Improving Animal Welfare and Data Accuracy in Primate Research Laboratories: Discussion and Recommendations for the USDA, the IACUCs, and the Research Funding Institutions

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January 1999 – September 2004

September 12, 2005
Submitted to the United States Department of Agriculture (USDA), Institutional Animal Care and Use Committees (IACUCs), and research funding agencies for the purpose of refining procedures and improving animal welfare in primate research institutions.

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I. Introduction

Overview

This report is intended to assist governmental regulatory agencies and research funding institutions in improving animal welfare in primate research facilities at a faster pace. As a former research specialist who worked with rhesus monkeys at a NIH-funded and USDA-accredited primate research facility, I provide a detailed account of my encounters with stressful data-collection and husbandry procedures, psychologically distressed rhesus monkeys, and obstacles to my attempts in improving animal welfare. My experiences as a primate researcher combined with scientific literature and Animal Welfare Act (AWA) regulations (USDA, 2002) are incorporated in this report in order to provide valid recommendations aimed at improving both nonhuman primate welfare and employee morale.

In addition to the AWA legislation, I also incorporate excerpts of the Public Health Service (PHS) policy (OLAW, 2002) into this report. NIH-funded institutions are required to abide by these guidelines and use *the Guide for the Care and Use of Laboratory Animals* as a basis for providing husbandry for the animals. The goal of the *Guide for the Care and Use of Laboratory Animals* is to:

> "…promote the humane care of animals used in biomedical and behavioral research, teaching, and testing; the basic objective is to provide information that will enhance animal well-being, the quality of biomedical research, and the advancement of biologic knowledge that is relevant to humans or animals." (National Research Council, 1996)

Although I worked at the USDA-accredited and NIH-funded laboratory for five years, this report is a personal account of events occurring during the last two years of my employment (2003 & 2004). By combining references to literature and legislation with my personal accounts, I hope to assist the Animal and Plant Health Inspection Service (APHIS) (a division of the United States Department of Agriculture (USDA)), the Institutional Animal Care and Use Committees (IACUCs), the National Institutes of Health (NIH) and other funding institutions in improving and enforcing the 'Refinement' aspect of the 3 R's: Reduction, Refinement and Replacement (Russel and Burch, 1959) more effectively across all laboratories. I am not disclosing the research institution that I worked at or the names/gender of the people involved in the incidents in this report because the problems I witnessed were a result of the flexible governmental policies and the stressful husbandry and data-collection practices still deemed acceptable by the IACUC, USDA, and NIH.

Loopholes in the Animal Welfare Act

There are several loopholes in the AWA regulations that enable stress-inducing procedures and species-inadequate housing (Reinhardt, 2004). One problem is that the AWA has flexible regulations and is meant to accommodate many types of nonhuman primate species (APHIS, 1999). Visalberghi and Anderson (1993) justify the purposeful vagueness of the guidelines:

> "Early experience, individual life history, personality, sex, age and species are all factors influencing what is 'good' or 'bad' for any particular individual. In other words, the diversity of

> primates is so great that precise regulations cannot seriously be expected to adequately accommodate all captive individuals."

The negative consequence of the non-specific regulations is a variation in data-collection and husbandry methods across research institutions who work with the same species of primate.

Many stress-inducing procedures such as restrained blood draws, hand-catching, chair-restraint, and orogastric gavages are classified as "noninvasive" and the full details of the procedures are overlooked by the IACUCs when reviewing the protocols, by the USDA(APHIS) when performing inspections, and by the funding institution when reviewing the grant proposals.

Inertia of Tradition

The Guide for the Care and Use of Laboratory Animals also has flexible guidelines and suggests using professional judgment regarding specific decisions:

> "The *Guide* is deliberately written in general terms so that its recommendations can be applied in the diverse institutions and settings that produce or use animals for research, teaching, and testing; generalizations and broad recommendations are imperative in such a document. This approach requires that users, IACUCs, veterinarians, and producers use professional judgment in making specific decisions regarding animal care and use." (National Research Council, 1996)

The "professional judgment" of the Principle Investigators (PIs) at the research facility I worked in involved employing traditional, stress-inducing handling techniques. Therefore, when new evidence of a less-stressful methodology arose, the mentality of "we have always done it this way" took precedence over the more innovative methodologies.

Reinhardt (2001) suggests that the unwillingness to conform to less-stressful practices is due to the inertia of tradition: "Many scientists resist any changes in the traditional husbandry practices of research monkeys, probably because of fear that historical data will be invalidated by different, albeit better, housing and handling conditions."

Stress-inducing Procedures

The stress-inducing methodologies in this report occurred contrary to the recommendation of *The Guide for the Care and Use of Laboratory Animals*: "Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors." (National Research Council, 1996) The current use of stressful data-collection methodology in some laboratory settings raises concern regarding the validity of data collected. It is well-established that even routine clinical procedures can raise stress levels of animals. Line, Morgan, Markowitz, and Strong (1989) examined the affects of stress on monkeys during routine events:

> "The persistent elevation in heart rate for several hours after cage changing and TB testing were completed was an unexpected finding. Though we anticipated that these might be aversive procedures, we assumed that the monkeys would have habituated through repeated exposure…

> Events that we tend to dismiss as potentially stress-inducing on the basis of their regularity may have more influence on our subjects than we suppose."

The differences between using negative stimuli and positive reinforcement when performing husbandry and data-collection procedures are profound. Research methodology can vary from stressful data collection by means of negative reinforcement (e.g. blood collection with leather gloves) to data collection by means of positive reinforcement (e.g. cooperative blood-draws and injections). Husbandry methodology can vary from the stressful removal of monkeys from the home cage by using poles, nets, yelling, and banging, to voluntary removal from home cage by using positive reinforcement training. Although there is evidence of refined methodologies by means of positive reinforcement training in some laboratories (Laule, Bloomsmith, & Schapiro, 2003; Reinhardt, Cowley, Eisele, & Scheffler, 1991), stressful data collection and husbandry techniques are still deemed acceptable by many research laboratories, by the IACUCs that review their protocols, by the USDA (APHIS) that performs the inspections, and by the funding institutions that review the proposals. The USDA (APHIS), the IACUCs, and funding institutions are needed in the thorough evaluation of various data-collection and husbandry techniques in order to make improvements in these procedures.

Section 2.131 of the Animal Welfare Act states: "Handling of all animals shall be done as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, excessive cooling, behavioral stress, physical harm, or unnecessary discomfort." (USDA, 2002, §2.131, p 52) Standardization of least-stressful methodologies across all research laboratories is necessary in order to improve data-collection and animal welfare in nonhuman primate research. Since APHIS, a division of the USDA, is the common regulatory body among extramural research facilities, it has the authority to evaluate and record different data-collection techniques and work with the IACUCs in implementing the least stressful methodology. The resulting standardization would ensure stronger compliance with the Animal Welfare Act and, as a result, behavioral stress and trauma would be minimized among all research facilities. The use of stressful husbandry and data-collection methodology must be phased out at a quicker pace. The only way to eliminate stressful animal-handling practices is for the regulatory bodies and funding agencies to evaluate the husbandry and research methods occurring at every research facility on a regular basis.

Background of Author

From 1999 to the fall of 2004, I worked as a student-researcher and then as a research specialist. During this time, the research facility I worked in housed approximately 450 rhesus monkeys. My situation was unique because I worked with the same 95 rhesus monkeys (approximately 90 of the monkeys were funded by two NIH studies) for the duration of my employment. This allowed me the opportunity to witness the individuality in the rhesus monkeys and to understand that each animal required its own set of unique care and handling procedures.

I learned the husbandry and data-collection techniques as a student and then completed my undergraduate degree in Medical Microbiology and Immunology. While pursuing other job

opportunities, a research specialist position opened at the laboratory. The PI and lab manager hired me because I was an experienced leader of the students who was always willing to work extra hours for the monkeys and the research project. I took pride in my job and trained the incoming students to be as humane as possible in handling the animals.

In order to share refinement ideas between research laboratories, voluntary email forums such as the Primate Enrichment Forum (PEF) and the Laboratory Animal Refinement and Enrichment Forum (LAREF) were available to researchers to share information pertaining to refining husbandry and data-collection procedures. The Principle Investigator (PI) of my laboratory did not have time to be on these forums and was not around the monkeys, because (s)he had classes to teach, grants to write, and data to analyze. I felt it was my responsibility as a researcher to improve the welfare of the monkeys and so I contributed regularly to these forums and read about other experiences of nonhuman primate researchers. It was on these forums that I learned about the variability of procedures that was occurring among research labs.

Collection of literature for the purpose of improving animal welfare is suggested in The Guide for the Care and Use of Laboratory Animals: "Relevant objective information regarding the procedures and the purpose of the study should be sought from the literature, veterinarians, investigators, and others knowledgeable about the effects on animals." (National Research Council, 1996) I learned from the literature and from my own experiences that animal welfare should have been given as much priority as the research being conducted.

I also discovered there were many efforts set forth regarding the refinement of husbandry and clinical procedures in the United States and other countries. Morton et al (2001) discuss the purpose of The Joint Working Group on Refinement, an animal welfare workshop funded by the British Veterinary Association:

> "The workshops are based on the important principle that whenever animals are used in laboratories, minimizing any pain and distress should be as important an objective as achieving the experimental results. This is essential for humanitarian concerns. It is also necessary in order to satisfy the broad legal principles in national and international legislation, and to produce reliable and reproducible scientific data."

The refined methods I found most compelling included positive reinforcement training (Pryor, n.d), increasing naturalistic behaviors (Reinhardt, 1993), and creating a trusting relationship between the researcher and the monkey (Waitt, Buchanan-Smith, and Morris, 2002). I frequently discussed the literature I had read with the lab manager and the PI. Usually, the feedback I received for not moving toward a less-stressful method was one or more of the following reasons:

- We have always done it this way.
- We can't change methodology mid-study.
- There is not enough money.

- It will take too much time.
- There is no empirical evidence indicating the benefit.
- Monkeys have a high-pain tolerance and are resilient.
- These monkeys live in captivity-of course they are going to be stressed out.
- You are thinking of the monkeys like they are human.
- You are being too emotional.

Despite this resistance to refinement, I was still able to implement minor changes that did not require additional money or protocol amendments. For example, I spent extra time many weekends clicker training (positive reinforcement training) monkeys for transport removal. I also encouraged trusting relationships between the students and the monkeys in order to create a less-stressful environment for blood draws or injections. Building a trusting relationship was difficult, however, since using negative stimuli was a common practice and so the monkeys developed a negative association with personnel.

We had the motto in the lab that "the monkey always comes first." This was true in the case of performing routine check-ups and treating sick animals. When one of our monkeys got sick, the animal care staff did not have the time to spend on treating the research-assigned monkeys. They were constantly cleaning cages, weighing animals, and treating colony (unassigned) animals. Therefore, the students and I assumed the role of treating the animals assigned to our studies. I spent approximately 35% of my time treating injuries and illnesses in the laboratory. Since I was getting paid to perform research, this time-investment was taking away from the time that I could spend assisting with the research project. The lab recognized that I needed to treat the animals because we could not perform research on them until they healed from their illness. As a result, there were several occasions where data-collection was cancelled due to illness or injury.

With the hope of improving the welfare of the monkeys at the lab, I attended the Animal Behavior Management Alliance (ABMA) in Baltimore, MD in April, 2004. I learned about several success stories of training various types of species of animals using positive reinforcement techniques to cooperate with clinical procedures such as blood draws or injections. I tried to incorporate the positive reinforcement principles I learned into the laboratory environment. When I justified spending time on clicker training to remove the rhesus monkeys from their home cages, my PI did not recognize the need for positive reinforcement training and insisted that the current practices in the laboratory were acceptable. As a result, both the PI and lab manager did not support my spending any "researcher" time training the animals. For a couple of months, I came in early, stayed late, and came in on weekends to train the animals. Although I witnessed some progress with positive reinforcement training, the task became too time-intensive for me since it was not part of my 40-hour work week. I tried to assist the animal care staff and other researchers in an attempt to incorporate positive reinforcement training techniques to remove the monkeys whenever I could during the work week, but it was difficult when the most of the laboratory staff did not see the need for positive reinforcement training. Despite my arguments supporting positive

reinforcement training, the majority of the laboratories (including my own) within the building soon started to believe that I was treating the animals like pets and started labeling me as "animal rights". I resigned from the laboratory because I could no longer make improvements and I felt that the data collected from the monkeys was not accurate given the stressful data-collection methods, the lack of naturalistic enrichment, and the species-inadequate housing conditions.

I decided to write this report because I want to improve the quality of data, animal welfare, and employee morale in research labs housing nonhuman primates. I am asking that this report be looked at thoroughly and hope that the following reported events will help in assessing routine data-collection and husbandry procedures that often go unnoticed in USDA inspections, protocol approvals, and grant proposals since these procedures are frequently classified as 'noninvasive'.

II. Excessive Use of Negative Stimuli

Removal of Monkeys from Their Home Cages

Discussion. Removal of monkeys from their home cages was often a stressful event for both the personnel and the monkeys. Negative stimuli such as nets and poles were frequently used to coerce the monkey to leave the home cage. Although the negative stimuli did ultimately get most monkeys out of their home cages, the responsiveness of the monkey varied based on their personality. The use of negative stimuli also reduced the ability of other researchers and animal care staff to use positive reinforcement training because any relationship of trust (or potential for a relationship of trust) between the staff and animals was jeopardized.

The amount and type of negative stimuli progressed as the monkey resisted removal, moving from small amounts of negative stimuli to large amounts until the monkey finally entered the transport. This procedure involved putting the transport up to the cage. If the monkey did not go in, I banged on the cage with a pole. If this didn't work, another person assisted and banged on the sides and back of the cage with a pole. If the monkey still did not come out, someone would get the net and bring it into the room. While the net was in the room, activity increased in the animals and random warning calls would erupt from other monkeys in the room (that contained 30-40 pair-housed animals). Most of the "difficult" monkeys exited at that point, but a few monkeys still needed the leather gloves or the net to be thrown into their cages. The time to remove these "difficult" animals would take approximately 15-20 minutes.

If the monkey did not come out after all of these methods were exhausted, a cattle prod was available for use. Although the laboratory I worked in never used it, an animal care employee told me that a male colony (unassigned to a research protocol) monkey in the building was so difficult to remove, he was shocked with a cattle prod one time and then came out from then on when shown the shocker. The animal care employee said that the shocker worked well, because the animal would just get it once and then bolt into the transport from then on. Even though this was a quicker technique, the animal formed a negative association with the cattle prod.

The concerns I had with using negative reinforcement were fully justified in terms of both animal welfare and data-collection efficiency. The students and I often spent 10-20 minutes removing approximately 30% of the monkeys for data collection. If 25 monkeys were being tested every day, approximately 5-7 monkeys would require extra time and effort. This meant that 1-2 hours of research time was wasted every day on a sub-par removal technique.

Recommendations. The initial time investment for positive reinforcement training when the monkeys are young may take 6-12 months, but there are many benefits for using positive reinforcement training as a standard throughout the monkey's life. Standardized, cooperative behavior will help to improve data collection, eliminate unnecessary stress, and save time on removal of animals. Since the monkeys do exhibit individual differences, the initial time investment will vary based on the personality of the monkey. The final result, however, would be a standardized removal of the monkey from his/her home cage. This would result in less time in removing the animals and eliminate unnecessary stress for the entire room of animals.

The APHIS inspector and the IACUC should inquire how the animals are removed from their cages and discuss refinement options for the technique. Researchers and animal care staff should be encouraged to join the Animal Behavior Management Alliance, so that they can learn about the benefits of positive reinforcement training (ABMA, n.d.). A clicker training program should be implemented at every facility. If all personnel worked together to create a training schedule for the animals, then all of the animals would have a standardized form of removal. In addition, the funding agency should allow additional money for at least one animal trainer per facility. The trainer can work with the monkeys, researchers, and the animal care staff in order to promote a refined, less-stressful methodology.

Stress-Inducing TB Testing Procedure

Discussion. Tuberculin skin testing (TB-testing) was performed on the rhesus monkeys twice per year via two different methods - once while under ketamine and once in a restraint apparatus. The TB test while the monkey was awake required the restraint apparatus, animal care staff, and negative stimuli. Typically, 2-3 animal care staff worked to get the monkey positioned in the restraint apparatus. A wooden stick was lodged in the monkey's mouth and their head positioned upward. The animal care manager would then administer an intra-dermal (ID) TB injection into the brow ridge. Some monkeys cooperated with the animal care manager, and didn't require restraint since they had formed a trusting relationship with him/her. Other monkeys would jerk while getting the ID shot. The day after, some monkeys had bruising in their brow region due to the struggling that occurred during the restraint. Despite the apparent distress of the uncooperative monkeys (i.e. struggling, fear-grimacing, and distress vocals), the procedure was still considered acceptable and necessary by all personnel in the research facility.

Recommendations. The IACUC and USDA (APHIS) must inquire about the method of TB testing occurring within the research facility. Clearly it is less-stressful and safer for the animal research

personnel if the animal receives the TB-test while under anesthetic. If TB testing must occur twice per year, then it should be timed with any instance that the animal receives anesthesia. The USDA (APHIS) needs to compile the TB testing methodologies from each nonhuman primate research institution. This information should be disseminated back to the IACUC to aid in implementing the least-stressful method at each nonhuman primate research facility.

Blood Collection in Macaques

Discussion. Obtaining blood from rhesus macaques was usually a stressful procedure for the monkeys while they were awake and being restrained. The monkeys at my research facility were typically restrained with leather-gloves (figure 1) or restrained in the restraint apparatus (figure 2). The method of obtaining blood can be done in other ways including positive reinforcement training (Reinhardt, Cowley, Eisele, & Scheffler, 1991) (figure 3), during ketamine anesthesia (Coe & Scheffler, 1989) and through a dexamethasone suppression—adrenocorticotropic hormone (ACTH) challenge (Schapiro, Bloomsmith, Kessel, & Shively, 1993). Blood was usually obtained from one of two areas on the rhesus monkey, the femoral or the saphenous vein. For clinical procedures, I was allowed to take my time with the monkey to collect the blood, because the amount of cortisol entering the bloodstream due to stress of the procedure was irrelevant in terms of data-accuracy. Sometimes, the monkey would already be under ketamine for an injury and then I would collect blood while the monkey was anesthetized.

It was well-understood by all employees at the laboratory that blood-collection procedures were stressful for the rhesus monkeys. For cortisol (a stress-hormone) measurement studies, I was instructed to obtain blood in less than two minutes from the time we entered the room to remove the monkey from the home cage. The time limit was imposed because I was told that cortisol from the stressful procedure could enter the bloodstream and confound the cortisol levels we were trying to measure. I was told that the cortisol levels were unaffected in the monkey as long as blood was obtained in less than two minutes.

At least two researchers equipped with a net and transport entered the room and retrieved the animal. Other monkeys in the room made warning calls while the monkey was removed. The response of each monkey to the restraint apparatus and surrounding researchers was variable-some monkeys were restrained with ease, while others struggled against the positioning of their leg. Often, the struggling monkeys would put the time of collection at over two minutes; this was when the lab manager (in addition to the senior researcher of the building) told me that up to five minutes was acceptable. Since the rooms of monkeys were stressed from the negative stimuli and abrupt removal, only two monkeys per day could be removed to have their blood drawn simultaneously by two different researchers.

The PI was writing another grant proposal that included timed blood draws and involved a stress assessment of the monkeys' reaction to a certain test. The stress assessment involved collecting cortisol levels via "timed blood draws", as stated in the proposal. Although the grant proposal said that blood samples would be collected within 2-3 minutes of entry into the room, the method used to perform the timed blood draw was not described in the grant proposal. I raised concern over the methodology,

because I thought the procedure would bring unnecessary stress into the monkey rooms (each room housed between 30 and 40 animals). In addition to the stress-inducting factor, I knew it would be difficult to collect blood in under 2 minutes from the 7-8 year-old female rhesus monkeys because a lot of the adult females had fat tissue in the femoral area. Furthermore, some of the monkeys took a long time to be removed with the negative stimuli.

Given these concerns, I presented the alternative less-stressful method of using positive reinforcement training to collect saliva, another accepted way to measure cortisol levels (Tiefenbacher, Lee, Meyer, & Spealman, 2003). My PI told me I should be "with the lab rather than against it." The laboratory manager told me "this is the way we have always done it and if it affects you that much you don't have to be a part of the blood draws."

Since I believed strongly in the refined methodology, I wrote down a list of pros and cons for saliva versus blood-draw procedures and approached the lab manager and PI with a literature review. I was then told a few reasons why we couldn't collect saliva including "you can't get a monkey to produce enough saliva", "we also want to measure ACTH", "they will take the cotton away from you", and "they will have to be anesthetized." I pointed out that with Tiefenbacher's (2003) methods, we could just modify the methodology a little and use flavor crystals without citric acid, then the monkeys would approach the flavor crystals and lick. Their approach to the cotton on the cage could then be clicked (reinforced) and then the monkey would be given a treat for performing the required behavior.

I was then told that the cortisol obtained from blood was more accurate than cortisol obtained from saliva. I said "Even if that were true, we have just worked so hard on keeping negative reinforcement out of the room unless it is absolutely necessary. The timed, restrained, blood draw will be hard to obtain 100% of the time since there are more resilient monkeys that won't want to come out of their cage." My PI said "We will just repeat the procedure on the monkeys again on a different day until the sample is obtained in less than two minutes."

Discouraged, I wrote to one of the country's leading nonhuman primate researcher who had done salivary work in monkeys to ask him/her for some insights. The researcher, having extensive experience with rhesus monkeys, wrote back and said (s)he understood my concerns but said if my lab really wanted to perform blood draws, I should not be concerned because "rhesus monkeys are very resilient creatures." I was surprised to hear that someone well-versed in less-stressful salivary methods was in the opinion that rhesus monkeys were resilient and I shouldn't be concerned because they spring back quickly when faced with a stressful situation. This reaffirmed my theory that my laboratory was not alone in its misconceptions regarding the stress and pain tolerance of rhesus monkeys.

The reason I was very insistent on implementing the least-stressful method was that I had performed the timed blood draws before for our laboratory and witnessed the stress and pain reactions in the monkeys. Sometimes, the monkeys struggled and freed their hands from the restraint device to try to grab the hands of the researcher drawing the blood. I have been scratched twice through double gloves

from a monkey trying to defend itself from me and the restraint apparatus (figure 2). I have also seen monkeys jolt in response to being stuck with a needle while the researcher quickly dug around the femoral area in search of blood so that it could be obtained in less than two minutes. I have seen extensive bruising in the femoral area due to the hematomas of hasty blood draws and researchers not discussing the notion of the monkey receiving ibuprofen, because it was considered a "noninvasive" procedure. Timed blood draws were stressful on both the animal receiving the blood draw and the other animals in the room (due to stress-inducing removal practices).

Recommendations. After researching the literature, I discovered the concern about varying cortisol levels with data-collection methodology was not a new concept. In 1976, Elvidge et al performed a study examining cortisol levels between trained-unanaesthetized, untrained-unanaesthetized, Ketamine-anaesthetized, and Phencyclidine-anaesthetized animals:

> "These results extend those of Setchell et al (1975) and show that significant differences may be found in the values for plasma cortisol between rhesus monkeys sampled under different 'basal' conditions. The present study shows that it is possible by long-term regular training to achieve mean cortisol values which are significantly lower than in untrained or anaesthetized animals."

Schapiro et al (1993) reviewed various blood collection techniques in juvenile rhesus monkeys and discussed the sensitivity of the plasma cortisol levels:

> "As a measure of psychosocial stress, plasma cortisol level is extremely susceptible to the effects of acute stressors, including the procedures involved in its measurement [Davidson et al, 1968; Herndon et al, 1984]. Therefore a sample collection technique that minimizes the stress brought about by measurement must be used."

After discussing various forms of blood draw methodologies through LAREF, I discovered that positive reinforcement training for blood draws was occurring in some laboratories. If the monkey is willing to voluntarily present an arm or leg to a person he or she trusts, then the two-minute time requirement for blood collection becomes obsolete. This allows the researcher to be gentler and take more time to collect the blood from the animal, thereby allowing for more accurate results.

My experiences lead me to conclude that restraint and the use of negative stimuli during the timed blood draws was a form of acute stress. Acute stress can affect the animal's immune system within minutes (Coe & Scheffler, 1989) and could lead to diminished welfare. The USDA and the NIH need to compile animal welfare literature and disseminate it to the IACUC so they can provide the proper oversight to minimize the pain and distress.

The Guide for the Care and Use of Laboratory Animals states: "Many dogs, nonhuman primates (e.g., Reinhardt 1991, 1995), and other animals can be trained, through use of positive reinforcement, to present limbs or remain immobile for brief procedures." (National Research Council, 1996) Although a blood draw is considered noninvasive, if done via negative restraint, it is often very stressful on the monkey. The AWA states that "the principal investigator considers alternatives to any procedure likely to

produce pain to or distress in an experimental animal." (USDA, 2002, § 2143, p 5) Timed, restrained blood draws cause distress to the animals regardless of the length of time it takes to obtain blood from them. If researchers write down "timed blood draws" in their protocols, I strongly recommend that the IACUC discuss the methods with the PI and address the feasibility of the 2-3 minute time frame.

The time limit to justify the stressful procedure also needs further examination by the USDA (APHIS), the IACUC and the funding institutions. Capitanio, Mendoza, and McChesney (1996) state:

> "Collection of blood samples within three minutes of disturbance to the individual animal (all but two of our samples fell within this time limit) is likely to result in reasonably true basal values for plasma cortisol, an interpretation consistent with data collected on laying hens [Lagadic et al, 1990] and squirrel monkeys [Lyons, Mendoza, & Mason, 1994]."

Although the aforementioned quote does not definitively say that blood collected in under three minutes is an acceptable value, USDA (APHIS) inspectors should still closely examine research records of the 'timed blood draws' to make sure that they are indeed under three minutes. The basis for these timed blood draws in the protocol should reference articles that state that cortisol levels are not affected in under a certain time period in the animal (i.e. three minutes).

Some researchers might argue that training a monkey to present a limb for a blood draw, instead of using forced restraint, requires too large of a time investment. Given that the negative stimulus induces unnecessary distress in the monkey receiving a blood draw in addition to the monkeys in the housing area, the time investment would be justified. Positive reinforcement training would standardize the monkey's behavior in response to a task, making it easier to replicate and verify data with other studies. This would also eliminate unnecessary stress in the housing area.

Less-stressful alternative methodologies such as collection of saliva or blood through positive reinforcement training of the animals must be discussed and standardized. Innovative, less-stressful methods of blood collection should be encouraged and rewarded by all governmental agencies, because it leads to improved welfare and data-accuracy. Positive reinforcement training literature including articles written by Stephen J. Schapiro and Viktor Reinhardt among other animal training experts should be included in informational packets when the IACUC encourages these truly noninvasive methods to the research facility.

Capturing Animals

Discussion. When a rhesus monkey escaped from his or her home cage it was usually due to the monkey removing the clip, a researcher not locking the clip properly, or the monkey popping the cage door out of its track caused by excessive banging. Time spent to catch the animal through negative stimuli varied from approximately 20 minutes to two hours. During the time that the monkey was out of the cage, several problems arose. Typically, we had 3-5 personnel in the room along with transports, nets, and leather gloves while trying to capture the animal.

I had been trained by my supervisor to chase monkeys around the room with negative stimuli when they escaped, because as (s)he noted, "we want to make it a miserable experience for them so that they are less likely to escape the next time." I had experience with catching monkeys and hand-caught at least four female rhesus monkeys when they escaped on different occasions with leather gloves in a room consisting of 30-40 pair and single-housed monkeys. The negative stimuli combined with the escaped monkey typically caused other monkeys in the room to fight with each other or with the monkey that landed on their cage. We usually called the vet in to suture wounds on the escapee in addition to the other monkeys who had been injured from the "chase". The monkeys would then get a padlock put on their cage to ensure they would not escape again.

In terms of safety of the personnel, male rhesus monkeys were more dangerous than females because they were larger (10-15kg), and had longer canines. Both females and males would become aggressive and dangerous if they felt threatened, however (Reinhardt, 2002b). On one occasion, two overweight rhesus female monkeys (approximately 10kgs each) got out into the room at the same time. I was called down to help a pair of students and one animal care personnel catch the animals. In the midst of running after the monkeys with leather gloves, I caught one female. The other female partner threatened me and then jumped on my back. I let the monkey go and the partner jumped off of me. I ultimately caught both animals and put them back home; however, myself, the animal care person, and the students were put into a very dangerous situation.

On at least three separate occasions, I witnessed tongue injuries in three pair-housed monkeys during the escape episodes, even though none of the injured monkeys escaped themselves. It was not clear in each case how the tongue injury occurred (i.e. monkey biting its own tongue, partner biting the tongue, or escapee biting the tongue), but these animals required extensive subcutaneous suturing, at least 10 days of antibiotics, soft food, as well as daily monitoring to ensure the animal was eating properly. Daily monitoring, feeding, and treatment took at least 1.5 hours and so treatment of the animal consumed approximately 7.5 hours of our weekly research schedule. In addition, there were typically 3-5 other monkeys per week (out of 95 monkeys) that needed treating for other ailments such as lacerations, conjunctivitis, or diarrhea. The weekend staff consisted of only one or two animal care staff per day, and so the additional dosing was overwhelming to them since they had to provide basic husbandry for approximately 400 other animals. Usually, two laboratory personnel spent 2-3 hours preparing doses on Friday for the animal care staff to administer on the weekend.

Recommendations. Bringing negative stimuli into the housing area caused unnecessary stress for every monkey housed in the research facility. If positive reinforcement had been used and the resulting trusting relationship had been established between the monkeys and the laboratory staff, then the aforementioned problems could have been prevented.

Everyone in the research facility recognized that when a monkey escaped, it created a very stressful situation and it was necessary to cancel data-collection. I have learned, through discussions with others

on email forums, that less-stressful methods are available that have been used successfully by other nonhuman primate researchers (See Reinhardt, 2005 for a LAREF discussion of removal techniques). If the monkey escapes, he or she should be rewarded for going back home. Two people that are involved in training the monkey should bring a piece of fruit and transport into the room. The person should try to lure the animal back to the cage with fruit or another enticing treat. If the monkey is running around on the floor of the room, he or she will usually run into a transport since a transport is also familiar to the monkey.

If the monkey is a large male with his canines intact, then perhaps a tranquilizer gun should still be used for the safety of personnel. Even with the tranquilizer gun, only two people have to enter the room, one person administers the shot immediately. They should make sure that the animal does not fall and then place the tranquilizer gun immediately outside the room so as not to stress out the other monkeys in the room. The monkey should be placed back in his home cage and allowed to wake up on his own.

The IACUC and the USDA (APHIS) should inquire about procedures that are used to capture monkeys. Procedures across facilities should then be reviewed by the USDA (APHIS) to determine which methods are the least-stressful. The USDA (APHIS) should compile the different removal techniques and disseminate the least-stressful technique to the IACUC committees so that they can work with researchers to refine capture techniques. These measures would ensure that pain and distress are minimized when monkeys escape.

Use of Restraint in Chairing Animals

Discussion. Typically monkeys were restrained in chairs so the researchers could test on them with ease. I saw two types of chairing restraint methodologies occurring among two different laboratories in the same research facility. One technique was significantly more stressful than the other one. This variation in chairing methodology occurred within the same building despite the fact that one method was clearly less-stressful.

The stressful chairing technique involved the researchers removing the monkeys from the home cages and placing them into a restraint apparatus (figure 2). The researchers restrained the monkey by squeezing the apparatus to immobilize the monkey and then put a muzzle on the mouth to prevent biting. The researchers removed the back end of the restraint apparatus while the monkey was squeezed and immobilized. They then grabbed the monkey from the restraint apparatus and one researcher carried the monkey down the hallway to a chairing apparatus. Since stress is known to alter physiology and immunological function (Balcombe, Barnard, and Sandusky, 2004), the restrained monkey indicates the animal is not a good research subject for measuring any physiological responses.

The moderately less-stressful method I saw involved the researcher removing the monkey with the pole and collar technique through positive reinforcement training and (s)he led the monkey down the hallway and then the monkey voluntarily entered the chair. The monkey in this situation was not as stressed in the restraint, because he was trained to enter the chair and was given rewards for complying. If

the IACUC had witnessed these two methods of chairing, they could have worked with the first lab to change their methods toward a more refined methodology by using positive reinforcement training.

Recommendations. Researchers who participated on the email forums frequently described their successful experiences using positive reinforcement techniques when chairing monkeys. Stressful restraint methods continued to be used, even in 2003 & 2004, when there were scientific articles available describing techniques of chairing which offer suggestions for refined chairing techniques. Furthermore, the Animal Welfare Act regulations state: "Handling of all animals shall be done as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, excessive cooling, behavioral stress, physical harm, or unnecessary discomfort." (USDA, 2002, §2.131, p 52) The stressful chairing technique still allowed to occur in the research facility was causing excessive behavioral stress, unnecessary discomfort, and questionable data.

Less-stressful practices and theories have been around for a long time. Prentice, Zucker, and Jameton (1986) reviewed principles from the University of Nebraska Medical Center (UNMC). When referring to restraint, the University's principles recommend:

> "Physical restraint procedures should be used on awake animals only after alternative procedures have been considered and found to be inadequate. If restraint will be utilized the animal should be trained or conditioned to the restraining device, using positive reinforcement, prior to the beginning of the experiment."

Fuchs (1997) discusses the challenges in using restraint chairs. The author notes:

> "Personnel must be educated not only in hygiene, animal care and health but also in training the animals to cooperate with the experimental procedures. This will reduce stress elicited by catching, handling, and transport. Restraint procedures (monkey chair) which are necessary for some neurobiological experiments elicited a lot of criticism since physical restraint is thought to cause discomfort to the animals. Based on the methodological and technological progress of the recent years, new developments in computer technology in combination with remote monitoring, and home-cage testing can in many cases replace the classical restraint methods." (p 45)

The differences produced by positive reinforcement and negative reinforcement in chairing equates to a difference in baseline physiology for the animal, and therefore the data that is collected. The animal's welfare is also compromised by involuntary restraint because it leads to an increase in stress, weakens the animal's immune system, and alters physiology. Since negative stimuli produce a variable response among the same species, it is inevitable that the animals react differently to the chairing (Fuchs, 1997). If positive reinforcement training is used, the animals, given time, would produce a standardized, predictable response to a chairing methodology-thereby increasing the likelihood of a more reliable, standardized baseline physiology in the same species population.

The more stressful method of chairing was allowed to be used in the research facility because less-stressful methods of chairing were not examined by the research lab and therefore were overlooked by the IACUC. The IACUCs and the USDA (APHIS) inspectors must require laboratories to provide the detailed methodology of the chairing technique and discuss standardization of the least-stressful methods across all primate research laboratories. IACUC members should ask to view the chairing methodology, so they can personally assess the stress involved in the procedure. In addition, funding agencies who review grant proposals should ask for elaboration on the use of positive reinforcement versus negative reinforcement when working to restrain the animals in a primate chair.

III. Enrichment Disparities

Feeding Standard Ration Once Daily Versus Twice Daily

Discussion. I was told by my laboratory supervisor that we only fed the monkeys once a day, because it was the minimum USDA requirement that the monkeys must be fed at least once every 24 hours (USDA, 2002, § 3.82(b), p 96). Instead of letting the animal care staff feed our monkeys, I asked if our lab could start feeding our own assigned monkeys because I wanted to pay attention to their eating habits and to dominance issues in order to make sure everyone was getting adequate chow. For two years, I witnessed at least 30 male rhesus monkeys (>6 years old) eat their full ration of chow in less than 15 minutes (female monkeys usually still had food in their cage by the afternoon or next morning). In the afternoon, all of the monkeys received a slice of fruit, peanuts, or flavored ice. This small afternoon snack was not adequate enough to prevent the male monkeys from eating the chow very rapidly the following day. Consuming one's daily ration of chow in less than a half hour meant that the monkey was incapable of rationing food for himself.

Members of email forums discussed the benefits of feeding the monkeys their chow rations at least twice daily. The benefits included controlled portion size, manual rationing of food, and increased naturalistic behavior through foraging devices. One researcher said it was 'barbaric' to feed only once per day. This statement prompted me to approach my lab manager with my concern regarding the feeding. (S)he asked how I could tell if they were hungry. I reiterated that they were eating their chow in less than 15 minutes. (S)he said since most of them had partners, they need to eat as fast as they can so they get equal portions of food and this also happens in the wild. I said I didn't think that argument was correct since they were in captivity and suggested implementing a chow-foraging technique similar to Reinhardt (1993) to both increase their enrichment and to make it take longer for them to eat.

Since the lab manager thought I had some good ideas in the past, (s)he allowed me to pursue the chow foraging. I developed a chow-foraging rating scale for all the monkeys and decided that we could break up one extra piece of chow to throw on top of their cage so that they could learn the foraging technique. I noticed that some males would not forage right away, but after they finished their ration of chow, they would approach and retrieve the chunks of chow when they were hungry later on. All of the

chunks of chow were gone from the tops of the cages by the end of the day. Even though some monkeys knocked the chow off of the cage instead of eating it, I felt that it was at least taking most of the monkeys longer to eat while simultaneously increasing their foraging behavior which meant increasing naturalistic behavior.

After monitoring each monkey's foraging behavior, I discovered there were many differences in how and when the monkeys (both male and female) would approach the food. My lab manager always said that we had to treat every monkey in the room the same, and so it was difficult to be able to adapt the foraging technique for each monkey. When I told the lab manager about a monkey that preferred to forage over eating chow from his chow box, (s)he told me to give him his whole ration on top of his cage. I said that, although we were trying to treat everyone the same, I liked the idea of assessing each animal individually. I felt like we were making progress in the lab. Despite the progress in this area, I resigned from the laboratory because I did not agree with some of the stressful techniques that were used for data-collection and husbandry practices (e.g. timed blood draws and removal from home cages). Shortly after I resigned, a former colleague informed me that all the chow-foraging had stopped. This removal of foraging meant that the males would likely be eating in less than 15 minutes and would be forced to cope with a less-enriching environment.

Recommendations. The USDA (APHIS) and the IACUC need to examine the feeding practices at every nonhuman primate facility they inspect. If laboratories feed their monkeys once per day, the possibility of chow foraging should be examined. At the very least, the IACUC committee should inquire how quickly the animals eat their chow. This is a very important issue since eating an entire daily ration in less than one hour can lead to storage of excess calories that are not burned off, which could also contribute to the overweight problem in some captive monkeys. The Guide for the Care and Use of Laboratory animals supports this theory, stating:

> "Moderate restriction of calorie and protein intakes for clinical or husbandry reasons has been shown to increase longevity and decrease obesity, reproduction, and cancer rates in a number of species [Ames and others 1993; Keenan and others 1994]. Such restriction can be achieved by decreasing metabolizable energy, protein density, or both in the diet or by controlling ration amount or frequency of feeding. The choice of mechanism for calorie restriction is species-dependent and will affect physiologic adaptations and alter metabolic responses [Leveille and Hanson 1966]." (National Research Council, 1996).

If monkeys are eating their chow very rapidly, then the research lab should ration the chow for them.

Adding a simple foraging device to the cage can solve the problem of quick consumption. As the Public Health Service (PHS) policy states: "The living conditions of animals should be appropriate for their species and contribute to their health and comfort." (OLAW, 2002) The lack of foraging devices should not be allowed to persist simply due to lack of funding, especially when there are cost-effective ways to implement chow-foraging techniques. If there is a problem with funding or labor, the funding

agency should provide more financial assistance to the organization in implementing more foraging tasks for the nonhuman primates. The USDA (APHIS) should require institutions to provide more money toward achieving a foraging option for feeding in the laboratory animals since it spreads food consumption over a longer period of time and increases naturalistic behavior.

Frequency of Providing Inanimate Enrichment

Discussion. The Animal Welfare Act regulations require that:

> "Dealers, exhibitors, and research facilities must develop, document, and follow an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates. The plan must be in accordance with the currently accepted professional standards as cited in appropriate professional journals or reference guides, and as directed by the attending veterinarian." (USDA, 2002, § 3.81, p 94)

Environmental Enrichment was given to nonhuman primates to improve their psychological well-being and to increase naturalistic foraging behavior. The research facility boasted about its enrichment program and followed the USDA draft policy of enrichment (Animal and Plant Health Inspection Service, 1999). Although some progress had occurred over time (i.e. increased amounts of enrichment and pair-housing), most of the monkeys still received minimal naturalistic enrichment and did not have a foraging option for their chow.

When I began working at the laboratory in 1999, the monkeys had wood in their cages. The wood was removed, however, because the shreds of wood (from being over-manipulated) were clogging the drains. At an annual meeting, I tried to ask if the research facility could start providing wood again since the monkeys used it so much. I mentioned that a branch was one of the few things the monkeys do not get tired of (Reinhardt, 1989). I suggested we could try to use a more durable wood to work around the husbandry issues. The majority of the researchers voted not to supply the wood, however, because it was too inconvenient and time-consuming.

A more convenient form of enrichment was to provide the monkeys with durable plastic toys. The laboratory had a standard of providing one toy for every female monkey and one toy and one chew toy for every male monkey. The toys were rotated every two weeks. In addition to the toy rotation, the rhesus monkeys received ice (containing foraging morsels such as corn, rice, dried apples, beans, and/or peas) once per week and had foraging devices for the weekends. Despite the toy and food enrichment, abnormal behavior was still present in many of the monkeys. The behaviors included pacing, back-flipping, rocking, self-biting, and fur-plucking.

Recommendations. The USDA (APHIS) must evaluate the frequency and type of enrichment given in primate research facilities. The information regarding the amount and type of enrichment should be gathered from each IACUC and be compiled by the USDA (APHIS). The most enriching and natural form of environmental enrichment should be recommended to the IACUCs to help implement the appropriate enrichment program. The standardization in turn, would help improve animal welfare and the

researcher's ability to extrapolate research results across institutions. In addition, the funding institutions should be required to provide more funds to improve psychological well-being in the laboratories that are in need of adopting other, more naturalistic enrichment options. It is important to provide as naturalistic an environment for the species as possible since it will help produce more naturalistic behavior (i.e. less abnormal behavior).

IV. Inadequate Housing Conditions

Early Weaning of Animals

Discussion. The monkeys were weaned from their mothers at six months of age. Weaning at six months was artificial weaning in that the infant was taken from the mother prematurely and placed into peer groups. Reinhardt (2002a) describes the principle of artificial weaning: "Under confinement conditions, artificial weaning is an abrupt occurrence which takes place several months prior to the biologically normal age of weaning." Although early weaning of monkeys may result in more offspring, artificial weaning often results in emotional distress. Reinhardt (2002a) discusses the affects of artificial weaning:

> "The event is particularly detrimental for the infant because the mother remains the primary source of comfort and reassurance well beyond the conclusion of nutritional weaning [Altmann, 1980; Lindburg, 1991; Sade, 1973]. The psychological trauma resulting from preweaning maternal separation is long lasting, perhaps permanent, and so severe that it has been used as a model of stress, immune deficiency, and depression [Capitanio, 1998; Kaufman & Rosenblum, 1967; Laudenslager et al, 1995; Capitanio & Reite, 1985; Laudenslager, Held, Boccia, Reite, & Cohen, 1990; Reite, Short, Seiler, & Pauley, 1981]." (p 153)

Despite the early evidence available discussing the problems with artificial weaning, some researchers continued to justify the premature weaning to increase the number of infants produced. Champoux, Metz, and Suomi (1989) state "In our laboratory, in order to allow lactating females to resume sexual cycling for the next breeding season, infants are removed from their mothers at 6 months of age." At the laboratory where I worked, the infants were separated from their mothers at six months, placed with peer groups of 5-6, and then paired in their juvenile years.

Recommendations. The Guide for the Care and Use of Animals discusses the importance of providing a species-typical environment (National Research Council, 2002). The 'species-typical' principle should be extended to the weaning process of nonhuman primates. Reinhardt (2002a) discusses the principle of natural weaning:

> "Natural weaning is a gradual process. It implies that the mother, over a period of several weeks or months, more and more consistently discourages her infant to suck on her breasts. Once the mother stops nursing the infant for good, the affectionate bond between the two is not broken [Altmann, Altmann, Hausfater, & McCuskey, 1977; Lindburg, 1971; Poirier, 1970; Roonwal &

> Mohnot, 1977]. The young usually remains in the maternal group at least until prepuberty." (p 151)

The weaning processes for each species in every laboratory should be noted by the IACUC and be compiled by the USDA (APHIS) so that the most naturalistic, least-distressful method is subsequently enforced at all facilities. This standardization would help ensure a more species-specific environment for housing conditions and also aid in preventing abnormal behavior.

Uniform Lighting in the Housing Area

Discussion. The monkeys in the research facility were typically housed in a two-tiered caging system. The top cages had significantly more illumination than the bottom cages. Most of the sides of the lower row cage were solid, making available lighting from the room limited in the lower-row caged monkeys (figure 4). The darkness in the bottom cage forced personnel to use a flashlight when examining the monkeys.

The Guide for the Care and Use of Laboratory Animals states:

> "In general, lighting should be diffused throughout an animal holding area and provide sufficient illumination for the well-being of the animals and allow good housekeeping practices, adequate inspection of animals-including the bottom-most cages in racks-and safe working conditions for personnel." (National Research Council, 1996)

This principle is not extended to the two-tiered caging system, however. Reinhardt (2001) suggests that the "traditional double-tier cage system makes it impossible to provide uniform illumination; it condemns half of the animals to live in a cave-like environment that is often so gloomy that personnel has to use a flashlight to properly identify them." Schapiro, Stavisky, and Hook (2000) confirm the differences in lighting and state:

> "Light levels were significantly lower in lower-row cages at all nine sites within the cage [see *Table 1]*, [figure 3] confirming Reinhardt and Reinhardt's [1999] hypothesis that lower-row cages are darker than upper-row cages. Not only did light levels differ significantly, but less than 8% of light readings in lower-row cages were higher than the *lowest* light level readings at the same position in the upper-row cages. As one would expect, light readings in the front of the cage were significantly higher than light readings in the back of the cage. There was therefore considerable within-cage variation in light levels."

Although the authors conclude that there were no behavioral differences between the top and bottom cages, the requirement and need for uniform lighting still exists.

Recommendations. The uniform lighting standards must be applied to the two-tiered caging system in all facilities. This is important for animal welfare and husbandry purposes. Reinhardt & Reinhardt (2000) provide a housing solution to solving the two-tiered caging problem:

> "Monkeys are housed in single tiers in well-structured cages (perches or shelves at different levels) that reach close to the ceiling of the room. This assures that all cages of a room are directly and

> equally illuminated, and that all animals of a room have equal opportunities to access the arboreal dimension of their environment and to retreat to safe vantage points above human eye level [cf. International Primatological Society, 1993].” (p 146)

The IACUC should investigate the type of housing and the USDA (APHIS) should research more innovative methods of housing of nonhuman primates. The funding institutions should be required to provide additional funding in order to renovate the two-tiered, antiquated method of housing so that a more innovative and standardized form of housing can be implemented.

V. Improving Employee Morale and Retention

The Paradox of Employee Emotionality

Discussion. Performing research on monkeys was an emotionally challenging experience for many of the animal care staff and research personnel. In my experience, the expression of feelings regarding the animals was discouraged. When I was initially trained at my laboratory in 1999, I was taught that the animals were not “pets”; they were research subjects, and I should not form a relationship with them. I did form a trusting relationship with the monkeys over time, however, because it was necessary to reduce stress when trying to perform data-collection and providing clinical care. My experiences were consistent with the literature I had read, which indicated that a trusting relationship should be encouraged for both the welfare of the animals and the safety of personnel (Waitt, Buchanan-Smith, and Morris, 2002).

I was able to suppress my emotions most of the time, but sometimes I was overwhelmed with emotions and had to cry. After hearing I had cried out of frustration from not being able to implement the less-stressful cortisol assessment technique, the principle investigator told me that crying was unprofessional the following day. I found it difficult to suppress my feelings when I had to cause unnecessary stress in the monkeys. Since I had formed a relationship with them to help minimize stress, stress-inducing procedures such as timed-blood draws and removal from home cages, became difficult for me knowing there was evidence available consisting of more refined methods, including positive reinforcement training.

I researched the literature pertaining to animal-personnel relationships. Morton et al (2001) discuss the importance of establishing a familiarity with the animals:

> “Many species become familiar with individual people, so it is helpful for the staff who will be carrying out the dosing to develop this familiarity beforehand. The importance of this for dogs and primates is recognized but other species such as rats and mice will also benefit.… The selection of staff to carry out procedures should take into account their skill in handling the animals as well as in performing the technique.”

Arnold Arluke examined animal researchers and the lack of encouragement regarding the moral concerns of the employee. Arluke (1994) concluded:

> "In all but two of the 35 laboratories, newcomers faced a closed moral universe—'a company town'—where issues of morality were defined institutionally, and hence rarely confronted openly by individuals….Newcomers quickly learned that moral questions were best left unstated, and were not to be 'publicly' aired and explored in the lab, although they could be shared privately with co-workers." (p 31)

Evidence from Arluke suggests that suppressing feelings in the laboratory is standard practice.

Recommendations. The *Draft Policy on Environment Enhancement for Nonhuman Primates (APHIS, 1999)* explains how a primate may need human interaction: "If individual primates are strongly socialized toward humans and distressed by other primates, the plan should provide for daily, extensive positive human interaction in addition to that associated with routine husbandry, medical care, experimental manipulation, training, or exhibition." (p 38148) Furthermore, relationships will inevitably form with the animals while the researcher works hard to minimize stress by gentler handling or by positive reinforcement training. Therefore the employee's feelings should be expressed openly, and their relationships with the monkeys should be encouraged by supervisors.

The American Association for Laboratory Animal Science (AALAS) (n.d.) argues that "Kindness and concern for animals are desirable characteristics in animal care and research workers." This mutually beneficial relationship is important in ensuring that animals are being treated as humanely as possible. In addition, the trusting relationship reduces unnecessary stress in the monkeys because they are not dealing with unfamiliar personnel. AALAS (n.d) provides some additional solutions on how the institution can improve the welfare of the personnel working with the animals. These solutions include:

- Learn to recognize stresses to personnel related to euthanasia.
- Institute an open door policy with supervisors/administrators.
- Provide a pleasant work environment.
- Supply a comfortable break area for resting and reflecting.
- Offer education relative to humane animal care and use and ethics.
- Recruit investigators to conduct informational seminars for the research team highlighting the various aspects of their particular study (especially desired benefits and outcomes).
- Request investigators to detail the significance of specific endpoints of the experimentation.
- Encourage group support meetings among laboratory personnel, enlist the aid of an outside professional to facilitate therapeutic sessions. By scheduling seminars and discussion sessions on this topic, some institutions have created an atmosphere that encourages employees to openly acknowledge their feelings on such issues and helps to establish an open environment.
- Rotate personnel to distribute job responsibilities and share difficult tasks.
- Insure that individuals are properly trained in the procedures of euthanasia. Individuals involved in euthanasia procedures must understand the mechanisms of action of each euthanasia agent or technique and how each contributes to ensuring a humane death.

- Initiate policies that do not require the technician caring for long-term animals to participate in the euthanasia of the animals. In some cases, however, the technician may feel a moral obligation to perform the euthanasia if there is an established relationship of trust.
- Honor the request of an individual to be excused from euthanizing an animal to which he/she is particularly attached.
- Allow homes to be found for research animals suitable for adoption (after soliciting institutional and IACUC approval). Consider designating technicians to serve as primary contacts. (p 3)

The IACUCs should inquire about the current training techniques in labs regarding the humane treatment of the animals. Humane treatment involves being calm and non-threatening toward the monkeys and also establishing a trusting relationship with them. Instead of discouraging the inevitable bond that will form between the researcher and the monkey, the incoming researcher and animal care staff should be educated in how to develop a relationship with the animal and cope with the fact that the monkey is in captivity and that it is being used for research purposes. Training regulations can be established and maintained by implementing an employee emotionality committee that meets monthly to discuss various issues regarding both the welfare of the research animals and the employees who work with them. This committee should involve the IACUC members and invite all personnel working with animals to give their input on how to improve animal welfare and employee morale. Ultimately, the goal should be to create a caring culture, so that the animals are treated with quality, humane care the entire time they are being used for research.

Eliminating Misconceptions about Monkeys

Discussion. It was often argued in the laboratory that rhesus monkeys were valuable research subjects because they were closely related to humans. When it came to the animal's welfare, however, statements were made by my PI that the monkeys "have a higher pain tolerance" and a "different pain system". In addition to these statements, the PI and lab manager viewed the monkeys as research subjects and not as sentient beings. By holding such a viewpoint, it was easier for the PI and lab manager to disregard my animal welfare concerns regarding seemingly "noninvasive" procedures. To give an example of disregard to pain and distress in noninvasive procedures, I discuss a Positron Emission Tomography (PET) Scan below.

Administering an intravenous line in a monkey was classified as a noninvasive procedure in our protocol. If done several times however, it became invasive. I assisted in PET scans where we administered an intravenous catheter in the saphenous vein while the monkey was under ketamine anesthesia. We then had to feed approximately two feet of plastic tubing through the saphenous vein to where it emptied into the vena cava. Often, we encountered blockage in the veins and would have to re-enter at a different site on the leg.

On one particular occasion, the lab manager attempted to put an intravenous (IV) line a total of fourteen times in one monkey. (S)he attempted the line four times in each saphenous vein. After the

failed attempts, the lab manager suggested we shave the arms for an attempt in the brachial vein. The ketamine was wearing off since it had been at least 40 minutes since the monkey's last dose. We put a mask of isoflurane over the monkey's mouth to maintain the anesthesia.

I shaved the monkey's arm, wiped it down with alcohol and iodine, and held out it in a position to receive the catheter. The lab manager attempted three times before moving to the next arm. While on the last arm, a graduate student assisting with the PET project walked past the room, saw all of the attempts on the legs and arms, and said "Poor monkey." While attempting the IV line, the lab the manager said: "Poor monkey? I would say 'poor monkey' if it were lying on an operating table with its guts hanging out for surgery!" A successful IV line was inserted on the 2nd try in the final brachial vein on the second arm. The disregard of the lab manager toward the multiple IV line attempts and the monkey's welfare inhibited me from raising the possibility of giving the monkey ibuprofen, since I was already labeled as too emotional.

Later on, when speaking to the PI (who was not present at the PET procedure), I brought up the instance of the multiple IV line attempts performed by the lab manager. I said I thought we should have stopped sooner. I also said I thought the monkey should have received pain reliever after an IV line was attempted 14 times, but I was too intimidated by the laboratory personnel to bring up the issue. The PI said "the monkey was knocked out, wasn't it?" I said "yes, but what about when he wakes up?" (S)he said "I think your problem is that you are thinking of these monkeys like they are human". I noted, "The Animal Welfare Act says to treat pain in an animal as if it were as painful in a human." (S)he said "so you think they are human, then?" (S)he went on to tell me that (s)he worked with monkeys for a long time and thought they had a "different pain system" and a "higher pain tolerance." The PI dismissed my concerns, implying that my perception was at fault, and not the methodologies used in the laboratory.

Recommendations. Principle Investigators need to consider the current literature on animal welfare and pain relief in animals as important as the literature pertaining to their study. It was apparent to me that a historical mentality was involved in the PI's assessment of pain and impeded the individual's ability to see any other testing methods as a viable option. Other researchers and veterinarians also told me on different occasions that the rhesus monkeys were able to tolerate stress and pain and were therefore good research subjects. Another component to this argument, however, is that these monkeys could be suppressing their reaction of pain and distress because their natural behavior is to prevent showing signs of weakness, which could make them susceptible to aggression from conspecifics as well as to predators in the wild (Personal correspondence with Kathleen Conlee, HSUS, April 2005).

Often, the PI did not participate in the procedures performed on the animals because the PI spent the bulk of his/her time writing papers, analyzing results, teaching, and preparing grant proposals. In the case of PET scans, the PI told me (s)he did not want to partake in the procedure because it was too stressful for him/her, and "it takes a special kind of person to be able to handle PET scans." As Morton et al. (2001) argues in order to ensure the animals are being treated appropriately, "Staff should have a

detailed knowledge of what is being done to animals in their charge and when it is being done. They should know the end-points and severity limits of the project and be able to recognize if these are exceeded." Since the PI did not partake in the procedures, at the very least, an attending veterinarian should have been present during these procedures to ensure that the research methodology was humane.

Although administering an IV line was considered a noninvasive procedure in the protocol, attempting a line 14 times in one instance into a monkey's veins while it was anesthetized, involved many punctures through the skin and vein. The researchers should have been concerned about the animal's wellbeing when the monkey awakened from anesthesia. The Animal Welfare Act defines a painful procedure as:

> "any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." (USDA, 2002)

The classification of many procedures including timed blood draws, injections, and intravenous lines as noninvasive and "minor procedures" leads to lack of oversight on behalf of the USDA (APHIS), the IACUC, the funding institutions, and the research personnel. If a person from the USDA or IACUC observed the stress from the negative stimuli used to remove the animal, the procedure being performed on the animal, and the animal's appearance when placed in the home cage, the inspectors would see that, in many cases, that pain and distress in noninvasive procedures are more than momentary.

Accommodating Psychologically Distressed Animals

Discussion. I observed many types of abnormal behavior in the rhesus monkeys while monitoring them in the laboratory. The behavior ranged from pacing in circles, rocking back and forth, finger-sucking, and saluting, to more severe forms such as self-biting, and plucking and eating fur. Many scientific papers have been written discussing the methods for reducing these abnormal behaviors. Relieving abnormal behaviors included providing social housing, medication, or environmental enrichment. When abnormal behaviors were not relieved through the aforementioned methods, then the researchers deduced that all efforts were exhausted and that relief of the undesirable behavior was impossible. In most cases at our laboratory, the abnormal behavior persisted in the monkeys while we tried to relieve it by providing extra enrichment.

I heard a colleague state that all of his/her monkeys involved in a terminal study at a different research facility displayed some form of abnormal behavior because they were "rejects" from the normal monkey population and were being used in the terminal study to "put them out of their misery". The re-use of these monkeys prompted me to ask questions about the sale and re-use of primates. Through discussions with a few primate research veterinarians, I discovered that the culture in the lab was that monkeys with incurable behavioral abnormalities should be sold to terminal studies since there is a high demand for them in research. The re-use of distressed primates raises important animal welfare and data-accuracy concerns.

The sale of the distressed animals to terminal studies raises two issues: first, the presence of a novel, more stressful environment containing unfamiliar personnel would increase the stress of the monkey. Second, the distressed monkey would not serve as a valid research subject in the case of physiological, neurological or immunological studies. In addition, some animals may be hyperreactive to stress and may not serve as appropriate research subjects:

> "The differences between reactive and nonreactive individuals may be reflected in heart period and respiratory sinus arrhythmia (RSA)…Identifying animals that have a hyperreactive response to stress would benefit both researchers and monkeys. Animals that cannot adapt may not complete the experiment or may provide inconsistent and uninterpretable data." (Bowers, Crocket and Bowden, 1998).

The condition of the animal can lead to diminished welfare. The authors stress: "Animals that engage in frequent hyperreactive stress responses are at increased risk for both acute and chronic illness [Manuck et al., 1986; Roberts, 1993]. These animals do not adapt well to experimental protocols and can be more aggressive than nonreactors [Krantz & Raisen, 1988]." (Bowers, Crocket and Bowden, 1998) The hyperreactive monkeys should require special accommodations such as placing them in a less-stressful environment where naturalistic enrichment is plentiful and disruption is minimal.

There is also evidence suggesting that abnormal behavior can be relieved by placing a nonhuman primate in a more naturalistic habitat. A few sanctuary directors I have spoken to have received monkeys displaying abnormal behaviors and witnessed the disappearance of the behavior over time in a more naturalistic setting. Furthermore, Kessel and Brent (2001) showed that single-housed baboons could be rehabilitated and resocialized when placed in a more naturalistic, enriched environment.

When I raised the possibility of creating a sanctuary to the attending research veterinarian at the laboratory, (s)he said that "Most sanctuaries are 'mom and pop' with limited funding. The sanctuary just fills up, and then what do they do?" I brought up the idea of providing special attention to the animals showing psychological distress through behavior or appearance. (S)he mentioned that the well-being should be accommodated in the research lab because there is a huge demand for all primates in research. My contention was that, once a research facility deduced that the behavior could not be relieved within the confines of the laboratory, the animal should be provided with special accommodations by being allowed to retire to a sanctuary. (S)he said the demand was too high for the monkeys and we could find ways to learn from them by allowing them to be put into acute terminal studies. Later that afternoon, I called him/her back and asked "couldn't you make the argument that distressed animals sold to terminal studies, such as Simian Immunodeficiency Virus (SIV) studies, would make poor research subjects?" (S)he stated "It is not a confound, Amy. Most people with AIDS are stressed out, so it does not matter."

Recommendations. Although some researchers and research veterinarians believe there is a high demand for all monkeys, including the psychologically distressed and hyperreactive monkeys, the AWA regulations imply that psychologically distressed animals should be provided with special attention.

Section 3.81(c) of the Animal Welfare Act states: "Certain nonhuman primates must be provided special attention regarding enhancement of their environment, based on the needs of the individual species and in accordance with the instructions of the attending veterinarian." One of the nonhuman primates who require special attention are "Those that show signs of being in psychological distress through behavior or appearance" (USDA, 2002, §3.81(c)(2), p 95).

The solution of retirement should be considered as an alternative to euthanasia or selling distressed and hyperreactive monkeys to more research. If the nonhuman primate's distress cannot be relieved within the confines of the research facility, then he/she should be allowed to retire to a sanctuary where the relief of the behavior can be closely monitored and the animal can be properly cared for in a naturalistic environment. APHIS inspectors, the IACUC, and the NIH need to be more involved in regulating the re-use of nonhuman primates. Each grant proposal and journal article should list the entire research history of each non-human primate that was included in the proposal/protocol. Removing the distressed primates from the research population will lead to improved employee morale, animal welfare, and data-accuracy.

VI. Conclusion

Nonhuman primate research is a complex issue that requires immediate attention from the governmental agencies and funding institutions involved, with full cooperation from the nonhuman primate research community. The USDA, IACUC, and funding institutions are necessary in the evaluation of the husbandry and data-collection methodologies to make sure the legislation is being followed closely and to increase the standardization of less-stressful methodologies. The Animal Welfare Act states:

> "…the standards described in paragraph (1) shall, with respect to animals in research facilities, include requirements-for animal care, treatment, and practices in experimental procedures to ensure that animal pain and distress are minimized."(USDA, 2002, p 5, § 2143(a)(3))

The methods that are discussed in this report indicate that pain and distress were not being minimized in husbandry and data-collection procedures at the NIH-funded and USDA-licensed research facility. This report is designed to assist the USDA (APHIS), the IACUC, and the research funding institutions by providing details regarding stress-inducing techniques that can easily be overlooked through the inspection process, protocol review, and grant proposal review.

Despite the presence of more innovative and less stressful techniques occurring in some research facilities, some research laboratories continue to use traditional, more-stressful techniques. The incidents in this report suggest that stressful methods seem to still be well-accepted by at least some members of the nonhuman primate research community. Evaluation and inquiry of stressful procedures should extend into the noninvasive categories in which many stress-inducing methodologies are included. Since there is literature available that covers less-stressful techniques, it is necessary to work toward standardizing the

least-stressful husbandry and data-collection methods in the nonhuman primate research community. In addition to the principle investigators, the USDA, the IACUC, and the funding institutions should be researching the most current literature emphasizing positive reinforcement training of nonhuman primates. If all regulatory bodies are knowledgeable in the area of improved refinement, they will be able to ask more detailed questions regarding protocol procedures and traditional, antiquated methods.

The current use of stressful data-collection methodology in some laboratory settings also raises concern regarding the validity of the data collected. When using negative stimuli and restraint, the monkeys within the same species react differently to the same procedure. If an animal is sick and receiving clinical care, given that stress compromises the immune system (Morrow-Tesch, McGlone, and Norman, 1993) the animal is taking more time to fight off the illness, because every time the animal gets an injection, he or she gets stressed out. This compromises both animal welfare and data accuracy. Positive reinforcement training standardizes this behavior because the desired behavior is captured and amplified by reward and a trusting relationship.

Some researchers argued that restraint was necessary for the safety of the animals and personnel. Involuntary restraint in an immobilizing apparatus (figure 2) does not equate to safety, however. The restraint is merely a convenience for the researcher. Since each monkey produces a variable response to negative stimuli and restraint, a researcher cannot predict how each individual monkey might react. I have been scratched and grabbed by monkeys that were restrained, because it appeared that they did not want me to touch them.

Perhaps the best method to improve animal welfare at a quicker pace is to require the animal research facilities to hire an animal trainer well-versed in the techniques of positive reinforcement. The trainer would work with the animals and staff on refining stressful data-collection and husbandry procedures. An animal trainer will ultimately save the facility time and money while reducing the stress of the animals and the research personnel.

If a researcher thinks a standardized method can be improved, they should be allowed to explore the technique with ease. This means that the PI has to be open to suggestions, the IACUC has to approve it, and additional funding is available to perform a pilot study. Semi-annual meetings should occur among staff where ideas of refining techniques are encouraged so that they can work toward a less-stressful methodology. Frequent training should occur with even the experienced researchers that have been working with monkeys for 20-30 years. New discoveries are frequently made in how to apply humane care to animals. Therefore traditionally more stressful methods should not be allowed to persist, simply due to convenience. Appendix D in The Guide for the Care and Use of Laboratory Animals states:

> "Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals." (p 118)

Even if personnel are "experienced" in that they have worked at the institution for a long time, they should be retrained in innovative refinement principles so that antiquated practices may be phased out at a faster pace.

The methodology occurring at primate research facilities requires urgent attention. If funding agencies are going to continue to fund researchers who employ the animal model, these agencies must make certain that the results of these animal based studies are as accurate and as reproducible as possible. Therefore, the funding and regulatory agencies must act now to reduce, if not eliminate, the most obvious sources of stress in the lives of these research primates.

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Figure 1. Routine handling of a juvenile rhesus monkey (left) and an adult monkey (right) for obtaining blood. In the laboratory, leather gloves were used to catch and restrain the monkey, thereby adding an additional stressor. (Reinhardt, 2001; Reinhardt and Reinhardt, 2003).

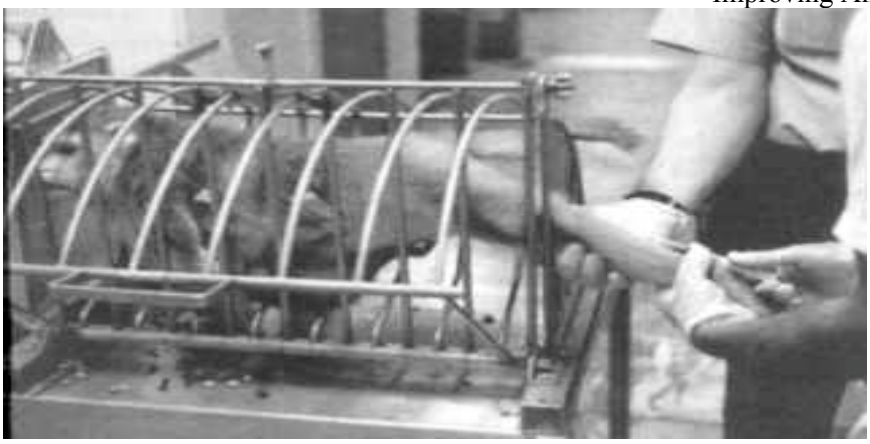

Figure 2. Restraint apparatus for a rhesus monkey (Reinhardt and Reinhardt, 2003).

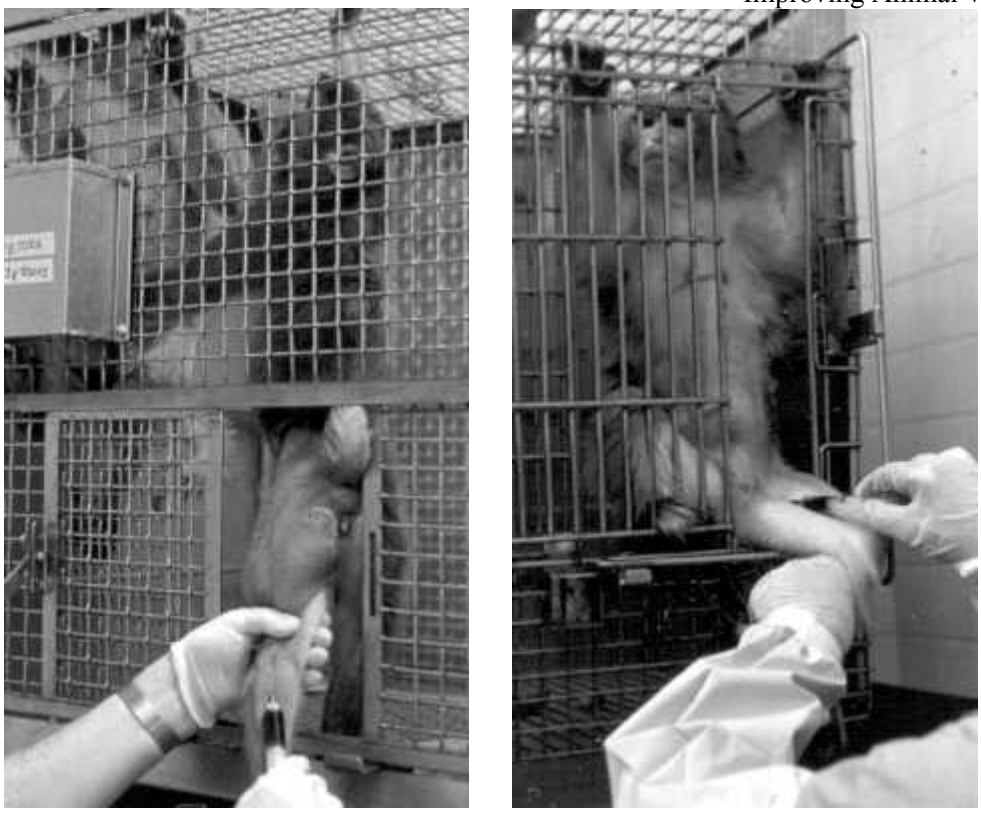

Figure 3. Cooperative blood draw from the saphenous vein (left) and the femoral vein (right). (Reinhardt and Reinhardt, 2003).

Figure 4. The two-tiered caging system still used today in some laboratories shows that there are large differences in lighting in the top and bottom cage. (Reinhardt, 2001).

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