit being euthanized, and this resulted from human error. Pairing rabbits is a difficult and time-consuming, but extremely rewarding job.

< Is **protected contact with a neighboring male** a safe compromise for adult, non-kin male rabbits to satisfy their biologically inherent social needs? >

I took care of 12 single-caged NZW bucks who had access to puppy pens clipped together for support every other day. In these little floor pens, neighboring bucks were separated by bars that allowed limited physical contact. Mostly the contact was peaceful. A few boys would engage in spraying each other, but they never took it to any physical aggression. I spent a lot of time with these animals during their play time in these pens. Whenever I sensed something was getting out of hand between two neighbors, I'd either offer a distraction—a new toy or myself, which most of the animals seemed to enjoy—or if this did not do the trick, the two antagonists got a time-out and had to go back to their individual homecages for the day. I had to deal with only one serious ear injury in the course of several months, when a buck attacked his neighbor while I happened to be out of the room for a few minutes.

There were two bucks who gave me the impression that they could get along with each other; one of them was a bit shy, the other very relaxed and easy-going. One day I decided to allow them to have playtime together without separating bars. The two stayed so close during their playtime

sessions that they looked like a two-headed rabbit! They spent the nights in their banks in separate cages but they played together every other day in the little floor pen. Fortunately, these two boys were adoptable at the end of their study. They went to a home together; to this day they are enjoying each other's company permanently!

Our group helped develop and test a double-wide cage with a special dividing panel. The front half of this panel is transparent with perforations allowing visual and minimal tactile contact between neighbors, while the back half is opaque and solid. Following a one-week acclimation, we monitored via remote video recording during a two-week test period the behavior of four pairs (eight animals) of male NZW rabbits in these refined cages, and eight male NZW rabbits in standard single-housing units.

We found a greater diversity of behaviors and an overall greater activity in the bucks who had protected contact with a neighbor. Much of this extra behavior was playful and exploratory. During the first few days, we had one buck who was thumping and charging his neighbor, but by the end of the week he had calmed down and stopped exhibiting these aggressive displays. The quasi-paired rabbits spent a significantly greater portion of their time in the quadrant of the cage closest to their neighbor—resting or sleeping peacefully side by side in contact with the clear, perforated half of the dividing panel—than in any of the other three quadrants of their cage. They remain in the front half of the cage when an unfamiliar staff member enters the room and approaches their cage. They also have a shorter latency to touch when that person introduces her hand into the cage than single-caged rabbits. This does suggest that they are less fearful of people (Lofgren et al., 2010).

We've used these refined cages for close to three years now for both NZW and Dutch Belted rabbits with only one major injury, an unexplained split lip—this could have been a bite through one of the small contact holes; we are not sure, as nobody saw the incident.

< What are the most effective, yet practicable **environmental enrichment options** for rabbits? >

Branches provide inexpensive enrichment. The rabbits spend quite some time gnawing at the bark, but once all the bark has been removed, the branch is of no more interest to them.

Hay is more attractive for the animals and more practicable for the personnel. Our rabbits do not get tired of nibbling and eating this natural foraging substrate. Presenting the hay on the top of the cage is a particularly

simple but very effective way of providing species-adequate environmental enrichment—strictly speaking "foraging enrichment," because the rabbits are given the opportunity to engage in foraging behavior. Offering the hay in a hanging manger is equally useful.

Wire whisks stuffed with hay and hung at the side or from the ceiling of the cage provide great foraging enrichment for rabbits. The wire whisks are inexpensive and easy to clean.

We stuff hay tightly into cardboard tubes and cardboard boxes and let our rabbits engage in foraging behavior; they pull the hay little by little from the tubes and rip the boxes apart to get to the hay.

All our bunnies receive hay every single day. I've learned that they enjoy a change in the type of hay; I keep several varieties on hand: timothy, botanical, orchard grass, oat hay and alfalfa.

We autoclave it for three minutes at 221ºF (105ºC) to make sure it's free of pathogens. The hay does change its color and takes on a smell that is difficult to describe, but this does not seem to bother the rabbits who still eat it with gusto. The main benefit of hay is to keep the gut working properly by providing a lot of fibrous material. The fact that it provides a natural way of foraging is complementary.

I was always taught to feed lab rabbits *ad lib* pellets, and that's all; hay was regarded as an extra treat—if it was mentioned at all. After adopting my two bunnies and researching everything I could find on rabbit health and diet, I discovered that for house rabbits it is recommended that they are fed *ad lib* hay and restricted pellets. I have now learned that it is the hay that keeps things moving internally. The pellets are important, but given *ad lib* can lead to obesity and can, probably, also cause inappetence.

At my laboratory, we feed our rabbits *ad lib* both hay and pellets. We met some initial resistance from investigators regarding the hay, but our veterinarian was firm and refused to allow any research to be done with rabbits unless they were given hay *ad lib*. The hay does keep the rabbits' digestive track working properly. Also, rabbits are hindgut digesters, meaning they use their cecum and large intestine for a very large part of their digestion; the hay is a very important factor for cecum health and normal cecum functioning.

Produce provides excellent feeding enrichment. Our rabbits get food fresh produce every day. We have approved lists for all food treats, so it can be a wide variety of options; apples, carrots and greens make up the dominant part of the produce.

Our rabbits get fresh produce on a daily basis. I order green beans, broccoli and lettuce for them but apples, carrots and sweet potatoes are the typical produce.

Rabbits love **noise-producing metallic gadgets**. The rabbits in my charge get a lot of entertainment by pushing metal jar lids along the floor and moving the shavings out of the way.

Yes, jar lids, either loose or suspended on a chain, provide great enrichment for caged rabbits. They show keen interest in these gadgets for prolonged periods of time. If one rabbit picks up the lid and drops it or if a person picks one up and drops it, within moments all the rabbits in the room will come and join playing with their lids. They push them around energetically, thereby creating quite a noise.

We give our single-caged rabbits each a small stainless steel bowl. They pick it up and throw it around their pen. I love to see the effort and fun they have when the bowl turns upside; they go to great lengths turning it around again and then fling it across their cage!

We used to fit to the front bars of all our rabbit cages large metal stainless steel rings, similar to a key ring. The noise of 40 rabbits all-playing with the rings was considerable, to say the least—the rabbits didn't mind it but it drove the staff crazy.

There's something almost comical about how much rabbits enjoy making noise. It sure seems to make them happy.

The funny thing with our rabbits is, while they don't like it if we are noisy, they sure love to make noise if it is their idea.

Our rabbits love the Rabbit Race Car. It consists of a stainless steel bolt with loose stainless steel washers and loops. I would have never thought it would be the most used rabbit toy in our inventory, but the animals get a kick out of picking them up and tossing them around, thereby creating quite a bit of rattling noise. They toss them almost immediately after they are placed in their cages and continue interacting with them for several weeks. What is interesting is that the rabbits don’t show any startle response when they are tossing them around in their cages, and other rabbits in the room seem to remain undisturbed, not at all startled when their neighbors are making such a racket.

Small bells hung from the ceiling of the cage, are similarly attractive. Our bunnies love these and will nose and push them during long play periods.

Hollow plastic cat toys with bells inside are also great enrichment gadgets. I guess, it is the noise of the bells that makes these toys so

attractive. Our rabbits play with them over long periods of time. I also have witnessed the domino affect, with one rabbit starting to play and the other(s) promptly joining. When I pick up the toy and toss it, sure enough, one of them will fetch it—just like a dog—and bring it back to me. I will toss the toy again, and this game can go on and on. These cat toys have been a big hit and the rabbits never seem to lose interest in them. The bell inside gets rusty after a while, and some of the rabbits chew on the toys and finally destroy it, but these toys are not expensive and we replace them as needed.

We give our rabbits autoclaved **cardboard boxes**, which the animals use not so much as hiding places but as lookout post. They spend much time sitting on top of the boxes and spy out the land, but they also tear holes in the sides and then spend hours playing tag in out of the holes. Nothing seems to make a bunny happier than a cardboard box to chew on! Our researchers do not raise objections that their rabbits have access to cardboard. When the box finally collapses after about a week, we just throw it away and replace it with a new one. Our staff saves boxes, so this kind of enrichment costs nothing, apart from the effort of collecting and distributing it.

We have been using cardboard for our rabbits for several years and have never had digestive issues. All cardboard items are autoclaved to avoid the spread of any potential pathogens. Our rabbits shred the cardboard; I never saw them actually ingest it.

Our group-housed bunnies have shelving and they love to lounge up there. I think it makes them feel more safe. **Elevated lookouts** are also used as a means of vigorous exercise, and the rabbits often jump up and down from shelves as they zoom around. Over the years I have never had to deal with an animal who had an injury or fracture that was related to a high lookout.

I was really nervous when I first put the shelves in the pens as they seemed pretty high to me—above the Lixit—but the rabbits figured it out with ease in no time. Rabbits seem to have a strong urge to overlook their environment from higher ground; this may give them a sense of visual control and safety.

Our group-housed rabbits also like to perch themselves at a higher level. We cut old barrels into halves to serve as shelters and lookouts. Occasionally we have a rabbit who will leap from the top of a barrel over the enclosure walls, but we have never had any injury resulting from this.

Our rabbits like to be visited by **friendly humans** who not only bring along treats such as banana chips or carrots but who also interact with them.

Not all, but some really like it when I gently scratch them, especially behind the ears. Those visits are probably one of the most appreciated enrichment options for caged bunnies. Our rabbits go crazy for plain Cheerios! They will run to the front of the cage, all of them banging the toys until you get to each one of them and hand out the treats.

Friendly contact with humans is very beneficial for caged rabbits. I have learned over the past couple of years that spending a good amount of time touching and gently handling them, not only provides enrichment but also allows for greater ease in handling them for procedures. I've seen bunnies go from thumping terrors to putty in my hands just by visiting them often and giving them something to throw and chew.

< Rabbits are biologically fearful of humans; this implies that being approached by and, even worse, being scruffed by a human is likely to stress a rabbit, hence influence research data collected from the animal. Based on your own experience, what is the most practical and effective way of **acclimating rabbits** to your presence and to being handled by you, without eliciting undue fear/stress responses? >

From the moment of arrival and for the rest of the acclimatization period, we get our rabbits as quickly as possible accustomed to our husbandry and procedure staff. From day one, staff announce their presence by knocking on the door before entering the animal room and talk to the animals, in order to habituate them to their presence and voice. Once acclimated to the staff, our the rabbits will readily come close to anyone who enters the room and calmly approaches their cages. Some of our rabbits have become so friendly that, when you open the cage door, they are right there, almost coming out at you in a welcoming manner.

We don't allow lab coats in our facilities so everyone wears disposable gowns over either their work scrubs (animal care/vets) or street clothes (research) so we (and the rabbits) don't have an issue with uniforms or what the person approaching is wearing.

As part of our husbandry procedure, all of the cages are opened at the same time and left open during the presence of the husbandry staff in the room. This entices the rabbits to come to the front of the cage and explore a different dimension of their living quarters. Most of them will sit right at the front of their cages, looking out into the animal room which has now become part of their cage environment, maybe even feeling less trapped by the physical limits of their cages but also realizing that their cage can be a

comforting retreat. We've never had rabbits jump out, maybe because they know how relatively safe their cages are.

In my experience, offering a treat every time a person approaches the cage is the best way to help a rabbit get used to the new living quarters and overcome his fear of people. Yogurt drops, fruit chewies, and hay are favorites that will quickly be associated with any friendly person who offers them. In order to make the animals feel relatively at ease when humans are present, it is very important that not only attending care personnel but also the investigator visits the rabbits and offers them treats on days when no experimental procedures are being done with them.

Rabbits are so easily stressed! Developing a trust relationship with them is extremely important when you have to work with them. The process from when they arrive to when they're ready to be handled involves a lot of patience and comforting interactions.

I work with them every morning, just using a soft voice (sometimes singing); slow, steady movements; allowing them to explore me. They are usually bug-eyed and nervous at first; gradually, they come around to get close and smell me, and then allow me to gently touch their nose. After a couple of days, I can pet their backs, but they're very tense about it.

After the first week, most of the rabbits will allow me to pet them on their backs without being tense; they are now relaxed when I am with them. I talk to them with a soothing voice; they seem to like it when I sing pieces of opera. By week two some of the rabbits will hop up to me and put their nose down to let me pet them. I've had a couple of rabbits who run up to me and lick me whenever I come into their room. These rabbits have learned that they can trust me and they will remain calm during almost any handling and treatment procedure.

There are always a few who don't quite get past just letting me pet them, but they're fairly calm for handling, which includes mainly PO meds, IM injections for sedation and eye drops.

Our rabbits LOOOOVE Fruity Gems (dried pineapple and papaya). When we get new animals, I give them these treats initially in their food hoppers. Once they have found out how tasty they are, I offer these treats through the cage bars, then open the door and place the treats in my hands. Unfailingly, the rabbits will go for the treats and I can then pet them while they eat. Most of our bunnies are pretty friendly and seem to enjoy it when I gently stroke them. I'm pretty sure they still don't like being scruffed, but they are so used to me that they relax very quickly after this disturbing procedure and take a treat from my hand.

When we get a new group of does, I will regularly spend some time sitting among them in their pen, gently talking to them and letting them approach me on their own terms; there is no hurry at all! I let them sniff and climb over me but will touch them only after they have made the first step and contact my hand. I don't think they will ever lose all fear of me, but they certainly tolerate me; this may be as good as it can get.

I like to hang hay balls in our rabbits' cages. They are suspended from the cage ceilings and look like round metal baskets; you fill them with hay and the animals will have to stand up to retrieve the hay from the basket. After the first few fills, the rabbits will eagerly try to get the hay out while you are filling the basket. Eventually it becomes easy to gently pat their heads or stroke along their backs while they are busily foraging; in this situation they will not dodge my hands, in fact some of them give the impression that they like it when I stroke them. I do this little ceremony every day; it does take a few minutes but it pays off greatly in rabbits who are relaxed when you touch them and who don't panic when you pick them up for a procedure.

< If you have direct experience with the care of rabbits, do you recommend **nail trimming as needed**? Is it practicable in the research lab to trim rabbit nails in order to prevent them from overgrowing? >

Yes, it is very important to clip their nails so they don't inflict injuries on themselves and scratch personnel during handling procedures; if you haven't got a good grip of your bunny, scratches from those hind nails can be painful when they haven't been trimmed!

All our rabbits are on monthly nail trimming and teeth checks. I think this is a very important part of caring for rabbits no matter what the setting. If you have properly trained staff and stagger groups of animals so they aren't all on the same schedule, it's not a daunting task.

Our rabbits are also on a monthly schedule for nail trimming, teeth checks, body weights, and brushing. Our husbandry staff is trained by the veterinarians to do this. They do a bunch each week, so that they are not overwhelmed with the entire colony at the end of the month.

Several years ago, our USDA inspector specifically asked to see our nail trimming records, which we keep in a notebook in the rabbit area. She noted that if we hadn't been doing this, we could have been cited for improper medical care.

I very much agree, nail trimming should be a part of the standard care of rabbits. For scheduling, it's not difficult to do a check at rack change.

Rabbits are weighed, given a quick look-over, and any nail-trims are done at that time. It's not overly problematic to schedule and if your rabbits are accustomed to the process, it can go relatively quickly.

We found an awesome way to trim rabbit nails! We do it on a regular basis and bought a baby bouncy seat for that purpose! The rabbits love the support. They will lie on their sides and allow nails to be trimmed; you can almost put them to sleep in the seats while gently petting them on their foreheads. One of our care staff came up with this idea and I was so excited to see for myself how well it worked!

< Rabbits can be quite **feisty** at times when you handle them during procedures. Do you take any precautions when dealing with such animals? >

I had to deal with the occasional attack-bunny; our most problematic one was a charger-scratcher-and-biter. We found the best way to remove her from the cage was to use a rat box and scoop her out. This was easy, if all we needed to do was give her a clean cage. But getting her out for antibody bleeds became a bit more challenging. On those days, we would, again, scoop her into a rat box, place the lid onto the box and move her down the hall into a quiet space. We would then—please note the use of *we* as it took more than one person—kind of pour her from the box into a towel or lab coat, quickly place the fabric over her face, firmly scruff and then move her front half into the crook of one person's arm who would very gently bounce her while keeping her feet on the counter top or floor. This would, after a while, calm her to the point where the other person was able to inject her with some ace (acepromazine) to calm her further for the purpose of bleeding.

So, long story short, I'm all for the use of a towel to calm a notorious *attack-rabbit.*

We've been doing lots of rabbit studies lately during which the rabbits need to be removed from their cages and handled a great deal. One of our challenges is that they cannot be scruffed as one would normally. At the start, we had several animals who were beyond feisty and would attempt to attack at the drop of a pin—I have the scars to prove it. In order to prevent further injury and hopefully reduce the animals' uneasiness, I started using a lab coat to remove them from their cages. I turn the coat around and put my arms through the sleeves in the opposite way in order to create a cover for my arms and a blanket-type cover under which I can scoop the rabbit up and which I can wrap around the animal, if need be. It works quite well.

I have now started to handle the rabbits upon arrival so that they get accustomed to being scooped up and subsequently manipulated, right from the beginning. I've found that taking the time to get the rabbits used to me and the handling procedure, prior to the actual testing, is much better for all involved. Petting them really helps with the handling process; I even had one buck recently who, when I opened the door to his bin, would butt my hand—much like a house cat—to ask for a gentle scratch.

By the time the study starts the rabbits are much more cooperative and we are able to do without the lab coat most of the time. However, we always keep a lab coat handy—just in case.

I use a blanket to retrieve aggressive rabbits from their cages. For the rabbits, the large blanket functions like a barrier, so most of the time—but not always—they don't try to charge me when they come out of their cage. The blanket protects my side once the rabbit is scooped in a football fashion. A few rabbits have given me a bite or two when I tried to carry them. In these instances the blanket was very useful; the rabbit got a mouthful of the blanket instead of me!

< What is the safest and for the subject least distressing method to **collect blood** from conscious rabbits? >

We place a catheter in the auricular artery whenever we have a study where multiple blood samples are required. As long as the restraining person is good and the individual placing the catheter is accurate, it is not really stressful for the rabbit. We *burrito* our rabbits in a lab coat. We then have the restrainer—usually me—hold the rabbit very securely and also cover the rabbit's eyes; this tends to have a relaxing effect. If the person placing the catheter is skillful, it takes only one prick and the rabbit usually does not even bat an eye.

We apply an almost identical method. We never use restraining boxes but always restrain our rabbits by wrapping them in a towel and having someone hold them firmly but carefully while talking to them and stroking them between the ears.

It is probably not the blood collection technique per se that determines the welfare implications for the rabbit but the technical and manual skills of the person who performs the procedure and, above all, the empathy of the other person who restrains the rabbit. A good rabbit handler is the most important, stress-buffering factor.

< What do you do when your **rabbits refuse to eat** with no other obvious clinical signs? >

At times, we have rabbits go off feed as well, even though they appear fine otherwise. We generally give them a product called Critical Care; the rabbits love it! It comes in powder form and you mix it with water. We generally offer it together with five or six hay cubes, first two times per day then one time per day. This regimen helps in pretty much all cases—including post-surgical animals—and the rabbits gradually start eating again properly.

We had this problem with our rabbits also and found out that giving them hay every day as a supplement not only keeps their bowels working but also entices them to eat properly. We used to only give hay as enrichment a couple times a week. Adding hay daily to the rabbits' regular diet solved the problem of inappetence.

At my previous job, we did not feed hay, so we had constant problems with rabbits not eating, and once a rabbit stopped eating it was mostly downhill from there. Where I am now working, we have very little incidence of rabbits going off feed and I think that's largely due to the hay. Even after our rabbits have surgery, the first thing they do, once awake, is start eating their hay.

Our rabbits are fed *ad lib* hay and pellets. We have had no loss of appetite problems since we started hay daily as routine food instead of occasionally, as part of the enrichment program.

I'm really happy to see that there are facilities that do provide their rabbits *ad lib* hay. That's what the animals deserve and what they need.

Discussion on Ferrets

< There is very little published information on the species-appropriate **housing of ferrets**. Can anybody share first-hand experience on this issue? >

Hammocks provide great environmental enrichment for ferrets! Our ferrets enjoy curling up in their hammocks but they also have a great time destroying them. I am curious if anyone out there has a good, ferret-safe hammock substitute.

We had the same problem and solved it in a very simple way. We had some old-style (shoebox) opaque mouse cages that were no longer in use. We drilled a hole in each end and hung them from the top of the cage using snap hooks. The ferrets cannot destroy them but love sleeping in them.

Yeah that's a great idea; thank you!

There have been six ferrets at our facility, who have since all been adopted out. It struck me that these animals had very short attention spans, so it was important to have a variety of **toys** and to rotate them frequently. You do not have to spend a lot of money to make ferrets happy. Empty bedding bags were a great hit! They also enjoyed rolling around small cat balls with bells in them, though they destroyed them rapidly and, hence, needed frequent replacements. They also enjoyed playing tug of war with a hanging rabbit-carrot toy. A large hanging bird bell fascinated them quite a bit. They seemed to be particularly attracted to the ringing, as they would run over to you, if you jingled the bell. One of their favorite toys was a green Gumabone. As soon as one of them would pick up the bone, the others would chase him and try to get it.

I am not sure you can do this any longer in today's facilities, but when I took care of ferrets in the late 80s, on Friday afternoons when the facility quieted down, we would take ferrets out of their cages and 'bowl' them down the hallway: carefully grasping them around the middle with a low and slow bowling swing, and releasing them a foot or so above the ground. They would stick out all four feet, land and slide along the epoxy floor for several feet and when they stopped moving they would return to us to go again; they really loved it. I called it **ferret bowling**. They had the best time!

I agree, they do love that! I had one as a pet many years ago and I used to call it "Bowling with Merlin!" He would run right back and wait to be picked up again for another go!

That's an awesome story! For those housing ferrets currently, is this something you could consider doing or do you think it would be frowned upon?

I don't see why it would be frowned upon; it is safe and the ferrets enjoy it! Just envisioning writing an SOP or approved enrichment strategy list that involves ferret bowling! *Ha ha!* I can only imagine the looks on the faces of the IACUC during the full committee review!

Does anyone know why the ferrets enjoy being thrown like a bowling ball? Does it mimic some of their behaviors? For example, would they be running, jumping and sliding during play? It might provide a good rationale to convince the IACUC!

They certainly display a play gait. My guy would do what I called the *sidewinder slinky* (which is hard to explain) and make a chuckling noise; really cute and in a way funny.

Ferrets love human contact!

Discussions on Dogs

< How are facilities **social-housing** their dogs? Specifically, how are you housing pairs, trios and larger groups? Have you found an ideal number of dogs to house together? Are you using bedding material and platforms? >

We keep most of our dogs in pairs or trios, but feed them individually to avoid food competition. They all have daily access to a spacious outdoor pen in compatible groups of five to ten dogs. All males are vasectomized. This allows us to house our dogs together regardless of gender, but we do take the precaution of temporarily separating our bitches when they are in heat. In each dog room, we have six or 12 individual pens that can be interconnected as needed. The floors of the pens are solid. We do not use any bedding. Each pen is provisioned with one platform.

The optimal number of dogs per housing unit depends on the breed, and most importantly, the dogs' temperament.

< I have the feeling that traditional cages are uncomfortable for a dog when he/she wants to rest. Dogs are likely to prefer to sleep in a partially closed-in area—against a wall or in a corner—giving them a sense of security. Does anyone of you offer some kind of **bed** for singly housed dogs? >

This is a great idea! I wanted to do something like that for a long time. I will never forget one experience that showed me that dogs want a bed to sleep in: We were switching some runs and it happened that we let the dogs stay a few days in the pig room that was vacant at the time. The pigs' empty food bowls were still there. When I walked into the dogs' temporary quarters the next day, I found almost all of them curled up inside these bowls; it was so cute! The dogs showed me very clearly that they appreciate this type of security.

I have in the past used Kuranda beds; they were a huge success! Often we would see the dogs sleeping on them; they were so comfy that our presence didn't really disturb the dogs. They chewed on the edges, though, and sometimes would dig through the beds. As a result, the beds got a bit worn out.

< Do caged dogs benefit from **elevated resting surfaces**? >

All our dogs have access to an elevated resting surface. We have mounted a little platform on one side of each cage. It can be flipped up against the wall, so that there is more room when we clean the cage. This simple system works well for us. The dogs seem to like their platforms, jump on them and have a good view of what is going on in the room or sleep on them. Access

to a raised platform makes them more approachable, friendly and playful. At times when their enclosure is hosed down and the animals can escape to a dry place.

Raised resting surfaces are liked by dogs. They provide some degree of security, increase the dog's ability to view outside the cage, and increase the overall area available to the dog. I have noticed that dogs, who have

Dogs want to see what's going on in their territory. They are less excitable and bark much less when they can access a raised structure from which they can see who is entering their room and what is going on in the room. For that reason alone, raised platforms or benches should be standard furniture in all primary enclosures of dogs.

I've worked at two facilities that had indoor runs for dog housing. One facility had a platform at the back of the cage, the other had no platform. While there was no obvious sign that the dogs without the platform felt they were lacking, I'd say raised platforms should be mandatory. The dogs who had platforms, all used them whether for sleeping, as a way to see more of the room, or to hide under when they were stressed or fearful. Had only a few of the dogs used their platforms I might say "make them optional," but seeing an entire room of dogs using them indicates to me that that the dogs have a need for a raised resting surface; so it should be a standard item of furniture of their living quarters.

The dogs I have worked with almost always used their raised platforms to sleep or rest on, hide under or jump up and down from them. We had random-source dogs of various breeds and I can't think of one dog who didn't make regular use of the platform. I firmly believe that dogs need an elevated resting area to feel at ease in their living quarters.

< How do you get a dog to **exercise** in the research lab setting? >

It is a legal requirement in the United States that dogs kept in research facilities are given the opportunity to exercise. There is, however, no consensus how this can/should be accomplished. To release a dog alone in a large but barren so-called exercise area would not be a sensible way of complying with the law. There is no reason to believe that a dog would actually run around alone and play with himself in such an empty, albeit large enclosure.

We allow our dogs to exercise and play alone or with other dogs in a designated area, with at least one technician being in the same room. The amount of time spent in the exercise and play area depends on the availability and willingness of staff to spend some time with the dogs.

I work in a high-paced CRO; we have a designated canine playroom that we refer to as ZenPen. Our dogs have ZenPen-time for 30 minutes, at least once a week. We can have 2-8 compatible dogs out for playtime. We move our dogs in a cart, the Beagle Bus. Currently our vet techs manage the exercise-play program for our dogs, but they are not solely dedicated to just this. Occasionally we have volunteers from other non-animal departments join us, which is fantastic.

At my former job we had 15 to 20 beagles. I would let all the girls out into the hallway while I cleaned their rooms in the course of the morning. Later I would do the same for the boys. The dogs absolutely loved coming out to run up and down the hallway, play with each other and check what I was doing. Often I would let them out again in the afternoon and sit and play with them. They sure were spoiled! Man, I loved that job!

We have an exercise program for our dogs, but I firmly believe it's as good as it is only because I have one of the world's best dog caretakers at my facility. She manages to run our dogs for at least 30-60 minutes per day. The dogs come out in compatible pairs/groups of the same gender inside the room. Once the pens are cleaned, games are played, or special run-time-only toys are used. If the rest of us have extra time during the day, we'll pull the pups out for play as well. Nothing is better than taking a tennis ball to a lab hound when you're having an otherwise lousy day!

My current facility has dogs from Class A vendors. For the most part these dogs don't do much running—unless you run with them. They don't pay much attention to other dogs, and rarely play with toys. They mostly enjoy either sitting next to people or being petted. Interestingly, the dogs we have adopted out settled into more typical dog behaviors in their new homes: sitting on furniture, running in the yard and barking at other dogs. They

never, or very rarely, displayed these activities while in the research facility. We've not been able to identify the source(s) of their discomfort that causes them not to express more expected canine behaviors. They just aren't rambunctious, and getting them to exercise isn't an easy task, when they seem much more interested in just sitting in your lap while you talk to them. Putting them on the floor while cage changing/cleaning, or leaving them in a room to play by themselves does not really help.

< If you are in charge of dogs, do you find the time to **interact with your animals** in a relaxed, playful way on a regular basis? >

Yes, Regularly interacting with our dogs is acknowledged as part of our enrichment program not only at the facility where I am working but at five other sites of our company.

We too have regular relaxed human interaction with our approximately 400 dogs incorporated in our enrichment program. The dogs are pair-housed; they are regularly released so that they can run up and down their rooms, but also sometimes in the long hallways when the rooms are sanitized. We have great animal care staff and depend on them to be the primary people interacting with the dogs. We also have a program where principal investigators and chemists can come over and play with the dogs of their projects in a designated playroom.

Our dogs get daily human attention in a play room. We teach them tricks for treats, groom them, play with them, or just sit with them quietly.

Each member of the staff is responsible for one or several dogs, and this includes walking each dog once a day for at least 30 minutes. I can’t overemphasize how important human contact is for these animals.

Our dogs were circling in their cage or jumping at the back wall of their cage over and over again. After much pleading, I was allowed to let the dogs run around and play on the room floor; they absolutely loved it! I was not surprised when I noticed a remarkable decrease of stereotypical activities after the dogs were given the opportunity to exercise and play.

We have also seen a remarkable decrease in stereotypies of our dogs who get regular play and exercise time on the floor of their room with other dogs. These animals tend to bark less, rest more, and do not display the pacing or circling in their homecages.

Regular relaxed interaction with the human caretaker(s) is so critical in obtaining quality research results that it should be an integral part of any dog enrichment program. Socializing with the dogs in their care has to be part of the technicians' job description so that they can really commit themselves to this important responsibility during their regular, paid work hours.

We have a dog play-room set up two days a week for the chemists who make the compounds. I have been surprised by the number of chemists signing up every week to come over to spend time with the dogs even though this is not part of their jobs. They say it's a stress relief for them to sit on the floor with the dogs, pet them, play with them and let them run around.

< Is anyone on our forum working with dogs who have learned to actively **cooperate during procedures**? Did the animals learn to cooperate through habituation or formal training? >

It is my experience that purpose-bred beagles are very compliant. They almost spontaneously cooperate with procedures, like blood draws; only minimal restraint is required. The dogs need no training other than a few seconds of petting before they cooperate without further ado. I can usually obtain blood samples alone. The dogs tend to object much more to the restraint than to the actual procedure—be it blood collection, rectal temperature, nail trimming or ear cleaning. Therefore, I think there is much more value in spending a few minutes with the dogs and making sure that they feel at ease rather than applying physical restraint that might not be necessary but is likely to distress the animals.

It is my experience that dogs very often **freeze during procedures** but that this is mistaken for a sign of their cooperation.

Not only dogs but many other species, including humans, *freeze* in terrifying situations. A creature who freezes may, indeed, give the

impression to cooperate when being handled or moved; but this kind of passive cooperation does not mean that the individual is not experiencing physiological stress and possibly extreme emotional distress.

Purpose-bred beagles seem to be extremely submissive. I'm guessing they are specifically bred for this trait; it certainly does make them quite compliant for procedures. I agree that they freeze and cooperate, but they are not actually desensitized to the experience. I think desensitizing would still be very valuable for their benefit. Desensitizing with positive reinforcement training (PRT) is useful for our convenience in getting the job done, but it is also very much about the dogs and their quality of life. I would love to see PRT implemented not only for non-human primates but also for dogs.

I have seen the freezing behavior only in beagles. There was one female who accidentally became pregnant at our facility. All the puppies showed this freezing behavior. One of the runts was rejected by mom immediately; I had to bottle feed the pup from day one. I brought the pup home and she has been a member of our household for over two years. The thing I noticed about the freezing behavior is that she only does it when she has to do something she does not want to do. It's like she is saying, *You're not my boss!* For example, when I want her to go to bed, she will just lie on her side and act paralyzed. If you tell her to move out of the way so you can get to the front door, she does the same thing. You could push her all around the room with your foot and she will just play dead. If you could see her, I think you would agree that it is not displaying submission but opposition, i.e., passive resistance. It really is quite funny. She probably thinks, if she looks all sad and pitiful you won't make her do what you want her to do.

< The barking of dogs can be a serious noise problem in research labs. Do you—and the dogs—simply put up with it or do you try to modify the environment so that the dogs have **less reasons for barking**? >

We house 40 to 60 dogs at a time in two rooms adjacent to each other. Whenever we enter a room, the dogs greet us with barking—of course—but they usually chill out and stop barking after a short while, except at feeding time! We require that everybody wears ear protection when working in dog rooms.

Our dogs are used to me, and get quiet very quickly once they have recognized that it's me who entered their room. The problem starts when someone else comes into the quiet room and sets everyone off. I have been working with the barking ringleader to remain calm when others enter the

room, but he is really stubborn; unfortunately, we have kind of trained him to bark by giving him attention whenever he gets all riled up.

The dogs at our facility bark much less if they are taken out and walked. We have a volunteer walking program. We also noticed a significant decrease in barking after we placed **elevated platforms** in all our indoor group-runs.

We have implemented elevated bed-boards in our facility. The first thing we noticed when we moved into this facility several years ago was the dramatic reduction in both the duration and the volume of barking. We attributed this to all the dogs of the room being able to see immediately and simultaneously the cause of the initial barking, i.e., personnel entering their room. In more traditional facilities, only those dogs closest to the door start barking when a person enters the room, and this then triggers a kind of chain-reaction from all the rest of the dogs who are unable to see the cause of the excitement and hence will continue barking until they have all seen the person who has entered their room.

At my previous facility, we combined **two basic rules**:

1. Only open the door when the room is quiet.
2. If the dogs bark, shut the door and wait—yes, looooooots of waiting in the beginning! Once you are able to enter and shut the door, and all the dogs sit and are quiet you go to each dog, click and hand-reward the behavior with a treat.

A number of great behavior interns conditioned our dogs to these rules. You were able to enter a room without a deafening din of barks and the whole kennel of hound mixes would greet you with wagging tails. What a difference!

When we had the opportunity to design our new kennels a few years ago, we put emphasis on creating a very **open visual field**, including windows in the animal-room doors and clear Lexan sliding panels in the kennels. The ability of the dogs to see the entire room and also what is going on in the access corridors literally stopped the barking.

We have found that the only way to get the barking settle down is to stay in the room and **not interact with the dogs** but go about the work that needs to be done. If the work involves some of the dogs then, for sure, take them out but ignore the others. In time, the barking will only last for the initial entrance into the room, and then you will be quietly but very attentively watched. Once quiet, say *Hi!* at your own risk.

I have used **Karen Pryor's clicker training technique** to reduce barking in the dog-shelter environment. It's like magic.

- If a dog is barking, approach the dog, wait until the barking stops even for split second, click, treat, and move on.
- If a dog is barking persistently, don't look at him or her; instead, click and treat the dog's quiet neighbor(s), just once, and move on.
- If a barking dog stops when he sees you coming, click and toss treat. Wait a few beats, watching the dog. Click and treat, again.
- Mark kennels of persistent barkers with a colored tag or ribbon; ask staff to click and treat any periods of quiet from those dogs.
- Click persistent barkers for any of the following behaviors: looking away, lying down, backing away from gate.
- Be patient. Habitual barkers may get worse, temporarily, before they give in and offer silence as the new way to get attention.
- In traditionally noisy moments, such as mealtime, click quiet dogs before feeding. Feed barkers last, and only after a click.
- Ask staff to click occasionally for quiet on an irregular basis; unpredictable clicks and treats will maintain behavior strongly.

We had an amazing caretaker who applied this clicker training technique and achieved a most remarkable difference in the dogs' barking habit!

< Are there any **commercial toys** that provide long-term enrichment for single- or group-housed dogs when no person is around to entice them to play with the toys? >

In my experience, it very much depends on the dog. Some dogs will readily play with the toys whether a human is involved or not while others aren't the least bit interested even if a human is there.

This has been my experience as well: the attractiveness of a toy depends greatly on the individual dog. However, the dogs in general make it overwhelmingly clear by the behavior they show when people enter their room that they much prefer the human contact and interaction to any other environmental enrichment. Often, this makes them pay attention to the toys even less, as they are far too busy trying to get attention from the human. I've secretly watched the same dogs, who seemed to have no interest at all in a toy, pick up the toy and play with it when they thought no humans were near or watching.

I find that dogs lose interest in toys that are not interactive, do not change their appearance and form and do not provide any social play reward.

Chase-n-catch the ball in the hallway is a game my dogs can't get enough of. They love it when I let the ball bounce back from the wall over and over again until I get tired, not the dogs!

Many of the dogs enjoy a tennis ball, but we don't leave it in the pen overnight since nothing would be left of the ball the next morning. This automatically enhances the attractiveness of the tennis ball: it's always new again the next day.

We have an elderly beagle who loves his blue hard plastic ball. He noses that around and flips it in the air all the while barking up a storm. It is really fun to watch. He does it on his own, no one is rolling the ball to him. Most of the other dogs show no interest in this type of ball, but this particular dog just loves it.

The majority of our dogs like the dumbbells. They show more wear than any other hard plastic or hard rubber toys they have in their kennels.

< Is it emotionally more challenging to work with **pound dogs** than with dogs who have been bred specifically for biomedical research? >

It is a lot more difficult for me to work with pound dogs, such as a Golden Retriever or a Labrador, than with the dogs who have been bred for research purposes. I know that the dogs from the pound were companion animals at some point. They exhibit many signs of a companion animal: knowing how to sit and give paw, wanting to play fetch with a toy, or just craving human attention. I can offer these pound animals, who have abruptly been turned into so-called research animals, some comfort by trying to re-create a home environment, as much as I can, while they are here. Because of my experience as a dog owner, it's easier for me to provide enrichment to ex-companion dogs than to purpose-bred dogs who are more aloof, although some do play.

I remember the time when we worked with dogs who were ex-pets. It was emotionally very disturbing, and all the techs and the majority of the researchers I worked with found this circumstance extremely difficult to tolerate, even though we knew that the owners had willingly sold their pets to our supplier. We were lucky in that the researchers used to turn a blind eye to our re-homing schemes and entered into the records that the animals, for whom we found a new home, had died from *natural* causes.

Since our facility has a fairly strong adoption program, I would rather that we use pound animals, as it gives these dogs a chance to be adopted into a good home. In addition, most pounds in the US hold animals for possible adoption only for about 5-7 days and then euthanize them. At my prior

facility, we actually removed dogs from the pound's euthanasia area just prior to them being killed—literally minutes before. In the two years that I worked there, we were able to return four dogs to their owners. While that's not a big number, you have no idea how good it felt to bring these animals back to their original homes!

If research laboratories could purchase pound animals, who are scheduled to be euthanized because no new home could be found for them in time, the pound would make enough money to allow for a longer stay of ex-companions animals, thereby increasing their chances to finally get adopted. I would rather see dogs used for research purposes than killed in pounds.

< Should researchers only be allowed to use **purpose-bred dogs** for their projects? >

In The Netherlands we are obliged by law to only use dogs who are purpose-bred; it is illegal to obtain dogs from shelters for research.

- Purpose-bred dogs can be prepared for research already at the certified breeders. Good breeders—and there are some—will train the puppies to undergo several common procedures without stress. You can even ask some breeders to train the puppies for specific procedures prior to sending them to your lab. These animals will already be acclimated to laboratory conditions when they arrive at the lab. For them, the laboratory environment will probably not be distressing.
- Dogs from shelters have an unknown history. They may have had diseases, were medically treated and had all kinds of experiences that can interact with your research; you may never find out. Having to take unknown variables into account in your experimental group, you are likely to need more animals than when working with purpose-bred dogs.
- Unlike purpose-bred dogs, dogs from shelters are not at all used to the laboratory environment and, having to go through experimental procedures, however mild, is probably extremely stressful for them.

I also feel that purpose-bred dogs, unlike dogs from shelters, are raised for and hence are familiar with laboratory-type living conditions. Dogs from shelters are not at all used to this, and they are probably extremely upset when brought into a research laboratory and subjected to procedures that evoke intense fear. Their greater variability, however, makes random source dogs probably <u>better</u> research subjects than purpose-bred dogs because they resemble much more an intrinsically variable human population.

Discussions on Cats

< Cats tend to be rather solitary animals, but seem to prefer companionship — with the option for privacy! — over being caged alone. Is permanent **social housing** a species-adequate option for cats in research labs?[0175] >

We house groups of female cats on a permanent basis; the animals do just fine. Newcomers get integrated without serious fighting. Our cats have access to airline crates, boxes, other hiding places and plenty of elevated resting surfaces. We give them several litter boxes that we exchange daily. In order to circumvent conflicts associated with food, and assure that each cat gets enough, we partition the daily food ratio of a group into more portions than there are cats and distribute them on different locations of the room.

For many years we have kept same-sex groups of up to 18-month-old cats without encountering serious aggression-related issues. Initially, we had more problems with the girls than the boys, but we were always successful in bringing order back into a group of females, by putting a castrated male into their group. We try to keep the groups as stable as possible and, especially, avoid removing cats whom we consider to be the main players in the group. Good care staff, who are encouraged to get to know all the cats in their charge very well and are given extra time to establish good relationships with them, is a major factor to assure that cats living in groups remain compatible over time.

< I am searching for ideas of **environmental enrichment**; it needs to be something that can be sanitized and is non-ingestible. The market for cat enrichment in the research laboratory setting is so limited; I'm just not sure where to look. >

We work with cats for vaccine research. All our cats are housed in groups, up to 15 animals per room. Besides scratching poles, elevated resting surfaces and typical cat toys, each group has access to a custom-made **running wheel**. The cats really enjoy it when they have figured out how the wheel works, and they run in it, especially when we entice them to do so with a laser pointer.

I love the cat wheel idea! Do they just figure out how to use it or is there some introduction involved?
Yes they just figure it out by themselves how the wheel works. They are adorable and often use it two at a time.

The room with our singly housed cats has a **window** that allows all the cats of the room to look out to the animal care hallway. They love to sit and watch the day's business go by. I'm sure an outdoor window would be even more popular but that isn't feasible.

Access to a window is one of the most attractive enrichment options for cats. They love to watch what's going on outside their room.

I recently brought in a **laser pointer** to play with our cats and soon discovered that I can prompt individual cats, and even pens full of kittens, to move wherever I want them to move, without catching them but simply by using the laser as a target. I bought our staff laser pointers, and we've found that it's an effective way to move cats for routine procedures. Not only that, but trying to catch the quickly moving laser prey is also entertaining for the cats. They get to exercise and play a fun game every day. I've only seen one male cat who is not interested in chasing the laser.

We hang the laser pointer outside the cat room next to the little window in the door, so that passing-by technicians can play with the cats by shining the laser into the room, and enticing the animals to chase the moving light dot. The technicians and the cats enjoy this game, which provides entertainment to both parties. Amazingly, no one walked off with the laser pointer.

Laser pointers work very well for cats living in relatively large quarters. To have them chase after the light dot provides species-adequate environmental enrichment for the cats and for the personnel. It's real fun! Unfortunately, the laser pointer gets useless when you are dealing with single-caged cats. There is just not enough space for chasing after a target.

Our cats make constant use of the **Fast-Trac toy**. Singly housed animals curl in the center to sleep; if startled, the ball moves, and the game begins. Group-housed kitties line up around the outside edge—with one sitting in the middle—and don't get tired trying to somehow get the ball out. Now, my cat at home has no interest in this toy at all. They are probably less bored than cats in laboratories.

We have a cat at our facility who made her own enrichment. She was kept alone in one of our cat rooms for about three weeks and finally decided to get out for a walk. After everybody leaves in the late afternoon she does jumping exercise at the door, pulls down the lever handle, allows the door to swing open, and out she goes, roaming the hallway all night until we come back to work in the morning. We have found her like this every weekend,

and now she has started to go for a stroll also during the week. **Pretty smart kitty**!

We finally decided to intervene and changed the door handle so that she can no longer open the door. As a compromise, we added two other kittens in the room so that our escaper is less bored.

I remember a similar story of mice, leaving their cages during the night, hoarding food from other mice and returning back home before personnel can witness the escapade.

I believe that the unobtrusive presence of the **attending caregiver** is the most effective enrichment that can be offered to single-caged cats.

Once they have overcome their distrust of humans, most cats like it when you interact with them in a friendly, cat-appropriate way. Under those circumstances, the attending care personnel can provide high-quality environmental enrichment, especially for single-caged individuals.

I would say that pretty much all of our cats actively solicit human contact. Our cats are handled in a friendly manner by the husbandry, clinical and research personnel several times a day. They are with us for several years, most of them have been here for about seven years; this allows us to establish affectionate and trusting relationships with them. As a result of this, most of our cats are more dog-like in that they initiate playful interactions with you; they would love to have you around all day long.

I strongly believe that the most effective enrichment for single-caged cats is contact with humans. Time is one thing that is in short demand in most facilities, but I do feel it can be worked around.

< Cats can be quite feisty when you have to do **hands-on work** with them. What is your trick to get the job done without being scratched or bitten and without unduly distressing the cat? >

I've worked with cats in specialty medicine for years. To work with a cat is more of an agreement between the two of you: You are working together, but it's primarily on the cat's terms. When you can get cats do something on their own terms, that's always better than trying to coax, or even force them to do it. I can't tell you how many times I've walked up on someone trying to grab a cat out of her cage for several minutes and then, finally got the idea of letting the cat do it on her terms: The person just held the carrier up to the opening of the cage and watched the cat walk into it without any ado.

Using the very least restraint possible is probably the basic condition to do hands-on work with cats without distressing them. Most cats have a

kind of free spirit, so they need to have the feeling of not being controlled in order to be/remain at ease with a given situation. Encircling them with your arms and body rather than holding them down usually works. A cat who feels forcefully restrained can turn into a fiercely self-defending creature who will show no inhibition to swiftly strike out at you even if you think you can trust her.

I had the luxury of being able to spend a good bit of time with cats. Typically what I'd do is go into the rooms in the afternoon and just sit on the floor and let the cats come to me. I'd do this every day and as they got more comfortable with me I'd start doing small things like picking them up, combing them, cleaning their ears, checking their teeth, etc. When it came time for me to restrain them for a procedure, it was just another day and nothing for any of us to be worried about.

A lot of stress can be avoided when you are well familiar with the cat's body language and cues so that you can see when she is frightened and when she is completely relaxed with you. If you find yourself having to apply more and more restraint, then it's time to take a step back and let the cat (and the human handler) calm down.

Cats tend to be particularly skittish when you want to take a blood sample.

The cat must be able to trust the person who is holding her; and the person must be able to read the cat's emotions properly and respond accordingly. Usually, I am able to hold the cat's head in a position that I can obtain a blood sample from the jugular without a second person restraining the cat. The cats do not show any signs of stress and the samples collected are great! If I do have an aggressive cat who needs to be restrained, I wrap the cat in a towel, almost like a kitty burrito. This method gives the cat a sense of security, which is likely to reduce stress, and I can take a blood sample from the jugular vein. I have come across a few cats who were so intractable that anesthesia was the only option to get a blood sample from them.

Discussions on Squirrel Monkeys

< What is your experience with **forming groups** of squirrel monkeys? >

We form and re-form new pairs and groups of more than 15 adult *saimiris* on a regular basis with no major problems: male-male, female-female and male-female groups. We always use a new cage for group formations and stand inside the cage during the first moments of group formation just in case there is trouble.

The most important thing is to use a new enclosure when grouping to avoid resident effects. Typically we group one or two individuals with a big stable group in a cage that is new for all animals. If one of the new animals is a male, we keep him in a cat transport cage inside the new enclosure of the group and release him after one or two days. This proved to be very effective in keeping new males quasi-under control and avoiding fights.

We established a trio of male squirrel monkeys at our institution. The animals lived in a generously spacious cage with numerous perches at different heights, platforms and hiding places. This group was a great success. We had one minor incident when the hierarchy seemed to change, but other than that these males were socially very compatible; they were always foraging, moving, and eating together as a coherent little group.

< Is anybody on the forum in a position to share experiences with **training** squirrel monkeys **to enter into a transfer box**? >

In my experience, adult squirrel monkeys are just as easy to train as macaques to move through connecting tunnels during cage change, but juvies tend to be devious. They'd run right to the edge of the tunnel, then sit there grooming themselves or picking at seeds, or sitting right in the middle of the tunnel rather than going all the way through to the new cage. Having a favorite piece of fruit that you place so that they have to go all the way through can help but if you're not quick, they can grab the fruit, turn around and run back into the tunnel to eat it. I've not worked with them in years, but I still laugh thinking about the little games they'd play to make me wait until they were finally ready and decided to walk into the new cage and stay there while relishing their food reward.

We have trained squirrel monkeys to jump cages and have started training them to enter a transfer box for cage change. Squirrel monkeys can be trained; it just takes a lot of patience because of their natural inquisitiveness and high energy levels. I really enjoy working with them but

man, can they be frustrating, just like trying to train a toddler! They know what is expected of them but nothing will make them move any faster.

I volunteered at a local zoo and worked with the squirrel monkeys. We used meal worms as rewards for shifting into their indoor enclosures or for entering into transfer boxes. They loved their rewards so much that they probably would have gone anywhere for them; they grossed me out completely! I think with squirrel monks, as with all other primate species, finding the right food motivation is the key for success!

Discussions on Marmosets

< Can you **train marmosets to cooperate during procedures**? >

Years ago I trained our marmosets to enter a transport box. They were mixed age groups (mom and 1-2 daughters). With daily 5 to 10-minute-sessions, in less than two weeks we were able train them to reliably enter a transport box. We baited the box with mini-marshmallows which are a real crack for marms. It was easier to get the whole little group enter the box than individual animals.

We have created a scale that has a T-Bar on it with a small cup on the top that contains a food reward. The marmosets voluntarily approach and sit on the T-Bar and take the reward while we record their weight. The only problem we encounter is that dominant animals tends to monopolize the scale, so we have to place a couple of T-Bars in the enclosure so that others can get on a free scale.

I have trained marmosets to accept oral doses from a blunt tip-cannula on a syringe; the PI didn't want to add stress via gavage. Splenda—a zero calorie sweetener available in various flavors—and sugar-free maple or sugar-free raspberry syrup worked well as flavor mask for the test compounds. The trick was to first find each individual animal's flavor preference. We patiently did many taste tests and finally knew which flavors to use for each of our marms.

The administration of the test dose was not at all a stressful procedure for the animals; they freely came to the cage front to get their test-drug dose.

< Does anyone have experience with **phased lighting** in marmoset rooms? It seems to me that the abrupt change of the lights going on and off might be stressful for the monkeys. >

We use a 12-hour light-dark cycle, with the animal room lights gradually coming on and then going back off in a series of steps, to simulate dusk and dawn periods.

When there has been a need for me to stay in the animal rooms until evening, I have noticed that when the first room lights switched off, the animals became subdued and quiet; by the time the second set of lights switched off, the animals had begun to retreat to their nest-boxes, so that by the time total darkness had descended, the animals had sufficient time to *close-up shop* and be settled into their nest-boxes for the night.

If the lights would go off all at the same time, many of these animals would spend the entire twelve hours of darkness on the floor or on the perches of their enclosures, not in their nest-box. I would strongly recommend simulated dusk and dawn periods as an integral part of the light cycle for marmosets, as a simple means to foster their welfare in the laboratory setting.

Discussions on Macaques

< How do you go about **pairing previously single-caged adult macaques** to address the animals' need for companionship? >

I have had great success with pairing **cynos**. For some reason, adult males have been much easier to pair than females. Cynos don't always group well, but they make pretty good pairs!

I usually start with a clear, transparent panel between the two intended partners. From this I can usually gage how the socialization will go:

- Attacking the panel = bad.
- Lip smacking or showing curiosity = good.

Most of the time, I know within the first 30 minutes whether things will work out when I eventually give the two individuals full access to each other.

We have a large colony of adult female cynos here and have had wonderful success with pairing new partners while they are sedated.

Using a novel housing room is probably key for success; we remove the sedated animals from their familiar living quarters and bring them together as a pair to a housing room that is unfamiliar to both of them and where they are strangers to the other animals in the room. This pair formation method has been 100% successful for our colony since we started using it a few years back, with the majority of the pairs remaining compatible for six months or longer.

Pairing previously single-caged macaques has always been one of my favorite, and most challenging thing to do! I do have to say though, as a former doubter, the sedated pairing here has done wonders for our socialization program success; we rarely have a single-housed animal for any extended period of time—and we have a large colony!

<What's your trick to keep pairs long-term compatible? How are the homecages designed? Are companions allowed to stay together 24h/day? >

I am not really sure of what the trick is, but I feel that living together in unfamiliar surroundings helps a lot that new partners bond quickly as a compatible pair. We typically keep them together 24/7 unless we notice that one partner is losing weight as a result of food competition; this happens only rarely. We house the pairs in one-over-one double-tiered cages with the center floor removed.

We had two adult male cynos who were housed side-by-side for about a week. No behavioral signs of incompatibility were reported during that

time. On the day of pairing, the two sat at the divider beside each other and calmly took food treats that we offered them simultaneously. There was no social tension that we could notice, so we decided to pair them. Within five seconds of removing the divider the two males had inflicted substantial gashes on each other and had to be quickly separated.

The case you are describing suggests that territorial competition triggered the instantaneous aggression between the two males. When I started pairing **rhesus macaques**, I also simply removed the transparent cage dividing panel that had allowed two animals to get establish their rank relationship without risk of injuring each other. After a few tests it became obvious that being introduced to each other in their own homecages can trigger territorial antagonism, with one or both partners fiercely defending her or his home-cage territory. After I learned my lesson, I made it a rule to transfer new potential pairs to another room and introduced partners to each other in a double cage that is new for both of them. Once compatibility of new pairs was evident, I moved them back to their original, now interconnected homecages.

When establishing new pairs, it is important to first allow two animals to establish a rank relationship, without risk of injury, in a double cage where they are separated by a transparent or steel mesh partition. It is my experience that most animals settle their relationships within a few hours; if I don’t see clear-cut signs of this within a week, I do not pair these animals but test them with other partners. Animals with a clear-cut rank relationship are then paired in a different double cage. Since they already have established a dominance-subordinance relationship, they really don't have any good reason to fight over dominance again.

I have tested in this manner 97 same-sex dyads of adult rhesus macaques. Shortly after introduction, fighting took place in only 2 cases. Of the 97 pairs tested, 92 turned out to be compatible. Over a follow-up period of 12 months female pairs remained compatible in 88 % of 77 cases, male pairs remained compatible in 80 % of 20 cases.

The attached photo shows the most senior pair: Sissa is 27 years old and Senila (right animal) is 36 years old. Both of these two female rhesus macaques have lived alone in single cages since they were removed as nursing infants from their moms (artificial premature weaning). The pair-formation procedure was easy-going; Sissa accepted Senila as her dominant partner without any ado. When they were introduced to each other as a new pair, it took only a few minutes before Sissa started grooming Senilia who's response left no doubt that she was happy having finally found a cage companion.

When considering the circumstances under which the animals are forced to live together, day-in-day-out with no real private space, their degree of long-term partner compatibility of about 80 % is truly amazing. How would our relationship with a loved person develop if we had to live under conditions similar to those of pair-housed macaques in research labs?! Human primates who chose to marry each other and live in an environment that allows for some private space become incompatible in approximately 50 % of cases. Humans could learn from monkeys!

< Is pair housing of **SIV-infected macaques** an acceptable option? >

Whenever possible, we keep non-human primates who are experimentally infected with SIV in pairs. The cagemates are assigned to the same experimental groups and receive the same infection and treatment regimen, thus minimizing the effect of cross-infection. The paired partners

are also manipulated for treatment and other experimental needs on the same schedule to avoid temporary separation.

Multiple experiments involving SIV infections have shown no adverse effects of pair housing.

That's fantastic news! These findings could sway the opinion of researchers who are still wary of addressing the social needs of SIV-positive animals despite published benefits of pair housing on the animals' behavioral and clinical health.

< Is it safe to house **macaques with head caps** as pairs? >

I worked in a colony with cranially implanted rhesus, and the major concern was removal of sutures by the partner before wound healing. Our compromise was that the animals were separated from their cage mates for a couple of days post-op and were then re-paired.

The concern of suture removal is probably not based on actual observations but on an assumption. As attending vet, I worked with well over 100 pair-housed, cranial-implanted female rhesus macaques; I do not remember a single case of suture removal after surgery by the cage companion. The surgery was always done in the morning; the operated animal was typically returned to her companion in the pair's homecage by late afternoon. Rather than jeopardizing proper healing of the implantation site, the familiar companion is likely to buffer post-surgical stress, thereby supporting the recovery process.

It has often been argued that monkeys with cranial implants should not be caged with another companion because of perceived risks of damage of the implant and local infections.

We are working with cranially implanted adult male rhesus macaques; most of these animals live with a compatible partner, some live alone. I have observed many times how the animals groom their partner's implantation site. They are very focused while grooming and pick up any tiny crust material. In fact, they do keep the implantation site very clean. Anecdotally it seems to me that we end up having more margin infections in singly housed animals than in pairs.

Almost all of our pair-housed macaques (cynos, rhesus and bonnets) have cranial implants of one style or another; so far we haven't had any trouble with any of them damaging each other's posts, wells, etc.

For at least ten years rhesus macaques with cranial implants have been pair-housed at our facility. We have never had an incident involving an

implant mishap or local infection that resulted from social interactions between cagemates.

We have paired rhesus males with cranial implants for several years. The monkeys tend to show no interest in their mates' implants and we have encountered no implant complications due to housing the animals in pairs.

I have worked with a large number of pair-housed juvenile and adult female rhesus macaques assigned to neurophysiological studies requiring cranial implants. In the course of more than nine years, no report was made of partners damaging each other's implants or partners causing local infections when meticulously grooming each other's implantation margins. Yes, it is true, the principal investigator initially showed very strong resistance to the idea of having her head-cap implanted animals housed in pairs. She finally became an ardent advocate of pair housing after having noticed for herself that her animals—especially the very young ones—were much more robust and able to withstand the challenge of the experiments when they had a companion.

Our university tries to pair all rhesus macaques regardless of cranial implants. Normally the pairs are established before they have undergone surgery for head caps, but we have successfully paired primates after surgery as well. Over a period of ten years, we have had no incidents of damage to the implants. We have more problems, with coils of head caps breaking, in single-housed than in pair-housed rhesus. The head caps of pair-housed animals are cleaner—as they groom each other—than those of individually caged animals.

We have ten pair-housed male rhesus and long-tailed macaques with head caps. The animals were 3 to 6 years old at the time of pair formation. They are presently approximately 10 years old. Some of them had head caps before they were paired, others got them afterwards. It didn't seem to make any difference. In my experience, pair housing does not create a risk factor when the animals have head-cap implants. In all the time I've been working with these monkeys, they've never damaged one another's head caps.

I have worked with more than 100 pair-housed rhesus macaques with cranial implants and encountered no clinical problems related to the fact that these animals shared a cage with another companion. I established the pairs always prior to surgery, but this was probably not a necessary safety precaution.

We mainly have monkeys with head caps and there was definitely resistance a few years back to pair house for fear the cage mate would somehow cause damage to the head cap. We have never had an issue with head-cap damage due to a cage mate tampering with it. For the most part, the pair engage in natural behaviors rather than paying any attention to the head cap.

The reason for wanting to keep the primates singly housed with head-caps was not justified, so we did not oblige the request and had the backing of the animal care committee. With time, the researchers realized it as well. We don’t really get anyone requesting this anymore—it took some time to achieve this!

< Do you notice a **species difference** in the readiness with which adult macaques can be matched up as compatible cage companions? >

I have found over the years that cynos can be paired much more easily than rhesus. They seem to be more tolerant, less suspicious when meeting a stranger. Sometimes I form pairs without any preliminary familiarization. I can do this with cynos from time to time without serious consequences, but I would not dare to do it with rhesus.

< What tells you that two animals have established a **clear-cut rank relationship** during the familiarization period? >

Cynos seem to have this *thing* where it just takes them a long time to really find out who is dominant and who is subordinate. It can go back and forth, and back and forth for quite some time. I'd rather they had it thoroughly worked out before putting them together. This is what I have learned over the years:

- If both partners are lip smacking and acting friendly all the time, THAT is a dealbreaker and I wouldn't even attempt the pairing.
- If both are acting dominant and are threatening each other, that is also a dealbreaker.
- If one or the other reacts strongly to room dynamics and exhibits a lot of re-directed aggressive behaviors, again that is a deal breaker.
- If it *appears* the two may do well together, we will put a food dish down in front of each monkey, real close together in front of their caging, so that each partner—while still separated by the mesh panel—can see the other approaching and eating the food. What happens at the dinner table is critically important and can really give you some good additional clues as to what could most likely happen when you pair. The clues are sometimes very brief and could easily be missed, so you REALLY have to watch every second during this event; don't blink. You want to see that one partner goes directly to the dish without any hesitation and starts chowing down. The other monkey should NOT be so quick, but should cautiously glance toward the monkey eating, and sort of ask for permission to approach his or her own dish; I call this *being polite*, and this clearly distinguishes the subordinate from the dominant partner. Then when he or she starts eating we watch every gesture of the first monkey, making sure he or she is a *giving-permission* type, that means a dominant animal who accepts the presence of the subordinate companion.

There are always exceptions to the rule, but it is my experience that the above criteria are helpful when you want to end up with a happy, i.e., compatible pair.

Rhesus partners are pretty straightforward when they establish a dominance-subordinance relatioship with each other. When the following behaviors are consistently shown by the same partner, I assume that a pair has established a clear-cut rank relationship, with the animal showing these behaviors being subordinate and the other animal being dominant:

- fear-grinning,
- withdrawing,
- looking/turning away when being looked at by the other partner,
- yielding when the other partner comes very close, and
- threatening against the observer or other monkeys in the room and looking back over the shoulder to the partner.

The rank relationship is ambivalent when both partners show these behaviors or when they both display threatening and aggressive gestures

toward each other. In this case I will not attempt to pair them but test them with other potential partners.

I am looking for the same behaviors. In most cases the animals show clearly who is submissive and who is dominant. If they don't, I offer them food treats right next to the transparent partition. The dominant animal will take the treats directly, stay in front of the cage and beg for more, while the subordinate partner will hesitate, timidly watch the neighbor while taking the food, or even not dare to touch the food at all but retreat to the back corner of the cage. I have found this treat competition test the easiest way to check if two potential partners have sorted out their social rank relationship. You may have to test the pair several times, but you will then have the assurance that the risk of injurious rank-determining aggression is minimal at the moment of pairing.

It is my experience that potential cage companions often focus their attention on me rather than display gestures that could show me if they have established a rank relationship. I found it very helpful to set up a **remote-controlled video camera** in such cases to get a better picture of the animals' undisturbed behavior. Usually, I find out very quickly what the status of their rank relationship is.

< Is **sedating potential cage mates** a good option for the establishment of isosexual macaque pairs? >

There are no publications on establishing new pairs of macaques with the help of sedation; this strongly suggests that some people tried it but the result was so disastrous that they did not publish the findings. Based on my own experience it would never cross my mind to sedate potential partners and have them gradually come to senses in one and the same cage. Yes, perhaps juveniles, but not adults who would first have to establish a dominance-subordinance relationship in order to share a cage with each other in peace. Imagine two adult males, both groggy but regaining more and more consciousness, getting entangled in a dominance-determining interaction!

At my last facility, care personnel tried re-introducing paired rhesus macaques who were separated for a longer period of time, after first sedating the partners; it was assumed that, since the partners had already been paired, they would have no problem waking up together. Wrong thinking! Monkeys wake up at different rates after being sedated; we learned the hard way that lower ranking monkeys will take advantage if they wake up faster than their higher-ranking counter parts. We had two serious wounding incidents from

using this method. After we had learned our lesson, we decided to no longer sedate adult monkeys in order to socialize or re-socialize them.

By sedating potential cagemates, you are just delaying the inevitable. Two macaques have to figure out their respective rank relationship. This is their top priority when they meet each other for the first time. I would prefer to see their interactions while they are awake and have their wits about them. How does a groggy monkey accurately display signals of subordinance or dominance? If they cannot communicate clearly with each other, there is little chance that they will establish a clear-cut rank relationship, but they may start fighting with each other even when they are not yet fully awake. At least with fully awake animals, you can watch for clear warning signs that a brawl is brewing and take action to prevent injury.

< How do you know that two animals are **reliably compatible** after you have paired them without incidence of overt aggression? >

I check right from the beginning that the new pair clearly confirms its rank relationship; there must be no ambiguity about who is dominant and who is subordinate. I find that unidirectional yielding and unidirectional grinning are good signs for that. Threatening and mounting are by no means reliable indicators of a new pair's compatibility! The same is true, to a lesser extent, for grooming. Huddling with each other is a good indicator that the two companions are compatible.

When I offer food treats to a new pair, and both partners retrieve a treat, I know for sure that the two are reliably compatible. The attached

photos show the two males Moon (left) and Peter (right) sharing a slice of bread; Peter, the dominant one of the two helps himself first while subordinate Moon waits in the back for his turn and then takes his share within being hindered by Peter to do so.

< We have several same-sex pairs of adult cynos and rhesus who will be assigned to a project requiring repeated 48-hour separations, during which one partner will be tested in another room. The question is: Will it be safe to re-unite the animals after the testing, and will the pairs remain compatible when the **partners are repeatedly separated and re-united**? >

Your animals will be separated only for relatively short periods, so I really don't think you have anything to worry about re-uniting them. I had no trouble re-pairing several adult male cyno pairs who were separated for weeks. The only animals I had consistent difficulties re-pairing were adult rhesus macaques of both sexes. When you simply put them together, the two compatible companions may not recognize each other quickly enough at the moment of re-pairing, but treat each other as strangers and start fighting. The consequences of this misunderstanding can be very traumatic. I finally discovered that you can avoid this risk by inserting a transparent or grated mesh cage divider, and then introduce the one who had been away into the empty half of the homecage. Let the two find out who they are, and then simply remove the divider. I have used this trick many times without failure.

< Do you have to keep male pairs in **male-only rooms** to avoid aggression possibly resulting from sexual competition? >

In my experience, overt aggression among compatible cyno males is not provoked when they can see receptive females. Our **cyno** males live in isosexual groups who are frequently exposed to the sight and scent of mature females. When this happens, the males may perhaps get frustrated, but they show no abnormal behaviors, no injurious fighting, no conspicuous hair loss, nothing really alarming that would render it necessary to keep the sexes separated.

It is also my experience with paired **rhesus** males that you can keep them in a room where mature females are housed, but there is a risk involved. Most male pairs will do just fine, but some will not. Those, who cannot cope with the challenge of facing females who are not accessible to them, can get so excited that they vent their frustration onto the other partner. The consequences can be devastating; nobody around, and two adult male macaques getting into a fight that is unlikely to end because the cause of the fight is still present! A very, very bad situation. I learned it the hard way and became strict in making it a <u>must</u> that all male pairs are housed in such a way that they cannot see mature females; a curtain between the cages of females and males will do the trick if there is not enough building space for male-only rooms.

Male rhesus pairs with females in the same room can trigger serious animosities between compatible cage partners. I have seen females who would actually taunt the males.

I have worked with quite a number of male **stump-tailed macaque** pairs who had visual contact with receptive adult females; the males' long-term compatibility was not noticeably affected by the females' presence.

< The PI who does research with our pair-housed rhesus insists that cage companions be separated during the night and on weekends, so that they cannot fight and injure each other while nobody is around. I would love to **keep partners together also during the night**, but cannot argue with the PI because I really don't know if that would jeopardize the safety of the animals. >

In our facility, compatible companions are allowed to remain together also during the night, on weekends and holidays. This applies for both female and male pairs, as well as for all animals who have head-cap implants. It never has happened that we found paired animals injured or bruised when entering their room in the early morning. I think there is no special risk when pairs spend the night together without being supervised.

We also keep our male and female rhesus pairs together 24/7 and encounter no problems related to aggression during the night.

At our facility, after pairs have been established, they are housed together uninterruptedly. This includes male and female isosexual pairs, and each species housed here, including rhesus, pigtails, sooty mangabeys, squirrel monkeys, chimps, and cynos. We have not noticed that paired companions fight during the night, on weekends and holidays when nobody is around.

Based on my own experience with a large number of pair-housed rhesus macaques, I do not recommend partner separation during the night; it's not necessary. When two compatible companions are separated during the night, you run the risks that someone forgets to re-unite them in the morning, or re-unites the wrong animals; and on top of that, it's a lot of work for the staff.

< Does anyone have experience with **pairing vasectomized males with females**? >

Vasectomizing male **cynos** to pair with females has been a game changer for our program since I don't have enough primates to find compatible same-sex partners for everyone. We've had a 100% success rate with vasectomized-male/female cyno pairs at introduction, with no preventative measures needed to keep the peace. Pairing vasectomized males

with females is a thousand times less stressful than pair housing adult, intact males. It's a nice change of pace to have a pairing situation that doesn't involve much, if any, aggression—which helps me sleep better at night!

The vasectomy procedure our vet staff uses is minimally invasive and they usually do it during quarantine; so once the males clear quarantine they are ready to be paired with a female.

We vasectomized four **rhesus** males after they failed to get along with other males as compatible cage companions. When we then paired them females, three of the males did fine with their girlfriends, but the remaining one didn't work out. He was aggressive with no matter who we tried to pair him with no matter male of female. He was a grumpy guy who, so it seemed, just wanted to be alone.

Successful pair housing of six vasectomized male rhesus with females has been reported by Weed et al. (2003).

< Is it necessary to also pre-familiarize potential companions when working with **young animals** who have not reached the age of puberty? >

With juvenile cynos, I usually don't take the trouble of pre-familiarizing them, but simply put them together. I have never had a pair that was incompatible.

I also skip the familiarization procedure with rhesus who are three years old or younger. These young animals spontaneously get along with each other, probably because dominance-subordination relationships are not yet firmly established. When they are over three years, they typically show dominance status ambitions—especially young males— which makes it very advisable to allow them to establish their rank relationships during a familiarization period before introducing them as a pair. I am always inclined to reduce the risk for the animals to an absolute minimum, even if it means that I have to invest a bit of extra time.

< Is there a **sex difference of pair compatibility** when you keep rhesus macaques in same-sex pairs? >

It is my experience with about 50 male pairs and several hundred female pairs that male pairs are equally compatible—and equally affectionate—as female pairs.

Single-caged males are generally as readily transferred to compatible pair housing as females are. It's true, if two animals get into a fight, the consequence is usually more serious in males than in females, because of the long canines, but this does not mean that males are more aggressive or more intolerant of companions. They simply have more dangerous social

weapons. There is, however, one age group of rhesus males that causes me quite a headache. Many—not all—rhesus males turn into real rowdies shortly after they reach puberty; they can remain rather fierce and intolerant animals until the age of 6-7 years. When I have to deal with such monsters, I first try to find surplus infants as cage companions for them. It always amazes me how gentle and caring these big guys behave with little kids.

This has also been my experience: even the most querulous adult rhesus male becomes friendly—even gentle—when you pair him with a little kid. Usually such pairs develop amazingly affectionate relationships that can last beyond the time when the kid has become sexually mature. When no surplus infants are available for rowdy males, I keep them alone. As time goes by, they become more mellow and ready to be tested with another potential cage companion.

< When monkeys are housed in pairs, is **competition over food** and perhaps even monopolization of food by the dominant partner a problem? If so, how do you deal with it? >

The regular food, for most **cyno pairs** does not trigger competition, unless you have an animal who loves the primate biscuits. We had one adult male cyno who loved ALL food! So no matter what it was, he had to be separated from his buddy. The rest of the time they got along great.

Even in the most bonded pairs, there always seems to be a little competition when it comes to the good stuff like raisins or fruit. In some cyno pairs that don't handle treat feeding well, I have been using the cooperative feeding techniques. This works well, but the training can take a bit of time. When you are in charge of a large number of monkeys, cooperative feeding isn't as convenient as temporarily separating paired partners so they can enjoy their food enrichment treats in peace.

We haven't had any issue among our cynos with their regular food; paired partners do share pretty well. I'm actually impressed because this was not so with the rhesus I used to work with. I ended up separating most rhesus pairs for feeding once they reached the age of about 6 years.

My experience is the same; paired cynos don't compete over their daily biscuit ration, while **rhesus pairs** very often get into a conflict over access to their food boxes.

I notice food competition in about ten percent of our pair-housed rhesus monkeys. I tried cooperative feeding for a while. It works if I come in early enough to feed the monkeys myself. Due to time constraints, however, I typically separate the problem monkeys with a solid panel. In many pairs

the submissive monkey either would not eat at all even when separated or would be attacked after being re-united with the dominant cage mate.

You don't really need to separate the partners. When I started pairing rhesus and stump-tailed macaques in double cages, I noticed very quickly that some animals had difficulties getting access to one of the feeders, because the dominant partner tried to monopolize the food. In some pairs, the subordinate animal got so intimidated that he or she no longer made attempts to get food while the dominant partner was eating.

In order to get the issue of food competition fixed, I had **privacy panels** installed in all the double cages of pair-housed animals. The panels are regular cage dividers in which a comfortable passage hole is cut in the back (not in the front!) of the cage. With the privacy panel in place the two paired animals can access either one of the two feeders and eat without seeing each other.

Once we got the privacy panels implemented, there was no longer any report of food competition between cage mates. It was a pretty simple and inexpensive refinement project for a large (700+ animals) macaque colony.

The privacy panels not only worked to avoid food aggression but it also gave paired partners the option of sometimes getting away from each other. I tested 30 paired female rhesus macaques in a control situation when companions had not privacy, and in an experimental situation when they were offered the option to move behind a privacy panel and be alone for a while. It turned out that paired partners spent significantly more time in close proximity (same half of double cage) when the privacy panel was provided. At the same time, they spent significantly more time engaged in

affiliative interactions while the incident of agonistic interaction had the tendency to decrease.

Basile et al. (2007) concluded from a study of 25 pairs of rhesus macaques both with and without the presence of a privacy panel "that the increase in proximity associated with the presence of the privacy dividers reflects an increase in social tolerance and/or attraction. A privacy divider may provide a safe haven and give monkeys the ability to diffuse hostile situations before they escalate."

I completely agree that the provision of optional visual seclusion is a very helpful tool in keeping the peace between cage companions! We also include a variety of visual seclusion options for our pair-housed macaques and have found it extremely useful in easing tensions. We have sliding floor-panels that allow top/bottom separation and sliding panels that provide visual break horizontally.

< There is scientific evidence that the presence of a compatible conspecific can buffer stress reactions in rhesus macaques. Do you make use of this **social-stress buffer** effect with the animals in your charge? >

It is my experience with rhesus macaques that it is advisable to pair-house an animal after surgery as soon as possible with his or her compatible companion. We do this especially with pairs, after one of them had head-cap implant surgery. It is the investigator's and my own impression that the animals **recover from surgery** quicker when their familiar companion is with them than when they are alone.

We re-unite rhesus macaques with their cage companions after cranial-implant surgery as soon as they are fully awake, alert and fully mobile. I don’t remember that this practice ever caused any clinical complications, but it is evident that these animals recover much better than in the past when all animals were strictly single-caged, so they had no companion while recovering from surgery.

Close to 95 percent of our cyno population is pair-housed. The animals are subjected to a lot of orthopedic procedures. There have never been problems with the re-pairing of the animals after surgery. We partition the pair’s cage with a transparent panel, which we remove after the treated companion has fully recovered from anesthetic effects (usually 24 hours). It has never happened that animals who had no surgery showed any negative behavioral reactions toward their temporarily probably weaker cage mates. In a small study we compared post-op recovery of paired companions when the two partners had surgery resulting in a full length cast on one of the legs. We found that there was less cast picking, faster recovery, and quicker

return to full range of motion after the cast has come off when the animals were re-paired with their partners after surgery, than when they were kept alone after surgery.

When I worked at a primate research facility, my primary motivation for implementing pair-housing was prompted by individual rhesus monkeys who were assigned to research protocols requiring chair-restraint. These animals were tested alone in sound-proof chambers. Their behavior made it very clear that they experienced anxiety and fear, not so much because they were restrained, but because they were alone—apart from the sporadic presence of the investigator or animal care personnel, who unknowingly frightened rather than comforted the monkey. These animals needed the **psychological support** of a companion.

It took me a whole year to coax the PI into pair-housing all 40+ monkeys assigned to her particular research project. What difference it made! Whenever an animal was chair-restrained, the compatible companion was now brought along in a mobile cage, allowing both partners to keep uninterrupted visual and acoustical contact with each other. This calmed the restrained monkey, who no longer exhibited behavioral signs of distress, such as open-mouth threat, teeth grinding, restlessness, depression and refusal of food treats.

If circumstances do not allow conspecific companionship, the attending care personnel with whom the restrained animal has a trust-based relationship can act as an effective stress-buffering substitute. When my monkeys are chaired during an experiment, I stay most of the time with them, talking to them reassuringly. I have the feeling that my presence has a strong calming effect on them, and this actually is the reason why I do it with consistency with all my monkeys.

It has been my experience that chronic diarrhea in single-caged rhesus macaques often stops when these animals are paired with compatible cage companions.

Alexander et al. (2003) transferred 80 single-caged rhesus macaques to social group arrangements. This change in housing condition reduced the yearly incidence of diarrhea from 20 percent to less than 2 percent. This suggests that companionship **boosts the immune system** thereby increasing an animal’s resistance to pathogens.

< If pair-housed macaques in your care have to be separated, what do you do to **minimize separation stress** for the animals? >

When companions have to be separated in their homecage, we allow them to keep visual, olfactory and limited physical contact (e.g., perforated panels). I've worked with several pairs who have remained separated in this arrangement for up to two months and longer. Re-uniting them after completion of the project was always uneventful, probably because the partners had been able to maintain uninterrupted contact for the duration of the required separation.

Wire-mesh separators allow our pair-housed macaques to keep visual, olfactory and limited physical contact while one or both of them are assigned to studies that require physical separation of the partners.

Grated cage dividers are used at our facility; they make it possible for paired companions to stay in their homecages and keep uninterrupted contact with each other while they are physically separated during certain tests.

When we have to separate paired monks for feces/urine collection or for food consumption measurement over a period of one week, the partners are always allowed to stay in their familiar homecages and keep visual contact with each other through a wire-mesh cage partition. It is our experience that partners do not engage in aggressive interactions but do get along well with each other once we remove the partition after termination of the study.

We did have a pair of girls who did not properly adjust to being separated by the mesh partition while the study was going on. One of them would not eat well in this situation; it made me sad and showed me very clearly that being physically separated from the familiar cage companion and not being able to groom each other during the day, and huddle together during the night can be really hard for macaques.

< We have a female juvenile rhesus macaque who could be paired with an adult female who has been paired before, but her cage mate was separated for research-related reason. I was wondering if anyone has experience putting **adult-infant pairs** together, and what advice there is on the best introduction method. The adult female is pretty quiet and shy, and doesn't show aggressive behavior. >

I have established many adult-infant rhesus macaque pairs, both adult female-infant and adult male-infant pairs without encountering problems. The pairs were formed by simply introducing the infant into the adult’s cage.

As long as the infants are not older than 18 months, they have typical baby features that inhibit overt aggression in psychologically healthy adults.

We do this type of introduction at our facility on a regular basis with good success, better than like-age pair formations, in fact. We always start with a short period of protected contact, just out of prudence.

< Occasionally, single-caged rhesus macaques are **pair-housed with macaques of a different species**. If you have dealt with such pairs, were the rhesus partners dominant or subordinate in cases in which both partners were of the same age group? >

Since we have a limited pool of potential pairing partners at our small university, we have done this a few times with rhesus and cynos (both male and female pairs). In every case, the rhesus assumed the dominant role.

There is one elderly female pair of a rhesus and a stump-tailed macaque at our facility. From what we can tell, the rhesus is dominant. They've been a compatible pair for several years now!

We have a 14-year-old cyno female who successfully paired with a 5-year-old male rhesus. This pairing turned out so well! The female is a strong, confident bully and has been hard to pair because of her unreasonable aggression toward other macaques; she weighs 22 pounds and is rather

large-boned for a cyno. This young rhesus explained to her that he was the king but that he would allow her to do his laundry. She asked him how he wanted his socks folded (grin). The two have become a great pair.

< How do you **form a new group of cynos**? >

Working with adult **males**, we first establish several compatible pairs, then a group of four and add pairs to this nucleus until there are 8-10 males per group. Each group is kept in a separate room and the appearance, appetite and body weight of each individual is monitored daily to make sure that the animals are compatible. This system works fine at our institution and we encounter no serious aggression-related problems with it.

Clarke et al. (1995) arranged the single cages of three adult males in such a way that the animals could have close visual contact with each other. After the animals were well familiarized in this manner, they were released into a new homecage. The trio established dominance-subordinance relationships mainly via submissive behaviors; no injurious fighting occurred. Relationships were primarily affiliative and the group lived peacefully together for more than two years.

We keep our cyno males in small groups of up to six animals in male-only rooms without contact with females. These groups typically remain compatible for many, many years. We had groups who remained compatible for more than eight years. Problems may occasionally occur when individual group members are removed for research-related reasons and are no longer accepted by their group when they return.

That some of your cyno male groups do remain compatible for eight years and longer is remarkable—unthinkable with rhesus males!

Yes, we are also proud that we can keep the males together in compatible groups for so many years. Visitors are often surprised when they see our mature cynos sharing the same enclosure harmoniously. Our attending care personnel can take credit for this!

I would like to caution on grouping adult **females**. In my experience, they have been some of the most difficult, violent animals I have ever dealt with. Initially, new group members may get along just fine, but then it is like a switch is flipped and the fighting begins. I would rather socialize adult males with intact canines than adult females. We have had so many problems with the females that we stopped socializing them in groups altogether.

In the laboratory adult female cynos often don’t get along with each other in a group setting, but they readily match up as compatible pairs. I have had a lot of success pairing them; so if grouping them doesn't work out, all hope is not lost to socialize them. I once grouped 14 adult female cynos. This resulted in disaster after about a month and the group had to be

disbanded. However, I was able to pair all 14 animals; each of the seven pairs were compatible and they remained compatible for a long time.

< What do you do with weaned macaque infants; do you raise them in a **kindergarten**? >

Yes, we form kindergartens with weaned rhesus and with weaned cyno infants. They live in spacious pens together with an adult animal who takes care of them. As soon as we begin to see sexual swellings and/or any overt aggression, we split the groups into same-sex cohorts or into new breeding groups.

There is one kindergarten group at our facility. Before we got the idea of making use of aunts or uncles, we saw a lot of fighting between the kids. These prematurely weaned infants were extremely disturbed; idiopathic diarrhea was a constant problem. In their despair, some kids were clinging to one another pretty much all the time, even trying to walk while not letting loose from one another.

The kids started clinging to one another much less often once we added an adult animal to their group. The trick was finding good aunts or uncles for the kids. We ended up with a few who we use every year at weaning time. These adults are very caring with the kids who were just weaned and missed the comforting presence of their moms. The aunts/uncles allow them to cling to them; one very tolerant male cyno even lets the kids steal food from his cheek pouch!

The kindergartens were a great way to socially house some of our older rhesus males who were retired from research but lived alone. Each of them adjusted very well to having a whole army of kids groom them—imagine one big male rhesus being groomed by five or six weanlings!

I have fond memories of a rhesus named Grandpa, a former alpha breeder who, sadly, lost his sight in his advanced age and was no longer able to breed in a harem setting. Because everyone loved him, and he had had several youngsters in his troop, our lab manager took it upon herself to see if Grandpa could be used for the weanlings. As it turned out, he was fabulous with them! After the height of breeding season, he would receive up to ten weanlings at a time and did a marvelous job with them. Not only would he allow them to be kids—jump on him, grab his ears and play with his tail—but he would also break up all kinds of disagreements, teach the little ones not to fight over food, and to wait their turn when the treats came around. It amazed us that, even without his sight, he was so wonderful with them. Every so often we would find everyone tucked away in a snuggle patch for

an afternoon snooze; and once the little guys started to grow, we would always find them practicing their grooming skills on Grandpa.

< When is **individual housing** called for? >

It sometimes happens that individual animals do not get along with each other. It wouldn't be right to force a persistently incompatible animal to live in a social-housing situation. It has been my experience that some males seem to get along with no other adult male when they are 4.5-6.5 years old; they are real **rowdies** and don't hesitate to provoke senior males … only to be beaten up. I think these guys need to be kept alone until they reach full maturity, or they can be paired with a naturally weaned surplus infant. Pair-housing otherwise incompatible young males with such infants is a good alternative to individual housing. Young males who are real rowdies with other males turn into a gentle, caring fellows when they get a naturally weaned infant as a cage companion. It's amazing to witness the abrupt shift in the demeanor of such belligerent males.

Primates assigned to **food-intake studies** are often removed from their social partners and kept alone in single-cages. This is not necessary.

The daily food ration is usually distributed in the morning and mid afternoon, and the cages are cleaned with water in the late afternoon; on this occasion all food leftovers of the day are removed. Since the animals have no food during the night phase, there is no good reason why compatible companions cannot be joined together at this time and allowed to stay together during the night. They can be separated prior to the morning food distribution with a grated/transparent panel, allowing them to keep social contact with each other during the hours when their food intake is monitored. In the evening, the panel is again removed, etc. This system helps to minimize, or perhaps even eliminate altogether, the extraneous variable stress resulting from social deprivation.

Yes, there is no good reason why paired animals cannot be put together after the last cage cleaning of the day and then separated again in the morning as you suggest. The trickier part of this schedule is to get the husbandry folks cooperate during the weekends and take the extra time to separate and re-unite the animals.

In our facility, social housing is the default, and a study director needs a very strong argument for single housing—that goes not only for monkeys but also for dogs, mice and rats. I assign all monkeys as compatible pairs to studies. We **plan necropsy schedules** around keeping pairs together throughout the process so that a cage mate is not left behind.

It took a long time to change study directors' minds to be amenable to this approach, but it does work well; it preserves established pairs, avoids separation distress and makes it unnecessary to spend time re-pairing animals who have lost their companions with new partners.

< I am working with several investigators who claim, that in order to get macaques to listen, they first have to teach them to be submissive—for example, by intimidating them through shouting. Only then, so they claim, would the animals be ready to learn certain tasks during experiments. The idea sounds quite barbaric to me. What's the best humane way of **training macaques to cooperate during procedures**? >

I have experienced with single-caged female and male rhesus macaques, who have been squeeze-back restrained in their home for routine blood collection, or have been transferred to a squeeze apparatus for this procedure, that some animals learn over time to cooperate. These animals will come to the front of the cage when you gently talk to them while

partially opening the cage door, and present for saphenous or femoral blood collection without the need for being physically restrained. This kind of **spontaneous cooperation** happens only under the condition that the handling person has a mutual trust relationship with the animal, is patient and kind and always gives the animal and food-treat reward at the end of the procedure.

It is my experience that macaques who are being gently pushed forward with a squeeze-back for routine injection sometimes start

cooperating without any formal training, making the squeeze-back unnecessary in the future. I think such animals learn from the repeated experience that being squeezed is unpleasant, but that the injection itself is not really painful, and that they can avoid the squeeze-back by voluntarily coming to the front of the cage and present a specific body part for injection.

Daily injections (e.g., diabetic animals receiving insulin) lend themselves to gently habituating the subjects to the procedure; if the animals trust you, successful habituation can be achieved with only a few preliminary sessions.

I have encountered quite a number of such animals, especially, but not exclusively, animals assigned to diabetes studies.

I work with 12 rhesus who behaved just as you describe: being approached by me, the monkey comes forward without me even touching the bars of the squeeze-back; I encouragingly say *come* and then *hold* and give the injection without triggering any fear or aversive reaction. These animals have not received any formal training other than receiving a food treat reward after the injection. They have learned on their own to avoid being squeezed by voluntarily coming to the front of the cage and accepting the injection.

The response of an animal during a common procedure, such as blood collection or injection is predetermined by his or her relationship with the handling person. If the relationship is based on fear, enforced restraint or a formal training program will be necessary. If the relationship is based on mutual trust, the disturbing element of fear is absent, and there is a good chance that the animal gradually learns through positive experience to cooperate rather than resist during the procedure; restraint then becomes unnecessary.

I am in the process of forming iso-sexual pairs from a group of nine adult male cynos. Thus far, I have established two pairs. One male was bitten by his partner and has extensive damage on his left hand. I am now waiting to get vet clearance so that I can try pairing him with someone else. In the meantime I am monitoring the male's hand very carefully.

The other day we were sitting close to each other and I was talking quietly to him while displaying my left hand in a way he would have to do with his injured hand so I could examine it. To my amazement, he copied me! He raised his left hand and I was able to inspect the wound. I had never asked him to do that before; he simply moved his left hand in the same

manner I had moved my left hand. I then tried with simple gestures to communicate that I would like him now to present the left arm through the feeder-box opening of the front panel of the cage. He seemed to immediately understand what I meant, and put his arm through the opening. I gently held his hand and looked it over and then rewarded him by grooming his wrist and lower arm and offering a treat.

Yesterday, I visited these nine males. When I approached the male with the injured hand, he started putting his arm into the feeder box so that I could check the wound! It was an amazing experience!

I cannot think of an example from the laboratory at the moment, but I remember several incidents of spontaneous cooperation during my time working as a humane society officer, in particular dealing with wildlife. A common call to attend to was skunks getting themselves tangled in hockey nets—as I live in Canada, a frequent encounter for skunks. Skunks are fairly gentle creatures, but of course have their secret weapon, and can still spray even in compromised positions. Yet, on the calls that I attended, after approaching trapped skunks slowly and gently restraining them, they were quiet as could be while they were cut free from the net. Then, once free, they would waddle off. Though shock is obviously a factor when dealing with wildlife, I have memories of skunks who truly seemed to connect with me during that moment, and surrendered themselves to the task at hand, allowing me to free them quickly; these were always rewarding calls to attend to.

I have managed to create some time for **target training** the monks in my charge. I began carrying the target around the facility in every monkey room during routine rounds for about one week. This second week I began bringing the target very close to each cage while asking the monks to touch it. I had 22 animals yesterday who figured it out! It's great to watch them as they pick up on the game, hear a click as soon as they touch and then promptly receive a piece of veggie or fruit.

Today the number increased to 39; some of the animals are outstanding and already touch the target every single time I ask for it. It's amazing to see them figuring out what you are asking of them, and once the food rewards start coming, they really catch on! Some of the more timid monks are watching their cage mates play with me and before long are observing intensely how their companions are getting all these lovely grapes, pieces of apple, pieces of cucumber and other goodies. It's a lot of fun!

I find myself spending at least two hours every morning playing this game with all of my animals. By doing so I am building a close bond of trust with them which, ultimately, is the foundation of any successful training program.

All it takes is to always carry a clicker and some treats with me. Once the monks have made the association between the click and the treat-reward, I can click whenever I see a behavior or posture that I want to reinforce for a specific training goal, for example unintentionally presenting a thigh ... for injection.

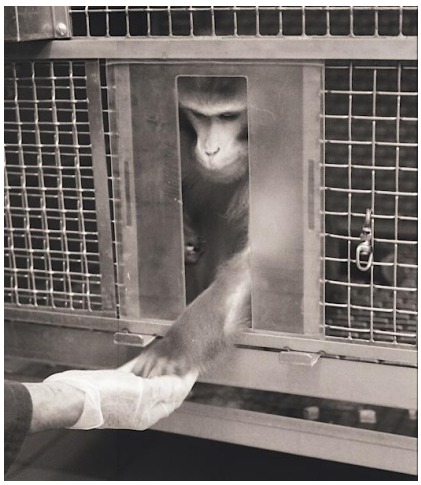
< Understandably, macaques don't trust humans, especially those humans who imprison them in cages and subject them to life-threatening procedures. How do you **prepare a macaque for training** with the goal that the animal cooperates with you during procedures. >

To prepare macaques for training does not require a lot of time but it offers valuable enrichment both for the animals and for yourself and it fosters a **mutual trust relationship** between you and the animals. Once an animal is no longer afraid of you, has been prepared to kind of work with the target and to associate the click with a favored treat, the training itself becomes less time-consuming and much more easy and fun. It's my experience that such preparatory training steps can be integrated into the daily animal-checking routine without undue extra time investment.

Rhesus macaques can learn many things once the bond of trust is there. The click and subsequent treat during routine rounds and clinical observations is a great trick; it's quick and easy and you will literally see the animals change before your eyes. I now have monks who exhibit all types of behaviors, including presenting the hind quarters for treat rewards. Some present for being petted; I could stay there all day grooming various body parts. They (and I) enjoy it so much!

Based on these routine informal interactions with my monks, I have trained many of them for pole-and-collar and subsequent chairing, blood collection, walking on the scale for weighing and entering and exiting a play cage.

The key for any successful training is mutual trust. You can shape various behaviors later on, once you have established that trust relationship with the monks; trust is so important! Trust also makes it safer for personnel handling the animals. A monkey who trusts you has no reason to be afraid and try to scratch or bite you in self-defense.

The two attached photos demonstrate in-homecage blood collection of an adult male rhesus macaque who has been trained to actively cooperate rather than resist during the procedure. His cooperative behavior is rewarded with vocal praise and a food treat.

It's important to emphasize that there has to be a mutual trust relationship to guaranetee that the interaction is safe for person and for the animal. Not only must the animal have learned through interactions with you that you are trust worthy, but you also must have learned through interactions with the animal that you don't have to be afraid of the animal. Just a trace of fear will be picked up by the animal and make her or him hesitant to trust you fully. If this happens the interactions between you and the animal are no longer predictable; the animal my get afraid, retreat and show self-defensive aggressive actions.

When you intend to make use of the squeeze-back during a training procedure, for example injection in the homecage, you will **desensitize the animal to the squeeze** back before you start the actual training. You will allow the animal to gradually learn through experience that being gently coaxed with the squeeze-back and subsequently rewarded (either with a treat or by you gently touching/grooming the animal) is not at all a fear- or anxiety-inducing exercise. It is my experience with rhesus macaques and stump-tailed macaques that you can desensitize an animal to the squeeze back in less than five brief sessions. Gently desensitizing a monkey to the squeeze-back will strengthen the animal's trust in you and shorten the cumulative time needed to achieve the final goal of the training. A well-acclimated monkey will come to the front of the cage without being pushed by the squeeze-back, when you approach his/her cage and ask him or her to come forward. The subsequent training for injection or venipuncture is no

big deal because the animal trusts you and is willing to work with rather than against you.

I have trained many rhesus and stump-tailed macaques to cooperate during various procedures and applied with strict consistency positive reinforcement. You as trainer or handler need to be dominant for your own safety. How do you get dominant? Not with a stick, not with shouting, not with impatient reactions, and not with any kind of punishment, but instead with patience and **gentle firmness**. It's a subtle process that I cannot translate into words, but it allows the animal to trust you. That trust is your safeguard against aggression and, I believe, gives the animal more space to comprehend the training tasks.

The animals I am working with should feel at ease when I am present; only then will I have a chance that they will understand what I want them to do. Yelling at them or threatening them with a broom, when they do not respond correctly to my training cues, would not only scare them and make them confused, but they would also lose trust in me and resist my attempts to train them any further.

I very much agree with you: Any kind of intimidation—be it shouting, showing a broomstick or even the net—is bound to have the opposite effect. The animal will feel scared and his or her trust in you will diminish or go down the drain altogether. An intimidated fearful monkey will not listen to you because he or she no longer feels at ease and safe in your presence. Your negative energy essentially blocks the animal's capacity to learn. A losing battle!

< Do you find it more effective to schedule **short or long training sessions**? >

Working with rhesus macaques, I definitively find frequent but short training sessions most effective. The more training you can get in, the faster the trainees learn, but you can't do it all at once, otherwise the animals get frustrated or bored.

I have found with rhesus and stump-tailed macaques that frequent short training sessions—two or three approximately 5-minute sessions per day—are most effective.

It is my experience with male rhesus that the animals work best with me when I keep the training sessions short; the males are more attentive and learn better during two 5-minute or shorter sessions per day than during one 10-minute session per day.

< If you have direct experience with the training of adult macaques, would you say that there is a **sex difference** with males being more difficult to train than females? >

A resounding *Oh heck no!* Male rhesus macaques are much more willing to be trained than females, who easily become determined not to cooperate.

No! In fact, I always found the males easier to train than the females as they tend to be less shy and will start taking treat-rewards sooner than the girls.

I agree, adult male rhesus are more at ease than adult females when you train them to cooperate with you during procedures; this implies that they learn faster to work with you.

I also think it is easier to train male cynos than female cynos for the same reason. I have many girls on a current study whom I can't get to come and take a treat. The boys are very different and all of them don't hesitate taking treats from my hand.

Adult males always gave me fewer problems than adult females, provided I had a very good relationship with them. My males have always been more willing to work with me and learn how to cooperate during various procedures.

Having trained numerous adult rhesus macaques of both sexes, I would definitely not say that males are more difficult to work with than females. I did get the impression that males learn the training steps more quickly than females. When I worked with males, I typically experienced that the trainee was really motivated to work with me, so the training progressed relatively smoothly and swiftly. Females had the tendency of being more hesitant, not so self-confident during the training; this often required numerous repetitions of training steps and hence more cumulative training time before the goal of the training was reached.

< How to minimize stress reactions to **intramuscular injection**, especially for sedation? It is my experience that the animals often show avoidance and fear reactions to this procedure, suggesting that their normal physiological status is altered even <u>before</u> the actual test or experiment is performed. Are there practical solutions to this problem? >

Based on my experience with macaques, I believe that the injection per se is no big deal for the animals; however, what they do fear is the proximity of humans who typically treat/handle them without much patience let alone compassion. Once you have helped them to overcome that fear and trust you,

injection becomes a little prick that has no alarming consequence apart from the raisin reward.

I have successfully trained two of my singly housed adult **rhesus** males to cooperate during intramuscular injection. As a first step, they learned to present their thighs to the front of the cage and then being touched with the target, consisting of a small plastic rod. Next, I started gently poking the thigh with the target, then switched to a syringe without needle, followed by a syringe capped with a large blunt needle, and then with a normal 25 gauge needle which I finally inserted into the muscle. I praised the animals at the successful completion of each training session.

Both males have learned to cooperate and neither of them reacts in any negative manner to this procedure. I should perhaps emphasize that the two get their injections in their homecages <u>without</u> being squeezed. They are in control of the situation, but they do cooperate very well. There is no doubt in my mind that the injection procedure is not a stressful event for them.

It's my experience that adult male rhesus react quite well to frequent (once a week) injections if I tell them what I am doing. I show them the needle and I tell them, *I need to give you a small shot*. I always talk in a calm soothing voice when working with them, and it is not uncommon that they spontaneously present for me, so that I can easily do the procedure. Since the animals show no signs of fear and resistance, injection is unlikely to be a stressful experience for them. After the injection, the animal is praised with *good boy!* or similar phrases such as *you are such a good monkey!* I believe, the animals deserve to be approached and handled with respect and trust. They definitely respond better to people they know and trust. Typically, they respond with fear and/or aggression to investigators and to the veterinarian. This implies that I am usually requested to first sedate the animals before the investigator or veterinarian handles them.

I have helped train many of our **cynos** to sit still for sedative injections at the front of their cages. For most animals we will pull the squeeze mechanism up half way. Applying clicker training, we first teach them to *sit* wherever they choose on the floor or on their perch. Some animals need the squeeze-back pulled up at about three-quarters, but they still have room to move away if they want.

I have the impression that activating the squeeze mechanism acts as a signal for the animals that *it's time to work*. They respond promptly and come to the front section of the cage without actually being touched by the squeeze-back. Once they have chosen their location to sit, we say *arm* or *leg*,

touch that body part, give the injection and reward the subject with a treat. Since they are going to be sedated, their treat consists of a piece of a Popsicle or cool pop. That way, even if the ice sits in their cheek pouch, it will be melted before they lose consciousness. We have a colony of only 52 cynos; of them 45 sit calmly for us every time we have to inject them.

With positive reinforcement, I have also trained adult female cynos to cooperate during intramuscular injection in homecages that are not equipped with squeeze-backs. When they can trust you, they readily learn to cooperate during this common procedure. These animals work with rather than against me, which automatically implies that they show no fear or stress reactions during the procedure.

< I intend to train rhesus and cynos to present for blood collection. So far, I have gained the trust of several animals, but I don't know how far I can trust them in return? It's a **safety issue**. I respect them very much—more than I respect some human primates—but the animals are under stress and, therefore, may turn on me for no apparent reason. >

It's true, if the animals are under stress while you are working with them, there is a great risk that they show aggressive reactions to you, in an attempt to get away from the stressful situation. One of the conditions of successful and safe positive reinforcement training is a stress-free work environment, both for the animal and for you. This means, neither the animal nor you should be under the emotional influence of fear, apprehension or frustration. These emotions are dangerous when your handle monkeys or, for that matter, any other animals.

You should reach a stage when you know that you can trust the trainee while you work with him or her. This does not mean that you should not be alert and perfectly present, but any traces of mistrust and fear puts you into a seriously dangerous position. Do not work with an animal, unless you have trust in him or her! You have to be very focused and you need to get the animal's uninterrupted undivided attention.

For your additional safety, you will always make sure that your interaction with the trainee will not be disturbed or disrupted by any unexpected event, such as personnel entering the room or loud personnel passing in hallways.

< Is it safe to train macaques to cooperate during **blood collection in their homecages**? >

I've been met with opposition about training the monks for blood draws in the cage for safety reasons; instead, our monks are sedated in the

restraint chair to facilitate blood collection. I would love to get them to cooperate in their homecage rather than sedating them so often.

The researchers I am working with will also not allow blood collection while the animals are awake. They said it is because the macaques are too dangerous.

I know from my own experience that many investigators, like yours believe in the myth of the “aggressive monkey” and deal with their research subject accordingly. You can read in professional handbooks and articles:

- "All monkeys are dangerous. ... Monkeys should not be handled in the fully-conscious state."
- "Primates can injure personnel severely if adequate restraint is not used. The risk of herpes virus B infection and other zoonoses transmitted by bite or scratch is minimized by appropriate restraint which may be physical or chemical or a combination of the two."
- "Adult male rhesus monkeys are aggressive animals and very difficult to handle. Hence experimental manipulations necessarily involve the use of restraint procedures, either chemical or physical, which may influence endocrine functions.”
- "One of the major drawbacks to the use of nonhuman primates is that they can be difficult and even dangerous to handle. Restraint is therefore necessary and desirable to protect both the investigator and the animal."
- "Despite rigorous observance of all precautions, bites and scratches are frequent" because the animals have not been trained to cooperate during handling procedures; they defend themselves whenever they can by scratching or biting the person who handles them with force.

The strongly upheld myth of the “aggressive monkey” is hindering efforts to train macaques to cooperate rather than resist during research and husbandry-related procedures. Macaques are not aggressive animals but they may exhibit self-defensive scratching or biting in a life-threatening situation such as being forcefully restrained by a human.

What I have learned over the years is that the risk of being scratched or bitten by a monkey can practically be eliminated when you have first allowed the animal to establish a relationship with you that is based on trust, mutual trust. The animal knows through direct experience that you do not intend to harm him or her in any way, and you know through direct experience that there is no reason to be afraid of the animal. In order to establish such a safe relationship that is free of any traces of fear, some extra

time is required in which you offer the animal your undivided attention and affection during encounters that are pleasant for the animal and enjoyable for you.

You don't really have to make a study to demonstrate that animals, just like humans, are less stressed by a potentially distressing procedure, such as injection or venipuncture, when the procedure is done at home. When people argue that training monkeys for blood draws in the cage is dangerous, they cannot have much first-hand experience with monkeys. When you train a monkey, you are creating a predictable, safe environment for the animal; so there is no need for aggression. Many people ignore the fact that personal safety is markedly increased when the handling person works with an animal who has no reason to defend himself or herself.

< How long does it take to train a macaque to present a leg for a blood draw? >

Before the start of the first training session I always spend an appropriate amount of time with the trainee to gain his or her trust and to feel safe in his or her presence. Once there is no trace of fear left in my relationship with the animal, the subsequent training becomes an easy-going interaction that we both enjoy. Training non-human primates to cooperate during blood collection provides valuable mental enrichment for both, the trainee and the trainer.

To achieve active cooperation during blood collection in the homecage, I invested on the average 40 minutes with 15 adult male rhesus macaques, and 34 minutes with 6 adult female stump-tailed macaques. After entering the animal room, it took 1 to 2 minutes to draw a blood sample from a successfully trained subject. Once trained, the animals showed no behavioral signs of stress or distress prior to and during blood collection; they also failed to show a significant cortisol response to this common procedure (Reinhardt et al., 1991; Reinhardt and Cowley, 1992).

I have worked with both single-housed and pair-housed rhesus and stump-tailed macaques and got the impression that the pair-housed animals learn faster, perhaps because of the reassurance provided by the companion’s presence.

Juveniles—unlike adults—have difficulties to overcome their fear of being handled. Yes, you can also train them to cooperate during blood collection, but the time investment is considerably higher than with adults; that’s my experience.

< How do you **train macaques to enter into a transfer box** in standard cages without squeeze-backs, or in pens? >

I am working at a biomedical research facility; this issue brings up a lot of frustration for me here. Almost all of our macaques are single-housed, and for the most part they are not trained for any cooperative behaviors. I do not know why the research staff does not take the time to train the animals before they work with them. It breaks my heart to see monkeys squeezed up and threatened with brooms in order to make them enter transfer boxes, because no time is allocated for training the animals to cooperate rather than resist during common husbandry procedures, let alone research-related procedures.

There should be no need to threaten the monkeys with brooms! The animals will have a very difficult time trusting anyone when the research staff treats them in this manner; monkeys are very smart but also sensitive. Only when humans have earned their trust will they be willing to work with them, rather than being filled with fear and apprehension when they see a person approaching them. I understand why your heart is broken, mine is, just reading your observations.

With the use of a banana or other fruit placed into the clean cage, our rhesus macaques learn quickly to exit their dirty cage and transfer via a tunnel into the clean cage. This works very well and most of the time the squeeze-back is not needed, except for a few exceptional animals. With some of the shy young guys, it requires about three cage changes—which are done every other week—to also get them to jump reliably.

We put a few Prima Treats or peanuts into the weigh box, which is temporarily attached to the cage front, to entice our young, inexperienced rhesus macaques into leaving their cage and entering the box. Most of our adults have learned the trick and enter the box spontaneously, knowing that they will receive their reward upon re-entering their homecage after their weight has been taken. I find adults are very nice to work with; they tend to learn faster than the youngsters.

I am working with rhesus macaques. What I like to do first is make the transfer box a part of the trainee's homecage that can be thoroughly explored by the animal. For that purpose I connect the open transfer box to the open homecage, lock it securely to the cage and place favorite treats—like frozen grapes!—in it. I will leave this arrangement for a few days so that the animal can find out that the transfer box is not scary after all.

Once the animal gives the impression of not being afraid of the transfer box, I lure her or him into the box with encouraging words, gently

close the door of the box, and let the animal settle down and eat the treats while I praise her or him. I will go through this little exercise several times, adding brief commands like *In!* to prompt the animal to enter the box, and *Hold!* to get the animal stationed in the box so that I can close the entrance without any ado. After a minute or so, I open the door of the box and let the animal return into the homecage where she or he gets another treat reward for good cooperation. It has been my experience that rhesus macaques can be trained easily to enter into transfer boxes.

When I have trained several animals, I ask the attending care staff to come with me in the animals' rooms so that I can demonstrate to them the steps of the capture procedure—including the treat-reinforcement of cooperation—and familiarize them with the vocal prompts. Everyone needs to be on the same page so that the animals can rely on what they have learned during the initial training. It is important to emphasize, never to use the squeeze-back in order to force an animal to exit into the transfer box; patience is a key condition to gain the animals' trust and willingness to do what is expected of them.

I have applied the same training technique you have described with very good results. For the macaque colony I worked with, all trained animals would cooperate during the capture procedure with any of the NHP staff—techs, investigators and vet. These animals were very food motivated, so their favored food treats were great incentives to do what was expected of them. I would like to add though that for some individuals we would move the squeeze-back forward in the cage just a few inches; this seemed to give them the signal that it was time to move, almost like our alarm clocks go off and we get out of bed.

For getting our cynos to enter transfer boxes, I used a lift-stand to hold the box. Those animals who promptly moved into the box and let me close the door were rewarded with an apple slice. For those who were hesitant to enter, I just moved a bit away and waited for them to enter the box. For some, I had to wait 10 to 15 minutes but usually it just took a minute or two until they decided to move. If these animals stayed in the box and let me close the door, they too got their fruit as soon as they returned in their homecage. For those animals who didn't stay long enough in the box but jumped back into their cage before the door could be closed, I started the session again by moving a few steps away and letting them re-enter and stay in the box until I had closed the door. Usually this exercise had to be repeated about five times before they would stay in the transfer box long

enough for me to close the door. I would then release them back into their homecage and give them their reward.

The transfer box training was rehearsed every other day for one or two weeks, depending on the individual monk's performance.

For the next step, I rolled the animals in the transfer box on a cart into their observation room or into their work chair where they were given, again, an apple slice. I waited with them for about 20 minutes; during that time, nothing was done with the animals; they could simply relax. After that, I boxed them up again and brought them back to their homecage where sunflower seeds were waiting for them as a reward for cooperating with me. We repeated this five times before the animals were signed off to begin their tests.

I didn't have a single monk who resisted the box transfer after the first test run. I definitely worked alone to avoid that the animals got frightened by too many humans in their room. The monkeys were used to daily procedures being done by a single person. Anything out of the norm is likely to make the animals suspicious, perhaps even alarmed.

The key for success, especially with older macaques, is to give the animals sufficient time to recognize that the transfer box is not a trap. So you can't be in a hurry to get them to move into the tunnel or jump into the box. If you're stressed, chances are the animals are also going to be stressed and both parties will have a hard time with the training. It's always a good idea to not even start a training session under such conditions.

With vocal commands, patience and fruit rewards I trained two rhesus breeding troops—the animals had names which allowed me to call them individually—to first shift into a holding area of their home enclosure, then exit one animal at a time into a transfer box which I lifted on a mobile scale, recorded the animal's weight and released the animal back into the home enclosure where an apple or a banana was waiting as reward for cooperating with me. Mean cumulative training-time investment per troop member was 18 minutes.

No extra person was required to do the routine weighing of the troops or to catch an individual animal for medical treatment. The procedure was not distressing for the animals; they cooperated without being stressed in any way. Both troops developed stable exit orders; an individual's position in this exit order was dependent on her/his age but not on dominance rank status.

< I am currently **pole-and-collar training** one of our adult pair-housed rhesus females and hope to get her to graduate to the chair in the next few weeks. Wendy does remain sitting when I move the pole towards her but squirms when I try to actually attach the pole to her collar. Can anyone offer some advice how to get over this hurdle? >

The adult rhesus monkeys with whom I work also go through an initial period of resistance when the pole is being attached and also, when they are then put into the chair.

To start the training, I always first make sure that the trainee is so comfortable with me that she takes treats from my hand. I subsequently include the **pole**, offering treats with one hand, while holding the pole close to the cage in the other hand. The animals usually get used to this little ceremony very quickly and seem to ignore the pole, while focusing more on the treats.

The poles come with that handy little clip, opening and closing for collar attachment. The clip is a great place to hook treats, which the monkey can retrieve directly from the dreaded pole. I stuff a marshmallow tightly into the clip. This makes it a little harder to get the treat and extends the time the animal is in contact with the pole. Once the treat is retrieved consistently without signs of apprehension or fear, I start moving the un-baited pole very carefully in the cage and, finally, also touch the animal with it. In subsequent sessions, I gently tap the collar with the pole, and when I am done hang it on the front door of the cage overnight, so that the animal gets more and more acquainted with it. Needless to say that extra rewards—jackpot if you feel it's deserved—always are distributed at the end of each training session.

I always collar my rhesus macaques at least two weeks ahead of the first training session, so that they get used to wearing a collar all the time. If they're not comfortable with the collar, it really sets you back, because they will spend most of their time pulling at the collar and scratching their neck. I do not apply any enforced restriction when I train my animals; there is no squeeze-back. The trainee is always in control over the situation. I believe this greatly helps the animals to stay relaxed, keep trusting me and learn quickly what is expected from them in each training session. I consistently reward cooperation with a treat and with praise. If the animal doesn't cooperate, patience from my part replaces the reward. This strategy creates a tension-free ambience.

The first few times the pole is actually attached to the collar can be quite dramatic. The trainees usually freak out the moment they realize what is happening to them. But there is no reason for panic. I simply leave the

pole attached and talk reassuringly to the animal who gradually will calm down, stop squirming and remain quiet long enough so that I can gently unhook and remove the pole. This interaction is immediately followed by a generous treat reward, which is never refused. During the next sessions, I get the trainee to sit still with the pole attached to the collar for progressively extended periods of time, until she or he forgets all about the pole and takes treats from me. I repeat this step until I get the impression that the animal is comfortable with it.

I think the biggest struggle for many of us is simply time. Cooperative training really needs to happen at the animal's pace, not ours, and many facilities don't have the time or staff to do it that way. If you can give yourself and the animals more time, that will always work in your favor.

< What's the time investment of successfully training an animal to cooperate when being caught with a pole and then guided onto the restraint chair? >

On average it takes me around two weeks to successfully train an animal. With the squeeze mechanism in place, I just start with briefly touching the collar with the pole. I use the verbal cue *collar*. As soon as the pole touches the collar, I use a clicker and give a food reward. I repeat this process in a single session 10-15 times. I do this every day until the animal does not show any fear of the pole.

If there is an overly fearful animal, I introduce the pole further away from the collar, just at cage front. I've clipped marshmallows to the pole to lessen the fear response. I then repeat the same steps but now clip the collar, always using the verbal cue *collar* when I clip.

Initially, I only leave the pole clipped for a few seconds, click, and reward. I repeat this a bunch of times in each session. I then just extend the time the animal is clipped on the pole while offering juice or fruit. The repetition of these exercises helps the animals to settle down and cooperate with me more and more. After they are comfortable sitting with the pole attached, I slowly release the squeeze back. Most monkeys get nervous when they realize they can move, but are still attached to the pole. I wait until they relax, then release and reward.

I have found most of the animal actually learn spontaneously to present their collars when I am using these training steps. For all of my guys, I now just say *collar*, hold the pole up and they lean in and allow me to clip the collar. I do not have to squeeze any of them anymore. I even have one boy who insists on holding the pole and placing it on his collar, LOL.

Coaxing the poled monkey to get out of the cage and **walk on the pole** is always a big challenge. It often feels like there's a rabid cat on the other end of the pole! I think it's a lot for them to handle all at once; the door opening, the pressure of the pole and having to contact the chair for the first time. It's an intense situation not only for myself and the poled monkey but for the whole room really. After all, the familiar homecage is a relatively safe haven for these animals. I find it very helpful to have a second person work the cage door while I have the animal on the pole without expecting him or her to leave the cage—just calmly opening and closing the door. With patience and many reassuring words, the trainee does finally stop resisting. Once the animal is comfortable sitting with the pole on and the door opening, you could put treats on the floor or on the chair and patiently wait until the monkey ventures out. I know it's time consuming and some of us have no choice but to do it the quicker route, but steady progress at the animal's pace is the absolute best way to accomplish any new behavior with as little anxiety as possible.

After a few sessions, the trainee will feel confident enough to walk—rather than struggle—on the pole and pick up treats from the floor. Should the animal begin to thrash about, I take the pole and carefully but firmly push the animal's head to the floor. To be clear, I do not throw him or her down, but rather use the pole to turn the collar up towards the animal's head and then apply some forward and downward pressure in a determined manner. The monkey is now fixed and can get his/her bearings while being safe from causing any serious problems, such as getting injured while jerking around. I have noticed over and over again, that you can help the animal to calm down when you speak to him/her reassuringly with a gentle whisper-like voice. When the animal has settled down, I carefully start to walk him or her again.

It takes about one week of training until a monkey will cooperate and walk on the pole in a reasonably calm manner and pick up treats from the floor as a reward for good behavior. I want to get the trainees to walk, because after they come out of their cages—or out of the chair—they have a lot of pent-up energy that they like to release, especially the smaller guys. Their legs get cramped sometimes, and they really seem to like the opportunity to stretch. But, I treat this as a reward for good behavior. If they can calmly walk around, I let them do that, but if they start playing super man, I pull them straight back into their cages. If you don't have enough space, or the racks are enticingly close for climbing and rattling cages, or if you are a little new at this and do not have a second person around who can

help you control the monkey if need arises, the pole walking isn't a good idea.

Now, onto the **chair**:

- First, push the chair up against a wall with the opening facing out and put all the brakes on. This keeps the chair stable and makes it impossible for the animal to walk straight through, a situation that is really not fun when you're on the other end of the pole!
- Allow the monkey to explore the chair, touching it, climbing on it, walking around it and perhaps retrieving a treat that you have placed somewhere on the chair.
- After a day or so, coax the monkey into the sitting position in the chair, and don't forget to reward cooperative behavior!
- Gently lift the neck into position and get the collar into place. If another person, who is also on very good terms with the trainee, can help you, the situation becomes less of a challenge, especially when you are dealing with one of these incredibly strong and sometimes extremely stubborn guys.
- Once you have your monkey in place, let him adjust for a few minutes. Don't forget the treats! Some animals will be initially restless and try to push your hand away, but with gentle patience they will all settle down and finally accept your food reward. Gradually extend the time the trainee remains in the chair, with you always being close by, serving as a comforting social support.

The first time in the chair is quite stressful for the monkeys, so I put them in for only 5 minutes. After one day off, I repeat the chair acclimation for 10-15 minutes. After another day off, the animals are chair restrained for 30 minutes; during this time, I will gently stroke their legs, shave the insertion site(s) and place vet wrap around the shaved area(s); this session is again followed by a day off. In most cases we have to repeat this session several times, depending on the individual animal's reactions. Once an animal is reasonably at ease with being restrained and handled, she or he will be kept in the chair during a final acclimation session for 4 hours. During this final session I give them their regular food, water and, as a reward, a favored treat. Generally I have a rhesus ready to go for a 6- to 8-hour study period with 4-6 acclimation sessions; cynos will usually require 8 or more sessions.

I need to emphasize that a lot of patience is needed to acclimate the animals successfully. They determine the speed of the process, and there is no problem when a session has to be repeated once or several times until I get the feeling that the animal is at ease with it.

I have found that each training step involves an initial struggle, but I have also found that with consistency and patience, the animals learn quite quickly what I expect them to do. I have a female who is fully trained and just comes up to the front of the cage without being squeezed and actually will move her collar, so the loop is exposed for me to attach the hook of the pole. This monkey also struggled a lot when I first started working with her. It is amazing how these animals can gradually relax into the training sessions and finally start working with you, rather than against you. I guess that **trust** in the trainer is the ultimate key for success. These monkeys are smart and, when they are free of apprehension or fear, quickly figure out that it is much easier and even rewarding for them to cooperate with you rather than resist. A successfully trained monkey will have developed so much trust in you that he or she will never fight against you when you pole and chair her/him.

When I train my animals, I work with them daily once or twice, five days a week, until the goal of the training has been achieved. If I don't work with them on a consistent schedule, they tend to get rusty quite quickly. The faster you can get them over the initial struggling, the easier will be the whole training. If you try to pole a monkey who vigorously resists on a Monday, and decide to wait and try again on Friday, chances are that the struggle will be the same; but if you are persistent and repeat this training step over and over again every day, you will definitively notice progress by the end of the week. I would imagine that without consistency and patience, the training would be a rather frustrating experience, both for the trainer and for the trainee.

To successfully pole-and-chair train a monkey is not necessarily a time-consuming process. My quickest subject took five days of consistent training to reliably cooperate. He was two years old and an angel! But I also have had some tough customers who have taken me well over a month to get going, especially cranky older females, who can be very stubborn and hard to food-motivate. Also, I have had some animals who were just never meant to be put in a chair. This is a reality that you and the investigators must acknowledge. You cannot force a monkey to cooperate and be relaxed in the chair. It's impossible. Sure, you can try, but you're not going to win.

I think we have to make it very clear to investigators who want us to train their animals that we cannot guarantee to be successful in all cases. Animals are not predictable machines. Yes, most monkeys can be trained but some cannot, or let's say they should not be trained because their personality—which is presumably conditioned through negative experiences

with people—is very difficult to deal with. A monkey who persistently resists during positive reinforcement pole-and-collar-chair training is not a suitable candidate for research involving chair restraint. No investigator would benefit from having his/her research subject forced into an experimental situation such as chair restraint. The data collected from such an animal would be of little or no "scientific" value.

I wish all investigators could read this, understand it and accept it!

< It's not uncommon that macaques imitate the behavior—for example stereotypies—of other monkeys. How feasible would this be as a training tool in the laboratory; **learning through immitation**? >

I chair-trained two pair-housed male rhesus macaques and started with one partner while leaving the other one in the same room so that he could watch and provide social support. It seems to me that the onlooking partner partially learned the training steps: When it was his turn to be chair-trained, he knew immediately how to sit in the chair, and he also accepted treats as training reward right from the beginning. Watching the training procedure may have encouraged him to imitate his companion's responses, which made him cooperate more readily.

I'm in the process of training two paired rhesus right now to pole/collar and chair, and I ask them to station themselves at the front door at the beginning of each session. A few days ago, the submissive partner started doing an adorable head bob-bow during this session; we actually reinforced this behavior with a treat because it is helpful when poling the animal. Today the dominant partner did the same bob-bowing gesture at the cage door, hoping to get our attention—and a treat. It was quite cute; he obviously had learned that behavior by imitating his submissive partner's rather dainty gesture.

That these guys do learn this quickly from watching another monkey's behaviors makes it very likely that a well-done video could serve as an effective learning tool for them.

I had two adult male cynos occupying pens across from one another. One was very curious and got into everything. He quickly learned how to let himself in and out of his homecage that was attached to the pen. He would open the door, slide under and then close it behind him. The door was usually propped open, but he seemed to prefer having control of the door, so we let him have his way. His buddy across the room was more of the couch potato type; he loved to watch what the other guy was up to as long as it

didn't require him to move. — One day I was wandering by and saw the *lazy* cyno messing with his door. After a couple of days the animal care staff reported that he had learned the same behavior as his roommate and started letting himself in and out of his homecage.

< Is it possible to train macaques to **touch the Lixit** of the cage so that attending personnel can see if the watering system is functioning? >

I wanted to get our rhesus macaques to test their water Lixits on command, instead of the caretakers having to put a pole through the mesh of the cage and push the tip of the Lixit. This routine procedure often frightens the animals quite a bit; they sometimes grab the pole in a self-defensive or aggressive reaction and struggle with the caretaker who then has to remove the pole by force. Even if they don't grab it, they often cower at the opposite side of the cage in a fearful posture.

Back when I was the enrichment tech at my last job, I trained one cynomolgus macaque to check his Lixit with a laser pointer. My current position doesn't give me the time to do this kind of work, but I think it could be incredibly valuable, plus would serve as a great form of enrichment for the monkeys ... and for the caretaker who does the training.

I trained 8 rhesus (6 males and 2 females) and 15 cynomolgus (12 males and 3 females) macaques to check their Lixits, using a laser pointer as the target. The macaques were already familiar with me, and 8 of the cynos had been pole-and-collar trained by me.

To start with, I conditioned each macaque to the sound of a clicker, by clicking and providing a food reward. This was followed by introducing the individual to the laser pointer either on the side of the cage or the back of the cage. When the animal touched the laser point, I clicked and offered a food treat reward. After the animal consistently touched the laser point on the cage wall or the back of the cage, the laser point was moved to the Lixit.

Training sessions lasted 5 minutes or less. After an average of four sessions, 7 of the 8 rhesus (88%) and 13 of the15 cyonomolgus macaques (87%) checked their Lixits on cue. In the beginning, the laser pointer was still needed but it was eventually phased out; a verbal cue was then sufficient to prompt the animals to check their Lixits (Haba Nelsen et al., 2010).

We have successfully trained with a laser pointer at least one cyno in each pair/group to target to their Lixits. Currently this stands at about 35 cages housing a total of 63 animals. We no longer need to check the Lixits with poles. Most of the animals don't even need the laser and will check the Lixit upon a verbal command and a simple hand gesture. It's fun to watch

the animals as the technician goes around the room and all the animals are checking their Lixits themselves (Ferraro et al., 2013).

< What's the least distressing way of **oral-dosing macaques**? >

When our rhesus or cynos come in, the husbandry staff and/or the vet techs visit them regularly in order to establish a friendly relationship with each one of them. During these visits yummy things such as Kool-Aid, juice, honey or applesauce are offered to the animals from syringes. Most of our current residents can and will accept oral meds via **syringe tip** if needed.

As part of my enrichment program, I started out teaching my rhesus macaques (males and females) to take Margarita Mix from syringes. I squirted the liquid at the monks to hit their hand and after they licked it off, they inevitably came to taste a drip from the syringe. It took two sessions of a couple minutes each to teach the guys that the syringe contained good stuff. I used 20 cc syringes because it was easy for the monks to pull smaller syringes with their teeth into the cage, and some monks got frightened when I approached them with small syringes, probably because these were commonly used to give them injections.

The training of the monks paid off when the researcher wanted to oral-dose them and I informed him that it could be done without much ado and without the need to anaesthetize the animals.

I have trained 43 marmosets to drink their doses via syringes with blunt tips. First, I tested many flavors and came up with the following favorites of our colony: Splenda in 5% or 10% solutions, maple syrup, blueberry and raspberry syrup—both also available sugar-free. We were able to obtain the cooperation of all 43 marmosets and dose them during studies—most of the animals right through the bars without missing a single drop! We dosed the colony this way for four years both during acute studies and chronic studies extended over a time period of up to 14 days (Donnelly et al., 2007).

We have really good luck with a **spoon**. We mix meds in various food items that best mask or complement the taste of the drug; strawberry yogurt works for most, commonly used drugs. If a drug is very bitter, we mix it in something else that also has a bitter flavor. Coffee does the trick in such cases; yes, ALL our macaques like coffee. Coffee with a tad bit of Coffee Mate fat-free vanilla creamer effectively masks bitter drugs, and the animals lick it from the spoon without much hesitation.

We first train the animals to lick the drug-masking liquids from a spoon without using their fingers; otherwise they may grab it, smear it on their body or my arm, or spill it altogether. It doesn't take much time to teach them how to use the spoon as intended. We then mix the med in the liquid of choice and offer it again with the spoon.

When I oral-dose one of our monkeys, I know for certain that the animal has ingested all of the compound when she or he has licked the spoon clean and swallowed one more time.

We rarely encounter a monkey who won't lick the spoon clean. Once in a blue moon a monkey will not lick everything from the spoon. In this case we mix whatever is left over with one or several different favored food items until the monkey has taken the entire dose. This may take some time.

Whoever would have thought of giving monkeys coffee? Have you experimented with regular and decaffeinated coffee?

We use such a small amount that it really isn't a concern; but FYI, we use regular coffee as that is what we have available around here.

Flagyl (metronidazole) is the reason why I started playing around with different compounding options. You see, in my earlier years I was a compounding pharmacy tech and learned about *what* goes with *what*. You wouldn't get a pleasant taste if you mixed sardines and ice cream, or vinegar and milk. Does that make sense? YUCK! The Flagyl is bitter and lingers in a bad way, so by adding something also bitter but in a good way, the two tastes blend; and then adding a hint of sweet that would also blend, making the cocktail palatable. I use 2/3 coffee with 1/3 creamer. In some cases I even add a drop of vanilla extract to that mix. Now SOME monkeys still will

not take it, but most do. For the ones who won't take it in the coffee, I mix the Flagyl with ketchup; a few accept this cocktail.

I need to mention: ALWAYS offer the coffee or whatever you are going to mix the meds with several times pure, before you add the meds! The animals will quickly develop a taste/craving for it, especially for the coffee, and will then put up with the little *extra taste* of the meds, just to get what they really like.

I would suggest that animals in laboratories should be given the chance to cooperate with the licking of the test concoction from the spoon or from the syringe tip. Only if they fail to lick the spoon clean or empty the contents of the syringe should gavage with a gastric tube be considered. Why not allow those who WILL lick from a spoon or from a syringe do so? Why would anybody use a nasogastric tube in preference to letting a monkey lick from a spoon or syringe tip, when nasogastric intubation is stressful for the handling person and distressing for the animal?

I love this discussion but it gets me so frustrated to read that others are implementing these better methods, which are far kinder to the animal, without PI issues, when I would be faced with such unreasonable road blocks. As I'm sure others have encountered, you spend months working with these animals, gaining their trust and cooperation, just to have one day a group of techs fly in, shove a tube down their throats and then leave. Needless to say, your animals are back to being terrified of any human they see, they are angry and perhaps even aggressive.

Orogastric intubation is inherently stressful for monkeys; in our experience, the animals are unable to adapt to this procedure. In this light, we were searching for an alternative refinement technique for conscious cynomolgus macaques. After implanting a subcutaneous port, a **surgically-placed gastrostomy** was completed to afford access to the gastric lumen and enable the administration of substances. The device was left in place for 2 to 12 months in 11 macaques. The procedure is compatible with the presence of another cage companion (Fante et al., 2012).

Your Refinement technique is ingenious. I am particularly impressed that this alternative approach of oral drug administration allows the monkey to remain in her or his familiar homecage, and that you are keeping these animals in a compatible pair-housing arrangement.

< How do you teach/train macaques to RELIABLY **allow saliva collection** for cortisol assessment? >

We did a study with two rhesus and seven cynos, assessing their cortisol responses to abrupt versus phased light changes. In order to avoid data-biasing stress reactions to blood collection via venipuncture, all nine macaques were trained to cooperate during saliva sample collection for the cortisol analysis.

We dipped cotton ropes in mango juice, and practiced with each monkey, training them not to touch the rope with their hands, but to just chew/suck on the end of the ropes. Mango juice doesn't have the right pH for the actual test but the animals LOVE the mango flavor, so we used mango juice first to get them conditioned to readily chewing and sucking on the ropes. Once they learned that, we stopped the mango juice and replaced it with coconut juice, which has the correct pH balance for the hormone test; we got that little adjustment down pat before we actually started the test.

The lab told me that a lot of times the saliva samples are compromised by insufficient volume and/or traces of blood—which will ruin the test. If a macaque chews too hard or too long, he or she may experience a bit of gum bleeding. It's tricky to get them to chew/suck just long and gentle enough. The lab suggested rope chewing times of 2-3 minutes. I think that's why many colleagues end up literally with bloody samples. To get a better idea of that procedure, I chewed on one of the ropes to find out how long I had to chew to produce sufficient saliva; I noticed that the special ropes from the lab seem to suck the saliva right out of your mouth within just a few seconds. As a result of this little experiment, I had all our monkeys chew/suck the test rope for only about 10 seconds, and all samples were useable and free of microscopic traces of blood!

Sucking on flavored cotton ropes became a really fun enrichment activity around here; it certainly also helps with human-animal bonding. The monkeys love this! So do our interns working with them!

< When training adult rhesus macaques to cooperate during procedures, do you find that the animals respond differently to **female versus male trainers**? If you notice a difference, what could possibly be the reason for it? >

Smell may play a role; women and men have different body odors, which may trigger different emotional responses in the animals.

From my experience, female trainers have more success with cooperative training than male trainers. I think it's a mix of variables—compassion and patience being important factors.

I've also noticed that the adult rhesus I've worked with responded very differently to me versus male co-workers. The monkeys are less nervous and less aggressive when I work with them. They quickly learn that they can trust me; this helped me very much forming a mutual trust relationship with them and implementing training for them.

In my experience, adult rhesus macaques respond better and are more willing to cooperate with female trainers than with male trainers. I think they are less afraid of women than of men and are more easily inclined to trust them. Men in general tend to be larger and broader in stature than women, and they have deeper voices; the animals probably find this more threatening, so they are more hesitant to work with a male trainer than with a female trainer.

Our macaques seem to be more accepting and less fearful of female humans. Male humans tend to be larger, louder, use less baby talk, and are less patient when working with the animals. The animals give the impression that they are afraid of male humans but feel relatively safe when I am around.

I give a NHP Recapture training class, where we cover how we should be acting in the NHP rooms, in order to be less intimidating.

- Talking in high-pitched voice, not squaring off shoulders and not facing the animal directly.
  This part of the class is VERY hard for many of the male students. Cultural misogyny has taught them to perform maleness with aggressive attitude and behaviors that they are often not even aware of. Some of them even refuse to use a higher softer voice because .... misogyny. Many just constantly forget.
- We do a practice mock trial with an imaginary loose monkey. They act it out and I give feedback in the form of the monkey's point of view.
  WITHOUT FAIL, the male students walk into the monkey room facing straight at the animals, hands on hips, shoulders squared.
  I say things like:
  Holy crap, 2 full grown male apes just broke into my home!! (they always laugh at this)
  OMG, that one is staring right at me and the other is making himself look larger, they are gonna attack me, for sure!
  Jesus....I think that one is growling. ... I'd better either fight or run!

We have male rhesus macaques who respond to women very differently than to men. He (Junior) allows only women—me, an investigator and two of our vet techs—to groom him through the bars of his cage or while he is in his chair during training. Junior is biased against men and does not want to be groomed by any of our male animal care employees. He invites us women by placing himself up against the bars of his cage and closing his eyes and then rotates himself around to make sure we get all of his favorite spots. He usually falls asleep when one of us is grooming him gently, but he does not like it when any of the men try to do the same.

The way most women approach animals and work with them in research labs differs quite a bit with the way most men deal with animals in research labs. The animals typically respond to the presence of women with much less apprehension and fear than to the presence of men. The differences, in my opinion, are based on gender-specific biological programming in women (patiently and compassionately caring mother) and gender-specific conditioning in childhood in men (to be a real man you have to be tough, aggressive and have no feelings).

< When you train your animals to cooperate during procedures, how do you find the time for this? Do you get **institutional support of your training programs? >**

At our facility, training animals to cooperate during procedures is an integral part of our daily work.

I have to sell a cost benefit analysis of training animals in my charge to the management of our laboratory. This brings out the salesman in me.

In order to implement a training program, I first had to train my staff to re-think their routine. Just a change in the way everyday tasks are performed. For example, to train macaques for transfer or injections, we teach the animals what the clicker is by using it during regular morning feedings. Click means biscuit as a reward. Any animal who naturally presents during that time will receive a click along with a biscuit and raisins as a reward. Whatever desirable behaviors we can catch will be rewarded throughout the day. Once the clicker has a meaning for the animals, the tech starts using a simple target—this can be the mirror already on the cage, or the plastic cage tag—to reward animals during regular feedings when they touch the target. Now we can begin targeting individual animals where we need them to move and station. This training program does not require an extra time investment, so it is endorsed by our facility.

My institution is very supportive of training our macaques to cooperate during routine husbandry procedures such as shifting animals. Unfortunately, however, our animals are not trained to cooperate during specific procedures such as injection and blood collection.

It will be great when all investigators understand the importance of training. All research benefits if animals are trained to some extent, even if it's only to prevent them from stressing out when someone approaches them. Published research data do show that working with animals who have been taught to cooperate during procedures is very beneficial, but it's odd how that research gets often dismissed so easily with the presumption that there is no extra time for conducting training sessions with the animals.

Not only that, but also many investigators are kind of stuck in the inertia of tradition. They interpret any attempt to change their traditional practices as a personal critique, so they have the tendency to stubbornly defend the *status quo* even if it implies that they resist well-documented progress.

My staff has the training/conditioning of animals built into the care schedule and, if things take longer than planned, someone else will pick up some of their chores so that the animals don't lose out.
Investigators and administrators at our facility understand that training helps produce better science, so I get very little opposition from the research team.

< When you are in charge of new monkeys, is it an option to **make use of quarantine time** to familiarize to familiarize the animals with you, perhaps even try to develop a mutual trust relationship with them and/or clicker-reward train them before they go on a study? >

In my experience, a lot of the **trust building** can be done while newly acquired animals are in quarantine. They can be spoiled with treats and given lots of human interaction during this time. I will also start some training with them, once they have become used to me. Just some basic commands such as stationing and shifting from their homecage to a play cage and back are very helpful and easy for them to learn. Usually within the time frame of quarantine (about 40 days at our facility), the macaques have established a trust relationship with the one or two of us who attend their room. When quarantine is lifted, the animals have lost their fear of humans; they are now relatively relaxed when a person comes into their room with whom they are not familiar.

When we get in a new room of 16 monks (rhesus or cynomolgus macaques) in quarantine, we start working with the animals to build trust,

having them come to the front of the cage and accept a treat. This can turn into a *vet check* at cage side, especially if you can prompt them to present various body parts. Then, when they come out of quarantine, they have some trust with us already built, and this will help when we train them for pole-and-collar and then for chair restraint.

The attending care staff plays a very important role when you want to have new arrivals prepared for research while they are in quarantine. Right now I have fantastic care techs who really enjoy being with these animals, so they spend extra time with them. This helps tremendously! The monks seem happier as well. They quickly learn to trust humans, which in turn is the basis of training them to work with humans. When I go in the room, I can see the difference it makes for those monkeys in quarantine, and how fast they learn to cooperate with the vet staff. I think it's really important to have care staff who truly love monkeys and enjoy working for and with them.

One of the most rewarding things we have done is **housing new arrivals in compatible pairs** as soon as possible; we do research with rhesus and cynomolgus macaques. Our facility is small enough so that we can quarantine monkeys in all-in/all-out fashion. Although we haven't scored it objectively, attending staff report that monkeys in quarantine are now doing much better, especially when subadults are brought into our facility where they are housed in pairs rather than alone. They almost immediately change their response from being terrified at the far corner of the cage to co-threatening with their cage mate against personnel looking at them. In general, they seem so much less apprehensive, even during TB testing. We have only recently begun this practice, with dedicated staff who also condition the animals to remain calm during husbandry procedures. For sure, research techs, vet techs, and animal care staff have noticed a positive difference.

Many years ago I started pairing and habituating all our new rhesus arrivals while they were in quarantine. It didn't take long for people to realize that the animals who had positive experiences through quarantine were easier to handle once they were on study. Pair-housing macaques soon after arrival is rewarding, and I believe, much better for the monkeys and for the staff; it is great!

< What is the best strategy to **capture macaques who have escaped** from their primary enclosure? >

I was told by my supervisor that you have to chase escaped animals until they get so exhausted that they will voluntarily go back into their cages.

Supposedly, such a stressful experience will make it less likely that they will escape again in the future. I remember a student who was scolded for using an apple—since it was a reward—to lure a female rhesus back home after we had chased her around for 20 minutes. The monkey ate the apple and finally walked into her cage. The problem with using so much negative reinforcement was that it typically created quite a chaotic situation. Sometimes the animals in the cages got so excited that they started fighting with the escapee or even with their cage companions. We then ended up, with the veterinarian not only taking care of the injuries of the escapee, but also of fight wounds of other monkeys in the room.

I am staggered to hear that you chase them until they drop. A far better approach is to remain calm and quiet, preferably with only one person in the room. Since monkeys normally retreat from you, it's quite easy to make them move away from you into the direction of their homecage. It is my experience that they are usually only too pleased to get back home. We had a singly housed rhesus male get loose this morning. He is one of our more grouchy monks with an attitude! He sat on top of the cages and made aggressive overtures towards me and my coworker. On two occasions, he instigated squabbles with some of the other monks in the room, but we were fortunately able to redirect his attention. Finally, through patience, nerve and a lot of praying to the macaque gods, we got the male to jump into an empty top cage into which we had thrown a bunch of fruit. It took about 15 minutes for this to happen. I am so relieved!

I have learned with group- and single-housed rhesus and stump-tailed macaques that catching animals, who got loose, can be a traumatic and chaotic event, but that it all depends on the personality of the attending care personnel. Some people freak out and create a real mess, shouting, scaring the escapee with broomsticks or trying to catch the escapee with a big net, while other people remain calm and quasi-mesmerize the disoriented monkey into entering a transfer cage, returning directly back into the homecage or jumping into an open empty cage baited with favored food.

Monkeys presumably escape not because they really want to leave their familiar home environment, but because something alarms them, such as an investigator trying to grab them with heavy leather gloves through the partially opened cage door. If they can trust you and you give them a chance to settle down, they will find their way back home without much coaxing—and you close the cage door, while praising the relieved monkey.

I had to deal with quite a number of escaped pair-housed rhesus macaques and found it easy to get an animal back into her/his homecage

where the companion was waiting behind a grated cage divider. I always worked alone with an escapee rather having another person help me, respectively distract the escaped animal from me. I think it helps a lot to get the escapee's full attention in order to be gently guided with non-threatening words and gestures back home where the companion and a food treat reward are waiting.

We have cages with up and down access, so we just place the cage mate "downstairs" so to speak and open the top cage, as the animals never want to enter the bottom cage. cages. We did have one animal who would only return into his cage together with his cage mate; so we kept his buddy from escaping as well by clipping him with a small pole near the front door, opened the side door and the monk went right back in to his buddy.

We had over 40 rhesus monkeys get out of a corral, because a big branch fell over the wall, creating a perfect ladder. We noticed the situation first thing in the morning, so no one knew how long they were out. The reaction of the caretaker crew was to grab nets and dart guns. My thought was "Are you crazy? The monkeys will all disperse; they know what nets are for." So, I convinced the crew to let me fill the corral with fruit and wait some time. And not surprisingly, within only a few hours, every one of the escapees jumped back into the corral and snatched a fruit. No one got distressed or injured. It was all so simple!

< How do your **macaques respond** when you approach them with a **face mask** during this COVID pandemic? >

When I first started wearing a surgical mask due to COVID, two of our macaques who had recently retired from research fear-grimaced and shrieked when I approached their enclosures. I spent quite a bit of time desensitizing the two boys to my masked face, and while they got to a point where they wouldn't show such fear, they showed no interest in approaching me for a friendly grooming session the way they had before I started wearing the mask.

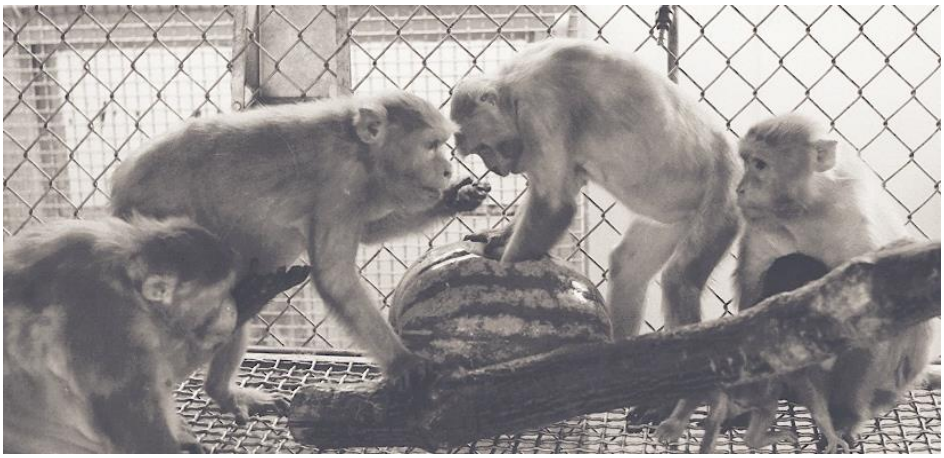
Because of their negative reactions, I designed a special mask using an image of *Keiki*'s face, a cyno the other macaques had always been comfortable to be around with. I was hopeful the macaques would appreciate it, and they did! All of them marveled, looked on with great interest, and became playfully curious when I entered the barns for the first time as *Keiki*'s cousin. Even my two boys who so disliked the stark white surgical masks seemed pleased!

As I thought about my ‘Keiki face’ and how the macaques seemed to accept and enjoy it, I wondered how it might affect the macaques initial behaviors toward new humans if the humans were to wear ‘Keiki faces’ as well. So I had a few more ‘Keiki faces’ made and then observed our newest human enter the macaques area wearing the mask for the first time.
The reaction was so unexpected and interesting to me! ALL of the macaques were curious as they had been with me, but they all immediately presented rumps to the new person wearing the ‘Keiki face’. Rump presenting as we walked through the barn from one enclosure to the next was like a domino effect!

It’s early in this Keiki-face trial but so far I think this is going to be a really positive thing!

< In their natural habitat macaques spend a large portion of the day searching for, retrieving and processing food; they have a strong drive to forage. What are practical **feeding enrichment** options allowing captive animals in research institutions to engage in foraging activities rather then quickly picking up freely accessible processed food? >

I have given whole **watermelons** to group-housed rhesus, cynos, bonnet and stump-tailed macaques for several years without any noticeable adverse effects. It would be a waste of time to cut the melons into small pieces. The monkeys first gnaw a hole into the rind and then dig into the



soft and juicy part. They really like this and are kept busy until the last morsel has been eaten. They usually discard the rind, but before they do so they thoroughly remove any soft material and eat it. This usually creates quite a mess, but I don’t mind cleaning it up, because the animals enjoy this type of foraging enrichment so much.

I get huge organic watermelons and **pumpkins** for a very good price from a local farmer at the end of the growing seasons in late summer and early fall. Both the caged and the pen-housed rhesus monkeys can't get enough of them. I distribute slices of melons and pumpkins to the caged animals. Animals living in pens receive the whole fruits; they would first bite a hole in the thick and hard rinds and then dig with their fists into the interior flesh, retract their hands and lick them with gusto. It always takes at least an hour until the melon or pumpkin finally breaks open, allowing the monkeys to finish all of its tasty contents.

We get a donation of pumpkins every year from local churches within our community. The larger pumpkins are cut up and put with the innards into Dixie Cups to freeze for a special treat later. We distribute the smaller pumpkins whole in several of our outdoor corrals and indoor/outdoor pens. Pumpkins provide amazingly attractive foraging enrichment for macaques. Some of the monkeys only like the 'guts' with all the seeds while others enjoy tearing apart the shell and creating quite a mess.

This year, we have decided to take the opportunity to do a pumpkin carving contest within our staff; so it will not be me and my technician carving them all ourselves. We're hoping this will enrich more than just our animals!

We give whole pumpkins to rhesus and cynos in both single- and group-housed environments. I would say that this is one of the most effective foraging option we have ever given our animals. All of them spend hours processing their pumpkin!

Our macaques absolutely love whole pumpkins, which we give them throughout the year, not only at Halloween. They carve little holes in the side and pull out the seeds. This makes a wonderfully fun mess! They also enjoy roasted pumpkin seeds that we bake in the oven. This year I painted faces on their pumpkins using non-toxic (safe for human consumption) paint; a few of these little darlings chew the painted face off before picking the pumpkin apart to get to the delicious seeds inside.

It's so interesting to watch how each individual responds to her/his pumpkin. Many we can predict, like Corney will have his *smashing pumpkin festival* while Jala will gently lip smack and carefully groom her pumpkin friend's bald head, before she takes it outdoors and floats it in the pool—she floats everything in the pool.

It would probably be fun for our macaques to get **corn on the cob**, but I am not sure if that would be a safe feeding enrichment option. I would be concerned if they ingested the cob.

I give whole corn with the husk to our pair- and group-housed rhesus and baboons. They love it, and I enjoy observing them peel and eat, leaving a big mess after they have finished. They gnaw the cob into little pieces that finally fall through the grid floor on the pans. I cannot say whether they actually also eat pieces of the cob, but we have never encountered any health-related problem. I don't mind cleaning up the mess; it's worth the treat!

We use corn on the cob for all our caged cynos, rhesus and vervets. The animals give the impression that they love processing and eating the corn. They typically pick the kennels both with their hands and their teeth. When they are done, they proceed gnawing on the cob. I don't know if they actually ingest pieces of it. Even if they do, we have never encountered any clinical problems.

Our rhesus and cynos eat the corn but not the cob; yes, they may nibble the cob but not ingest it. We use drain covers to catch the leftovers and prevent them from clogging the drain.

Our macaques also love corn on the cob; we are set up in such a way that drains can't plug. Our animals get the corn right off the stalk without shucking it, so they get to peel away all the layers ... and make an amazing mess! But it's so much fun for them!

Yes, they LOVE it unshucked. That's how I managed to get it banned at my facility. I handed it out once—only once! I knew it would be a mess, so I purposely went into the rooms early in the morning to pull as much of the 'waste' from the cages as I could. But, I didn't get to our third room before one of the service folks found out. The complaining and bellyaching that ensued was one of the most ridiculous arguments I've ever had. Mind you, all of our drains are covered to avoid blockage and our cages have open back flush pans. The complaint came from the fact that it took 'too much time' to hose out the cage and the silks made 'a huge mess' in the pans. When facility management came down on me, you'd think it was the 100th time I gave them unshucked corn and someone suffered a life altering injury because of it. Long story short, it's at the top of the 'Never allowed to give the monkeys' list. Bugs me like you wouldn't believe!

< How practicable is it to feed caged macaques whole produce that is bound to create quite a mess, for example corn on the cob, melons, pumpkins, oranges and bananas. The animals love it, but is it worth the extra time spent **cleaning up the mess** before you can leave for the day. >

I always let the care staff choose which day is most convenient to give the animals whole produce, and then I help them with the cleanup. That way

it's not such a chore. We feed corn on the cob with the husks, whole bananas and sometimes whole squash.

Yes, whole produce is so worth the effort, you just have to ensure the drain covers are in place so nothing like corn cobs or banana peels goes down the drain. Otherwise, I find the animals clean up pretty well. We open our cages daily to pull the biscuits, so it's not much more effort to remove pieces of produce. The amount of time the animals spend processing and eating the produce is well worth the little extra time for cleaning up leftovers.

In my opinion, the benefit for giving whole produce far outweighs the cons of cleaning up after them. I also feel that it's our job to do that. After all, at the end of the day, I get to go home, they don't. I am the one to give our animals the produce, and I am also the one who cleans up after them, so I understand both sides of the argument, but still feel the cleanup is worth offering the animals this kind of enrichment.

I very much like your work ethics! The simple fact is, it not only benefits the animals in your charge but it also makes you happy

I grow **wheat grass** right in the window of my office. Our rhesus and cynos love it. It's a great treat for them during the winter months when fresh browse is not available.

I have sprouted wheat grass seeds for rhesus and cynos at my last job. We purchased clean soil and wheat for human consumption. The wheat seeds germinate quickly and grow rapidly under fluorescent lighting conditions. Within 2-3 weeks the grass is ready to give to the animals. I

would pull the grass sod out of the tray and cut it with a knife into squares—like brownies—and hand them out. The monkeys loved it! First, they ate the grass then broke up the sod and pulled the roots apart to get to the seed sprouts. Most monkeys spent a good amount of time with this destructible, healthy and edible foraging enrichment. The wheat grass turf breaks up into small dissolvable fragments that pass through the drain without any problem.

Dry whole **coconuts** are favorite foraging/feeding/grooming/play objects for stump-tailed macaques. They don't get tired working on them until the last morsel has fallen through the mesh of the cage floor. It has been my experience that the animals do not get harmed/injured in any way while processing coconuts.

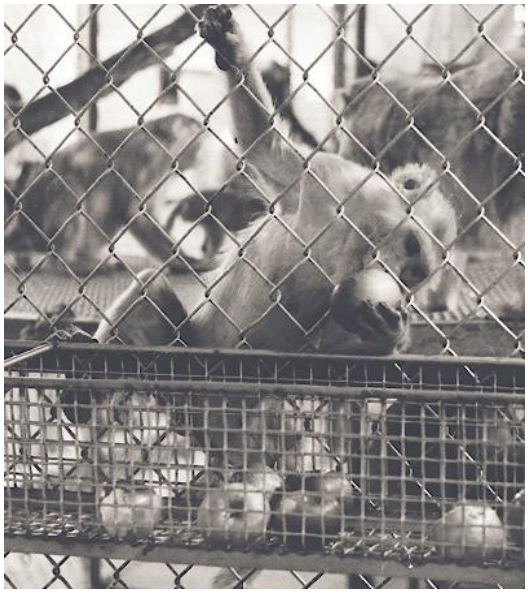
When our rhesus macaques get their first coconuts, they probably crack them open unintentionally. After a few experiences with these tasty objects, many of our animals take their coconuts to a higher ledge and appear to deliberately drop (or throw) them to the floor, and immediately come down to the floor to eat the coconut meat inside the broken coconut shell.

I give whole coconuts to our individually caged cynos. More than anything, they like them for grooming purposes. It gives them something else to do besides bite themselves. I also had a female who carried her coconut around as if it was a baby, constantly clutching it to her chest, and lip smacking to it, grooming it, etc. She was a chronic alopecia case. The coconut alleviated some of her stereotypical hair-pulling behavior.

I've given coconuts to rhesus, cynos and pigtails. All of them go bonkers! However, occasionally there are older animals who do not manage to crack a coconut open. I let them groom the fuzz off for a few days and then retrieve the coconuts and make a BIG production by throwing them onto the floor to break them open. All animals in the room hoot and holler; it's a great experience for everyone! When everybody has settled down again, I hand out the broken pieces to the original owners of the coconuts; they sure seem to be happy! Now they can gnaw and eat the good stuff without having to make undue efforts.

Our cynos also get whole coconuts; they love quasi over-grooming the nuts until all fibers are pulled and picked off the shell. Many of our cynos will pick out the coconut eyes and then drink the milk. Some—mostly our larger males—will throw the coconuts repeatedly on the ground until they crack open; once they have achieved this, they will relish the coconut meat and finally leave quite a mess that needs to be removed from their cages before the end of the day.

All our group-housed rhesus receive whole **apples** on a daily basis.



In order to make it more interesting for them, I place the apples into troughs that are attached to the chain-link wall of the pen 1.2 m off the floor. The animals have to climb up to the trough, reach into it and get hold of an apple, maneuver the apple up to the chain-link, press the apple towards their mouth while nibbling off pieces until it fits through the mesh of the chain-link barrier. In this way, the monkeys spend a considerable amount of time retrieving/processing apples every day. Whole apples provide an excellent daily foraging enrichment also for animals who live in cages.

My boys (rhesus macaques) love **mangos**. I like to keep the produce as natural as possible for our animals, so I do give them whole mangos. They enjoy gnawing on the pits; once the pits get funky I discard them. I never encountered any problem associated with the pits.

We give mangos about once a week and have also given the large seed inside to some animals without any ill effect. So far, none of our macaques have actually eaten the seed, but they all spend a lot of time getting all the fruity and juicy stuff from it.

We give whole mangos to both rhesus and cynos frequently and have never encountered a problem. The animals definitely enjoy them!

I distribute whole mangos to our rhesus and cynos. We want to give them more to do, so we give them the whole fruit. It is a hit! The animals play with the pit and shred it but it never happened that an animal actually ate a pit.

Here is a funny mango story: I provided fresh mango with the monkeys' breakfast as that is one of their most cherished foods. With Holly being paired with little Louise, I wanted to make certain there was plenty for both. Louise hasn't been here very long and only had mango once before, and that was before she was paired with Holly. She was beyond crazy about it! I cut the mango into smaller pieces and put several pieces in each food bowl. But as soon as I put their food bowls in front of their enclosure, little Louise ran down and quickly like a thief in the night grabbed every single piece of mango from both bowls, shoving them into her mouth with lightning speed! Holly, still on the ledge above, made a quick and single leap to the ground, landing almost on top of Louise. With a profoundly annoyed look on her face like none I have ever seen, she reached out and grabbed Louise by her ear, giving her a quick pinch and a little assertive jerk to the ear. She was angry and looked at the little mango hog with piercing eyes while keeping hold of her ear; Louise looking back at Holly with the most pathetic innocence, slowly opened her mouth and let several of the mango pieces fall to the ground, while maintaining eye contact with Holly.

The funniest part of this whole thing was the look on Louise's face that I just can't describe well enough! When she opened her mouth to spit out the mango, her tongue was sticking out of her mouth and the mango pieces just sort of rolled off her tongue onto the floor. She knew exactly why she was getting her ear pulled! It was one of those special moments that I will always cherish! I waited until I went outside and then laughed myself silly.

< What is a reasonable **serving size of fruits and/or vegetables** for a macaque as part of the environmental enhancement program? >

We offer each of our caged rhesus, bonnets and cynos a half-cup serving of banana, apple or orange pieces at least four times a week. The produce is fed in the late afternoon so as not to interfere with consumption of the daily chow ration.

Each of our adult rhesus macaques gets the equivalent of half an apple or comparable sized fruit and, at a second feeding, receives a vegetable, like a stalk of celery or a quarter of a green pepper every day. Immature animals get about half of that daily portion. These supplemental food items do not interfere in any noticeable manner with the animals' normal consumption of their daily biscuit ration.

All our caged rhesus macaques (approximately 950 animals) receive every day—including weekends and holidays—one piece of produce, which may be half an apple or orange, one whole banana, one corn on the cob, one sweet potato, a generous section of a watermelon or a generous section of a

pumpkin, as an integral part of our environmental enrichment program. The monkeys get these supplements in the late afternoon after they have finished their daily biscuit ration. I don't remember a single case of an animal being adversely impacted by processing, enjoying and ingesting her or his daily fruit or vegetable. There is not much time required to prepare these foraging items and they are easily distributed, but some animals may leave a mess behind that needs a little extra attention when cleaning the cage.

Based on species/age/weight, our monkeys receive quarters, halves or whole oranges on a daily basis. In addition we feed them fresh vegetables and fruits based on seasonal availability—such as bok choy, tomatillos, banana leaves, celery, kiwi, lettuce, cabbage, kale, mustard greens, herbs, turnips, onions, bell peppers, fresh corn, cucumbers, cauliflower, broccoli, carrots, pears, melons, apples, bananas, and grapes—three times a week. Our animals also get a variety of herbs—such as basil, chives, oregano, mint, thyme, rosemary and tarragon—that we grow ourselves all round the year.

We feed our macaques one whole fruit or one veggie per day, depending on availability. I distribute fruits or veggies on top of the cages so that the animals can spend some time foraging, i.e., retrieving and processing the produce.

Each of our 700 pair- or single-housed rhesus and stump-tailed macaques gets one whole apple, orange or banana each day—including weekends and holidays. Group-housed animals in pens receive daily more whole fruits than there are adults in the group so that low-ranking animals can also get their share.

Rather than wasting time chopping the fruits, our caretakers are encouraged to take some time and offer whole fruits by hand to each caged animal. This fosters a friendly, trust-based relationship between the animals and their caretakers and allows the animals to forage.

Your phrase “caretakers were encouraged to take their time distributing whole fruits to each caged animal” warms my heart. Time is what the staff needs, to establish important bonds with the animals in their charge; this time should always be available. Unfortunately, some facilities regard time as money, so animal care staff is often overworked with too little or no time to spend in *nonproductive* friendly interaction with their animals.

< When you provide fruit and vegetables to primates on **toxicology studies**, are there any **precautions** you need to take? >

I work at a CRO with primarily GLP tox studies and the thinking here is that, if the produce has been purchased through an approved vendor—one that sells for human consumption—it is acceptable for the monkeys. Of course, the produce is washed and most of the fruits are peeled as well. Our clients are aware of our guidelines and if there is any concern on their part we will modify our feedings to accommodate the study. As of yet I have not had anyone raise objections to the feeding of fruits or vegetables.

We're also a CRO; about 90 percent of our primate work is GLP. We have an IACUC approved standard section in all our primate protocols that states: “Certified Primatreats and fruit and food suitable for human consumption are also given as a supplement at least once each day and documented.” If clients want to opt out, they must present written scientific justification to the IACUC. In 13 years I've had this questioned only one time and, when told what must be done to opt out, the client said *never mind.*

The majority of our work is GLP and we do supplement feed our monkeys with fruits, vegetables and foraging mix. We have a list of seven fruits and veggies and foraging mix that we can choose from; the amount to offer the monkeys is predetermined. A fruit or veggie is offered on Monday, Wednesday, Friday, and Sunday; foraging mix is offered on the other days. The animals do not receive the same fruit or veggie more than once a week. The fruits and veggies are considered fit for human consumption and we do not wash or peel them before giving them to the monkeys. During a study, the technicians document what is given to assigned monkeys every day.

It is rare for an investigator to express concerns about our supplemental food program. The only time that I recall us modifying the amount or kind of food supplement offered during a study was for monkeys who were having GI (gastrointestinal) issues. If an investigator wants his or her animals excluded from the supplemental food program, he or she would have to submit a written explanation to our IACUC and get the committee’s approval.

< Can any members on the forum share experiences with feeding their monkeys **frozen fruit or frozen juice** as a form of environmental enrichment and, if so, how did the animals respond? Were there any discernible adverse impacts on their health and/or food intake? >

It is my experience that feeding frozen juice or drink mixes to macaques entertains the animals quite a bit without negatively impacting their health and standard food-intake. My only recommendation is to not use red juices or red-colored drinks. You can really freak out your vet staff if they walk into the room and see red everywhere—on the floor, on monkey faces and in drop pans. We bought a bunch of funky shaped ice cube trays. They are silicon so they clean really well in the cage wash.

Our rhesus and cynos get frozen treats very frequently; it's a favorite item here! I use ice cube trays or paper cups and freeze Kool-Aid, juice, yogurt and applesauce mixed with fruit and veggie bits in them. I also freeze chunks of cantaloupe, watermelon, pineapple, banana, strawberry and other fruits of the season for our animals. All of them seem enjoy the frozen stuff which does not affect their health or well-being in any noticeable way. We haven't had anyone who wouldn't eat their regular feed ration due to frozen treats.

Our rhesus and cynos love crushed frozen Prang that I throw into their cages by the handfuls. We haven't had any health issues or eating problems related to the frozen juice and fruits that we give our animals on a regular basis.

Kool-Aid frozen in ice trays and frozen fruits are favorite foraging enrichment items for our monkeys. We have never had any diarrhea or a sick animal due to these frozen enrichment items.

I give frozen certified treats—such as Fruit Crunchies, Fruity Gems and Fruity Bits—mixed with fruit, and veggies in Dixie paper cups at least once a week to our cynos and rhesus. I haven’t had any issues with the animals not liking these frozen treats or with health problems.

Popcorn is one of my favorite types of enrichment for our macaques. It is so entertaining and fun not only for the animals but also for me! Popcorn, unlike most other treats, is low in calorie, which is pretty cool.

We offer popcorn to our rhesus and cynos on weekends in addition to their regular foraging. We have a diet kitchen where we keep our popcorn machine. Usually one of us will make popcorn on a Friday afternoon, store it in a container for weekend staff to distribute. Our macaques love popcorn. It’s a healthy and cost affective treat.

Have you ever considered preparing the popcorn in the animal rooms rather than in the kitchen? Preparing the popcorn in front of the animals is bound to provide highly valued entertainment (enrichment) for them ... and for you!

I'm gonna pop it in the room! Great idea.

I've introduced the hot air-popper here at our facility also. It's the best thing ever. I love seeing the monkeys' (cynos) faces as they smell and watch the corn pop up and out of the machine; then best of all they get to eat it. We bring the popper in the animals' rooms once a week on the day when they also get a TV; it's awesome.

I try to pop popcorn once a month. I usually pop it in the room with the monkeys as they enjoy the sound, smell and visual of the popcorn falling into the bowl. Once it's done, I go from cage to cage and let everyone grab a few handfuls right out of the bowl; it's really fun to watch. Some try to shovel as much as they can into their mouths and others daintily pick a few pieces.

We air-pop corn several times a week in the rooms of our cynos and distribute the popcorn directly to the animals. The whole process is perhaps a bit time consuming, but the animals' enthusiastic response makes it pay off. They attentively sniff the air and stare at the popping machine when the kernels pop and eagerly, first investigate each popcorn they get and then nibble at it, and finally eat it. Popping and distributing popcorn in the animal's room provides great entertainment not only to the animals but also to the attending personnel.

Our macaques get air-popped corn once a week in their rooms; sometimes it coincides with movie time, but not always. Most of the monks like the popcorn; some don't care much about it, however, all of them like to watch the air popper in action. We have *melted* one air-popper, so we have all learned to unplug/turn off the poppers when you stop hearing it pop.

Our cynos <u>love</u> popcorn. I pop the corn right in front of them. They always get so excited when they see the popcorn machine! Sometimes popcorn will fly out in unpredictable patterns to the great delight of the monkeys. I mix raisins or nuts with the popcorn and let them grab their share directly from the bucket. Some of the monks are greedy, of course, and take several handfuls, but then you also have those few who are very picky, sorting through the bucket at great length until they finally find that perfect piece of popcorn; it's very cute! Of course I have to play movies while they are eating their popcorn too! It's a fun time not only for them but also for me. I always enjoy seeing them get so excited and happy.

We offer popped corn in the room several times a week, especially in the cold winter months. Our cynos and rhesus love it and don't seem to tire of it, no matter how often we provide it. We snack on it as well—note, we never eat in front of the monkeys, but we do eat with the monkeys!

Popping corn in the animals' room does provide enrichment in which neither the monkeys nor the attending personnel lose interest over time. It's easy to provide plus it doesn't cost much; the perfect environmental enrichment!

We routinely give all of our primates **cardboard with treats**. We stuff large boxes, paper towel and toilet paper rolls with dry treats and crunched-up magazine paper. Our labs regularly save glove and mask boxes for this monkey foraging-program. To make the treat harder to obtain, I roll the dry treats inside magazine paper and use painter's tape to secure it and then stuff it in the boxes with other crunched-up paper, making the monkeys work their way through a variety of materials to get their treats.

I use empty glove boxes, fill them with shavings mixed with dried fruit and other treats. The monkeys absolutely love them! When they see me coming into the room with treat boxes, they get all excited. Within seconds of receiving the boxes, the monkeys have pulled everything out and proceed eating the treats, leaving the boxes alone for a while. By the next morning, the boxes are completely shredded. Cleaning up the mess is not a big deal for me; it's worth it, since the animals have such a great time with these enrichment gadgets.

I use almost every type of recyclable cardboard item from home and from the lab, such as cardboard boxes, paper towel rolls, toilet paper rolls, glove boxes and surgical mask boxes. The boxes get stuffed with dried treats and then taped. The animals seem to enjoy trying to get into the boxes and often times, if taped firmly, they simply rip enough to get an arm into the box and grab treats. By the end of the day the boxes are torn to shreds!

< Commercial food treats for non-human primates are usually quite colorful. I am wondering why do we **add artificial colors to the treats**? If we do it for the benefit of the animals, do they show preferences for specific colors? >

From my personal experience, I believe the animals do have a color preference. I give our rhesus macaques fruit loops cereal as food treats. When I was a kid, I used to eat them with my eyes closed to guess the flavor. I later found out that the fruit loops have all one flavor despite their different coloration. When I give monkeys the cereal, some actually do selectively

pick out loops of the same color; for instance they would consistently first choose all the loops that are red before taking others of a different color.

We had a female rhesus macaque who, for a period of more than a year, would only eat green items. She had no interest in food treats unless they were green.

There was a female cyno in my care who loved red things in general, not just food treats. This conspicuous color preference came in handy when I had to administer some of the study compounds; when these were red, she promptly accepted them. When relocating her to a different cage, a red toy made her quickly feel at home in the new cage.

< With Easter upon us, I was thinking it would be fun to give my monkey friends some colored **eggs**, but I am not sure if it would be safe to have them perhaps ingest segments of the egg shells. >

I give **hard-boiled eggs** in shells to rhesus and cynos. Most of the animals like them, but we have a few picky eaters who refuse them. Those who like the eggs, carefully peel off the shells. I am not sure if they digest bits of the shells, but even if they do, it doesn't not harm them.

Our macaques get sometimes boiled eggs. for enrichment purpose. At first they were afraid of the eggs, which they have never seen before. However, it did not take a long time before they explored them and learned that they are edible. Most of the animals liked to eat the eggs; some cracked them by throwing them on the floor, others squeezed them apart with their hands. Once the eggs were open, some monkeys ate them right away—egg and shell together. Others ate only the soft egg material after picking it off the hard shell. Since we have no stove on site where we could boil eggs, employees do it at home and bring them to work. Since we take care of about 200 animals and want to make sure that each one of them gets her/his egg, we organize a boiled-egg-party occasionally as a special treat for all the animals.

I remember when wild monkeys (vervets) stole hard-boiled eggs from our breakfast table and ate their treasure with great gusto and no signs of guilt. It was so funny to watch the scene that we did not chase the bandits away but allowed them to finish all the eggs; when they were done, they tried to help themselves to the bread and the bananas, but we told them *It's enough and you better go!* They came back next morning!

I have given raw—yes **raw—eggs** to several hundred cynos and rhesus without any adverse effects and truly believe that raw eggs provide

perfect food enrichment. In their natural habitat, raw eggs are a highly valued source of protein for non-human primates. I did much research regarding the safety of raw egg consumption for cynos and rhesus and came to the conclusion that the health risk is miniscule, especially since most commercially available eggs are ultra-pasteurized and our animals are not artificially immunocompromised.

It's amazing and amusing to watch macaques exploring a raw egg for the first time. Instinct tells them how to handle this treasure. Some use a canine to carefully poke a hole into the shell and then drink the contents; some crack the egg lightly enough to open it perfectly and then first lap the yolk and then the runny and wiggly, yet tasty whites; others shove the whole thing into their mouth, crunch down and savor it; a few get so excited that they drop the egg, but they will lick up every last droplet of the splattered treat.

It's also my experience: Raw eggs are a favorite of non-human primates! I have never seen a monkey turn a raw egg down. However, I have found hard-boiled eggs are not as readily accepted. They get rolled around, smacked about, sniffed and looked at as if they were covered in bitter herbs.

We had some very ill cynos a couple of years back, and they wouldn't eat much of anything. I suggested we try feeding them raw eggs, but there was great concern regarding to the mess that would be made, so providing them was initially frowned upon heavily. Then, one weekend, I snuck in some raw eggs and fed them *ad nauseam*. Those little guys went ga-ga and consumed the raw eggs whole. Everyone was quite surprised how much more energetic they were on Monday. I was then allowed to make 'meatballs' using crumbled chow and raw eggs. Initially I made them like ordinary meatballs, cracking in the egg and discarding the shells; they didn't go over as well as I had hoped. Then, I just crushed the whole egg—shell and all—into the chow and it was like magic. It's now part of my arsenal for primate critical care.

Recently, on our forum, someone mentioned using **paint rollers** with a thin coating of a flour/water slurry that dries and flakes off. This keeps the NHPs busy, picking at the little flakes coming off the roller's wool.

Our animals get the paint rollers with smeared peanut butter, almond butter, fluff, agave nectar, jelly, and other syrups; the monkeys love them. I smear these tasty things on the rollers, then cover them in trail/foraging mix, coconut flakes, foraging crumbles, freeze dried meal worms, dried

fruit/veggies, etc. You can make the device more challenging and last a bit longer by first putting it in the freezer.

I like the paint rollers because they are safe and withstand cagewash; they are inexpensive and easily disposable. You can hang them on the outside of the cage with a short chain to make it even more challenging for the animals to forage, and you can stuff the center plastic tube with treats if you decide to put the device in the cage. I have successfully included them in a large enrichment program for rhesus and pigtail macaques.

I've used paint rollers in the same way as you have described and yes, the monkeys love them!

I also use the paint rollers, usually with peanut butter, sometimes just plain. Either way, the monkeys seem to like them and they spend considerable time manipulating and picking them. I'm in a CRO and I've had no push back from giving the animals paint rollers. I hang them on the outside of the cage with a short chain.

We are using the paint rollers with great success; especially the cynos like this kind of grooming-related enrichment! I take flour and mix it with just enough water to get a sort of pancake-like batter which I spread all over the roller; I let the roller dry overnight. Next morning I will make a monkey happy with it!

In order to keep the animals interested in the paint roller, we don't give it to them every day, and we change the color and taste of the spread periodically. Sometimes we add nothing special to the flour-water slurry; at other times we add seeds or currants. Occasionally, we replace the flour-water mix with plain peanut butter and press trail mix into it.

We've been using the paint rollers for many years and haven't encountered any health-related issues. I have never seen an animal tearing at the wool/fleece or eating sections of it.

We have commercial **foraging boards** for our caged rhesus and cynos. I have difficulties keeping the boards clean, especially when they have leftover peanut butter and seeds stuck in the little crevices of the Astroturf. This can be very frustrating and time consuming!

We don't use peanut butter with the foraging board, because—as you have found out yourself—it's too messy and our animals don't seem to like it all that much. We use cracked corn, white millet, whole wheat, sunflower seeds, and sweet feed—a horse feed — on a rotational schedule. As for cleaning, we just bump the boards upside down into a trash can, line them up

against the wall and high pressure-hose them. Then they run through the cage washer.

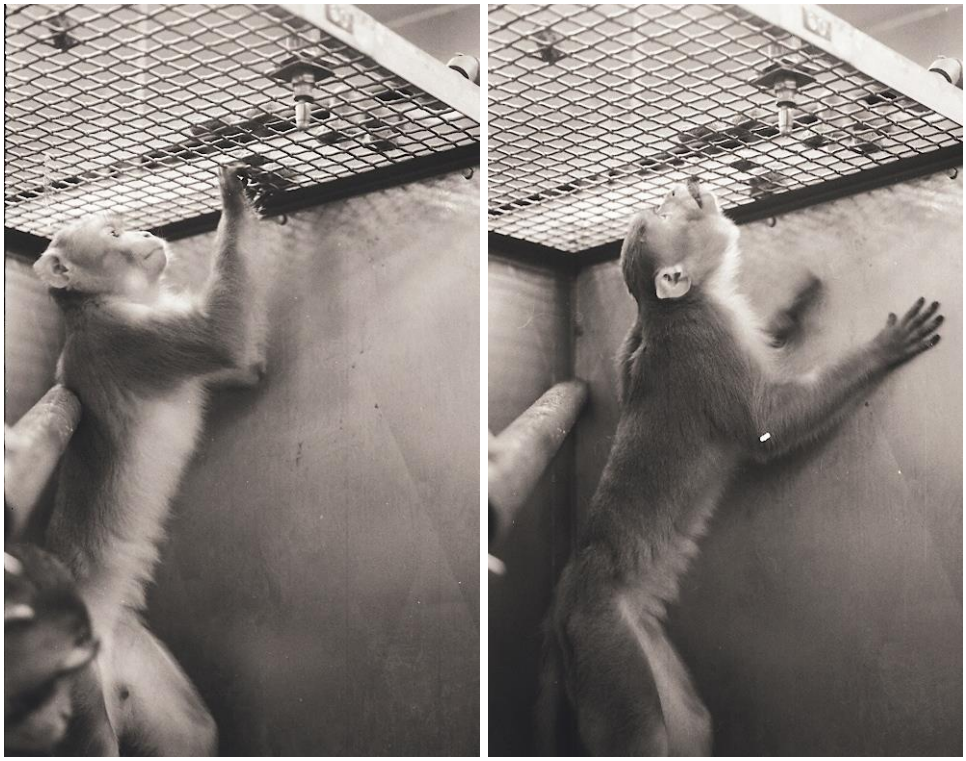
I sprinkle the seeds and other small foraging items on the board, then soak it with water and freeze it. When the foraging surface of the board is frozen, the animals spend a lot more time picking the seeds and crunching on the ice. Our monkeys are having a great time with this kind of foraging enrichment.

Our monkeys get excited when the foraging boards get filled with treats such as oats, nuts and seeds, but they are quickly cleaned out; I think these boards provide little in the way of actual foraging. Puzzle Balls baited with frozen fruit—unfrozen fruit gets foraged and eaten up too quickly—are more challenging for the animals, allowing them to really use their foraging skills to retrieve the food. Altogether, I have found self-made foraging devices to be of greater use than the commercial ones.

< Feeding enrichment for macaques often implies the provision of special food presented in foraging gadgets. It would be less labor intensive and less expensive to make the animals work, i.e., **forage for their standard food** ration. Do any of you present the ordinary standard food to your animals in a way that promotes foraging activities? >

In a previous facility, we distributed the regular food rations of cynos on the **top of the cage** and have the animals manipulate the food through the bars.

I also used the wire mesh ceiling of the cage as a food puzzle for pair-housed rhesus macaques on a routine basis. Retrieving their daily biscuit ration through the mesh rather than collecting it from ordinary food boxes resulted in a 80-fold increase in foraging time for small, bar-shaped biscuits and a 290-fold increase in foraging time for large, star-shaped biscuits. This kind of feeding enrichment does not cost anything but it is very effective; you simple toss the biscuits up on the top of the cages or pens rather than distribute them in the feeder-boxes.



We have been doing this for several years and it's been a great success. It is especially beneficial for the heavier animals who now have to work for their food rather than grabbing it out of the feeder and stuffing it in their cheek pouches all at once!

I took records of 8 pair-housed rhesus males on two different days, during 4-hour observation sessions when 66 bar-shaped biscuits were placed in two standard feeder boxes versus on the top of their homecages. It happened occasionally that an animal unintentionally pushed a whole biscuit or a fragment of a biscuit over the edge of the top of the cage, but it also happened that an animal threw biscuits out of the food box. Food wastage was 1.6% when the animals received their biscuit ration on the cage top versus 0.3% when the biscuits were placed in the food boxes.

We attach one-inch-high stainless steel rings on the top of the monkey cages, place the biscuits in these rings and let the animals manipulate the biscuits until they can pull them through the mesh and eat them. We use the rings to make sure that the biscuits don't fall over the side of the cage and down to the floor. This foraging enrichment is very effective; it keeps the animals quite busy retrieving their food.

We toss the daily biscuit ration of our group-housed rhesus macaques on top of the wire-mesh ceiling of their enclosures. The enclosures are 9 feet tall, which means the primates must climb to the top to access the chow and

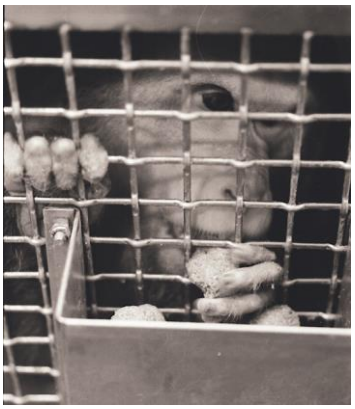
manipulate it through the mesh. The pens are reinforced with a metal edging, which makes it impossible for the primates to push the chow over the edge. If pieces of chow fall down, they drop to the wood shavings of the pen floor.

We too throw the daily biscuit ration on the large chain-link fence ceilings of our two rhesus breeding troops to promote skillful foraging behaviors. The animals do push some of the biscuits over the edge, but this does not really create a mess. When you throw the biscuits directly on the floor, food wastage is probably much higher because of contamination with fecal material and urine. When the animals have to retrieve their food through the chain-link barrier, they eat it rather than drop it on the floor, so there is hardly any food wastage in the pens.

< I've tried to find a foraging feeder that works in place of the regular feeder but I haven't come up with anything useful yet. Any suggestion? >

You may check whether you can simply move the regular feeder away from the large access hole; in this position, the animals would have to skillfully manipulate and pull biscuits through the mesh or the bars. I did this many years ago and implemented it with success for several hundred rhesus and stump-tailed macaques without any extra material expenses. I used structural elements of the cage and turned them into **food puzzles** by





simply moving the standard food boxes a few inches away from the big access holes. This little modification gives the animals a chance to spend about 45 minutes foraging for the daily biscuit ration rather than simply collecting the biscuits from the food boxes in less than a half minute. In fact, they will prefer working for most of their daily ration rather than collecting all their biscuits without effort from an open box or from the floor.

When you mount the food box on the front mesh panel of the cages, the animals will not create a mess. They will work for the biscuits and actually eat them rather than drop some of them on the feces/urine-contaminated cage floor. Working for their biscuit ration, rather than having

free access to it has no noticeable effect on the animals' body weight maintenance.

This simple food puzzle would be a wonderful idea, but our feeders were installed in such a way that many monkeys were able to dismount them and throw them to the floor. As a result of this, the management decided to weld all the feeders onto the cages. So, simply moving them away from the access holes is no longer an option; unfortunately.

To make our permanently attached, stainless steel food boxes more puzzle-like, we simply added a bar in the access opening to make it more challenging for the monkeys to remove the chow.

I turn clear shoebox plastic containers into food puzzles. I cut a hole in the top of the container for loading the chow and attached the box vertically with cable ties to the outside cage front. The monkey now has to work each piece of chow through the cage mesh. Since the feeder is transparent, care staff can easily see how much chow has been eaten. Unfortunately, we have to remove these containers for cage wash.

< Can we please discuss useful and safe **inanimate enrichment possibilities** for macaques? >

I give our 16 group-housed rhesus macaques one **empty cardboard box** once a week and made observations after a habituation period of eight weeks. During the first 120 minutes after cardboard distribution, individuals spend on average 78 minutes tearing the box apart and chewing pieces of it. At the end of the 2-hour observation sessions, the cardboard box is shredded into pieces that are so small that they don't cause problems with the routine cleaning; no clogged drains! The cardboard box has became a standard enrichment item for all our group-housed animals.

When I started working for the macaques of a primate research center, all animals were not only singly caged but their little cages were barren—no furniture whatsoever; it was a really depressing sight! I found this situation disgraceful and initiated the most simple and inexpensive thing I could do: Furnishing ALL 700+ cages with a high red-oak perch and with a red-oak gnawing stick regardless of the fact that it was an unwritten taboo to place unsealed wood into macaque cages. Fortunately, the director of the primate center and the chief veterinarian allowed me to take a risk.

Doug, one of the animal care takers gave me permission to cut the material from dead read oaks of his farm. I spend many weekends cutting thousands of branches with a hand saw .... but it was worth the effort. I

tested the usefulness and safety of the perches and of the gnawing stick, presented the findings at national conferences and published them in scientific journals. At a 1989-conference in Boston, administrators and scientists reached a consensus that unsealed wood can be used as enrichment material for macaques if commonsense is used to keep the material reasonable clean and replaced when it is worn down. In the course of the years I phased out the red oak perches and replaced them with PVC pipes; I simply ran out if big-enough dead read-oak branches.

It is my experience that **wood sticks** cut from dead red oak branches provide inexpensive enrichment for macaques. The animals do not get bored by these sticks which, due to gradual wear and progressive dehydration, keep changing their texture and configuration, thereby retaining novelty. They use these natural wooden objects about 4% of the observed time for gnawing, manipulating and playing, without recognizable health hazards. We toss the branch segments and replace them after one to three months when the animals have worn them down so much that they are almost small enough to pass through the mesh-floor of the cages. The shredded flakes are so small that even large quantities pass through the sewer drains without causing clogging problems.

We have one monkey who enjoys not so much gnawing on the wood, but throwing the stick up in the air and catching it. He hides it, buries it in the shavings, and then finds it again and starts all over. It's a game for him.

Wood is a very popular with all our caged NHP species—rhesus, cynos and African Green Monkeys. The animals get 6-cm long wood sticks that have a diameter of 1-2 inches and have little holes drilled into them to hide treats such as raisins. The animals LOVE to dig the treats out, and then will often try to peel strips off the wood with their teeth as if it were a Polly-O string cheese.

Our macaques LOVE their wood sticks; they really enjoy peeling each and every speck of bark off the sticks. I sometimes smear peanut butter or honey on the sticks to turn them into treats. The best part is, the sticks can go through cagewash! Once they get too brittle or start to break apart, we throw them out and replace them with new sticks.

We have an offsite facility of 200 acres of mostly wooded area. I go on occasions and cut wood sticks and limbs in varying sizes from a variety of trees. Our macaques go nuts over the fresh bits of wood. We have not had any problem with the covered drains.

I give our caged macaques manzanita sticks to chew on and play with. The animals are used on GLP safety studies; we had no research-, health- or husbandry-related issues with this kind of enrichment. Manzanita wood is so hard that the animals can gnaw off only small pieces at a time and the bark does not strip off, so there is no danger of drain clogging when the room is hosed down.

Gnawing sticks cut from manzanita are, indeed, very user-friendly albeit a bit hard for some monkeys, especially the older ones.

Our macaques get manzanita sticks; these rarely need to be tossed because they are very hard and are used by the animals not so much for gnawing but for playing and carrying around. We clean the manzanita sticks in a cagewasher.

Our guys ignore manzanita sticks. I give them softer wood sticks cut from red oak, maple or birch. The macaques—especially the cynos—go ga-ga for them and some turn them to shreds in less than a week! We hose the debris out of the pans and collect them over the covered room drains.

We also use gnawing sticks made from birch, maple, and red oak branches. Our macaques definitely prefer them to the manzanita sticks.

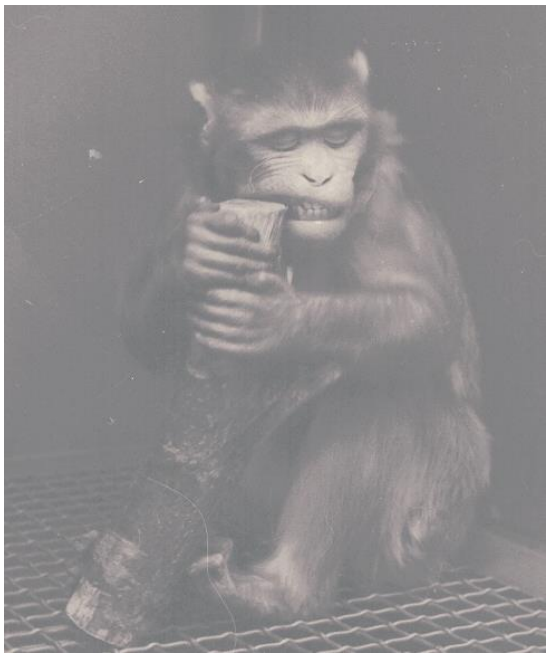
Our macaques have also access to branches and gnawing sticks. All the wood first goes through a quarantine period and remains in a cool dry place indoors for approximately 2 to 3 weeks. We have been giving our animals natural wood for maybe over a year now, with no clinical incidents, but we witnessed a behavioral problem associated with the branches: A juvenile rhesus male decided he was a chimp and chased his mates around the pen brandishing the branch as a weapon.

We encountered a similar complication when one of our rhesus male started using small branches as a beating stick for the rest of the group! We switched to PVC pipes and fence boards because of this brat.

We have used small branches for enrichment with our monkeys and it has been really good for them as natural objects for gnawing. We did encounter problems when we gave the gnawing sticks to diabetic monkeys; these animals often have a real craving for food and eat everything they can get hold of. We had one diabetic monkey who swallowed so much of the shredded branch material that she bloated and died. Needless to say, we no longer give branch segments to any diabetic monkeys.

< When you have made use of gnawing sticks for macaques, has it ever happened that regulatory inspectors raised concerns regarding the **cleanliness of the wood**? >

No, right now we use either red oak or manzanita; when soiled, the wood is thrown out. We are just about to receive a large order of gnaw sticks for the colony.



We have been using wood sticks for our macaques for eight years and, so far, have never had any problems with them during inspections. The wood segments are cleaned daily during the cage cleaning process. I don't remember that we ever had to throw a gnawing stick away because it was unduly soiled, but we replace them regularly before they become so small that they can pass through the mesh of the cage floor.

Our animals have received wood sticks for nine years; it never happened that an inspector raised concerns regarding the wood items in the animals' cages.

< **Wood shavings** can provide great enrichment for macaques, but how do you prevent problems with the sewage drain system? >

Unfortunately, here in this facility for our pen rooms we've not had success with keeping the drains clear and thus have had to eliminate floor bedding. We tried a few things to keep the drains clear, but after several months of having to come out multiple times a week, the campus facilities department basically told us to stop or they would have to fine us.

It really comes down to how diligent your staff can be and what protections you can take to keep things out of the drains. If your staff just hose everything into the drain, it's going to be problematic. So you'll need their buy-in for whatever solution you try with your drain.

I think you're absolutely right about husbandry diligence. If we can convince them to place more items directly into the trash as opposed to straight down the drain, we would have much better luck avoiding drain problems.

We use a dry system for this reason. Husbandry techs will change out those NHP cages every two weeks by dumping the shavings into a bag, and taking the cages down to cage wash areas to clean. The common misconceptions are:

- *The shavings make the cages smell*. I personally haven't really noticed this. It smelled the same when we had the wet system and were hosing down the cages daily.
- *It's more work on the technicians.* It's labor intensive when they have to change out those cages, but at least two technicians are working on it. They aren't changing them every day, so it's just a little more labor intensive on change out days.
- *NHPs eat the shavings.* They don't. I've never seen our monkeys eat the shavings.

Honestly, I love the dry system and having shavings as bedding in the cage to catch the urine and feces. It has expanded our enrichment program tremendously. We place more destructibles (bags, boxes, etc.) in the cages. All of our monkeys have full hair coats—no alopecia. I love just throwing seeds and cereals in the cages during the day so they can forage. They stick their fingers through, and start sifting through the shavings and eating the seeds. We don't worry about the drains at all; a little wire mesh cup inside the drain catches any big material. We have had drain problem with the wet system where the drains would get clogged due to enrichment items and technicians trying to spray those down the drain—which is part of the reason we moved to a dry system.

< Do **commercial toys** have any enrichment value for macaques? >

I have seen some of our monkeys maneuver all kinds of toys through the feeder, until they drop out of the cage, and then try to reach out to get them back again; it's probably an entertaining game for them. The toy itself was of no interest to them other than fitting through the opening of the feeder box.

Some of our monkeys will also push their toys through the feeder and then try to get them back inside. When they do this maneuver, they sometimes lose their grip and the toy ends up on the floor. Others seem to drop the toy on the floor on purpose so that they get the attention of the personnel who will fetch the toy and return it.

I assume that your monkeys find this little game quite entertaining, especially when the attending person is cooperative and picks up the toys over and over again, to the delight of the critter.

We had quite a number of individuals in our rhesus colony who used their biscuits as a toy substitute, and with great perseverance kicked one biscuit after the other out of the food box. Not only that, but the monkeys would attentively look out of the cage and check where the biscuits had landed on the floor. For them, it was all fun, albeit a waste of food.

< **Blankets** and **stuffed dolls** are items that are highly valued by human toddlers, and I would guess also by macaques. I am tempted to give one of our single-caged rhesus male such a doll as a substitute of a companion. He doesn't get along with other rhesus males; will accept a doll? >

There is a rhesus male in our facility, who is very attached to a purple stuffed monkey. He grooms his buddy daily, becomes fiercely protective when the stuffed monkey is removed for cleaning, and even tries to take it along to the restraint chair. Patch never attempts to rip the stuffed animal apart, but acts as if it is his social partner. It's so funny! The technician responsible for his care has to hunt for other purple stuffed animals in order to replace them when Patch has worn them out. Brown and yellow *stuffies* will not do, they have to be purple!

We have a really aggressive single-housed male named Hodor. I would have bet a million dollars that he would attack the doll on sight and therefore didn't try it for a while, until I started noticing how much his demeanor changed when baby macaques were on the TV. He didn't take his eyes off the screen and would occasionally lip-smack; so I did the usual acclimation process with the doll in the room for a week, carrying it around myself and grooming it, before finally holding it up to Hodor's food hopper opening. He lip-smacked like crazy and gently petted and groomed the doll, so I gave it to him. He's had it for a month now, and God HELP anyone who goes near it. He 'cleans' it regularly and carries it around. It's so cute to see such a large intimidating male be so gentle and sweet.

At one of my previous facilities there was a young rhesus who had to be separated from her mother and couldn't be paired with another cohort. She was provided—and she definitely used—a giant stuffed toy. She spent most of the day cuddled with it. The toy was about two to three times her size, so it was easy for her to just completely sleep on it. She was amazingly gentle and never ripped or pulled pieces off the doll.

Many of our rhesus and cynos either shred blankets or *stuffies*, or they are deathly afraid of them. We had a male rhesus who was donated to us for retirement. Ozzie came with a *blankie* that he loved dearly. He groomed it and carried it around. He was extremely protective of *blankie* and only gave it up after he was successfully integrated into a group.

I used to give my single-housed guys stuffed *friends* all the time. Some would tear them up; but others would carry them about their cages, cuddle with them and keenly groom them. I had one monk who would take his little friend along to the testing booth. If he forgot to bring it along, he wouldn't do the test! I would have to go back and fetch the friend for him. After a few times I learned to just make sure we had the friend before leaving the main room; it was easier that way ;o)

When we use stuffed animals, we turn them into *flatties* before we give them to the macaques; we remove the stuffing inside first. I can say that about 3/4 of our macaques love *flatties*; they tear them up and make a nice mess. The other 1/4 of our macaques never damage or tear their *flatties*; they enjoy holding onto and snuggling with something soft.

Instead of a *flattie*, we sometimes use a piece of polar fleece material and tie a big knot right in the middle of it. The animals love hanging on to it, dragging it around and cuddling with it.

We don't sanitize the doll. It stays in the cage together with the monkey until it is deemed gross—which takes months because it is getting groomed/cleaned very often—and then it is replaced with a clean one.

We have used stuffed animals with macaques for several years. There were never issues with ingestion of the stuffing causing troubles. We re-used the toys by either washing them in a washer—with towels and drapes—or by hosing them and then running them through the rack wash cycle. As long as they are kept flat while washing in the rack washer and then dried either flat or in a dryer, they hold up remarkably well.

We did always remove any eyes or other decorative additions from the toys since those could be easily removed by an animal and then inadvertently (or purposefully) ingested.

Monks will tear things to bits, stuff the occasional new substrate in their mouths to fiddle with, but never ingest anything foreign. I think they just like experimenting with the mouth feel.

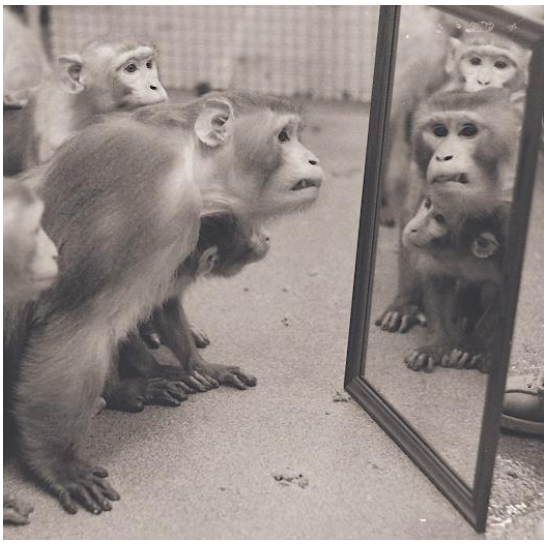
I learned this when I got a shipment once and after uncrating, I noticed one of the guys had swiped a staple on the ride. He twirled that two

inch sharp object around his mouth and cheek pouches for hours, would occasionally remove it to inspect it, but shove it back in. I was so worried he was going to swallow it and puncture an intestine. But, at the end of the day I found it in the drop pan. Guess he got bored with it!

< Can anybody of you share first-hand experiences on the usefulness of **mirrors** as enrichment objects macaques? >

All of our single-housed cynomoglus macaques have mirrors mounted on swivels that are attached to the outside of their cages, low enough so that an animal can chose to either bend down and intentionally look into the mirror or to make no extra effort, hence not being confronted— bothered?— by the mirror reflection. Our monkeys use their mirrors frequently.

I tested the response to a mirror in group-housed rhesus macaques. The animals' facial expressions left no doubt that they related to the reflection in the mirror. The male in the attached photo (left figure) tries to impress the other male, but he isn't quite sure of himself (right figure).



Our rhesus love mirrors too. They like to check us out by looking at us through the mirror. I guess they don't feel so threatened when they can look at us without being seen. They also check out the room, by looking at the reflections in the mirror. We have one male who never looks at people directly, but holds up a polished stainless steel mirror to watch people who have just entered the room. Of course, we named him Mirror Man.

We hang stainless steel mirrors right into the cages of our cynos. Some monkeys will cling to them and look at them for long periods of time, often lip smacking or making other facial gestures, while others will threaten

their own reflection and bang the mirror onto the side of the cage. There are a few animals who attack their own reflection in the bottom of the stainless steel cup when it is empty. It's quite hilarious!

Most monkeys use their mirrors to look around the room at other monkeys or at people, whom they could not normally see. I assume that the animals feel more at ease when they can avoid direct eye contact with personnel and other monkeys, yet can observe them without being noticed. It's fascinating to watch them moving the mirror in the right position so that they can look at a person, who is not in their field of vision.

We have found an acrylic sheet mirror that we can cut into different size pieces. Some get hung on the walls, using double sided tape, while other pieces get hung right inside the enclosures, using zip ties. We also cut small pieces and give these directly to the primates. Our rhesus macaques often combine the wall and hand mirrors to get extra viewing advantage! It's really fun to watch them. The acrylic leaves no sharp edges when it brakes; this means it is safe for the animals. We never encountered a problem.

< If you give your monkeys **Stainless Steel Balls** for environmental enrichment, how do the animals make use of the balls? Do you leave them in the cage all the time? >

Our macaques love these reflective balls! They enjoy watching themselves in the shiny mirror covering; they also like to push the ball back and forth on the floor, apparently enjoying the wobbly effect from the sand inside the ball! But like anything that is not alive and not edible, the animals lose interest in them after a while. When we notice this, we take the balls away and give them back a few months later, and the animals love them again!

We give the Stainless Steel Balls out in a rotation as well. The animals get fascinated when they are new and shiny. They hold them and shake them—as they contain a bit of sand—and look at their reflections; they also sit on them. They really like the balls.

Our monkeys also like them. It's fun to watch them push the balls and watch their faces when these roll back. Before long, the animals start to get bored by the balls. We, therefore, rotate them just like all the other toys with cage change out. This implies that our monkeys get their Stainless Steel Balls once a month for a period of two weeks.

These balls are not that heavy, and I catch our cyno pairs throwing them around all the time. They can get quite dull-looking but a good

cleaning makes them shiny again; that's how the animals like them most. We have encountered no injuries associated with the balls.

I currently have an adult male rhesus who loves his Stainless Steel Ball so much he tries to take it everywhere with him. I ran into problems with this obsession when he would bring the ball along into his training chair and hold it very attentively with his feet. He was so distracted by his toy that he was no longer able to concentrate on the computer task he works with. We finally had to attach the ball with a chain to the inside of the cage so he can cuddle with it at home, but he can no longer take it along to the training chair. When he is on *vacation*, we take the chain away and give him the ball so that he can carry it around and do with it whatever he pleases.

We also had a male rhesus who was very attached to his reflective ball. It was rather cute; he would take it to the corner of the cage with his biscuits and eat with it. After we found a compatible cage mate for him, his obsession for the ball abated.

< How do you introduce an **iPad** to caged macaques? In the beginning they are probably apprehensive or even scared to be exposed to such a strange gadget. >

I'll bring the iPad into the room for a few days, and play with it myself. When the animals show some interest or curiosity, I'll point it to them so they can see it. I find pictures are best for the intro phase; pictures may be interesting but not intimidating. Then as curiosity overrides their neophobia, I bring the iPad closer to them and allow them access to it via the food hopper opening. When they touch the screen I say *Good job!* If they

lose interest in the pics, I open Fruit Ninja, because the slow action of it draws and keeps their interest. They often try to look behind the iPad to see where the fruits are going; kinda funny!

We use iPads at my job all the time because our entire system is computerized. Our iPads come in OtterBox cases, so they are very easy to disinfect and are well protected from impact. I had the Netflix/Prime set up on my iPad just to make sure it worked in the room, and noticed all the NHPs staring at the iPad. I held it up to one of the males via the food hopper opening; he very gently explored it. In the beginning, he was watching movies on the iPad. To try some other stuff, I downloaded Fruit Ninja for free, and he was ENTHRALLED. He kept pulling the iPad towards him so he could see behind it to figure out where the flying fruits were going; LOL! I showed him how to swipe them; he copied the action almost immediately! He was so funny, swiping the fruits and *HOOing* in excitement. Later I tried baby tapping action games, but he had a really hard time with aiming and tapping. His dexterity just couldn't handle that. Swiping was much easier for him. I had a bunch of pics and videos in my iPad of other NHPs in the facility for him to swipe through; one video showed the male cyno pair Biggie & Tupac; Biggie was very dominant. I thought it was interesting that this male would swipe to Biggie's picture and then lip-smack and present his hind end to him in the iPad! I showed him Biggie & Tupac repeatedly on different days; his behavioral reaction was consistently the same. It was rather hilarious to watch his display.

< We have been using **television and videos** for visual enrichment of our macaques for years. I was wondering what you all do at your facilities for visual stimuli for your animals? >

They used to play cartoons at our lab. The monkeys couldn't care less most of the time. The enrichment team did not give up and took a bunch of videos of the outdoor monkeys and played those for the indoor monkeys: the monkeys were fascinated! They would watch intently, sometimes lip-smacking and grunting, and sometimes threatening. To watch other monkeys on the screen was very entertaining and engaging for them.

Our rhesus macaques respond just like yours; they love the vids of other macaques, but couldn't care less about cartoons.

We recently showed one of our macaques a YouTube clip of a show called Monkey Thieves, that is based on rhesus macaques in India. He absolutely loved it, lip-smacking at the screen and very intently watching; it was great!

I purchased the documentaries Clever Monkeys and Romeo & Juliet: A Monkey's Tale for our macaques. Of all of the movies my guys can watch, these two are definitely the ones that captivate them most. They must love to see the monkeys in Thailand breaking into homes and running away with sweets; it's funny to see them getting so excited while watching the movies.

Our favorite in-house video is an old special on the torque macaques in Sri Lanka which contains bonus clips of monkeys stealing things—like pies—from window sills in India. Our rhesus macaques watch the video with full attention and lots of chattering as if it's the best thing in the world to do!

Many of my monkeys exhibit a definite interest—through orienting toward the TV screen—in the Lion King series, Madagascar, and other Disney films. We have a group of cynos who all chime in and sing along to the scene in Cinderella when the mice are singing while making the dress *Cinderella, Cinderella, Night and Day it's Cinderella!* Some of them sound really good and seem to know the melody note for note!

I have one rhesus male who sits back, puts his feet up and munches on a biscuit while watching movies. He needs a Barcalounger! He really loves Elmo. LOL!! You seriously can't pull his attention away from it when it's on. He's the only one I've seen respond that way to movies though.

We provide our single-housed cynos with **balconies**. Balconies serve as a look out allowing the animals to see more of the room and visually interact with more individuals. We videotaped 18 single-caged cynomolgus macaques who had access to a stainless steel-mesh balcony attached over the open doors of their cages for a period of one week. The balconies were used for an average of 75% of the daytime.

How is the balcony attached over the open cage door?

They're fairly easy to attach. There are hooks that line up in the grids of the cage at the top and a latching mechanism on the bottom. It's just a matter of lining up and lifting. The door to the cage can then be slid open and should be locked open to avoid the monkey trapping himself in the balcony. We also use locks as an extra measure of security to attach the balcony to the cage. You can unlock and slide the cage door closed when you need to remove the balcony. Unfortunately, they do need to be removed to access the animal or to put anything in the cage that won't fit in the food hopper. However, I would say it takes less than five minutes to get one back on and locked into place.

We have Carter cages with balconies built-in to the top of each cage, as well as the detachable ones that attach to the bottom door. We have a few troublemakers who will sit in the *bump out* (as we call them) and shake them to try and dislodge them, so in addition to locking the door open to prevent them getting stuck in there, we also use a padlock to attach the bump out to the main part of the cage. It does not interfere with catching animals or placing them in chairs for our work. The monkeys are removed from the upper door of the cage and often will use the bump out as a step on the way into or out of the chair.

When we got these new cages, it was immediately obvious how much the animals enjoyed the upper balconies. I remember looking in the peephole of a room that had just been changed over and every single animal was in the upper balcony of the cage. They do love these high lookouts. Even now (3+ years later) most animals will prefer to sit there.

< Based on your own experience, what are practical options for making use of **water** as environmental enrichment for monkeys kept in standard cages? >

We have started doing this with some of our singly-housed guys—mostly adult male cynos and a few young-adult male rhesus. We use horse-feed tubs (relatively flexible thick plastic tubs, about 18 inches in diameter), fill them with a few inches of water and toss in a handful of foraging mix or baby carrots. This is placed on the bottom of the cage once weekly (removed after ~24h). Most of the cynos enjoy picking the treats out. I haven't seen them splashing around.

I've just started introducing water enrichment to our guys. I am using the cage bottom of a mouse cage as the bucket and fill it with about 3-inch-deep water. Chunks of floating produce are added along with some raisins. So far I have exposed three adult cynos (one single animal and one pair) to these little pools.

The two paired animals stayed away from the water while I was in the room. When I came back 4 hours later, the two had eaten all the raisins and all the apple pieces and had dumped the water out into their waste pan.

The third, temporarily singly housed cyno, came to the water right away while I was standing there watching him. He splashed with the water, retrieved and ate all the apple pieces and raisins and kept dunking his head into the water. When I came back 4 hours later, he had done the same as the other two, i.e., poop soup!

I will say that emptying the big waste trays with all the water in it was cumbersome at best. I'm wondering if even less water will have the same enrichment effect; otherwise husbandry is going to hate me, LOL!

We also use clean shoebox-style mouse caging, fill them with water and put them inside our 4-bank caging. I add floating toys or produce. The monkeys love it!!! Our juvenile rhesus macaques have been seen splashing in their 'pools' which are, however, not big enough for them to actually get in.

Just a bucket of water will do for our cynos. It's fun for them; they splash with the water, climb into the bucket and dunk their heads into the water. There is not enough room for them to actually swim but I definitively think that a big bucket filled with water provides great enrichment for caged macaques.

We give our pair-housed cynos bathtubs filled with 30 to 40 cm deep warm water, a few times a week, and have never encountered any problems other than a lot of splashing. Some monkeys take luxurious baths, others climb a perch and jump into the water, others sit on the side walls and drag their hands in the water, and others wash their fruit in the water. Usually the monkeys make a real mess within the first half hour, and yes they do urinate/defecate in the water. We empty the tubs after about two hours, if the monkeys haven't done it already themselves—which is often the case.

< The center where I work has several cyno breeding colonies housed in large outdoor enclosures. I am interested in using stock tanks to provide **swimming opportunities**. I have heard that cynos are adept swimmers, but is there a risk of drowning, particularly for infants? Is there danger of one monkey inadvertently drowning another monkey? >

The stock tanks we use have a lip half way up the inside of the tank, so if an infant fell into the water it could easily get back out. We used these tanks all of last summer and half of the summer before without ill effects. The monkeys who do go under water hold their breath for a surprisingly long time. I have watched juvenile cynos swim with no difficulty. I have never come across one who can't swim. It seems to be an inherent skill they don't have to learn.

Our indoor group-housed cynos get access to a 5 x 5 feet (1.5 x 1.5 m) kiddie pool several times a month. The water is about 3 feet (1 m) deep. The animals pick up small floating treats from the surface of the water; they swim and dive for grapes and other fruit items. They like it very much. We

exchange the water as needed and have encountered no hygienic problems; also, it has never happened that an animal got harmed in any manner in the

We had a near accident in the little swimming pool for our cynos, when an adult female was swimming underwater and a big male started playing around, like a cat chasing after a mouse, from outside the pool and, finally, jumped on the female's back. He put his hands around her neck and appeared to be deliberately holding her under in the 1 meter deep water. After about 15 seconds, I panicked, since I thought he was actually drowning her. I rushed to the scene to interfere, but just at that point he released her and retreated. She shot out of the water like a rocket and was <u>really</u> angry with him, screeching and with rage in her eyes. He looked surprised, and like he had made a significant error, ran screaming away from her as she chased him down and bit him a good one and repeatedly slapped and pinched him. The whole time, he was acting submissively toward her, lip smacking wildly, and ducking as she continued to clobber him.

The two have been in the pool together many times since then, but the female never takes her eye off the male even when she's under water (as cynos dive with their eyes open).

< I am in the midst of trying to expand **enrichment options at a tox facility** and would love feedback on what is offered at your facility(s). Right now we have a pretty strict and limited list for our tox NHPs, but an endless list for our other colonies. >

I work in Tox/GLP and our monks are given just about any produce; we did stop beets as the stool was just too red with it, but they are given tomatoes and strawberries for regular daily enrichment, and dried cherries, cranberries and strawberries for training (I think that's all our red things). We have pretty much free rein with produce and finally got permission also for cereal, other dried fruit mixes, nuts and mini marshmallows for training only. We give lots of toys, all certified except the wood, which is now even allowed for GLP studies. The vets felt wood was an important enrichment item after reading articles about it. They asked the study directors to okay giving it. We use sanitizable manzanita wood. The animals just love wood so much!

We also use wood in our tox facility for our monkeys. It is a hardwood, but I am not certain exactly what type. We give it out in the cages and also have them drilled so that they can hang outside, with foraging holes stuffed with small food items.

Our main restriction in our GLP environment is citrus fruits. Produce that is safe/intended for human consumption is typically allowed for the animals. The scientists are expected to specify restrictions and justify why a particular food item is not allowed. Wood products have been accepted because they are sold as being certified.

< How important are **elevated resting surfaces** for macaques? >

In their natural habitat macaques spend the night on high resting surfaces as a safeguard against ground predators. This biologically inherent need for elevated resting sites is alive also in captive animals. Our group-housed rhesus macaques have access to perches at about 1.2 m off the ground. On some occasions, I have checked on them during the night and have always found them **sleeping on the perches.** I have never seen them sleep on the ground.

We frequently observe caged animals for 24 hours, using a remote camera system at our facility. Our cynos are pair-housed; during the night, companions always sit together on a perch, never on the floor.

We have also used video cameras for overnight observations. Our paired cynos also always sleep huddled together on their perches. The perches consist of three parallel, ~3/4-inch-diameter bars with a combined width of ~5 inches mounted from the front to the back of the cage. I have occasionally seen the animals leaning on the side or back wall of the cage while sleeping, but most of the time they are leaning on each other. I have never observed our monkeys sleeping on the cage floor. Our cages are also slightly unique in that we do not confine animals restrictively on the bottom quad. All animals have full vertical access permanently. And even though there are identical perches in the bottom half, I have never observed them sleeping on them.

A **low perch** has little or no value as a safe resting location from the monkeys' point of view. Yet, the placement of resting surfaces at a too low level is legally condoned by Animal Welfare Regulations (United States Department of Agriculture, 1991, page 6499) and also by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, page 26). Both texts have included the following clause: "Low resting surfaces that do <u>not</u> [emphasis added] allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space." This loophole is probably the reason why built-in perches or ledges are usually installed at a height of only 20 to 30 cm, regardless of the fact that such low resting surfaces not only have little value for the animal but

they also block part of the minimum floor space of standard cages that is required by an animal to turn around freely and make normal postural adjustments.

One of my students compared the effectiveness of **PVC swings versus PVC perches** as environmental enrichment objects for 14 adult female rhesus macaques who lived in upper-tier double cages equipped with a PVC perch in one half of the cage and a PVC swing in the other half of the cage. The animals spent an average of 10.8 percent per 60-minute observations on the perch but only 1.4 percent on the swing. The preference for perches was probably related to the fact that perches, unlike swings, are fixed structures permitting continuous relaxed postures rather than short-term balancing. Moreover, perches unlike swings make it possible for the animals to sit right in front of the cage with optimal visual control of the environment outside of the cage.

In the small standard cage, a swing cannot really be used for swinging —there is just not enough room for that—but macaques typically use them to produce a lot of noise, by slamming the swing against cage walls. This is perhaps a great acoustical enrichment for the animals but certainly not for the attending staff!

It has also been my experience with pig-tailed macaques that the animals—even juveniles—do not benefit from swings when housed in 'small' standard cages; they simply don't use them.

We used to hang numerous swings and movable raised structures into the enclosure of our group-housed cynos, but we could see very clearly that they prefer the stable perches or platforms. Our animals very rarely used ropes or swings. The only ones using those elements were babies and juveniles.

I conducted behavioral observations in two large rhesus breeding troops; the pens were provisioned each with a big platform along with several branch swings. Even though I didn't collect data on that issue, I can say that the swings were used very often by young animals but only rarely by adults.

Dexter et al. (1994) noticed in single-housed rhesus macaques that subjects manipulated the swings but showed little inclination to actually use them for swinging. Phillippi-Falkenstein (1998) observed pig-tailed macaques in large living quarters and found that swings were primarily used by young animals, while adults rarely used them.

< When macaques are housed in a **double-tier caging** system, are the animals affected by the level of their cage's position? >

Given the fact that macaques are arboreal animals who avoid ground predators—such as humans—by spending all night and most of the day at elevated sites, and who flee from ground predators by climbing up into trees, it is unlikely that they feel particularly safe in **bottom-tier cages.** Based on my own experience, I would even argue that the majority of bottom-tier caged monkeys feel cornered when a person whom they cannot trust approaches their cage. Some rhesus males get so frustrated in such a situation—they can neither flee nor can they attack—that they bite themselves to the point of self-laceration.

When I managed a colony of macaques, I hung my hat on the generalization that the animals are arboreal, and I still feel that most prefer high places. Back in my import/export days it was noticed that if you put adult male macaques in bottom-row cages on arrival they would often become depressed—poor activity, poor appetite and withdrawn. Move them up top and they would snap right out of it. Height is status!

The problem is that the design of the double-tier caging system is based on human convenience and cost mitigation rather than species-specific behavioral needs of the animals who have to live in bottom-row cages.

Working in primate facilities for quite a number of years and visiting numerous primate facilities in different countries, I got the impression that personnel tend to give more attention to animals in the top rows than to animals in the bottom rows of the cage racks. If this is correct, the two-tier caging system—the prevailing caging arrangement in the United States—would bear an important variable that could affect not only the health status of the animals and the hygienic conditions of their living quarters, but also their stress response to being handled by personnel.

I would agree with your observation. Unless there is an animal in the lower tier who is particularly outgoing, the same amount of attention is not given.

It is my experience that males and heavy females tend to end up in lower-row cages for obvious reasons: It is not so hard on your back when you remove a heavy animal from a bottom row, plus it is easier to force a big animal to exit the cage into a transfer box when the animal is housed in the bottom row. Animals in the upper tier tend to flee from you upwards—not into the transfer box—while animals in the lower tier tend to escape from you right into the transfer box; that's their only route of escape.

I have witnessed more than once that bottom-caged primates with health problems did much better when we transferred them up to a top-row cage.

Cages in the top row provide much better **illumination** than cages in the bottom row.

Unlike the cages in the upper tier, those in the lower tier have very little direct light. This makes health observations more of a challenge. I agree that bottom-caged animals do have a disadvantage when it comes to the daily health checks of the attending staff or vet.

It is not uncommon that attending care personnel make use of squeeze-backs to push monkeys in dark bottom rows to the front of the cage to facilitate routine health checks and correct reading of the tattoos.

When I worked at a preclinical toxicology facility, flashlights were a necessary component to daily health checks for the cynomolgus macaques. Those lower-row cages were very dark—like caves. I am not sure what is more stressful for a monkey, being pulled forward with the squeeze-back to have the ID tattoo read by a person at very close quarters, or having a light shine in your face by a human once a day. I think this circumstance provides evidence against the use of double-tier caging.

It's almost ironic when considering the fact that Animal Welfare Regulations mandate for the Humane Care of non-human primates, "Lighting must [emphasis added] be uniformly [emphasis added] diffused throughout animal facilities to aid in ... adequate inspection of animals, and for the well-being of the animals" (United States Department of Agriculture, 1991, page 6498). Keeping non-human primates in the traditional two-tier caging arrangement makes it impossible to follow this unrealistic mandate.

In a survey of 96 primatological articles, I found that the cage location of research-assigned macaques was not mentioned in 98% of cases, in spite of the fact that the environment of upper- and lower-row housed animals markedly differs in terms of light quality, light intensity and living dimension. Not accounting for these uncontrolled variables may increase variability of data and, consequently, the number of experimental animals needed to obtain statistically acceptable results.

I feel that the outdated **two-tier turkey caging system**—dating back to the time when large numbers of monkeys had to be accommodated in laboratories for polio vaccine research—is counterproductive both in terms of animal welfare and scientific methodology. It's time to get this caging system phased out not only in Europe (Council of Europe, 2002) but also in other countries, especially the United States.

It may be difficult to completely phase this caging system out, as space is at a premium, especially in a country like the U.S. that keeps large numbers of monkeys in research labs. Researchers defend the prevailing two-tier caging system not for scientific but for economic reasons; it certainly is cheaper to keep 100 monkeys in a two-tier system than in a single-tier system.

As a compromise solution, we rotate the animals from top to bottom during cage transfers. When we first implemented the **rotation schedule**, there was a lot of push back from the techs, so we had our facility department fashion a mobile tunnel connection between lower and upper cages to make the rotation process less strenuous for the personnel.

Rotating the animals does not solve the problem; it literally rotates it. Even if you take the trouble to rotate your 100 animals on a regular basis, there will always be 50 of them who are forced to live in dark bottom-row cages. This is not a satisfactory solution.

< Does anyone have experience with **phased lighting** in macaque rooms? The abrupt change of the lights going on and off might be a daily disturbance for them. >

From my experience as an Animals in Science Inspector most facilities in the UK have phased lighting. The phasing of light intensity (early-morning dusk phase and late-evening dawn phase) is meant to be less stressful and startling for the animals.

We have a staggered light system for our rhesus macaques. At 6 a.m. only half of the lights are turned on. Shortly before our technicians enter the room to do their morning checks, they turn on the remaining lights. In the late afternoon the techs turn off half of the lights after they have checked the animals, and they turn off the remaining half of the lights at 6 p.m. We also have large windows on our doors, so there is some light filtering in after lights out. I don't have any info on the impact on the animals, but it is a relatively simple procedure that can avoid the drastic light change.

Recently we conducted a study that compared traditional lighting to that which replicated dawn and dusk phases with two rhesus and seven cynos. We observed, documented and compared behaviors with both lighting systems and found that with the gradual lighting system, all nine macaques were significantly calmer and more relaxed. They all appeared in much better moods especially in the morning during feeding.

In addition to our behavioral observations, we monitored the animals' cortisol levels throughout the entire study. We used saliva cortisol samples

to avoid possible stress reactions associated with collecting blood. The endocrine data mirrored very clearly our behavioral findings. The saliva cortisol concentrations were significantly lower during dawn and dusk phases compared to the same time when the lights were instantly switched on and off. Every one of the nine test animals showed this difference in cortisol concentrations. The results were truly amazing.

Something that has traditionally been completely overlooked in the scientific literature—namely instant versus gradual change from day light to night darkness—can have such a tremendous impact for captive macaques. I imagine myself sleeping peacefully in the dark and all of a sudden bright lights come on and someone says *Time to make the donuts*. I'm saying *ugghhh* at that moment versus feeling a warm sunbeam across my cheek and hearing birds twilling in the distance; no bright lights in my eyes, stretching and waking up at my leisure; I get it.

< When you work with rhesus macaques on a regular basis, you may develop **affectionate relationships** with individual animals and want to touch, stroke or groom them. This is a very nice experience, but it can be dangerous if you misunderstand the subject's feelings and motivations. How do you know for sure—and you must be sure for your own safety— that an animal wants to be touched, stroked or groomed by you? >

There are several female and male rhesus of various ages at my facility who will seek out our attention from within the cage and **solicit to be groomed**. Some will place their entire arm out of the cage and let you groom them for several minutes, while others press their chest or hind legs against the mesh of the cage front hoping that you touch or, even better, groom them. I believe these particular animals enjoy being groomed by humans as they (a) seek it out, (b) relax as the grooming goes on, and (b) will start grooming my jacket or latex gloves after I've groomed them for a while.

Some of our rhesus macaques—both females and males—seem to really enjoy being groomed by humans. They will approach the front of the cage and present different body parts for you to groom and then rotate so that you move on grooming another part of their body. They get that very typical glazed, blissed-out look—eyes at half-mast and completely relaxed body. This kind of human-animal interaction is enriching for both parties involved, the animal and the tech who does the grooming. Some of the rhesus males like to be scratched on their heads and necks while sitting in their restraint chairs with blissed-out faces. I worked with a large male who, while sitting in the restraint chair, would rather have me scratch his rear and

hips as a reward than getting juice or treats. Similarly, a younger cyno female had me regularly hold her hand while chaired for blood draws.

I have one girl, Meera, who loves to have her bum rubbed and her face groomed. She actually asks for it by presenting herself. This is a very clear signal that the animal is not afraid of you and wants you to come closer and, as in this case, start a grooming session.

We had another monk, Kiwi, who absolutely loved human contact. She would pretend to be asleep after a procedure, so that I would hold her longer before placing her in the recovery cage. I used to watch her squint her eyes slightly open to see what was going on, only to quickly close them if someone was looking at her!

The key signal that tells me that a monkey likes to be touched is when she or he presents, i.e., entices me to do so. A chair-restrained rhesus monkey, for example, will twist her body in an attempt to present her rear, thereby letting me know that she wants to be groomed. Under such a condition, the monkey will show no fear or aggressive-defense reactions but rather be relaxed and calm.

Currently, there are two aged rhesus males who compete for my attention when I am in their room. First, the more dominant partner will put his rear end up against the cage bars and, when I approach, will settle down so I can groom his entire back. The other partner will then saddle up to the front of the cage and present his body so that I can scratch his chest/belly/face and other parts of his body—he's even presented his tongue to me. When either of them is really happy, they'll go into that trance-like state and have a glazed look; the less dominant guy has even sighed a couple of times while I groomed him. There are days that, once I get done with these two, the rest of the room—all aged rhesus males—start presenting body parts for grooming.

I remember a female pigtail on a viral tox study who wouldn't show friendly behavior towards people other than lashing out from time to time, but for some reason she really liked me. I never figured out why, but whenever I entered the room, she would stand, hoot, and duckbill in my direction until she got my attention. Then, when I would walk to her cage, she would calmly settle and present her hips to me for grooming. We got to a point where she would reach out of her cage and attempt to groom me. I really don’t know why she has only taken a shine to me and not to other staff members.

I know quite a number of rhesus macaques who will present their chests, only to get mad when you touch them.

What you describe is a quite common scene in primate labs. I believe that, what the animals are doing is **teasing**. These guys are bored in their cages—who wouldn't be?!—and they are looking for some action. They present their chests or rear ends to the naïve caretaker or visitor, knowing beforehand what the reaction and the outcome of this little game will be. You have hardly touched them, and they will turn around, bang against the cage wall like a devil and/or threat-yawning like a lion. You may be shocked and react accordingly, and that's what they are after: your reaction to their display. Your reaction will reinforce the teasing. Once you no longer participate in this game, you will no longer be invited to groom.

Some monkeys don't like people for legitimate reasons; they will use the grooming invitation to tease you. Our Holly is such a brat. She's clever and will lure her victim by deception to get close enough to accomplish her mission. Once you are in her reach, she will perform that lightening fast turn, pinch you and give you some attitude. I am sure Holly enjoys these little attacks and the victim's surprise reactions.

Your story reminds me of a male rhesus who lives in a top-row cage and will pee on you when you add an enrichment device to the cage below. I don't think it's funny but he probably does. You have to watch out for him; he will kind of causally put his hand in the urine stream directing it straight to you. You will feel little sprinkles on your scrub pants and look up and voilà: he is getting the attention he was looking for. Pretty smart guy!

< Let me share a funny story with you: **Thieves by nature.** >

It was a warm summer day that started out like most. But this day, my husband had to go to a meeting nearly 70 miles from the sanctuary and didn't expect to be back until evening. This was one of two days a week that we didn't have volunteers scheduled so I was on my own for chore duty. It was early morning, about 6 o'clock. This wasn't a problem as I was more than able to complete the daily tasks on my own. I thought this might actually be a relaxing day for me, as the day before every enclosure had been deep cleaned and there wasn't really much to clean up that day anyway. After feeding, I planned on cleaning the special needs area and then spend a few quiet hours interacting with the 'primitives'. After that I might read a book or watch TV; maybe I could catch up on computer work.

I left the house with the monkeys' food bowls, my laser light, and a pocket full of treats. As always I locked the house and drove up the hill to the macaque buildings. The macaques greeted me with the usual array of

hoots and grunts, anxiously anticipating their breakfast. I hadn't eaten my breakfast yet, as I always feed them before myself. I was rather hungry, looking at all the lovely food in their bowls. Fresh mangos, apples, sweet oranges, grapes and even the tamarind sparked my appetite. As I passed out the breakfast bowls I could hear grunts of satisfaction and approval. That always brings a smile to my face and makes me laugh. What a great way to start the day!

Everyone had their breakfast and I could hear the crunching sounds of food being eaten all around me. I remember thinking how fun it would be to make an audio recording of macaques eating peanuts, fresh carrots, and other foods that are noisy when being processed and eaten by them.

I was indeed getting hungry so I started to leave the area, but when I did, Eve, a juvenile cyno, quickly approached the front of her enclosure and extended her arm out toward me, begging for a quick groom from her human. I stopped to appease her and sat down on the ground in front of her enclosure. Eve is such a sweet girl! She reached out and ever so gently began grooming my arm with her right hand, and as Eve always does, firmly gripped onto my sleeve with her left hand so I wouldn't get away until she was finished. This always makes me chuckle.

I was about to end our grooming session and I think that Eve sensed my intent to stand up. It was at this moment that she noticed my keys on a ring that were barely visible in my pocket. Or perhaps she had known they were there all along. The rule is that nothing, absolutely nothing when near the macaques is to be kept in pockets or anywhere else on a person's body that one is not willing to share with the macaques. They are all thieves by nature and much quicker than any human. But I hadn't planned on stopping to visit until after my breakfast and I simply forgot to remove the keys.

There I sat on the floor, watching Eve proudly examining my key ring on a high ledge furthest from the front of her enclosure where I helplessly sat. She had my key ring that had not only keys to every macaque's enclosure on the property, but my car keys and my house keys. My phone was in the house. My breakfast was in the house. My spare set of keys was in the house. My husband was 70 miles away and wouldn't be back until evening.

As I sat and watched, Eve ate her breakfast as she maintained a tight grip on my keys with her foot. A very tight grip, as she knew I desperately wanted them back! No matter how hard I tried to act like I didn't care, she knew I wanted them back!

Eve toyed with me most of the day. She would bring the keys within just a few inches of her enclosure front, and if I so much as looked at the

keys she would pull them back, and seem to enjoy my desperation. She was so deliberate in the way she teased me with the keys.

For the rest of the day I snacked on trail mix, I was thankful we have a restroom in the special needs barn, and thankful we have a Sierra Springs water dispenser in the macaque building. I didn't have a chair to sit in, those are kept in the shed. The shed was locked. That key was also in Eve's possession. But I put a pile of clean polar fleece blankets on the floor and sat on those for part of the day.

It had to be close to dinner time when I heard a car pull up to the building. It was my husband! As soon as he opened the door, I began telling him what had happened. Before I could finish, I heard a clang. I glanced over, and realized that Eve had thrown my keys out of her enclosure into the aisle way. She was sitting there with her arm extended out toward me, begging for a quick groom from her human. I laughed until I cried!

< Has anyone encountered monkeys who are repeatedly **smearing feces on cage walls**? >

Oh yes, we have a lot of macaques who do that. We call them Picassos!

We too have quite a few rhesus Picassos—or poop-painters. Some of our original painters have inspired others in the room, so now we have a room of juveniles who paint their cage walls with feces almost daily—unfortunately. We have tried various enrichments in an attempt to stop them from making such a mess; it works for a while, however, once they are done with whatever foraging-type of enrichment we give them that day, they resume the painting. Oddly enough, all these animals are pair-housed. It is very difficult to deal with this weird behavioral habit. Our cages are hosed daily—twice during the week, once during weekends and holidays—and I sometimes wonder if this circumstance might encourage them to repaint their walls over and over again after these have been washed and the familiar odor removed from their cages.

Poo-paining is perhaps a kind of creative behavior that helps the subject cope with abnormal living quarters. Extreme boredom and frustration resulting from enforced confinement can trigger an array of behavioral coping strategies, feces smearing is probably one of them, just like hair-pulling and self-biting.

I have found fecal smearing a difficult habit to stop and worse, monkeys teach other monkeys in the same room how to do it. We've been

working with adding extra enrichment but never saw change, right now we've added the verandas; so far no change is occurring.

< I have a pair of juvenile cynos who are **throwing their biscuits out of the food boxed** onto the floor. Although it is great enrichment for them, it is resulting in food waste and we are unclear if they are able to eat enough throughout the day as most of it ends up on floor outside of their cage. We have attempted hanging toys on the outside of the cage in hopes that it would distract them and reduce the behavior but they are still tossing their biscuits. Have you seen this issue at your facilities and have you come up with any techniques to improve the situation? >

I've had many monks over the years that will toss the biscuits out for whatever reason. Currently we have two pair-housed rhesus who are both tossing biscuits out on the floor the moment the food boxes have been filled with their daily ration. To make sure that both eat enough I am now throwing their biscuits directly on the cage floor.

Perhaps you can find a way offering the biscuits in such a way that the animals have to work for them? Putting them into a container in the cage they need to fish from?

Oh, that is a good idea. Right now the biscuits are freely accessible in the feed hoppers. I can see if we can rig something up to the cage that would take more time to get hold of the biscuits. When they have to work for their biscuits they are probably not inclined to throw them out of the cage but will rather eat them.

I also think that having the animals work for the food ratio will stop them from playing with the biscuits by throwing them out, even though it may be a fun entertainment for them.

I did a study with 8 single-caged rhesus males who had the choice of collecting biscuits through the large access hole of a standard food box that contained 33 biscuits or retrieving biscuits from a food-box puzzle that also contained 33 biscuits.

During one-hour observations, the animals collected an average of 15 biscuits from the food box; they retrieved an average of 11 biscuits from the food box-puzzle. It took them an average of 1.1 second to collect one biscuit from the standard food box and 60.2 seconds to retrieve one biscuit from the puzzle box.

- When feeding from the food box, the males were rather careless and dropped 7 (48%) of the collected biscuits on the floor.

- When feeding from the food-box puzzle, they were more concentrated and focused their dexterity on the retrieval of one biscuit at a time which they immediately consumed in almost every instance; they dropped on average only 0.2 (2%) biscuits on the floor.

< Can you cure individually housed non-human primates from **self-directed hair pulling** through environmental modifications such as foraging enrichment, inanimate enrichment or social enrichment? >

This is one of the most frustrating behaviors to deal with, in my opinion. Despite having little success in treating it, we do spend a lot of time enriching the cages of our rhesus macaques who persistently engage in hair-pulling. It seems hopeless but we keep trying anyway.

It is my personal experience with caged macaques that alopecia resulting from hair-pulling-and-eating is impossible to treat effectively with inanimate environmental modifications. It is true, you can temporarily distract a subject from hair pulling, for example with enrichment devices, but this is not a cure. Once the distraction gets weak or stops, the hair-pulling appears again just as before.

I have the impression that hair pulling—like feces smearing—is often triggered by watching another monk showing this behavior in the cage across the aisle. Once an animal gets the hang of it, the compulsive hair-pulling—like feces smearing—is very difficult, if not impossible, to eradicate.

Social facilitation probably explains the overall progressive increase of hair-pulling behavior in primate research facilities. After all, nobody will argue that it must be extremely boring to sit in a cage every day all year round; so why not imitate the behavior of the neighbor across from you? This probably is true not only for hair pulling but also for all other compulsive behavior patterns and stereotypical movement patterns.

Our cynos stopped pulling their hair within two weeks when they were moved from individual housing to social housing. I have had quite a few single-caged bald girls who grew back beautiful coats in the company of another cagemate.

At a previous institution we had a cyno—Grandpa—who suffered from severe hair-pulling. He had removed practically all hair from his body. Grandpa was not shy about his idiosyncratic behavior and would contort into strange positions to remove the hair from his body; all that was left was a patch in the middle of his back! He was not an active animal and gave the impression of being a bit depressed. The veterinarians tried various

treatments to alleviate the problem to no avail. We gave him various enrichment devices; they would only keep him occupied for a day or so. We pulled all the dividers from his quad cage to give him more space; no luck. We were reluctant to pair him as he was an older male who had been singly housed for so long; he didn't seem to be a promising candidate, but we saw no other option to address his behavioral problem.

The first two pairing attempts with two other adult males were not successful. We finally settled on a newly acquired juvenile who was very rowdy and active. This was the little guy's second pairing attempt; during the first attempt with a young male, all he did was start a fight. We were a bit surprised and so relieved, when his pairing with Grandpa turned out to be a success. This truly odd couple got along great right from the start. Grandpa responded correctly, brought the little guy in line, and he actually perked up. The most surprising part, however, was that Grandpa stopped hair pulling—completely! He didn't even over-groom the little guy; we were monitoring them closely, just in case.

We had an adult male cyno with severe hair plucking of the face, head and arms. You could observe him performing this behavior, and his wincing proved how uncomfortable it was, but no amount of enrichment or training seemed to help this male to stop pulling his hair.

We recently moved into a new building where we had to combine some rooms and separate others; this resulted in one female cyno being moved into this male's room. The male's behavior changed almost immediately with the introduction of the female; the hair plucking stopped completely. We were worried the hair wouldn't grow back but after almost a year, you can barely distinguish him from the other males. It's amazing what boys will do to impress a girl!

Hair pulling, or obsessive grooming, just screams mental distress arising from being denied full contact with a compatible companion. Plenty of things can be done to help curb this behavioral pathology, but unless the animals' social needs are addressed it is probably impossible to effectively deal with this problem. I have seen hair pulling very often in rhesus and cynos who have lived alone in single cages, but never in animals living in pairs or groups. Being imprisoned in a small, barren cage, without the possibility to touch and interact with another companion may well constitute a stressor significant enough to trigger and promote behavioral pathologies such as hair pulling, resulting in alopecia.

Trichotillomania (comulsive hair pulling) in people is generally thought to manifest itself during stressful situations. From personal experience, I know this was true for my sister, who struggled with trichotillomania in high school. She was diagnosed with obsessive-compulsive disorder and the hair pulling was a symptom of the mental problem, not the problem itself. So my understanding is that she had an underlying mental disorder, which made her more sensitive to stressful situations, and when faced with stress, resulted in compulsive hair-pulling almost to the point of baldness.

< Has anyone of you observed **partner-directed hair pulling** in a pair- or group-housing setting? >

When I studied the behavior of a rhesus breeding troop in a spacious indoor pen, hair pulling-and-eating was a rather common behavior. The troop comprised 22 adult and subadult animals and one infant that was not included in the observations. In the course of 162 hours, hair pulling-and-eating was shown 302 times by 19 of the 22 troop members, with one animal pulling hair from another member of the troop.

To have one's hair pulled out must have been painful—the attached photo demonstrates this—as it triggered squeaking, fear-grinning, crouching or fleeing. It's therefore no surprise that partner-directed hair pulling was shown in 95% of cases (287/302) by a dominant partner.

I concluded that hair pulling-and-eating between group-housed rhesus macaques is an aggressive behavioral disorder reflecting adjustment problems to confined living conditions. Being forced to share closed living quarters with other conspecifics on a permanent basis may be irritating, and the resulting build-up of aggressive tension is then released in aggressively

pulling tufts of hair from subordinate group members. The attached photo shows such a scene; the alpha male pulls hair vigorously from the scalp of a low-ranking subadult male who submissively screams but cannot escape.

< How do you deal with **self-injurious biting**? >

I had two male rhesus here at our facility who lived in the same room, each one alone in a separate cage. Since Big Guy suffered from self-injurious biting I decided to try pairing him with Theo, hoping that this would stop Big Guy's behavioral pathology. The two turned out to be a compatible pair. Once Big Guy lived with a social companion, he stopped the self-biting. Unfortunately, the two were separated many months later. Not surprisingly, Big Guy resumed his old habit of self-biting after he was kind of condemned to live alone again.

I have noticed over and over again that our cynos show less behavioral pathologies when they are on a training schedule. We have one male named Rock who would constantly scratch and bite at his testicles, fingers, arms and legs. He was doing a lot of damage to himself; we found him a compatible cage companion, and this greatly diminished his pathological behavior. However, when his partner was on study and the two needed to be separated for the day, Rock would go back to hurting himself. We tried providing foraging devices and puzzles to keep him busy, but none of it worked for very long.

Once we began training Rock for pole-collar-and-chair whenever his partner was on study, he stopped hurting himself. He is always ready to work with the trainer and really seems to enjoy sitting in the chair and getting attention.

To our great relief, Rock no longer hurts himself when he is periodically separated from his partner during the day, but a full day plus night separation from his companion turned out to be too distressing for him and he would invariably start biting himself during the night. So we do make sure that he and his partner are always paired overnight. Everyone who works with our cynos knows that Rock and Ray are the exception to the rule: they are always pair-housed during the night, no matter what. This extra attention to Rock's special needs for social companionship is worth all the effort: Rock has become a behaviorally healthy animal and no longer engages in self-injurious biting.

I had to deal with seven rhesus macaques who engaged in self-biting. The animals were all housed alone in barren cages. In an attempt to treat them, I first provisioned their cages, each with a perch so that the monkey

could at least access the arboreal dimension of the enclosure. The perch did not change the self-biting behavior in any way. I then gave each monkey a food puzzle to allow for more foraging related activities. While the monkey was engaged with these gadgets he or she did not self-bite, but the self-biting was resumed once all the food was retrieved and eaten. Environmental enrichment, obviously, was not what the animals needed to stop the self-biting.

After some hesitation, because of the potential consequences, I decided to try pairing each of the seven monkeys with another behaviorally healthy partner of the same sex. To my great relief all new pairs turned out compatible with no incident of injurious aggression. The new social-housing arrangement cured all seven subjects from self-injurious behavior within the first four months after the transfer to pair housing.

I concluded from this experience that lack of social companionship is a cause of self-biting and, therefore, committed myself to transfer as many as possible—several hundred—single-caged rhesus macaques to compatible pair housing arrangements in order to prevent the development of this serious behavioral pathology in any other animals. During the remaining four years I worked for this rhesus colony, no new case of self-biting was recorded.

People rarely take the time to find out what the animals are trying to tell us with these so-called abnormal behaviors. A single-caged male rhesus monkey resorts to repetitive self-destructive biting not because he is crazy but because he is desperately lacking social companionship.

Making it a standard practice to house all macaques in compatible social settings would be the best preventive against self-injurious biting.

At our facility are three adult male rhesus who had a history of SIB (self-injurious biting). The animals were treated with various drugs—diazepam, fluoxetine, guanfacine—which did alleviate but not eradicate the self-biting. Once the treatments were discontinued, the animals resorted to SIB as before. All three males self-inflicted repeatedly serious laceration that required surgical care. When it was considered to euthanize these males, because the SIB could not be stopped with pharmacological therapy, we were finally given permission to pair them with other compatible companions. This *treatment* brought the self-being to an end in all three cases. Carl, however, had a relapse when his companion was removed for research-assignment reasons after 14 months. Fortunately, the PI was considerate enough to drop the companion from the research protocol and

allowed us to re-unite him with Carl, who promptly stopped again self-biting himself.

Mojo was a very smart 6-year old rhesus male who was biting his thigh, hand and foot. His fits of self-biting appeared to be associated with frustration, when waiting for something he wanted. Addressing his behavioral pathology, we offered him more space (3 duplexes) and added a Prima-Hedron. We gave him water enrichment once every month; he loved it and was fascinated looking at the water sideways at eye level to see how it distorted the light. We gave him a monkey doll but he destroyed it immediately—doll guts everywhere, LOL! We gave him wheat grass and tried all kinds of versions of Biscuit Play daily, which he LOVED. We increased friendly human interactions and groomed him at least once a day, even though his cage mate did plenty of that already. We also added 2 females to his room which he found VERY interesting and engaging.

Mojo's SIB resolved after just a month. We would only occasionally see it when the 3rd duplex cage was being cleaned in the AM and had been returned to the room from cage wash in the PM, but the tech was taking longer than usual to attach it back to his house. Majo would demonstratively engage in self biting without actually injury himself and look at us like *Dude ... don't make me call the IACUC.*

< We have a rhesus who keeps removing her sutures from surgery. We've tried all kinds of enrichment to distract the animal but had no success. What do you do to prevent **suture picking**? >

If you have a suture picker, he or she can be one tough customer, and 9 times out of 10, no amount of enrichment will do the trick. I learned there were a few ways to take care of this:

- First, and this is the easiest method: subcutaneous closure. However, some will find the teeny tiny knot buried at the one end and go to town anyway.
- Second, and this is the solution I always used with the 'problem children': Wrap the bloody daylights out of the area if you can (limbs, feet, etc.). Make it with LOTS of layers of gauze, tape, vet wrap, more tape, so they just pick at that and not the sutures. Heavy duty tape takes some doing to come undone, and once they have a piece stuck to their fingers, it's hours of enjoyment. If they get through that in a hurry (and some will) there is one sure-fire way to get them to stop:

- Third, a simple interrupted closure using a stainless steel suture. It doesn't behave as nicely as other sutures, but I promise they will only pull on it once.

Our vet uses blind sutures followed by Nexaban skin glue to close any surgery site; the monkey can't see any sutures. In addition we always place 'decorations' (distractions) away from the surgery site. Distractions we've successfully used are things like random sutures in the palm of the hand and/or forearms, or a small clump of Nexaban skin glue randomly placed that dries before they wake up. They spend hours fussing with it and picking it out of their fur; sometimes we just make a flour/water paste to rub through the fur on their forearms and dry it with a blow drier while they are still sleeping. It dries flaky and they spend a lot of time picking it all out of their fur. Keep those monkeys occupied!

< We have a 10-year-old male cyno who spends most of the time sitting on his perch (usually in the front of the cage) with his feet up grasping the front of the cage and his head down between his legs touching the floor of the cage. Have any of you ever seen this or a similar behavior and do you think that this may be a case of **depression**. He is caged without a social companion because he is assigned to an infectious study that precludes contact with another conspecific. >

I have seen depressed monkeys sit in such a posture for hours on end. They get up and are able to ambulate without any signs of an issue, but only if presented with the motivation to do so, for example to get food or to drink.

I took the attached left photo of a rhesus macaque who shows the behavior you are describing. This 33-year-old female has been single-caged

all her life, i.e., since the time when she was taken away from her mother and prematurely weaned. The persistent hunched posture was for me a clear sign that the animal was suffering from severe depression. The solution to this miserable situation was simple: I paired her with another previously single-caged senior female. Rather than kind-of hiding from life, this aged lady embraced life again and eagerly interacted (right photo) with her companion and once and for all stopped taking the now-forgotten weird hunched posture.

Since your male cyno cannot have a social companion for research-related reason, you could substitute for such a companion by visiting him often, talking to him, giving him treats and perhaps even grooming him through the bars of the cage.

< I would love some input on a problem we are encountering. Our monkeys are having a field day with the automatic **watering system**. They can reach through and pull out the hose attached to their cage, thereby disrupting the watering system. Has anyone had to deal with this? >

I have only had a couple monks do this. To stop them from creating a problem, we took zip ties and tightened the water hose down to the attachment so that they get out of the monkeys' reach.

Ugh, we had this problem too. We fixed it by pulling the cages off the wall about 2 feet, just so hoses are taut enough that they can no longer be reached by the monkeys. It actually works nicely because then techs can walk behind the cages easily, checking and maintaining the water lines.

< Is anyone on the forum **remote-video recording** non-human primates in order to evaluate new enrichment items, to check the compatibility of new pairings and to assess possible stereotypies during times when the animals are not disturbed by human activities? >

We have cameras in every NHP room, recording all the time; the momentary recordings can be viewed on computers at the vet staffs desk; they can also be rewound and viewed at a later time. This remote monitoring system works great for behavioral and pairing assessment.

I also find video recording to be amazingly valuable to the whole process of pair formation, both in protected contact and free contact. Our presence really does affect the animals' behavior. On many occasions, I saw zero interaction while in the room, but the minute I left they'd start grooming. If it's a pair I'm a bit nervous about, I'll set up the camera and sit

quietly out in the hallway; that way I can see if things go wrong and I need to intervene.

I video record frequently during the evening hours, after everyone has gone home for the day, to assess pair compatibility and abnormal behavior. Video recording has been a great tool since so many monkeys behave differently when humans are in the room.

Video recording has allowed me to keep together compatible cage companions that everyone thought should be separated. We had one pair who had issues whenever certain individuals entered the room: the dominant would stare at the subordinate partner, who then would scream loudly. The two rarely fought and if they did, it never had serious consequences. So, I recorded them at various time points and discovered that the two were one of the most bonded monkey pairs ever; they slept together, ate together, played together, and groomed each other endlessly. The video made it very clear, it was certain people who were setting them off. With a little investigation, I found out that some people felt bad for the subordinate male and always tried to give him treats, but not to the dominant male because they thought he was being a 'bad' monkey. We changed how people interacted with the pair and everyone was happier for it! So yes, I would say remote video recording most definitely provides a wonderful tool to improve animal welfare.

< For those of you who perform **ECGs** on your monkeys, I am curious what type of clip you might use. We use the alligator-type clip and then flatten the teeth on them, but they still seem to pinch the animals' skin quite a bit. It would be nice to find an alternative that is less uncomfortable for the animals. >

A few years back I went to Ace Hardware and tried all of their alligator-type clips, trying to find something that was less harsh. I ended up with a little alligator that has no teeth, just fine lines, similar to a small hemostat. It also didn't clamp down hard in the closed position. I removed the existing ECG alligators, soldered them on and shrink-wrapped some plastic around the junction. I apply lube and attach this instrument between two toes. There are no adverse effects when using these little alligators. I got several extras at that time but have not needed to replace any yet.

Since there are lots of little grooves, I soak the gel off and use an eye spear to clean between the grooves. Works great. Happy toes!

We used those traditional alligator clips for a long time but in the past few years have switched to the sticky pads for all species. They work really well, and are much more comfortable than the alligator clips.

We also use these adhesive snap pads. They don't seem to bother the animals at all.

I have never seen any irritation resulting from the pads on any of our animals—monks, pigs, dogs. We have seen irritation due to shaving though. We shave just a little bit more than the space needed to place the pad. All of our ECGs are performed on conscious animals, so the pads really seem to work better than the alligator clips.

One of the PIs at my facility does a lot of work using ECG pads. He himself had an allergic reaction to the adhesive, but he said it's like 1% of the human population or something. He felt special :o)

Discussions on Pigs

< Pigs have a very strong social disposition and suffer in a similar manner as primates and sheep do when they are kept without a social partner. How do you address the **need for companionship** when the pig is housed alone during an experiment or test? >

We require regular human interaction for our single-housed pigs, just as we do for our dogs. Someone would go in and sit, pet, brush, even walk the pig.

Our pigs are kept in dog runs or pens and can hear, see and smell their fellow swine. I keep them side-by-side and they always lay beside each other even though there is wire between them. They have a conditioning period to become comfortable with human contact. Once a pig likes you, she <u>really</u> likes you and social time or playtime with you then becomes a must. I spend a certain amount of time every day with each pig, scratching, petting and giving the animal lots of attention. Often we let our pigs out in the hallway to interact with their neighbors while the pens are being cleaned.

When they find out that you are nice to them, pigs readily socialize with you and, if single-housed, will value any time you can spare to be with them. I visit each of our pigs at least once a day, talk to them, groom them a bit and distribute food treats.

Our single-housed pigs are well socialized with humans; I spend a certain amount of time with each one of them every day, scratching, petting and giving them lots of attention. A good scratch with a toilet brush and lots of petting goes a long way! While I am cleaning their pens, I let two pigs out at a time so that they can interact with each other and play if they feel up to it.

We use mesh-sided pens so that pigs can maintain contact with each other even if they can't mix. They also get lots of human contact and I do mean contact: lots of scratching, brushing and rubbing, and playing. On the odd occasion we only have one pig in the room, the human contact is increased even to the extent of just sitting in the bed alongside the animal for 10-15 minutes several times a day; pigs do seem to like lying, so they will typically lean up against you. I think we humans can provide—and should provide—highly valued companionship for pigs who have to be kept alone.

< How do you go about **establishing new pairs or groups** of unfamiliar pigs? Does it help to sedate the animals first and then allow them to wake up together? >

It certainly works both in agricultural pig units as well as in the lab situation. It does seem to be important that future pen mates have body contact while they recover from **sedation**. They will stir and stumble over other pigs, settle back amongst the others and by the time they are fully recovered, all the pigs will have the same smell mix, distinguishing each one as a member of the same group. Our pigs wake up and just get on with things as if they've always been housed together. We have gilts, but my pig technician who is from a commercial background says that this technique also works with adult females, young intact males as well as adult castrated males.

When we take delivery of new farm-reared pigs, we always sedate the animals so that they fall asleep; we then pair them and place the partners in such a way that they are touching each other (thereby transmitting each other's personal scent). When they finally wake up together, they seem to be quite happy with each other. We give them enrichment in the form of deep straw, cardboard boxes and portable objects along with lots of human contact to keep them entertained; we rarely encounter problems.

Our new pigs are transported all together in a truck, so we have no idea which pigs are littermates. The vendor has worked with us to mark the pigs that come from the same pens, and that is very helpful. We basically just put the animals together as pairs upon arrival in quarantine. Our technicians find that the main factor for compatibility is to pair pigs of **similar size**. It's common that they will scuffle for dominance but we let them work it out. Occasionally two pigs engage in prolonged fighting; when this happens, they are separated to avoid serious injuries and tested with other partners for compatibility.

We have so-called enrichment panels in between cages, allowing neighboring pigs to see, touch and smell each other. I like to pair pigs who have been **familiarized** in this manner for at least a few days. It's very rare that we don't see some fighting when the partners are then introduced to each other in the same enclosure. They need to work out who is dominant; once they have done that, they calm down. I typically watch them over the course of the day. If the fighting gets too rough I separate them, otherwise I let them stay together. The problem is that the pigs are randomized into studies, which means pairs are often split because partners end up in different studies. I keep track of who has already been paired with whom; so if the opportunity arises these animals will be allowed to live together again.

We also house new pigs in such a way that they can see, smell and touch each other. I will know they are ready to be tried together in the same

pen when they are lying beside each other even though there is chain-link fencing between them. When they are put together they have access to a few feeding and watering places; this makes it harder for one pig to hog those spots.

We follow the same protocol. New pigs first get access to one another through roll bar fence panels before we try introductions. It is important to first let them establish a social relationship with each other; this may take some time but it is necessary to let them figure out who is alpha and who is beta before they are introduced in the same pen as a new pair. The success rate using this method is pretty high but not absolute; I witnessed rare instances where potential partners simply are not amenable to pairing.

Most of our pigs are domestics, and we have very good success with getting them together without overt aggression and keeping them together as compatible pairs. The situation with Yucatans is very different; they have a strong sense for being dominant, so they are unwilling to tolerate the presence of another conspecific; aggressive interactions are almost inevitable. Most of the **Yucatan pigs** we get end up being singly housed.

< Do you make attempts to **socialize pigs with personnel** so that anxiety and fear do not become uncontrolled variables during experimental procedures? >

Absolutely! It helps calm the researchers as well as the pigs. Our work would be impossible if we did not spend the time, getting our pigs really well used to people and to being handled.

On arrival, mature adults are primarily concerned with their food supply; they tolerate being petted and scratched and are quite easy to handle for their size. Once they tolerate my presence and contact has been made, it is all downhill from there. They love to be scratched and have their bellies rubbed; they can't resist it. Good hard pats to injection sites will prepare them for those later. I use jam, peanut butter and large marshmallows for treats; medications can easily be hidden these treats.

Well-treated pigs don't get tired of human contact. Our animal techs spend quite a lot of time just popping in to say *Hallo!* and to give their animals a scratch, which they always appreciate a lot. I have worked many years with pigs and can affirm that they do enjoy being scratched very much. I purchased a toilet brush for scratching the pigs in my charge. They seem to like being scratched just about anywhere. When I need to obtain a rectal temperature, I scratch them around their tails quite a bit. They like this and stand still, allowing me to get their temperature without any ado.

< Do pigs readily learn to **cooperate during procedures**? >

I find that farm animals, like pigs, are not looked at the same way as companion animals. Since they are still treated a certain way on the farm, some people in the biomedical industry feel that this should be good enough in research. I think this is hogwash and I strongly disagree. No animal used in research should fear the caregiver, the environment or any procedure. Pigs are very easily stressed and a stressed animal cannot be a good research model. I have found in most cases with pigs that major stress can be avoided, but it takes time. Unfortunately, in a lot of facilities time means money and that seems to be more important than compassionate animal care.

Pigs used to be injected at our facility by simply lifting them off the floor by their hind legs and inserting the needle. Obviously, this was very stressful to the pigs and very disturbing for me while holding them. I knew there must be a better way to work with these intelligent creatures; so I got to thinking. I developed a simple conditioning program—based on mutual trust and scratching the pig at her favorite spot—that allowed me to give the pigs **injections** without any special ado and without eliciting noticeable stress reactions. It takes time and patience but it pays off in better animal welfare and better science, i.e., stress-free research data. Happy animals equal good research; I don't think that can be questioned in any way!

I worked with a highly food-motivated boar of about 200 kg. His kind nature made him popular with people, so he got very much attention. This is probably one of the main reasons why I could train him successfully to

cooperate during **blood collection**. Training took place daily for 5-10 minutes. I used juice in a squirt bottle as positive reinforcement and a fruit as a bonus at the end of each session. It took about a week to shape his behavior to allow for his first jugular bleed with no restraint.

Having a trust-based relationship with you is probably the key factor to making the blood collection procedure stress-free for the pig and stress-free for yourself. I don't think that the venipuncture per se is a big deal for the pig, provided the pig trusts you and you know what you are doing and how to do it swiftly and correctly.

We frequently bleed pigs and usually apply a numbing cream to their ears, then while one person distracts the animal with petting, food or treats, the other person obtains a blood sample from the ear. The pig has to be very well acclimated to humans in order to cooperate during this procedure. Since most of our pigs are very friendly, we've even been able to take blood samples from other peripheral vessels like the cephalic, using the same handling technique.

We had a project with daily bandage changes for 14-21 days. At first this required injectable anesthesia and a prolonged recovery. This was unpleasant for the pigs and for the technicians. Pigs are easy to train for a food reward; so I have used a trail of whipped cream to teach them to walk down the hallway to the OR and get a mask put on and cooperate during **inhalation of the anesthetic**. I give the pig a treat before and after the procedure. Eventually I fade the first treat but continue giving a second treat as a reinforcing reward. I worked with each pig twice a day for a total of about 30 minutes. It took four days for the smartest pig and nine days for the dumbest to get successfully trained before the actual research project started.

Our pigs have to ingest about 12 tablets twice daily. In the beginning they took the pills mixed with something sweet without much ado, but quickly got smart to our shenanigans. We did outsmart them all by making use of numerous different sweet goodies to which we first had acclimated each one of them. The tablets are then crushed and mixed with a different flavor mask for each treatment. Our pigs cooperate with this **oral dosing** scheme very well; they seem to really enjoy yogurt (Stonyfield Banilla, Vanilla), grape jelly, vanilla pudding, Nutri-Grain bars, Fig Newtons and canned dog food. Some also like molasses, apple sauce, apple juice and Boost Plus (chocolate, strawberry and vanilla).

Pigs love apples. The taste of apples seems to cover up the taste of most drugs/medicines. We cut the apples into two pieces in which we hide

the pills and then offer the pieces by hand. It has been my experience that minipigs accept the baited apple pieces without any ado.

There were over 100 pigs to be treated with a pill, the taste of which they didn't like at all. Many treats were tried to hide the pill, but the pigs stubbornly refused to cooperate. Finally, frozen cookie dough was tried. The pigs really liked it and swallowed the test pill while eating the tasty dough.

< I'm wondering if there are any folks on the forum who have any advice or written protocols for **acclimating pigs to wearing jackets**. These will be 30-50 kg farm pigs; the jackets are custom-made. The animals will have to wear them for about two weeks. Typically we acclimate all our pigs for one week; is this perhaps not long enough? >

Our plan on jacket acclimation for pigs is as follows:

1. The jacket is introduced to the animal with positive human contact and/or treats.
2. The jacket is partially placed on the animal for a short time (i.e., no less than 10 minutes). Once acclimated to partial placement of the jacket, the jacket will be zippered and adjusted and left on the pig for longer periods of time (i.e., 4 hours). Manipulating the jacket can be very stressful, so it is very important to thoroughly acclimate the pig to zipping the jacket and adjusting it. Positive human contact and/or treats are given.
3. The jacket is placed on the animal and kept on for the day.
   These first three steps may be repeated if necessary.
4. The jacket is placed on the animal and kept on as needed for study.

I have utilized jackets for a number of swine studies. In my experience the animals adapt to the jackets quite well, with larger breeds doing better than smaller breeds. I think it's because they tend to have calmer personalities. Yorkshires do particularly well with the jackets; so much so, we actually stopped acclimating them during one project because we found they didn't need it. Some of these pigs were quite sizeable—close to 80kg!

I find females to be more suitable, as you can use a longer jacket without fear of them urinating on it and developing skin irritations. Additionally, the longer jackets tend to ride up much less. If you're going to keep the jackets on the pigs for an extended period, you must be sure to loosen them occasionally to avoid irritation of the skin.

We typically place the jackets during anesthesia recovery and the animals do surprisingly well. The issues we've encountered with long-term use of the jackets are:

- If you use males, watch for soiling of the jacket's underside.
- Irritation of the forelimbs at the jacket armholes. There are two types of jackets available, one of which has fabric covering the mesh edges; the fabric helps to cushion the armholes and seems to be more comfortable for the pigs.
- Group-housed pigs tend to chew on each other's jackets and damage the zippers.
- Some of the jackets tend to ride up around the neck of the animal; this can become a source of great discomfort. It's therefore important to make sure to check the placement often and slide the jacket caudally as needed.

< Has anyone **socially housed jacketed pigs**? There is an investigator who will be jacketing his pigs with external defibrillators attached. >

Sadly, no; we've tried without success. The pigs root and chew at the jackets and end up tearing into them and/or causing damage to the equipment. We've tried to keep them in adjacent pens for tactile contact, but the same thing happened (we have wide bars between the pens). I hope others have not had the same experience, but I fear it's a universal attribute of piggies; they love rooting anything that's not too heavy for them to move around.

We made an attempt for supervised social-time, but all of our animals are way too curious about the jackets and equipment—lots of pockets with interesting good stuff inside! Nothing is quite like seeing a $5K piece of equipment turn into a piece of enrichment; yikes! So, instead, we allow one pig out at a time to roam the floor, and if she so desires, make visits with the other pigs. The front doors of the pens have the same wide bars as the sides, so they are able to have some snout-to-snout contact if they choose. Once they're used to the system, they're very cooperative, and some become quite excited during play time. It's always a delight to watch them trot towards their favorite friend, wagging their tail while happily oinking down the run.

We just recently had eight jacketed pigs; we had to keep them all single housed due to the pulling/biting of each other's jackets. The bars of their pens were close enough to allow snout-to-snout contact only, but some did manage to pull at each other's jackets. Not wanting to frustrate them too much, we offered them supervised open-floor play with each other a few

times a week so they would be able to satisfy their social needs at least occasionally. During these social outings, we'd scatter treats such as forage pellets and cereal all around the floor and hide bits of food in hard-to-reach places in order to distract the animals from snuffling around at the jackets. The pigs were so intent on finding the food that they forgot all about the jackets. I even had two pigs work together to flip over an upside-down kiddie pool to get at the treats underneath.

Our jacketed pigs had vascular and or GI-tract catheters/cannulae. They were individually housed in adjacent pens so they could hear and see each other. The technicians would do the catheter maintenance and/or sampling at least once a day, usually twice. As part of the process, the jackets would be temporarily removed and the pigs would be groomed with a small stiff bristled brush where the jackets were in contact with the skin but also down their backs. Usually the pigs would get to the stage where they would lie down with their head on the technician's lap whilst being brushed and getting their tummies rubbed.

< Pigs are intelligent, highly inquisitive social animals. What can we do to mitigate boredom when they are assigned to research in laboratories? What kind of **environmental enrichment** works best for pigs? >

We house our swine on raised flooring. To encourage the natural behavior of rooting, we purchased a heavy stainless steel **chain** and placed that in the pigs' pens, three links for the small Göttinger pigs and six links for the large swine. The pigs appear to really love the chains, as the techs hate them. With the pretty much constant pushing and banging of heavy metal there is so much noise in the room that hearing protection is required. The large pigs are literally throwing their chain across the room, making it quite an adventure for the staff to be around.

We cover suspended chains with garden hose or fire hose—for durability and as noise buffer. Sometimes we hang Jolly Balls or other toys from the end and hang a second chain/hose off the side. I saw one pig spinning around on it; quite adorable!

After hearing a recommendation of chain-toys for pigs, I made my own: I use about 75 cm lengths of heavy metal chain with assorted dog toys attached to the middle or end of it. The toys include Booda rope, the pigs' favorite toy, Nylabone rings, Kong toys and rubber bones. I attach these toys to the chain with metal clips so that they can be easily removed and rotated from pen to pen. I can hear the pigs rattling their chains and toys when I leave at night and when I come in on the weekends. Whenever I enter the

pig runs, I can always see a pig or two with the toys or chains in their mouths. I gave our pigs their toys four months ago. They still use them, and there are no signs that they have lost interest in them. It just makes sense that they need something to mouth as they chew on each other all day long —and chew on me when I enter the pen!

Our pigs get Buster Food Cubes which dispense treats when moved around. Pigs are persistent when there is food involved; ours are entertained for hours with these **toys**. They also enjoy to play with rope toys that are long enough to play tug of war with another pig or—and that is even more fun—with a human playmate. You might think these rope toys are hard to clean: you just throw them in the washer and then autoclave them; no big deal!

It is my experience that pigs display far more species-typical behaviors and are less restless—no longer bang at the door—when they have access to Kong toys, hanging rubber tires and cloth strips than when they are kept in barren enclosures. The Kong toys are a great hit. We replace them twice per week so that they can be cleaned; pig saliva tends to be very difficult to wash off once it dries onto/into the rubber material. I have to come with a new Kong toy, plus scratch a pig, so that she reluctantly releases the Kong that I need to get out for cleaning. Rubber tires or cloth strips also provide great enrichment, however, I have noticed that pigs housed on crates, rather than bedding, tend to lose interest in these items. For these pigs we rotate the tires and strips about every ten days to enhance novelty.

Pigs love to root for goodies that are hidden in **cardboard boxes**. I always close the boxes by weaving the flaps to avoid using tape. Every single pig has found his or her way through to the goodies and then had a blast tearing up the box.

Each of our pigs has access to a 15 cm deep wooden tray filled with sawdust that we top every day with fresh **straw**. The animals spend more time rooting, chewing the straw and playing with the straw than they do with any of the toys we have ever given them. Straw seems to be the perfect enrichment substrate for them, and there is no indication that they will ever get bored from it. Spoolder et al. (1995) showed that the provision of straw prevents the development and reduces the incidence of stereotypical oral activities, such as chewing pen fitting.

Our pigs are housed on solid floors with separate sawdust-substrate bed trays that have raised sides of about 4 inches (10 cm) and are

provisioned with fresh straw daily. We hide food treats in the straw, but our pigs engage in rooting quite happily even when given fresh straw without any incentive. Straw tends to repel water to some extent and although it gets wet, it doesn't stick—a big advantage over paper-based materials!

We have attached a **scrubbing brush** on the side of every pen, so that our pigs can scratch those itchy spots! Since most pens are smooth stainless, or the walls are smooth tile, the pigs usually do not have an opportunity to rub against anything. The scrubbing brush does not lose its attraction over time, probably because it offers the pig great relief from itching.

Pigs enjoy the **company of humans.** To be scratched by a friendly person or playfully interact with a person is perhaps the most favored environmental enrichment for a pig. I'm lucky in that I have a first class technician, superb staff in general and pretty much a free hand—any experimental reasons for not providing enrichment have to be justified in the appropriate project license. Probably the best aid to pig welfare are our rather progressive researchers who appreciate that contented pigs are so much easier to work with and, in addition, yield better results.

Discussion on Goats

< There is a rumor that we are going to get goats at our facility. I have no experience with goats other than petting zoos or friends' farms. Can anybody share advice on **species-adequate housing** for goats? >

You are in for quite a treat: goats are fabulous animals. Depending on where you source them from, they may be quite skittish or already humanized to chew all your clothes.

I used to manage a research herd of over 300 goats; they are fantastic animals to work with and I still miss them.

Goats investigate everything with their mouths and can be very destructive if bored. They are browsers rather than grazers, so if you can provide woody material for them to browse on, it's the best form of enrichment, but obviously you need to be careful about toxic trees and herbicides. If you can't get browse, then red clover hay or Lucerne hay (alfalfa) or straw will keep them busy; they prefer rough hay to good quality meadow hay. Mineral licks are also popular.

Goats like things they can climb on; if you have the space, old wooden packing cases or concrete blocks—as long as they are sturdy and won't topple—provide great enrichment. They will happily stand on their back legs for long periods; if you can suspend browse or hay at a good height, it will make them work. An adult Saanen type goat can easily reach up to six feet when standing like this; this means, any fittings in the room need to be made goat-proof even at this height.

Plenty of rubbing posts will be welcomed; goats are a bit like pigs in this respect and enjoy a good scratch. If they are housed in groups, the lead animal will start the rub and then the rest of the group will take their turns; so posts, gates and other vertical structures need to be quite robust. A daily brush, just like you'd groom a horse, is usually enjoyed and helps keep the goats clean.

Space is quite important to goats; if you can provide plenty, it will act as enrichment. They love to be outdoors. If group-housed, they will develop *Follow The Leader*. This activity doesn't seem to be a stereotypy as the goats will play this game in a variety of ways out in the field.

I don't think footballs or similar toys would last five minutes, but a large puzzle ball is enjoyed by goats, providing it is made of very tough material; Goats don't have a particularly strong mouth but they are very, very persistent; same effect as dripping water on stone, but quicker!

Discussion on Sheep

< Can anyone of the forum share experiences with **mirrors** as enrichment for sheep? Our head vet heard somewhere that sheep are fearful of mirror reflections. I actually can't find any published research supporting this. >

We routinely use mirrors for sheep when we must singly house them. It seems to me that when watching the mirror reflection they do see a pen mate; they spend most of their time nose-to-nose with the mirror, which probably has a comforting effect on them. I would definitely recommend using mirrors if you have to house sheep singly.

We've used mirrors when we had solitary sheep in the unit. Based on my own observations, I have no doubt that the mirror reflection calms a lone sheep and offers social comfort. Typically, a lone sheep stops calling and being agitated when you put a large mirror in front of her/him. We used sheets of polished stainless steel or full length ordinary mirrors.

We were amazed at the calming effect of mirrors when we got new sheep. The animals were quite timid and disturbed after being unloaded from the delivery truck and it took a lot of coaxing to have them walk down our animal housing hallway. But as they turned the corner into their housing room, they saw the mirrors and ran to stand as close to the mirrors as they could. Sheep really do find comfort in other companions even if these are mere reflections of themselves!

Parrott et al. (1988) found that the presence of mirror panels markedly reduces endocrinological (cortisol and prolactin) stress responses in single-housed sheep. McLean & Swanson (2004) observed that mounting large mirrors on the sidewall of isolation units has a calming effect on sheep. “Vocalization stops completely and the sheep remains completely calm. It seeks out its own mirrored image, stands close and occasionally nudges at its mirrored partner. Consumption of food and water remains unchanged and the risk of injury is eliminated, as the sheep no longer tries to jump or escape the enclosure.”

Miscellaneous

< What expectations motivated you to make a living as animal care technician in an animal research facility? What’s your **job satisfaction**? >

It was always my dream to be with animals. Working with wildlife in the field at Parks Canada was in my mind, but I ended up in an animal research facility because I needed a job at the time. When I got here 35 years ago, I realized that research was a place where I could help make a real difference in the way animals are cared for, and needless to say, today I still feel the same. It has been a tough journey at times; I thought of leaving at one point because of the emotional aspects of the job but moved past those.

I love my job because it allows me to make long-lasting positive changes in our animals’ lives. In my 4.5 years here I have transferred over 1,500 single-caged macaques to compatible pair-housing arrangements. I have made it my mission to make sure that I leave no stone unturned when it comes to socialization options for the animals under my watch.

Even though the evidence is extremely clear that macaques can easily be caged as compatible pairs rather then kept alone, a lot of people still hold an old school view that non-human "primates are not likely to tolerate each other when placed together as adults" (Line, 1987) so they are best housed in individual cages. It is a constant uphill effort, but one that I find to be the most rewarding. I continue to be the squeaky wheel, updating SOPs and policies to enact change, and get on everyone’s nerves by pestering them until they let me socialize their animals. After demonstrating that pairing can be done safely in a wide variety of configurations, most people have eased up and trust me to do my thing.

You can and should be proud; over 1,500 animals paired! That's really amazing and very encouraging.

I have witnessed a lot of positive change and have been very fortunate to be a part of that, but with all the scientific results that are out there, especially those pertaining to environmental enrichment, I expect that we should do much more to implement truly species-adequate living conditions for all animals in research laboratories; this includes mice and rats and all cold-blooded animals.

The problem is that many — fortunately not all — biomedical researchers don't respect animals as sentient creatures but rather use them as inanimate objects, i.e., research tools. They have hardly any or even no feelings for the animals of their research projects and consider refining their living conditions only when it is mandated.

My very first job was actually at a small animal clinic. I needed a job and didn't really like working with people so thought working with animals was a better option. My expectations were that it would be hard work but ultimately rewarding. Unfortunately those expectations were not met. The practice was not the most ethical, which is just a long, ugly story. When I quit, I still wanted to work with animals rather than people and, by sheer chance came across a job ad in the paper to work at a primate research facility. Being very new to research, I was apprehensive about what I'd see when I went for the interview, but the staff and veterinarian were wonderful people. I was hired to work with a colony of marmosets and a very small colony of tamarins. Yes it was a very physical job and at the end of the day I smelled horrific, but I loved those little guys with their expressive faces and grabby little hands.

I'd say that more of my expectations about the care of animals are met today than 30 years ago. Back then, enrichment was still hit or miss while today it's very standard and the ideas revolve more around the question "How can we improve the enrichment?" rather than "Do we need to provide enrichment?" As with all things, there is room for improvement but I think the culture has shifted so that people do not instantly think NO when a change or addition is proposed. I still do miss those very smelly marmosets!

I always wanted to work with animals and I was always interested in medical science. I fought to get in the vet tech program.

The past 25 years have been very rewarding and I have seen great changes. The idea that we can work co-operatively with the animals in our care has grown to be standard. With patience, skill and dedication, the animals we work with learn what to expect from us, so they aren't as

frightened of the procedures we must do with them. Even the way we house the animals has changed—very much for the better—from when I started and we thought we were advanced back then.

I'm mostly administrative now and I miss working with the animals but I'm confident that the techs taking up the reins will learn things I never dreamed of—keep learning!

When I was getting ready to graduate, our instructors set up many tours of private veterinary practices large and small, and a few research facilities nearby, helping us to find our career path and make contacts. I swore to myself NEVER, after touring the research facilities, not because I saw anything awful, I just thought working with animals in a research lab wasn't for me. My instructors tried to help me see differently, assuring me that I would probably find far more cruelty and suffering out in veterinary practice than in the biomedical research industry. Well, I was young and stubborn and went into a largely exotic veterinary practice; and my instructor was right, I did see much cruelty and suffering. After several years I quit under duress.

With a mortgage and no job, I found myself turning to the university to find a job and finally entered the research animal world. I became the primary caregiver for a large group of long-term rabbits. I learned a lot about myself, and where I stand in caring for and loving those girls. I still remember each and every one. I did end up going back to private practice for a bit, but quickly came to the realization that, while I function well in that capacity, caring for the lives of research animals is truly where my satisfaction lies. Medicine only goes as far as nature will allow, but the care one can give for the research animal lies in your own hand—with facility budget a limiting factor at times. Presently I am caregiver for macaques.

I am anxiously waiting for the time when animal research ceases to exist. I wish for it, but in the meantime I come home every night with a sense of fulfillment. Knowing that I make a big difference for the animals in my care; showing them affection and respect is an honor and one of the greatest satisfactions in my professional career. Making positive change for these animals is intoxicating. I can't get enough. Until biomedical research moves on to non-animal models, I'm staying.

I've been in my current job for almost 10 years. Before working at this facility, I was a zoo keeper for 15 years. When I applied for my present job, I had almost no expectations other than I would be doing primate enrichment and social housing. Then sometime during the first week of this

new and unfamiliar job something completely unexpected happened: I was called to look at some abnormal mouse behavior! WHAT???

Then I saw this mouse in the barren cage with nothing but a white square of so-called nesting material; that made me realize I had a lot to learn about helping research animals. Ten years later, I'm on the IACUC and in charge of rodent breeding here! Who could have predicted I'd be making mice and rats happy?!

I started as an animal technician back in 1968 and have never regretted my choice. I originally was going into agriculture but very quickly realized that there wasn't really a career for a woman. Working with animals had been my dream since I was a small child; it didn't matter which animals, so long as they were not spiders.

Yes I've been frustrated in the job, never with the animals or the work but with some people along the way, but show me a job that doesn't involve that blot on the landscape! I have the satisfaction of knowing I've done my best for all the animals who were in my care.

< When you work as a technician, caretaker or veterinarian in a biomedical laboratory you are bearing the **risk of getting stigmatized** by outsiders as a vivisectionist who does not care about animals. How do you deal with this often rather frustrating situation? >

It is my opinion that we need to inform outsiders what actually does go on in research facilities. They should be able to form their opinions based on facts, not on some stories that contain only half-truths. I tell critics that I wish animals would not have to be used in research, but since they are being used I feel an obligation to give them all the care, respect and humane treatment they deserve. I am not hiding the fact that I am pro-research but I am also not hiding that I am pro-animal; both positions can complement each other when you work in a biomedical research lab.

I talk about what I do fairly freely, stressing the controls we have in the U.K., the fact that I very much care about the well-being of the animals in my charge, and the benefits of medical research with animals. There are sometimes sharp intakes of breath when I first tell people that I look after research animals but, generally, I am given a fair hearing and most people will accept what I do as being necessary. Of course you'll never convert the hardened *anti*.

I used to hide what I did for a living from folks but then one day wondered, *why am I hiding?* After all, I care for animals and do what I possibly can to make life easier for them. Nowadays, I have no problem

talking with interested people and critics alike about my work in the research lab. I always start by making it clear that, as far back as I can remember, it was always my dream to work with animals and to make a positive difference in their lives. When I first came into the animal research environment, I didn't know how long I would stay, but it didn't take me long to realize that here was the place where I could make a big difference; that was 23 years ago. I explain to people that, while I do find research interesting, it is the animals that brought me here and keep me here. I have been fortunate enough to have been able to bring about positive changes in the way animal are kept and treated. This has been, and still is, very rewarding!

I once was asked how an animal lover, such as myself, could ever do what I do. My reply was, "would you rather have someone who doesn't love and respect animals work with them?" This was not rude, but it ended the conversation about my job.

I don't say a lot about my job unless I can trust the other person, and even then I do not go into details. The few people with whom I have discussed my work were at first rather critical, even judgmental, but after listening to me changed their view and told me that they do understand that research laboratories need caring technicians who do the daily work with and for the animals.

It is my personal experience that most people—not all!—quickly stop their accusations when you tell them honestly what you are actually doing, and how your presence alleviates rather than causes suffering to animals in research labs.

< Many people think that working as a caretaker, technician or veterinarian in a research laboratory implies that you **condone animal research**, even if it may cause suffering and death to animals. How do you respond to this assumption? >

When I am drawn into a discussion on biomedical research and testing, I do my part to steer away from the question of pro or con animal experimentation. Yes, I do have a personal opinion on that issue but it is of no relevance, simply because I do not perform invasive experiments with animals myself. My mission is to care for animals assigned to such experiments, so I do my best to make sure that the animals are, at least, properly housed and handled.

You have to be careful with that attitude. If you come across as not agreeing that the research being conducted on the animals in your care is

beneficial, you are going to send a negative message to the public about research in general. I can hear comments like, “she is actually working in the lab and does not even believe that the research is necessary.” That can hurt all of us in the field. I cannot imagine being able to justify to myself the use of animals for projects that I don't believe in.

Exactly! That’s the reason why I categorically refuse to be actively involved in a project in which I don’t believe, either because of its adverse implications for animals, or because of its scientific weakness.

For me, the answer to the question if I believe that research is necessary is neither a Yes nor a No. Based on my own experience and based on the literature that I have read, my answer would be that it all depends on the particular research protocol:

- yes, there are certain invasive research projects that have significant scientific merit and, hence, are justifiable because no alternatives are available;
- yes, there are certain invasive research/testing projects that are necessary or legally mandated, but I am not in the position to argue for them because I am not an expert in that particular area of scientific research;
- no, there are certain invasive research projects that have insufficient scientific merit and, hence, are not justifiable;
- no, there are certain invasive research projects that are repetitive, hence are not justifiable because they are likely to cause unnecessary animal suffering, and
- no, there are certain invasive research projects for which alternatives are available; they are not justifiable because they are likely to cause avoidable animal suffering.

< Imagine, I am applying for a position at your Animal Care unit. What qualifications would give me the best chance to be hired by you? As part of the **job interview** would you give me a tour through the animal quarters? >

When I interviewed potential candidates, I first spoke with them on the phone and then brought them in; I needed to see them around some animals. I actually had them walk rounds with me; it helped a lot. Especially when you are dealing with primates, many people think they really want to work with them until they come face to face with them in the laboratory setting and reality sets in. It is very frustrating when people look so good on paper and in person, and then you get them around the animals and notice that something is missing—you don’t see that sparkle in their eyes that says *WOW! This is awesome, when can I start?*

What is true for monkeys holds true also for any other animal species found in the research lab. If a person applies to work with rodents, rabbits, dogs, cats, frogs, fishes, birds or farm animals, she has to convey her fascination of these animals in some spontaneous manner, otherwise there is a risk that she regards animals as things and will interact with them accordingly.

Rather than focusing on diplomas, I check if the job applicant has a positive connection with animals. For this purpose, I do make the necessary arrangements so that I can take the candidate on a tour of the animal holding areas. If I have any doubt about an applicant's relationship with animals, I take her or him to a rabbit room and keep asking questions about his/her background. If the rabbits stampede or thump, the person does not get the job. The animals are the best judges of a good or bad team player and animal welfare enthusiast.

It seems important to me not to take the animals' initial reaction to the presence of an unfamiliar person, but wait and see how quickly they settle down and how they react when the person tries to communicate with them. I take it as a good sign when monkeys come to the front of the cage to get a better view of the stranger, and the stranger can look at the monkeys without scaring them away. I take it as a bad sign when monkeys kind of freeze in the back of their cage while the stranger talks to them.

< How many of you on this forum **serve on the Institutional Animal Care and Use Committee (IACUC)** at your particular facility? >

My title is Enrichment Specialist; my duties include the management of our enrichment program and the behavioral health of our animals. I am an ad-hoc, non-voting member of our IACUC, and serve to keep the rest of the Committee informed of the state of our enrichment program. I review all protocols involving non-human primates and exemptions from any aspect of our enrichment program for all animal species during the approval process.

Your situation is a fair compromise, even though it is my personal opinion that a qualified animal caregiver or animal technician should be a voting member of any Animal Care and Use Committee. This person would quasi-represent the animals who, after all, are in most cases at the mercy of people who have very little understanding of their research subjects' biological needs. It is my experience that most animal caretakers and most animal technicians are better qualified to assess their animals' needs to be free of stress and distress prior to and during experiments/tests than principal investigators and chief veterinarians.

I think your idea makes a lot of sense since we, the animal care technicians, are the ones who are in direct contact with our animals every day. I could tell you specific behavior/personality traits of each monkey in my room and in a lot of the other rooms I work in.

A professor who knows his/her animals only as computer data is certainly in a much less qualified position to assess the impact of the housing conditions and the actual data-collection procedure on the animals' well-being and stress status than the caregiver and technician who work with the animals on a daily basis and collect the data directly from them.

I feel the same; no one knows these animals like the folks who care for them daily but, sadly, in many places they are not yet recognized for the important jobs they perform. They often feel overlooked and insignificant in the big picture.

Here in Canada, the Animal Care Committee is similar to the IACUC in the United States. I am the lab animal technician who looks after all animals listed in our research protocols. I review the protocols prior to the ACC meetings and discuss any issues that I may have with the committee members before they vote. I am also a non-voting member but like to attend the ACC meetings, as it gives me a chance to find out what's going on with the protocols under review and get first-hand knowledge of anything that could be an animal welfare concern.

In the United Kingdom, it is a standard protocol to have appointed animal technicians serve as full, voting members on the ethical committees—the equivalent to the IACUCs in America.

The situation is similar in Switzerland where lab technicians, dealing with the animals on a daily basis, are full voting members of the IACUC.

Discussion Participants

Adrian Smith, Alan Bonner, Alexandra Bakarich, Ali Moore, Alison Grand, Allison Reiffer, Alyssa Foulkes, Amanda Carlson, Amanda Harsche, Amy Kilpatrick, Angelika Rehrig, Amanda Knight, Amy Krikorian, Amy Robinson-Junker, Angelika Rehrig, Anita Conte, Ann Labans, Anna-Linnea Fernstrom, Anna Olsson, Anthony Ferraro, Arianna Manciocco, Audrey Brown, Augusto Vitale, Autumn Sorrells, Becki Brunelli, Betty Hoots, Bill Felts, Brianna Gaskill, Brianna Parkinson, Brianne Stein, Britney Morea, Brittney Armitage, Carol Barriere, Carolyn Allen, Carolyn Waugh, Casey Coke Murphy, Catherine Breault, Catherine Brochu, Catherine Hagan, Cathy Liss, Chris Sherwin, Christina Winnicker, Christine Thaete, Cindy Buckmaster, Claire Castellanos, Corri Witt, Crystal Johnson, Cynthia Roberts, Cynthia Santaniello, Darlene Potterton, Darren Minier, David Cawston, David Morton, David Seelig, Dawn Abney, Dawn Haida, Deborah Hartley, Eileen Boehle, Elva Mathiesen, Emily Bethell, Emily Patterson-Kane, Erica Griffith, Erica Watson, Ernest Davis, Eva Waiblinger, Evelyn Skoumbourdis, Fabio Fante, Faisal Guhad, Francine Dolins, Gail Heidbrink, Genevieve Andrews-Kelly, Hank Davis, Harriet Hoffman, Hazel Johnston, Heath Neville, Heather Doviak, Heather Harris, Heater Kirby, Heidi Denman, Heleen Van de Weerd, Holly Carter Anderton, Huw Golledge, Iliana Quintero, Inger Marie, Jacqueline Schwartz, James Love, Janneke Arts, Janette Wallism, Janice Parker, Jann Hau, Janneke Arts, Jas Barley, Jason Allen, Jeannine Cason Rodgers, Jennifer Defosses, Jennifer Green, Jennifer Lofgren, Jennifer Deutsch, Jennifer Penny, Jennifer Scott, Jessica Leclerc, Jessica Mikels, Jessica Peveler, Jillann Rawlings O'Connor, Jo Keeley, Joanna Cruden, Joanna Makowska, Joanne Edgar, Jodi Scholz, Joe Erwin, Joseph Garner, Judith Schrier, Jürgen Seier, Kaile Bennett, Karen MacLeod, Karline Elder, Karolina Westlund, Kate Baker, Kate Cherry, Kathleen Conlee, Kathy Clark, Katie Chace, Katie Mahoney, Kathleen Banks, Kathleen McDonald, Kay Stewart, Kayla Shayne, Keely Harding, Keely McGrew, Kelsey Neeb Lambert, Kendra McCafferty, Kim Moore, Kimberly Rappaport, Kris Alaimo, Krista Beck, Kristina Carter, Kurtis Swekla, Krystle Allison, Larry Williams, Laura Poor, Laura Richardson, Lauren Cresser, Larry Carbone, Lars Friis Mikkelsen, Lace Lively, Lesley King, Leslie Jenkins, Lisa Abbuhl, Lori Burgess, Louis DiVincenti, Louise Buckley, Lori Burgess, Lorraine Bell, Luis Fernandez, Lydia Troc, Lynette Chave, Lynette Hart, Marcie Donnelly, Marion Smith, Marisa Hall, Mary Feurtado, Marloes Hentzen, Martyna Abbatello, Mary Lu James, Masaki Tomoaga, Meagan McCallum, Meagan Shelter, Melissa Ann James, Melissa

Timm, Melissa Truelove, Michel Emond, Michele Cunneen, Michelle Martin, Michelle Walsh, Misty Williams-Fritze, Monica Carlson, Monica Keith-Luzzi, Moshe Bushmitz, Ngaire Dennison, Natasha Down, Octavio Presgrave, Paula Austin, Paula Bazille, Pascal VanTroys, Pascalle van Loo, Polly Schultz, Rachele McAndrew, Rebecca Brunelli, Renée Gainer, Richard Duff, Richard Weilenmann, Robert Hubrecht, Robin Minkel, Rolan Plesker, Rosemarie Einstein, Russell Yothers, Sam Izzard, Sarah Murphy, Sarah Rzewski, Sarah Thurston, Sarah Shannon Gray, Sarah Whitehead, Sharon Bauer, Sheila Roberts, Shelley Lower, Shirley Seamman, Sonja Banjanin, Stacie Havens, Stacie Perry, Stefanie Haba Nelsen, Steven Ortiz, Sue Howell, Sue Rubino, Susan Rubino, Sylvie Cloutier, Tadatoshi Ogura, Talia Acosta, Tara Lang, Tara Shelton, Theresa Faughnan, Theresa Mathiesen, Thomas Ferrell, Terri Hunnicutt, Toni Trahearn, Vanessa Herring, Vera Baumans, Viktor Reinhardt, Wendy Clarence, Yoshi Yoshikazu Ueno, and Yvette Chen.

References

Ader DN, Johnson SB, Huang SW and Riley WJ 1991 Group-size, cage shelf level, and emotionality in non-obese diabetic mice — Impact on onset and incidence of IDDM. *Psychosomatic Medicine 53*(3): 313-321

Alexander S and Fontenot MB 2003 Isosexual social group formation for environmental enrichment in adult male *Macaca mulatta*. *AALAS [American Association for Laboratory Animal Science] 54th National Meeting Official Program* : 141

American Association for Laboratory Animal Science 2001 *Cost of Caring: Recognizing Human Emotions in the Care of Laboratory Animals*. American Association for Laboratory Animal Science: Memphis, TN

American Psychiatric Association 1987 *Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition*. American Psychiatric Association: Washington, DC

Basile BM, Hampton RR, Chaudhry AM and Murray EA 2007 Presence of a privacy divider increases proximity in pair-housed rhesus monkeys. *Animal Welfare 16:* 37-30

Beynen AC 1992 Communication between rats of experiment-induced stress and its impact on experimental results. *Animal Welfare 1*: 153-159

Canadian Council of Animal Care 1997 Transgenic animals, animal welfare and ethics. *CCAC Resource Supplement Spring/Summer*

Carbone L 2021 Estimating mouse and rat use in American laboratories by extrapolation from Animal Welfare Act-regulated species. *Scientific Reports 11*: 493 https://doi.org/10.1038/s41598-020-79961-0

Clarke AS, Czekala NM and Lindburg DG 1995 Behavioral and adrenocortical responses of male cynomolgus and lion-tailed macaques to social stimulation and group formation. *Primates 36*: 41-46

Clough G 1982 Environmental effects on animals used in biomedical research. *Biological Reviews 57*: 487-523

Crockett CM and Gough GM 2002 Onset of aggressive toy biting by a laboratory baboon coincides with cessation of self-injurious behavior. *American Journal of Primatology 57*: 39

Council of Europe 2002 *Proposals for the revision of Appendix A of the Convention: Species-specific provisions for Non-human Primates*. Council of Europe: Strasbourg, France

Di Gangi BA, Crawford PC and Levy JK 2006 Outcome of cats adoption from a biomedical research program. *Journal of Applied Animal Welfare Science 9*:143-163

Donnelly MJ, Wickham A, Kulick A, Rogers I, Stribling S, Strack A, Doerning B and Feeney W 2007 A refinement of oral dosing in the common marmoset (*Callithrix jacchus*). *American Association for Laboratory Animal Science [AALAS] Meeting Official Program:* 45

Emond M, Faubert S and Perkins M 2003 Social conflict reduction program for male mice. *Contemporary Topics in Laboratory Animal Science 42*(5): 24-26

Fante F, Baldan N, De Benedictis GM, Boldrin M, Furian L, Sgarabotto D, Ravarotto L, Besenzon F, Ramon D and Cozzi E 2012 Refinement of a macaque transplantation model: application of a subcutaneous port as a means for long-term enteral drug administration and nutritional supplementation. *Laboratory Animals 46*: 114-121

Ferraro A, Brunelli R, Nelsen SL, Andrews-Kelly G and Schultz P 2013 Making use of a laser pointer as training and enrichment tool: a discussion by the Laboratory Animal Refinement & Enrichment Forum. *Animal Technology and Welfare 12*: 195-196

Garner JP, Dufour B, Gregg LE, Weisker SM and Mench JA 2004 Social and husbandry factors affecting the prevalence and severity of barbering ('whisker trimming') by laboratory mice. *Applied Animal Behaviour Science 89*: 263-282

Gebhardt-Henrich SG, Vonlanthen EM and Steiger A 2005 How does the running wheel affect the behaviour and reproduction of golden hamsters kept as pets? *Applied Animal Behaviour Science 95*: 199-203

Gentle MJ and Corr SA 1995 Endogenous analgesia in the chicken. *Neuroscience Letters 201*: 211-215

Greenman DL, Kodell RL and Sheldon WG 1984 Association between cage shelf level and spontaneous and induced neoplasms in mice. *Journal of the National Cancer Institute 73*: 107-113

Guhad FA and Hau J 1996 Salivary IgA as a marker of social stress in rats. *Neuroscience Letters 27*: 137-140

Haba Nelsen SL, Bradford D and Houghton P 2010 Laser Lixit™ training: an alternative form of target training that can be utilized in the daily husbandry care of rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*). *American Journal of Primatology 72*(Supplement): 27

Hassler CR, Moutvic RR, Hobson DW, Lordo RA, Vinci LT, Dill GS, Joiner RL and Hamlin RL 1989 Long-term arrhythmia analysis of primates pretreated with pyridostigmine, challenged with soman, and treated with atropine and 2-PAM. *Proceedings of the 1989 Medical Defense Bioscience Review* pp. 479-482. Johns Hopkins University: Columbia, MD

Huang-Brown KM and Guhad FA 2002 Chocolate, an effective means of oral drug delivery in rats. *Lab Animal 31*(10): 34-36

Kirchner J, Hackbarth H, Stelzer HD and Tsai P-P 2012 Preferences of group-housed female mice regarding structure of softwood bedding. *Laboratory Animals 46*: 95-100

Krohn TC, Salling B and Kornerup Hansen A 2011 How do rats respond to playing radio in the animal facility? *Laboratory Animals 45*: 141-144

Lagakos S and Mosteller F 1981 A case study of statistics in the regulatory process: The FD&C Red No. 40 experiments. *Journal of the National Cancer Institute 66*: 381-399

Line SW 1987.Environmental enrichment for laboratory primates. *Journal of the American Veterinary Medical Association 190*: 854-859.

Lofgren JL, Wrong C, Hayward A, Karas AZ, Morales S, Quintana P, Vargas A and Fox JG 2010 Innovative social rabbit housing. *American Association for Laboratory Animal Science Meeting Official Program*: 131

Mantel N 1980 Assessing laboratory evidence for neoplastic activity. *Biometics 36*: 381-399

Mason G, Wilson D, Hampton C and Würbel H 2004 Non-invasively assessing disturbance and stress in laboratory rats by scoring chromodacryorrhoea. *ATLA (Alternatives to Laboratory Animals) 32*: 153-159

McLean CB and Swanson LE 2004 Reducing stress in individually housed sheep. *American Association for Laboratory Animal Science 55th National Meeting Official Program*: 144

National Research Council 1996 *Guide for the Care and Use of Laboratory Animals, 7th Edition*. National Academy Press: Washington, DC

National Research Council 1998 *The Psychological Well-Being of Nonhuman Primates*. National Academy Press: Washington, DC

Organisation for Economic Co-Operation and Development 2000 *Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane*

Endpoints for Experimental Animals Used in Safety Evaluation [OECD Guidance Document No. 19 on Humane Endpoints]. OECD: Paris, France

Parrott RF, Houpt KA and Misson BH 1988 Modification of the responses of sheep to isolation stress by the use of mirror panels. *Applied Animal Behaviour Science 19*: 331-338

Patterson-Kane EG 2003 Shelter enrichment for rats. *Contemporary Topics in Laboratory Animal Science 42*(2): 46-48

Pines MK, Kaplan G and Rogers LJ 2004 Stressors of common marmosets (*Callithrix jacchus*) in the captive environment: Effects on behaviour and cortisol levels. *Folia Primatologica 75 (Supplement 1)*: 317-318

Reinhardt V 1991 Training adult male rhesus monkeys to actively cooperate during in-homecage venipuncture. *Animal Technology 42*: 11-17

Reinhardt V and Cowley D 1992 In-homecage blood collection from conscious stump-tailed macaques. *Animal Welfare 1*: 249-255

Reinhardt V and Reinhardt A 2000a Blood collection procedure of laboratory primates: A neglected variable in biomedical research. *Journal of Applied Animal Welfare Science 3*: 321-333

Reinhardt V and Reinhardt A 2000b The lower row monkey cage: An overlooked variable in biomedical research. *Journal of Applied Animal Welfare Science 3*: 141-149

Rourke C and Pemberton DJ 2007 Investigation of a novel refined oral dosing method. *Animal Technology and Welfare 6*(1): 15-17

Sarna JR, Dyck RH and Whishaw IQ 2000 The Dalila effect: C57BL6 mice barber whiskers by plucking. *Behavioural Brain Research 108*: 39-45

Scales M and McDonald KM 2011 Factors influencing the preferred nesting location of laboratory mice. *American Association for Laboratory Animal Science Meeting - Abstracts of Poster Sessions*: 31

Schnell CR and Wood JM 1993 Measurement of blood pressure and heart rate by telemetry in conscious, unrestrained marmosets. *American Journal of Physiology 264*(Heart Circulatory Physiology 33): H1509-1516

Schreuder MF, Fodor M, Van Wijk JAE and Delemarre-van de Waal HA 2007 Weekend versus working day: differences in telemetric blood pressure in male Wistar rats. *Laboratory Animals 41*: 86-91

Shyu WC, Nightingale CH, Tsuji A and Quintiliani R 1987 Effect of stress on pharmocokinetics of amikacin and ticarcillin. *Journal of Pharmaceutical Sciences 76*: 265-266

Spoolder HAM, Burbidge JA, Edwards SA, Simmins PH and Lawrence AB 1995 Provision of straw as a foraging substrate reduces the development of chain and bar manipulation in food restricted sows. *Applied Animal Behaviour Science 43*: 249-262

Traystman RJ 1987 ACUC, who needs it? The investigator's viewpoint. *Laboratory Animal Science 37*(Special issue): 108-110

United States Department of Agriculture 1991 Title 9, CFR (Code of Federal Register), Part 3. Animal Welfare; Standards; Final Rule. *Federal Register* 55(32), 6426-6505

Van Herck H, Baumans V and de Boer SF 1994 Assessment of discomfort in laboratory animals. In: Cohen J and Miller A (eds) *Auto-immune Disease Models, a Guidebook* pp. 03-320. Academic Press: New York, NY

Van Loo PLP, Kruitwagen CLJJ and Van Zutphen LFM 2000 Modulation of aggression in male mice: Influence of cage cleaning regime and scent marks. *Animal Welfare 9*: 281-295

Van Loo PLP, de Groot AC, Van Zuthpen BFM and Baumans V 2001 Do male mice prefer or avoid each other's company? Influence of hierarchy, kinship, and familiarity. *Journal of Applied Animal Welfare Science 4*: 91-103

Van Loo PLP, Blom HJM, Meijer MK and Baumans V 2005 Assessment of the use of two commercially available environmental enrichments by laboratory mice by preference testing. *Laboratory Animals 39*: 58-67

Walters SL, Torres-Urbano CJ, Chichester L and Rose RE 2012 The impact of huts on physiological stress: a refinement in post-transport housing of male guineapigs (*Cavia porcellus*). *Laboratory Animals 46*: 220-224

Weed JL, Wagner PO, Byrum R, Parrish S, Knezevich M and Powell DA 2003 Treatment of persistent self-injurious behavior in rhesus monkeys through socialization: A preliminary report. *Contemporary Topics in Laboratory Animal Science 42*(5): 21-23

Wolfle TL 2002 Introduction. *Institute for Laboratory Animal Research Journal 43*(1): 1-3

Young SS 1987 Are there local room effects on hepatic tumors in male mice? An examination of the NTP eugenol study. *Fundamental and Applied Toxicology 8*: 1-4

Photo Credits

page 1	M.K.Meijer, Utrecht University, The Netherlands
page 24	Bob Dodsworth
page 41	Ali Moore
page 42	Mary Rambo
page 43	Marji Beach
page 72	Viktor Reinhardt
page 106	Pehpsi Altemark
page 118	Max Resdefault
page 127	Michele Cunneen
page 143	Lyudmila Izmaylova
page 144	Don Burkett
page 145	Merel Ritskes-Hoitinga
page 162	Viktor Reinhardt
page 164	Viktor Reinhardt
page 169	Viktor Reinhardt
page 173	Viktor Reinhardt
page 177	Polly Schultz
page 181	Viktor Reinhardt
page 186	Bob Dodsworth
page 201	Polly Schultz
page 210	Viktor Reinhardt
page 213	Marcie Donnelly
page 216	Viktor Reinhardt
page 218	Viktor Reinhardt
page 227	Viktor Reinhardt
page 228	Viktor Reinhardt
page 235	Viktor Reinhardt
page 236	Polly Schultz
page 255	Viktor Reinhardt
page 260	Viktor Reinhardt
page 273	Tamara Godbey
page 274	Louis DiVincenti