

- We typically put two hamsters in a cage and encounter no real aggression-related problems. I let the two wake up before I reach in the cage and handle them. Some folks like the saying *let sleeping dogs lie*, mine is let sleeping hamsters lie. It occasionally occurs that just-waking-up hamsters are—understandably—grouchy. When this happens, I take a PVC tube section and simply herd them into the tube and pick them up.
- We house six to eight males in large cages where they receive sunflower seeds and have access to gnawing blocks. Generally, there is no fighting, but minor scuffles at cage change occur regularly. They result in a few squeaks and nips, but everyone calms down quickly and goes back to sleep most of the time; after all, they want to sleep

during daytime when we do our work with them. The hamsters often sleep on top of one another, suggesting that they do seek close contact with each other.

Occasionally, wounding does occur, and you can typically see the troublemaker. While you change the cage, remove him, and the others quickly resume harmonious communal life. My policy on hamsters is to try housing them in groups but to always be on the lookout, as some animals may get aggressive and need to be single-housed.

As for attitude, hamsters must be handled a lot or they will become feisty. This can range from screeching and teeth barring to actual bites and bloodshed. It appears to me that when left alone, a hamster remembers that he or she is by nature a curmudgeonly, solitary animal, content to be alone.



- These days, most commercially supplied hamsters—in the U.K. at least—have been selected for temperament and seem to tolerate each other if they have been reared together. Previously, we have overcome any aggression towards personnel by regular handling although, in fairness, most of the aggression is just bravado and, if you handle hamsters correctly, they rarely bite.
- In my experience, hamsters are usually aggressive to personnel, but not to each other. In order to minimize aggression against personnel, we gently handle the hamsters regularly before we conduct experiments with them.



pair housing

Is it easier to keep female hamsters than male hamsters in social settings?



- We keep five females, older than 30 days, per cage. They seem to get along with each other quite well, and there is hardly any overt aggression. They always sleep very close together, often one on top of the other. Even when they are eating, we do not see any antagonistic behavior.
- Our male hamsters live in pairs; they are not siblings. They may have a few arguments when the cage is changed, but they work it out; in almost all instances no serious stuff that requires separation.

guinea pigs



group housing of males

By transferring our pair-housed male guinea pigs to groups in pens with more opportunity for social interaction and exercise, we hope to keep their body weight at around 500 g. Our animals get fed chow ad lib, together with hay and a daily ration of carrots and apples; they have access to chew sticks and tunnels. What would you consider to be the optimal housing environment for these animals to help them satisfy their species-specific behavioral needs while also managing their body weights?

- It seems to me that you are already providing your animals relatively species-adequate living conditions. If you offer them the same feeding and structural enrichment when kept in groups, you are already doing your best pretty much.

However, 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 probably 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, you already do an excellent job with enrichment, but 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 achieve the same effect by changing their diet.
- I would not be too concerned about aggression but make sure that environmental factors don't trigger unnecessary aggression among the males.

I find that the presence of females in the same room is the main trigger for aggression among male guinea pigs. Refuges will not change the males'



behavior; once fighting starts, the integrity of a group can usually no longer be maintained, which is not an ideal situation.

We try to keep our females in a separate room, but if this is not possible, the two sexes are always in separate racks and the males are cleaned out before the females. Even though all our cages have solid floors, it is not uncommon that aggression among males is set off when females are kept in a tier above them.

proper diet to prevent obesity

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 to 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.



- Yes, it is frustrating at times to deal with PIs and administrators who stubbornly cling to the traditional way animals in labs have been kept, fed, treated and handled. It can be quite a task to break this inertia of tradition; it is worth the effort because it is possible!
- Our guinea pigs only get a pelleted diet and autoclaved hay if the study allows. I would love to see them eat fresh foods, but I guess the time allowance for that may be a problem in some facilities. The washing and preparing of the green stuff for the first feeding, then a second feeding at night, all takes time. Unfortunately some facilities just won't go for it, when it is so much easier and less costly to simply supply pellet food.

- Although it's true that guinea pigs adore their greens, I have been taught that animals housed indoors should be fed a staple manufactured diet in addition to greens so that they get the proper amount of vitamin C. If one were able to provide the animals with veggies rich in vitamin C, there might be a chance that one could lower the amount of manufactured food in the diet. However, I am not sure if the guinea pigs would be willing to eat enough of the veggies to obtain the needed nutrients; this would be a condition for such a diet regimen.





- Wild guinea pigs don't get a balanced commercial diet but they thrive well on natural plants and seeds supplemented occasionally with rain water.
- In order to feed greens, hay and vegetables as a staple food for captive guinea pigs, it is essential to make sure that the foodstuff provides the animals the necessary vitamin C. If this is not possible, supplemental vitamin C is needed.
- In my experience, guinea pigs like a simple vitamin C solution a lot. We dissolve one vitamin C tablet (for human adults) in water. The animals literally suckle it from the syringe voluntarily.

straw bedding

Is it a practicable and safe option to keep breeding groups of guinea pigs on straw?

- I would prefer hay for guinea pigs, as straw may cause eye damage. Hay is softer.
- You can use straw—we give it when we don't want the animals to obtain too much nutrition from foodstuff. However, the straw needs to be wheat or oat straw not barley; the awns in barley straw do, indeed, cause eye problems.





rabbits

shelter

Rodents have a need for a refuge area, be it a shelter or a self-constructed nest. Do rabbits have a similar need; if so, how can we address this need for animals who are caged alone and for animals who live in groups?

- Our group-housed rabbits regularly use an old metal rabbit cage—with the door removed—as shelter. 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.

When the rabbits are housed individually, we furnish each cage with an old plastic mouse box. The animals often sit on top of the box; some turn it upside down and sit in the box instead. The rabbits can push the box around until it's in a spot they like.

- We use huts for our group-housed rabbits who seem to like them. We make the huts by slicing large, very thoroughly cleaned, recycled chemical barrels in half, and then cutting out a couple of entries.
- Gerson (2000) modified traditional rabbit cages by linking two cages vertically by means of a ramp and installing in each cage a 30-cm high platform that the pair-housed animals regularly used as a shelter or lookout.

enrichment for single-caged rabbits



How do you enrich the environment of single-caged rabbits?

- What my rabbits really like with great consistency is autoclaved hay. It serves them both as a foraging substrate and a hiding place. Sanitary paper rolls entertain them quite a bit. They play with them and chew them, but the material gets soiled quickly and the rabbits then lose interest in the rolls.
- Most of the time we give our rabbits toys that make noise, such as hollow objects containing one or several bells, or stainless washers inside of plastic balls; the rabbits can move the item—usually by nudging it with the nose or picking it up with the mouth—and make noise. Typically, not all rabbits in a room are ringing the bells at the same time, so the noise is not much of an issue.
- When we first started giving our rabbits items to manipulate, we used canning jar lids as they were inexpensive and, at the time, we had a very large number of rabbits but a very small budget. The rabbits loved to pick the lids up, drop them on the

cage floor and push them around. If we went into the room and the rabbits were relatively inactive, all we had to do was drop a lid or tap it against a cage door to make the familiar tink sound and soon, most or all rabbits in the room were making quite a racket with their jar lids.

We have since expanded our enrichment toys to include small plastic barbells, and a variety of hanging toys such as metal chains with metal rings or bells attached to them. While inside the room the rabbits can be quite noisy with their toys, the noise level outside the room is not an issue.

Preference for the type of toy varies by rabbit but nearly all of them seem to enjoy any toy that makes a metal-against-metal noise.

- A little stainless steel bell, attached to a suspended foraging ball filled with hay, turned out to be a real hit for my rabbit at home. Both the hay and the bell exert an amazing attraction, but I must admit that the constant ringing can get on your nerves when you try to focus on something other than the cute rabbit.
- 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.

- Yes, rabbits love to make noise. We give them 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 down; they go to great lengths turning it around again and then flinging it across their cage!

- My favorite single housing set-up for rabbits was at a university in Canada. The rabbit room was subdivided by a series of baby gates that formed a grid. The animals had plenty of room for several hops across their floor space, could stand as tall as they wanted to, could hide in a big tube, and they could also touch noses with their neighbors and lay down on either side of the fence with direct body contact. The gates were too high for them to jump over.

When it was time to clean, the rabbits were temporarily placed in cages, the grid collapsed, the floor swept, and finally the grid and the bunnies replaced. Everyday the tech came in and fed them Cheerios; they would stand up to retrieve their treats. This served as enrichment but, additionally, the animals' reaction, or lack of reaction, was a reliable and early indicator of any illness.

- That's the way to do business with rabbits in the laboratory setting. So simple, but at the same time smart and rabbit-appropriate.



- Our rabbit banks have six cages, three rows of two. There are panels between the cages but they have a space at the top where neighbors can see each other and touch noses, if they so wish.

All our rabbits get autoclaved hay daily. When distributing the hay, I pet each rabbit while talking to him or her gently.

Each rabbit also gets a cardboard box. Some animals use it as a platform, some hide their toys or themselves in it while others use it as an outhouse. They all love to chew on them and tear them apart. We keep a steady supply of recycled cardboard boxes.

- We have no proof that the rabbits do not ingest any cardboard but have never had a problem. The cages are always full of the shredded cardboard but that is not to say they don't snack on it. We do take care to only use plain cardboard to avoid potential problems with ink from printing.



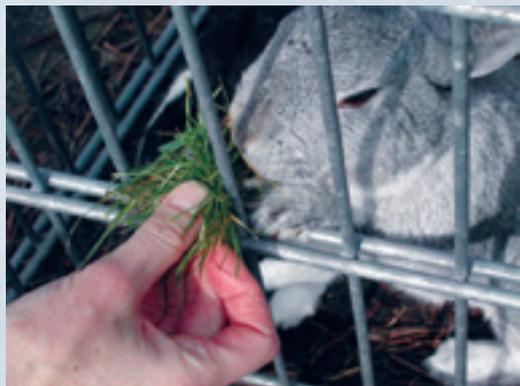
- Our single-caged rabbits also each get a small cardboard box, big enough for them to fit in or lie on top. We do not autoclave these boxes; we have been using them for many years without encountering any hygiene- or health-related problems. Nothing seems to make a bunny happier than a cardboard box to chew on! Our researchers do not raise objections that their rabbits have access to cardboard.

- If we single-cage rabbits, we arrange the cages in such a way that the animals can see one another. They are provided with hay on a daily basis including weekends, cardboard boxes—unprinted variety garnered from various sources and autoclaved—and a redundant plastic mouse cage that they can use as a platform. Cardboard boxes last an indeterminate time, some rabbits destroy them on a daily basis, and with other rabbits they may last a week. We have also tried metal rings fixed to the cage front which the animals can jingle—staff wear ear-protectors as noise can be deafening—but we found that these can cause problems as they become sharp; we had rabbits get legs stuck in these rings. We also supply food treats if protocols allow.

Researchers are encouraged to visit the rabbits at least once a day.



- Our single-caged bucks also have visual contact with each other. For entertainment, we give them empty plastic bottles, which they enjoy pushing around and throwing against the cage walls, thereby creating some noise.



- All of our bunnies are singly housed for both research and safety reasons. I have noticed that the more attention they are given, the calmer they are and the easier they are to work with.

We provide our rabbits with toys—jingle balls, plastic barbells, hay foraging balls and occasionally Kong™ toys. I've been trying to keep up a steady rotation as, like all other animals in labs, rabbits quickly become bored with the usual commercial toys.

- I have recently begun using large cardboard tubes—following investigators' approval of course—which the animals really seem to love. Some rabbits see how far they can fit into the tube, others knock it and roll it around the cage, and still others simply just chew the edges of the tube.

Finally, I try to find the time to stop by each bunny's cage every day. During those visits I give the animals treats and, if they want, a gentle scratch. Our long-term bunnies have gotten quite accustomed to this little ritual; I now have several who will bang their toys around in the cage or try to climb the cage bars to get my attention. I have to say that I really enjoy my rabbit rounds and have become quite attached to this little routine. I find my day is just a little empty if I don't get a chance to visit my bunnies.

handling

Rabbits can be quite feisty at times when you handle them during procedures. Do you take any precautions when dealing with such animals?

- It is my experience that most rabbits like to have their eyes covered when you handle them and choose it when they have the opportunity. When we hold rabbits for blood collection, using the saphenous vein, we place them on a counter with one hand holding the scruff and the other hand holding the hind leg. The rabbit can rest her head on my forearm or, as most of them do, tuck it under my arm against my side and elbow.
- 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 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 hold her by the 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."
- It seems to me that an animal—not only a rabbit but any animal—who is excessively intractable and, hence, suffers extreme distress when being handled and forcefully restrained, should be acclimated to the handling personnel and to the handling procedure especially thoroughly or be exempt from participating in that particular research protocol. After all, a distressed animal is not a good model for biomedical research. I also believe that we have an ethical obligation to avoid or alleviate suffering of the animals in our care whenever this is possible; and it is possible in almost all instances.



- I remember working with single-caged rabbits years ago at another facility. As soon as you entered the animals' holding area, all the rabbits freaked out because they were so frightened by people. To help them overcome their apprehension and fear, I developed the routine of brushing the fur of as many rabbits as I could during a week, clipping the animals' nails regularly and, most importantly, during my frequent visits, turning on a radio channel that played beautiful Gregorian chants and classical music in the rabbit room.

When the animal facility supervisor entered the rabbit room after a few months of this routine, his jaw literally dropped. He couldn't believe how calm the rabbits were

when he or even a stranger came into the room. I told him that I didn't consider the rabbits just a piece of meat.

The rabbits came to appreciate me. They enticed me to pet them, whenever I came into their room, by tapping on the floor of their cages until I gave them a big body scrub. Needless to emphasize that any handling procedure I had to conduct with these bunnies was easy to accomplish because the animals had gained trust in me.

- Rabbits do have the potential of becoming attached to humans when they have learned through experience that they can trust them.

- 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 do normally. At the start, we had several animals who were beyond feisty and would 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 animals 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.



- When I used to look after rabbits, I always petted each animal daily while giving them their hay. I started with just gently placing my hand on the neck area and then gradually stroking them from the top of the head down to the rump. I always talked to them while doing this so that they get used to my voice as well. By starting out this way I have encountered only a few aggressive rabbits.
- 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!
- I keep forgetting that I may be in the minority when it comes to talking to the critters—some of my coworkers will catch me in the act and ask to whom I'm speaking! Regardless, I always talk to the bunnies as I'm getting them used to common handling procedures. I enter the room with a greeting and then speak in a soft voice throughout the process, so they might associate my voice with the fact that what they're going through isn't all that bad.
- It is also my experience that talking to animals—this includes also wild animals/birds—is an amazing tool to establish positive communication with another critter. Many people shake their heads when they hear you talking to animals, but they overlook the simple fact that words per se are meaningless—just symbols—but words are accompanied by certain emotional vibrations and those are beyond verbal language and, hence, can be properly interpreted by other people and animals.
- I talk the moment I walk into any animal room until I leave. It is my impression that the animals get calmer and less startled when I talk to them in a gentle tone.
- In my experience, animal “users” who don't talk to the animals need to be watched carefully, as I feel it's a sign that they are not seeing the animals as living beings but more as some sort of machines, and they will most likely treat the animals accordingly.

blood collection

What is the safest and, for the subject, the 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 gentle and firm 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.
- When we bleed our rabbits, we give them a small shot of ace and, once that has kicked in, place them into hard-sided restrainers. We use the restrainer rather than a lab coat because it allows the animal to be bled by one individual rather than two—we have a small staff, so we often need to fly solo on procedures such as this. We then insert a 21- or 23-gauge butterfly extension into the ear artery and collect directly into tubes.

- For once weekly blood draws, we first acclimatize the rabbit to being handled, then place the rabbit on a counter and hug him or her with one hand while inserting a butterfly in an ear vein and collect the blood sample. It is my experience that the animals get used to this procedure very quickly and basically stop flinching after a few times and sit there, while you hug them for insertion, and then the rabbits relax and quietly sit on the table while you finish. If clotting becomes an issue I use the 3-inch tubing butterflies with vacutainer; the speed of collection will prevent clotting.

This method probably does not work for multiple blood collections per day. I think the rabbits would get fidgety.

- We normally sample from the saphenous vein. A 21-gauge needle without hub is inserted into the vein; the blood then drips from the needle into the collection container. If the rabbit dislikes this route, we use the ear vein with the same mode of collection.

A good rabbit handler is the most important, stress-buffering factor. If the rabbit is held properly, then all are safe and hopefully the sample will be obtained with one try, which means less discomfort for the animal.



- 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.

inappetence

What do you do when your rabbits persistently 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.
- It is my experience that allowing any rabbit showing signs of inappetence some additional exercise—such as running around the floor of the room for an hour—often helps.
- We also had this problem with our rabbits 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.
- Two years ago I adopted two dwarf bunnies. What I find very interesting is the



differences between the diet of a lab rabbit and a house rabbit.

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 most of their digestion; the hay is a very important factor for cecum health and normal cecum functioning.

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 working now, 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 also fed ad lib pellets and hay. The group-housed animals do not have weight gain issues, presumably, because of the exercise they get. The single-caged ones receive the same food but are kinda chubby. The chubbiness has not been a concern as of yet; even though these guys get little exercise, their bowels still are kept quite regular due to the hay! 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.

How do you make sure that the hay is free from pathogens?

- We autoclave it for three minutes at 221°F (105°C).
- We do the same.
- Does the autoclaved hay have any nutritional benefit, or do you provide it simply for enrichment? Also, how do you autoclave the hay? We typically wrap it in surgical drape material, but that smells so bad; I imagine it could add a bad taste to the hay.
- I doubt if there is much in the way of vitamins left after autoclaving, but then isn't the main benefit of hay 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. Our rabbits also get pelleted diet but only in small amounts.

We autoclave the hay either in strong paper sacks or autoclavable nylon bags that our sawdust is supplied in.
- At our institution the hay is autoclaved in cloth laundry bags and we always use the same ones over again. We no longer use the dry cycle after it burned the hay on occasion! We now dump it into big plastic bins and let it air dry. It works, and our rabbits seem to like the autoclaved hay.
- Since autoclaving destroys vitamins, we use irradiated hay at our facility.



pair formation of does

We will soon receive female Dutch Belted rabbits for an upcoming project. The PI would like to have these rabbits pair-housed. I have hardly any experience with rabbits, so I am wondering if anybody can share some practical advice on how to establish pairs of compatible does.

- We order our rabbits as early as possible and have them paired up already at the breeder station when they are still very young. This means, we get them at our facility as compatible pairs, so we don't need to worry about pairing them.
- 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 my 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.
- In my experience, Dutch rabbits can be pretty aggressive both to each other and to humans, but it depends very much on whether or not the breeder has selected for relatively docile animals and has already socialized them.

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 [Love & Hammond, 1991]. 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.

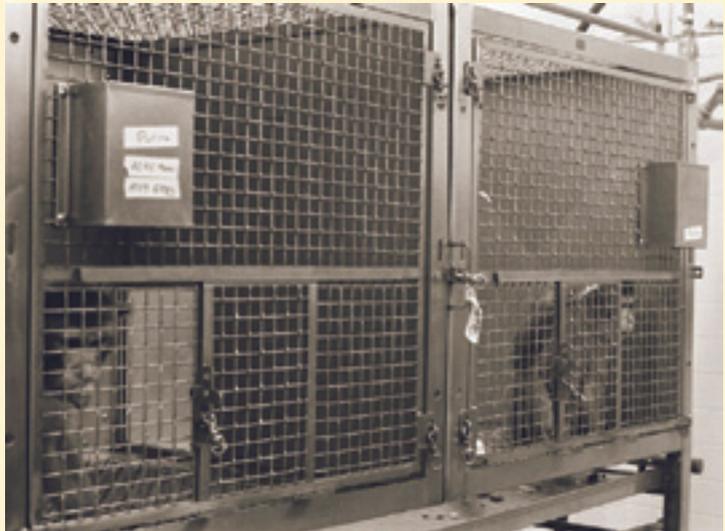
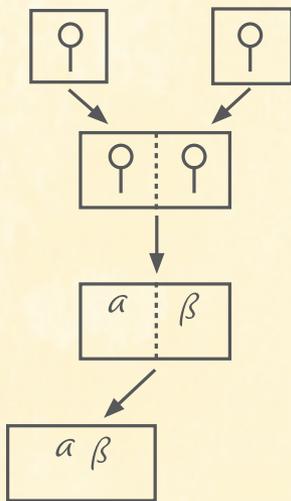


{Chapter 3}

Refinement and Enrichment for Primates



pair formation and pair housing of macaques



pair formation

How do you proceed when you want to establish a pair of adult macaques of the same gender who have lived most of their lives alone in single cages?

- In the past, we have pair-housed adult rhesus macaques with varying results. We are, therefore, in the process of changing our pair formation protocol hoping to increase our success rate. The biggest issue we have is pairing adult females who have lived alone for many years. In the past, we used to transfer a potential pair to a quad unit where the partners were separated from each other by transparent or steel-mesh cage dividers. We gave the two animals some time to get to know each other in this housing arrangement. When no overt aggressive gestures were witnessed in the course of several days, we pulled the cage dividers, thereby giving the two partners full access to each other.
 - I believe that this method allows the macaques to develop territorial feelings, which decreases our chance of success when we pull the diving panel. This is how we plan to refine our pair formation procedure:
 - (a) Pair potential partners in a new cage after their non-contact socialization period; this should eliminate territorial-related hostility.
 - (b) Make systematic efforts to observe the animals during their non-contact familiarization, with the intent of determining if the two partners have established a dominance-subordination relationship.
 - (c) Form a new pair only if the two partners have established a clear rank relationship.
 - (d) Give the new cage companions the option of breaking visual contact with each other, either by installing a privacy panel or by pairing them in a whole quad unit where one partner can be out of the other's sight in the top section and the other partner in the bottom section of the cage.

- It's true, pairing adult female rhesus macaques can be a big challenge; they are often quite crabby. I have worked with ten single-caged females and managed to match up only one compatible pair.
- I, too, had this problem with 15 female rhesus. Of 14 different pair combinations tested, only three turned out to be compatible.
- We had two adult male cynos [long-tailed macaques] 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.

- It is sometimes not possible to find out what triggered a fight between two animals who had given the impression of getting along well with each other. The case you are describing suggests that territorial competition triggered the instantaneous aggression between the two males.

When I started pairing rhesus macaques 24 years ago, I also simply removed the transparent cage-dividing panel that had allowed two animals

to get familiarized with each other.

After a few tests it became evident that being introduced to each other in their own home cages can trigger territorial antagonism, with one or both partners fiercely defending her or his home cage. After I learned my lesson, I made it a rule to transfer a new potential pair to another room and introduce them to each other in a double cage that is new for both of them. This required some extra time but solved the problem of initial aggression related to territorial feelings. Once the new pairs were settled and their compatibility was evident, I moved them back to their original, now interconnected home cages.

- It seems important to first allow two animals to establish a dominance-subordination 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 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—to avoid possible territorial antagonism and interference by other familiar animals in the room. Since they have already established a dominance-subordination relationship, they really don't have any good reason to fight over dominance again.

I have tested in this manner several hundred same-sex dyads of adult rhesus

and adult stump-tailed macaques without running into problems related to serious fighting (Reinhardt, 1989; Reinhardt, 1994a; Reinhardt & Reinhardt, 2008).

Pairs were compatible in most cases:

- 95 percent of 77 female rhesus macaque pairs tested;
- 95 percent of 20 male rhesus macaque pairs tested;
- 100 percent of 10 female stump-tailed macaque pairs tested; and
- 100 percent of 6 male stump-tailed macaque pairs tested.

- I formed a pair of two adult male rhesus macaques who had lived, each by himself, in the same room for over ten years. I was very nervous because they were 13 and 16 years old animals, and at that time I had only paired juveniles and young adults.

I monitored their behavior on video for about a week during which I moved their cages closer and closer each day. They lived in ordinary baboon cages that allowed them to see each other only when they were up on the perch. I looked for unidirectional dominance or submissive behaviors.

Once I was sure that they figured out who is dominant and who is subordinate, I opened the doors so that they could visit each other. Initially, I left the doors open only during the day and separated them for the night. The two males did very well together, so I decided to leave the doors open permanently.

The two buddies got into a fight after several months, but we figured out what caused it, fixed the problem and allowed the two to stay together as a compatible pair.



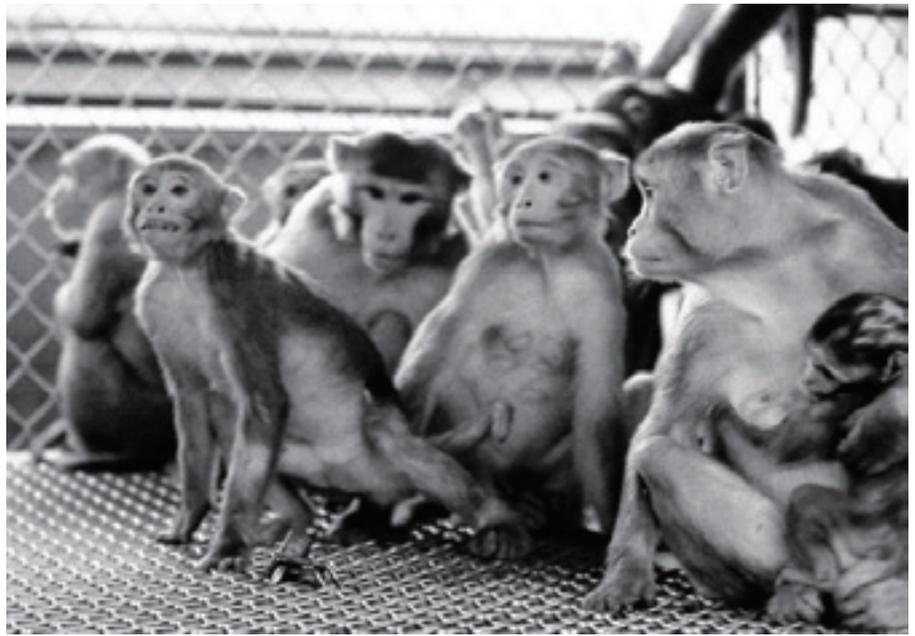
Could you please tell us what the circumstance was that caused your pair to have a fight after several months?

- Big Guy and Theo, the two older males I had put together at that time, shared a room with two single-caged *teenagers*. One of the youngsters appeared to challenge the older guys; he was really a very wild fellow who made a lot of aggressive displays towards the two seniors. I had started to notice this behavior before the fight and, every once in a while, I would see Big Guy making intense visual contact with this teenager when he was strutting his stuff. I think Big Guy got fed up by the constant provocation of this young male and finally just snapped and took it out on Theo which then led to this pretty bad fight. I still have the big canine teeth that Theo lost during that fight! Even though Theo was the dominant male in this pair, he actually was injured the worst.

I removed the two teenagers, with the thought that they had indirectly triggered the conflict, and replaced them with two single-caged seniors, Jay and Ross. This eliminated the problem, and Big Guy

and Theo continued to live together as a compatible pair for about two years. Unfortunately, the two were then separated for husbandry-related reasons. When Big Guy started to engage in self-injurious biting, we got permission, fortunately, to pair-house him again with Theo. The self-biting stopped, and the two are still living together as a content pair.

- I think it's great that you took the risk of allowing Big Guy and Theo to continue living together after this extraordinary fight. It is more than fair to carefully assess the background that may have caused a spat between two animals who have been compatible for a long time and then consider leaving them together, if the cause of the conflict can be removed.



What tells you that two animals have established a dominance-subordinance relationship during the familiarization period?



- When the following behaviors are consistently shown by the same partner, I assume that a pair has established a rank relationship, with the animal showing these behaviors being subordinate and the other animal being dominant:
 - (a) fear-grinning,
 - (b) withdrawing,
 - (c) looking/turning away when being looked at by the other partner,
 - (d) yielding when the other partner comes very close, and
 - (e) threatening the observer or other monkeys in the room and looking back over the shoulder to the partner—to make sure that the partner sees that he/she is *defended*.

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.

- 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 not even 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.

Threatening the other partner is not a gesture that reliably reflects dominance. I have seen subordinate animals threaten dominant animals—who usually overlook such silly behavior.

- What seems to be crucial is that submissive behaviors are shown strictly only by one partner *before* you introduce the two as a pair in a cage without a dividing panel. If you cannot verify this, it's good advice to wait another day or two, continue observing the animals and test them with food treats. If you don't get a clear picture of the partners' rank relationship, it is better to give up and test another combination rather than take the risk of introducing them and possibly having them fight over dominance.

There are situations that I take as warning signals that two partners have not yet come to an agreement on who is the boss and who has to submit. Typical scenarios for such ambivalence are when:

- (a) both partners threaten each other;
- (b) both partners show fear-grinning after being looked at by the other, and
- (c) both partners sit next to each other—with the familiarization panel between them—and calmly take treats from my finger tips.

- Consistency of rank-indicating behaviors is also the most important thing I am looking for before introducing two animals as a new pair. New partners are familiarized with a mesh/grid dividing panel. Only when I am pretty sure that the two have sorted out their rank relationship will I test them by drawing the panel just a bit so that they can touch and groom each other during a 30-minute and later during a 60-minute supervised session. When they pass these tests without noticeable antagonism, I remove the panel and allow full contact. This protocol has worked great for all my adult female rhesus macaques.
- It is my experience that potential cage partners 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 sedation a safe 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 their 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. Can you 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 reintroducing paired rhesus macaques who were separated for a longer period of time, after first sedating the partners; it was assumed that, since the two companions 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 counterparts.

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 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.

privacy panel and grooming-contact bars

What is the privacy panel good for?

- A privacy panel is a regular cage divider with a passage hole close to the back wall. Two animals can access the two feeders in the front of each cage section separately without having visual contact with each other. This makes food monopolization impossible, or I should say almost impossible. I remember one dominant guy who, during the first few days after pairing, tried to eat from both feeders kind of simultaneously, until he finally gave up this rushing around and allowed the subordinate partner to eat quietly. Fortunately, the subordinate didn't get depressed during the first days. He may have anticipated that the other guy would, one day, get so exhausted as to stop his

silly racing back and forth. He was right; this pair also turned out compatible.

Privacy panels have proven to be so useful in facilitating long-term pair compatibility that they were installed in all cages of the more than 300 pairs of macaques at our facility.

- We have two adult cynos who matched up very easily as a pair, but when they were fed for the first time in their new double cage, they tore each other up pretty badly; we have not been able to pair-house them since. Our cages don't have privacy panels. I wish they did, since the feeders are in the front of the cages and partners can watch each other eating and become competitive.

- In the journal *Animal Welfare* there is a great article by Basile et al. (2007). Based on their observations of 25 pairs, the authors conclude that the increase in proximity associated with the presence of the privacy dividers reflects an increase in social tolerance and/or attraction, and that a privacy divider may provide a safe haven and give monkeys the ability to diffuse hostile situations before they escalate.

Encouraged by these results, we are now trying such privacy dividers for our adult macaque pairs to mitigate possible social tension.



Who can share experiences with the grooming-contact bars housing arrangement for monkeys?

- We tried the grooming-contact bars with duos of juvenile and adult cynomolgus macaques of both sexes and found that they caused more problems than benefits.
- It is my experience that paired rhesus macaques interact at lower levels and show fewer behavioral improvements when they are separated by grooming contact bars compared to when they have free access to each other [Baker et al., 2008]. However, it is clear that the welfare of the animals is improved in the grooming-contact bar housing arrangement relative to single housing.
- Crockett et al. (2001, 2006) tested adult same-sex pairs of several species by housing them in double cages in which partners were separated by widely spaced vertical bars that permitted grooming but not aggressive pursuit. The following pair compatibility was found:
 - (a) female cynomolgus macaques, 89 percent,
 - (b) male cynomolgus macaques, 67 percent,
 - (c) female yellow baboons, 57 percent,
 - (d) male yellow baboons, 64 percent,
 - (e) female pig-tailed macaques, 53 percent,
 - (f) male pig-tailed macaques, 57 percent, and
 - (g) male rhesus macaques, 16 percent.

signs of partner compatibility

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—first the dominant partner and then the subordinate partner—without being hindered by the other companion, I know that the two are reliably compatible, even in a potentially competitive situation.

males

Do you find that males are less tolerant of each other than females and, therefore, less suited for pair housing?

- In my own experience with rhesus and stumps, I can say that 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. When no surplus infants are available, I keep them alone. As time goes by, they settle down and become more mellow. I managed to establish compatible pairs with all of them that I can remember.

- I've found that a large proportion of the 6-7 years old, pair-housed rhesus males at my facility become intolerant of their companions at this age, resulting in injurious fighting and separation. I should add that the fights typically occur when a previously compatible pair has been separated for several weeks, and is then reunited after a brief familiarization period. Despite this, I've found that adult rhesus males are easier to socialize and keep in a social housing setting than adult rhesus females.

- From personal experience, I know that pair housing previously single-caged male rhesus macaques can be a highly successful procedure, if the exercise is well planned, executed, and the individual characteristics of the monkeys are considered.

I first test the rank relationship between two potential cagemates by arranging their home cages in such a way that they face each other and then offering a piece of food midway between the fronts of the two cages:

- (a) The dominant monkey will take the food without any hesitation, while the subordinate monkey will not dare to pick up the food.

(b) The dominant animal will often redirect aggression towards the subordinate monkey when anyone is approaching them; the subordinate monkey will look away rather than retaliate with aggressive gestures.

If the two animals show no indication of a clear dominance relationship, we do not proceed with the pairing but test them with other partners until we find the right match of a clearly submissive and clearly dominant male.

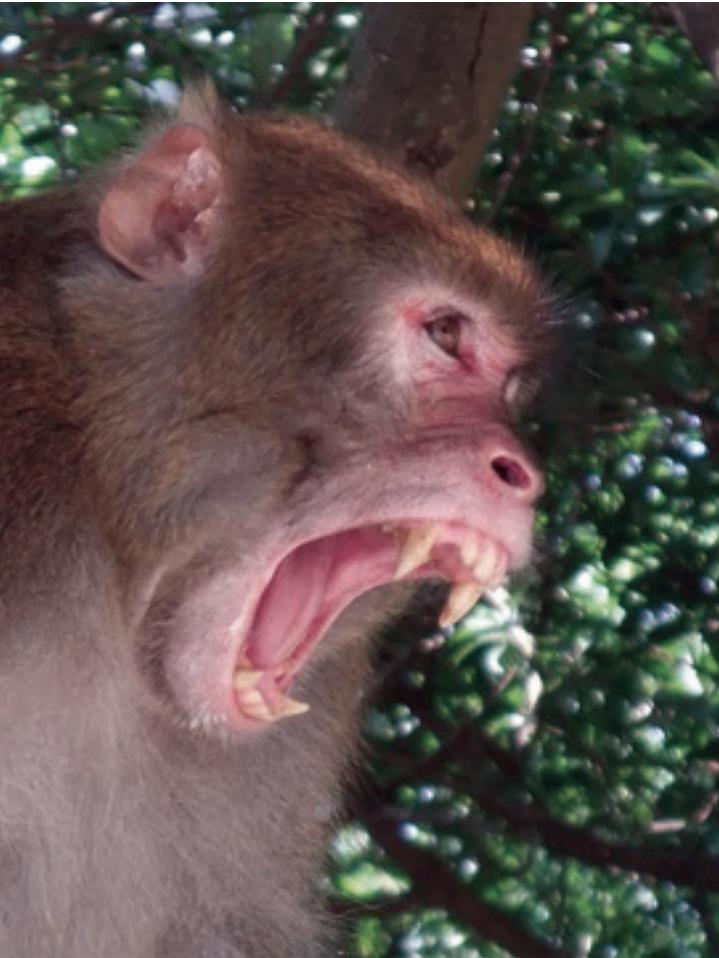
Providing visual barriers and taking care that the males have no contact with receptive females has probably helped us to make the pair housing of our rhesus males a success in most cases. When we deal with a male for whom we have difficulties finding a suitable companion, we pair him with a juvenile male who always takes the role of a subordinate animal; these pairings always work well.



- 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 [Reinhardt, 1992].
- I do believe that male rhesus macaques are suitable for pair housing. It takes some time to match the pairs well and monitor their long-term compatibility, but it is worth the effort; to be housed with a compatible companion seems to be so important for these highly social animals.
- One of the LAREF members has recently co-authored an article that seems to be tailored for our discussion on pairing adult male rhesus macaques (Doyle et al., 2008). Behavioral and physiological data were collected on eight adult male rhesus macaques before, during, and after pair formation. Partners were first familiarized with each other during a 24-hour period via a panel consisting of bars spaced 2 cm apart. They were subsequently paired by removal of this panel. All four introductions were successful and subjects showed no physiological or behavioral signs of stress, such as increased heart rate, or psychological indices of distress. Aggression was minimal. Fecal cortisol levels were lower in the compatible pair housing situation than in the single housing situation. Obviously, living with each other as pairs was not a stressful housing arrangement for these adult males.

Do you have to keep male pairs away from receptive females 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 *must* 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.
- This is exactly what I have observed and it is the reason why I recommended, at our institution, to keep male pairs in male-only rooms.
- I have also worked with quite a number of male stump-tailed macaque pairs who had visual contact with receptive adult females; the males' compatibility was not noticeably affected by the females' presence.



There is published evidence that the blunting of canines of male vervets reduces the incident of serious trauma related to aggression (Knezevich & Fairbanks, 2004). Based on your own experience, would you recommend the blunting of canines of adult male macaques as a preventive procedure against serious laceration resulting from overt aggression?

- We don't blunt the canines of our males because we had some bad experience with males that we received from other facilities. Their canines were blunted and so badly infected that we had to remove them altogether. We want to avoid this with our own animals.
- There was a time when we blunted canines of subadult and adult male rhesus, hoping that this would reduce the incident of bite lacerations. We stopped this program after about a year because quite a number

of males developed abscesses, which made it necessary to extract the roots of the amputated canines. On top of that, males with blunted canines will continue delivering bite lacerations that also require surgical treatment. Bite wounds inflicted with blunted teeth tend to be more tissue damaging than bite wounds inflicted with pointed, intact canines.

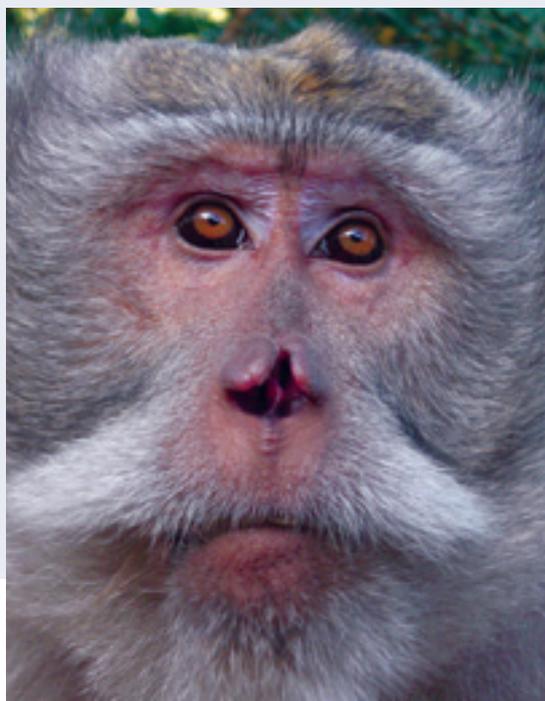
- The practice of blunting canines was stopped many years back at my facility. Some of the macaques who had the procedure done developed abscesses as well.

I have also seen one case of a macaque who needed sutures after getting in a fight with another male with blunted canines.

- We have never blunted the teeth of our macaques. I believe the males could still do a great deal of damage even with blunted canines. I've also been taught that the removal or blunting of canines can affect species-typical behavior, as the males would normally use their canines to display dominance.

- I would recommend blunting the canines of rhesus in a group-housing situation. With blunted teeth there can still be serious injuries, and I have seen some. However, I think it helps to avoid life-threatening injury.

Having said this, we pay for a veterinary dental specialist to blunt the canines. This way we minimize the chance of complications. Dental radiographs are taken, and can be retaken at a later date, to ensure the integrity of the teeth. I think this is one of the main points: if canine blunting is done, it has to be done correctly!



aged animals

How safe is it to try pairing old animals who have always lived alone?



- I have isosexually paired quite a number of over 25 years old rhesus males and females who have lived most of their lives alone. These animals created no special problems and I paired them in the same manner as younger animals. They must first show me that they have established a clear rank relationship during a non-contact familiarization period; they are then introduced to each other in a new double cage [Reinhardt, 1991a].

Pairing aged animals was an especially positive experience, because these poor critters had spent so many years in single cages with nobody to groom and nobody to hug them. It was amazingly easy to establish compatible pairs, and you should have seen how new companions groomed each other! Finally they could be *true* monkeys; some of them reached the age of 35 years!