RESEARCH ARTICLE

Positive Reinforcement Training Affects Hematologic and Serum Chemistry Values in Captive Chimpanzees (Pan troglodytes)

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Positive reinforcement training (PRT) techniques have received considerable attention for their stress reduction potential in the behavioral management of captive nonhuman primates. However, few published empirical studies have provided physiological data to support this position. To address this issue, PRT techniques were used to train chimpanzees (Pan troglodytes) to voluntarily present a leg for an intramuscular (IM) injection of anesthetic. Hematology and serum chemistry profiles were collected from healthy chimpanzees (n = 128) of both sexes and various ages during their routine annual physical examinations over a 7-year period. Specific variables potentially indicative of acute stress (i.e., total white blood cell (WBC) counts, absolute segmented neutrophils (SEG), glucose (GLU) levels, and hematocrit (HCT) levels) were analyzed to determine whether the method used to administer the anesthetic (voluntary present for injection vs. involuntary injection) affected the physiological parameters. Subjects that voluntarily presented for an anesthetic injection had significantly lower mean total WBC counts, SEG, and GLU levels than subjects that were involuntarily anesthetized by more traditional means. Within-subjects analyses revealed the same pattern of results. This is one of the first data sets to objectively demonstrate that PRT for voluntary presentation of IM injections of anesthetic can significantly affect some of the physiological measures correlated with stress responses to chemical restraint in captive chimpanzees. Am. J. Primatol. 68:245–256, 2006. © 2006 Wiley-Liss, Inc.
Key words: chimpanzees; *Pan troglodytes*; positive reinforcement training; acute stress response; hematology; blood serum chemistry

INTRODUCTION

Nonhuman primates, and specifically chimpanzees (*Pan troglodytes*), serve as essential models in a variety of areas of biomedical research [Carlsson et al., 2004; Skiadopoulos et al., 2002; Wieland et al., 2004]. Primate subjects that are trained to voluntarily participate in management and research procedures are thought to yield superior data compared to those obtained from untrained subjects [Laule et al., 2003]. Positive reinforcement training (PRT) techniques, used as a part of nonhuman primate behavioral management programs, can be beneficial for both the practicing humans and the animals. The use of PRT can 1) increase effective human–animal communication, 2) help gain the animals’ trust by giving them increased control and choice, 3) enhance the animals’ well-being [Bloomsmith et al., 1993, Laule et al., 1992], 4) facilitate husbandry practices [Bloomsmith et al., 1994; 1998; Laule & Desmond, 1990; Reichard et al., 1993], and 5) improve veterinary and research procedures [Desmond & Laule, 1987; Lambeth et al., 2000; Laule et al., 1996; Perlman et al., 2001, 2003, 2004; Schapiro et al., 2005]. Training animals to voluntarily cooperate in management, husbandry, and veterinary procedures can also enhance health and safety by decreasing stress and reducing the risk of injury to the animals [Anzenberger & Gossweiler, 1993; Baker, 1991; Bunyak et al., 1982; Grant & Doudet, 2003; Knowles et al., 1995; Luttrell et al., 1994; Moseley & Davis, 1989; Phillipp-Falkenstein & Clarke, 1992; Priest, 1991; Reichard et al., 1993; Reinhardt & Cowley, 1990; Rogers et al., 1992; Vertein & Reinhardt, 1989].

To fully understand the effects of behavioral management techniques, including training, on the physiological responses of nonhuman primates, one must attempt to understand the complicated relationships that exist among stress, behavior, and physiological responses [Boinski et al., 1999; Capitanio et al., 1998; Clarke et al., 1996; Gust et al., 1996; Kaplan et al., 1991; Laudenslager & Boccia, 1996; Lilly et al., 1999; Line et al., 1996; Maninger et al., 2003]. While some behavioral data suggest that PRT in particular is beneficial for nonhuman primates [Bassett et al., 2003; Bloomsmith, 1992; McKinley et al., 2003; Prescott & Buchanan-Smith, 2003; Reinhardt & Cowley, 1990; Schapiro et al., 2001, 2003], only a few studies to date have provided physiological data to illustrate the potential benefits of training [Clarke et al., 1988; Dettmer et al., 1996; Elvidge et al., 1976; Reinhardt, 1991; Reinhardt et al., 1990], and especially PRT [Bassett et al., 2003]. For example, in studies using both positive and negative reinforcement techniques for venipuncture, lower serum cortisol levels were demonstrated in trained rhesus and capuchin monkeys [Dettmer et al., 1996; Elvidge et al., 1976; Reinhardt, 1991; Reinhardt et al., 1990]. Common marmosets were trained with positive reinforcement techniques to urinate on command, and showed less stress behaviorally when exposed to mild husbandry-related stressors than untrained animals. However, in another study, trained marmosets showed no significant changes in excreted urinary cortisol compared to untrained marmosets [Bassett et al., 2003]. Furthermore, Clarke et al. [1988] demonstrated that three species of macaques differed in their responses to training and to transport-cage confinement via measurements of corticosteroid concentrations in blood samples.
There are humane and ethical reasons for enhancing the welfare of captive nonhuman primates, but there are also several practical reasons. Any behavioral management effort that successfully enhances welfare also improves the quality and utility of the animal as a biomedical model, and thereby supports experimental procedures and conditions that can in turn more directly address experimental hypotheses. This increases our ability to generalize research findings to normal populations. It seems quite plausible that data obtained from animals that experience distress due to management, handling, and/or research procedures may be affected by physiological confounds that could reduce the validity of the data. For example, one would expect that values obtained from blood samples of chimpanzees that voluntarily presented for venipuncture would be less likely to be confounded by the effects of acute stress compared to values from samples obtained by involuntary means.

The present paper is an attempt to provide empirical data to support the claim that using PRT techniques to train chimpanzees to voluntarily participate in husbandry, veterinary, and research procedures results in enhanced welfare for the animals, as measured by physiological indices that may be indicative of lower levels of acute stress. Specifically, the present study was designed to examine whether PRT of chimpanzees to present voluntarily for an anesthetic injection reduced the stress (as measured by specific hematological and serum chemistry values) associated with involuntary chemical restraint in these animals. While enhancing the welfare of captive chimpanzees is a significant goal on its own, the resultant potential benefit of enhancing the quality of the chimpanzee as a biomedical model, and thus facilitating investigations to more directly test experimental hypotheses, is perhaps even more significant.

MATERIALS AND METHODS

Subjects and Housing

The subjects of this study were 128 chimpanzees of both sexes (55 males and 73 females, 3–41 years old) that were socially housed in indoor/outdoor runs, Primadomes, or corrals [Riddle et al., 1982] at the Michale E. Keeling Center for Comparative Medicine and Research, University of Texas M. D. Anderson Cancer Center (UTMDACC), Bastrop, Texas. The social groups ranged in size from two to 15 animals. All animals in the colony participate in a behavioral management program to ensure their mental health and well-being. This program includes comprehensive daily environmental enrichment procedures, compatible social groupings, and PRT [Bloomsmith, 1995].

All animals in the colony also participate in a veterinary management program to maintain their physical health. Included in the multifaceted veterinary management program is a complete annual physical examination. The animals are anesthetized (Telazol®, 3 mg/kg) for their annual physical after a 12-hr fast in order to facilitate the examination. To ensure the safety of the animals, the standard operating procedure is to administer the anesthetic when the chimpanzee is temporarily isolated from its group. Therefore, on the morning of a subject’s scheduled examination, the animal is separated from its social group and is not fed. Shortly thereafter, the chimpanzee is asked to approach the front of the enclosure and present a thigh for an intramuscular (IM) injection of anesthetic. Animals that voluntarily present for the injection are injected and provided with a positive reinforcer (typically a secondary reinforcer, such as a click or a whistle). Animals that choose not to present when asked are then shown the dart gun (negative reinforcer) and given another chance to present. If the
animal presents at this point, the animal receives the injection. If the animal still
chooses not to present, one of two traditional methods are used to inject the
chimpanzee involuntarily: the CO₂ dart gun (Telinject USA, Newhall, CA) or a
wire stick (which entails enticing the chimpanzee to the front of the cage while
hiding the syringe and injecting the anesthetic without any warning). In general,
animals are given at least two chances to voluntarily present for the injection
before darting or the wire stick is used.

PRT Program

The chimpanzees in the UTMDACC colony have participated in our PRT
program since 1991 [Bloomsmith, 1995], and many animals are trained to
voluntarily perform a variety of behaviors that are important for husbandry,
clinical, and research purposes [Schapiro et al., 2003, 2005]. The overriding goal
of the PRT program is to enhance the quality and utility of the chimpanzee as a
model for biomedical research. PRT facilitates this goal by enriching the lives of
the chimpanzees and enhancing the management of the population by reducing
the stress they and their caregivers experience during routine husbandry,
Veterinary, and research procedures. The training techniques used in the
UTMDACC colony were adapted from previously described methods [Bloomsmith
Laule & Desmond, 1995; Whittaker et al., 2001]. Some of the procedures with
which the chimpanzees are trained to cooperate include urine collection
[Lambeth et al., 2000], moving or shifting between enclosure areas [Schapiro
et al., 2003], presenting the perineum for parasite monitoring [Perlman et al.,
2001; Schapiro et al., 2005], and voluntarily presenting for an injection (IM
[Schapiro et al., 2005, in press] or subcutaneous [Perlman et al., 2004; Schapiro
et al., 2005]). Training the chimpanzees to present a leg and accept an injection
has been a primary focus of the training program, since this behavior should have
a large overall effect on the reduction of stress associated with anesthetic
injections during medical or research procedures.

Training Method

Training complex behaviors (such as presenting a leg and accepting an IM
injection) using PRT techniques requires multiple steps. Establishing a secondary
reinforcer and shaping the desired behavior are basic operant conditioning
techniques, and more detailed treatments of the processes involved with PRT can
be found elsewhere [Laule et al., 2003; Prescott & Buchanan-Smith, 2003; Pryor,
1999; Whittaker et al., 2001]. The point of this paper is to discuss the effects of
PRT rather than the process of PRT; therefore, the reader is directed to the
sources cited above for further discussions of PRT techniques.

Data Collection

Data for this study were retrospectively gathered from records of physical
examinations performed at the UTMDACC chimpanzee colony between 1996 and
2003. This database contains physiological information (serum chemistry and
hematology values) as well as the method used to deliver the anesthesia to the
chimpanzees. A total of 575 records for 128 chimpanzees in the database were
analyzed for the present investigation. The mean number of physical examina-
tions for each chimpanzee in the database was 4.5 (range = 1–7). Data regarding
the research protocols or pregnant, wounded, ill, or chronically infected chimpanzees were not included.

The method used to administer the anesthesia for the physical examination of the chimpanzees was recorded as one of four mutually exclusive categories. The first category, voluntary presentation of the thigh for an IM injection (Present), used positive reinforcement training solely, while the other three categories involved at least negative reinforcement, and for the purposes of this analysis were considered involuntary (additional explanation provided below). The three involuntary categories included presentation of the thigh after seeing the dart gun (Present After), darted with the dart gun (Dart), and unexpected injection through the wire (Wire Stick; see Table I).

Blood samples collected during each animal’s physical examination were analyzed using standard techniques for hematology and serum chemistry. The complete blood count (CBC) and chemistry data were entered into the animals’ permanent medical records. From these assessments, specific measures that were thought to be indicative of acute stress, based on stress leukograms from other species, were chosen for analysis [Stockham & Scott, 2002; Thrall et al., 2004]. The specific variables included the total white blood cell count (WBC), absolute segmented neutrophils (SEG), blood glucose (GLU) levels, and hematocrit (HCT) levels. Although HCT has not been established as an unambiguous stress marker, it has been shown to increase during acute stress situations in rhesus monkeys [Lilly et al., 1999].

Within-Subjects Analysis

In the majority of the chimpanzees in the database (n = 79), samples were obtained after both voluntary (Present) and involuntary (Present After, Dart, or Wire Stick) injections, allowing for within-subjects analyses of the dependent measures described (WBC, SEG, GLU, and HCT). The within-subjects data set included 34 male and 45 female chimpanzees, ranging in age from 3 to 36.5 years.

Collection of Physiological Data

Samples for hematologic and biochemical analyses were processed through the Department of Veterinary Sciences’ Clinical Pathology Laboratory. Venous

<table>
<thead>
<tr>
<th>Category</th>
<th>Method of administration of anesthetic</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary</td>
<td>Present for injection</td>
<td>Present</td>
<td>Voluntary presentation of the leg for injection without being threatened by the dart gun or the presence of the gurney</td>
</tr>
<tr>
<td>Involuntary</td>
<td>Present after seeing the dart gun</td>
<td>Present after</td>
<td>Present leg for injection only after seeing or being aimed at with the dart gun</td>
</tr>
<tr>
<td></td>
<td>Wire stick</td>
<td>Wirestick</td>
<td>Injected at the wire mesh by a syringe that was hidden from sight (dart gun may or may not be present)</td>
</tr>
<tr>
<td></td>
<td>Dart gun</td>
<td>Dart</td>
<td>Anesthetized using the dart gun (Tel inject with CO₂)</td>
</tr>
</tbody>
</table>

TABLE I. Definitions of Methods of Anesthetizing the Chimpanzees
blood was aspirated from the femoral vein with an 18-, 20-, or 22-gauge needle into commercial vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ). Blood was collected into K2 EDTA tubes for hematologic evaluation. The Cell-Dyn 3500 hematology system (Abbott Laboratories, Abbot Park, IL) was used to determine the red blood cell (RBC) count, total WBC count, and HCT. A manual microscopic WBC differential was performed by the laboratory technician. Blood for the biochemical evaluation was collected into red-top serum tubes that did not contain anticoagulant. The blood was allowed to clot for 30–60 min at room temperature and then centrifuged at 3,000 rpm for 10–15 min. Serum was removed from the clot and processed with a Cobas Mira chemistry analyzer (Roche) to determine the blood serum chemistry (GLU).

Statistical Analyses

The primary focus of this study was to assess whether voluntary presentation for an IM injection of anesthetic would affect the physiological variables that are indicative of acute stress. We expected that values associated with voluntary injections would be indicative of less acute stress than values associated with involuntary and more traditional anesthetic techniques. Preliminary three-way analyses of variance (ANOVAs; Present vs. Present After vs. Dart plus Wire Stick) revealed that the dependent measures differed significantly across these three categories. However, planned comparisons revealed that values in the Present After condition differed significantly from values in the Present condition, but did not differ significantly from values in the Dart plus Wire Stick condition. Therefore, to maximize the clarity of this paper and to emphasize the effects of voluntary presentation/PRT (Present) on physiological responses, all methods of anesthetic restraint were grouped into two categories: 1) voluntary (Present), and 2) involuntary (Present After, Dart, and Wire Stick). Only analyses of voluntary vs. involuntary categories are included and discussed below. Students’ t-tests were used to compare WBC, SEG, GLU, and HCT levels.

Of the involuntary methods of anesthetic restraint examined, darting was the predominant category. It is also the most traditional method of anesthetic restraint used for chimpanzees (see Table II). Therefore, additional Student’s t-tests were used to compare the physiological variables of interest between Present and Dart.

Additionally, paired Student’s t-tests were used to compare dependent measures from the subset of subjects (n = 79) with data points from both a voluntary present and an involuntary injection.

### TABLE II. Distribution of Subject Cases in the Database for Each Method of Anesthetic Restraint Category

<table>
<thead>
<tr>
<th>Method of Administration of Anesthetic</th>
<th>Total cases for overall comparison</th>
<th>Total cases for within-subjects comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>222</td>
<td>79</td>
</tr>
<tr>
<td>Present after</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>Wire stick</td>
<td>37</td>
<td>5</td>
</tr>
<tr>
<td>Dart</td>
<td>268</td>
<td>69</td>
</tr>
<tr>
<td>(Total = 575)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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RESULTS

Voluntary vs. Involuntary Methods of Administering Anesthesia

The subjects voluntarily presented for an anesthetic injection 222 times (38.6% of cases) and had to be involuntarily anesthetized 353 times (see Table II). Comparisons of the levels of WBC, SEG, GLU, and HCT by the two general categories of anesthesia administration (voluntary vs. involuntary anesthetic injections) revealed statistically significant differences. Subjects that voluntarily presented for injections had significantly lower WBC (t(573) = 3.55, P ≤ .001), SEG (t(573) = 3.86, P ≤ .001), and GLU (t(573) = 5.13, P ≤ .001) levels than subjects that were involuntarily injected, but there were no significant differences in HCT (t(573) = −1.44, P > .05; see Table III).

Voluntary Present vs. Darting

Significant differences also existed between the Present condition (n = 222) and the Dart condition (n = 268), the most traditional method of administering anesthesia to chimpanzees. Subjects in the Present group had significantly lower WBC (t(488) = 3.16, P ≤ .005), SEG (t(488) = 3.76, P ≤ .001), and GLU (t(488) = 4.87, P ≤ .001) levels and higher HCT that bordered on significance (t(488) = −1.92, P ≤ .06) compared to subjects that were darted (see Table IV).

Within-Subjects Comparison (Voluntary vs. Involuntary)

The within-subjects analyses also identified interesting differences. Paired Student’s t-tests revealed that the chimpanzees had significantly lower WBC (t(78) = −2.05, P ≤ .05) and GLU (t(78) = −4.41, P ≤ .001) levels when they voluntarily presented for the injection than when they did not (Table V). Neither SEG (t(78) = −1.70, P > .05) nor HCT (t(78) = 0.34, P > .05) differed significantly in this comparison.

DISCUSSION

Persistent exposure of research subjects to involuntary participation in management and medical procedures, such as traditional administration of anesthetic, may exacerbate acute stress and potentially reduce the validity of

<table>
<thead>
<tr>
<th>TABLE III. Comparison of Relevant Dependent Measures for Subjects Receiving Voluntary (n = 222) or Involuntary Injections (n = 353)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological variables</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>WBC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLU&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCT&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>(× 10<sup>9</sup>/μl).
<sup>b</sup>(mg/dl).
<sup>c</sup>(%).
<sup>d</sup>denotes statistical significance (P ≤ .05).
research findings. The analyses in this study empirically demonstrate that using PRT techniques to achieve voluntary acceptance of an IM injection of anesthetic can significantly affect specific physiological measures that should be correlated with acute stress responses in captive chimpanzees. Total WBC counts, blood GLU levels, and SEG levels were significantly lower in animals that voluntarily presented than in animals that received an injection involuntarily or were darted. Decreased HCT values were previously reported in rhesus monkeys subjected to an acute stressor [Lilly et al., 1999], but compared to the three other physiological parameters, HCT is less well validated as a stress marker. The results of the present study do not suggest that HCT should be used as an additional measure of acute stress in chimpanzees.

Perhaps the most noteworthy finding in this study comes from the within-subjects comparison. Since the subjects served as their own controls in this analysis, the previously identified age/sex differences in hematologic and blood serum chemistry values for chimpanzees [Herndon & Tigges, 2001; Howell et al., 2003; Ihrig et al., 2001] were at least partially controlled for. Therefore, the dependent measures in this analysis were free of many potential confounds, which allowed us to more directly test the hypothesis that PRT can affect physiological variables. Our data suggest that the total WBC count and blood

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**TABLE IV. Comparison of Relevant Dependent Measures for Subjects Receiving Voluntary Injections (n = 222) or Dart Injections Only (n = 268)**

<table>
<thead>
<tr>
<th>Physiological variables</th>
<th>Present</th>
<th></th>
<th>Dart</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>WBCa</td>
<td>.002d</td>
<td>10.20</td>
<td>± 4.9</td>
<td>11.49</td>
</tr>
<tr>
<td>SEGa</td>
<td>.001d</td>
<td>6.54</td>
<td>± 4.7</td>
<td>8.08</td>
</tr>
<tr>
<td>GLUb</td>
<td>.001d</td>
<td>82.45</td>
<td>± 18.1</td>
<td>90.96</td>
</tr>
<tr>
<td>HCTc</td>
<td>.055</td>
<td>42.53</td>
<td>± 4.2</td>
<td>41.86</td>
</tr>
</tbody>
</table>

*a(× 10^9/μl). b(mg/dl). c(ⅹ%). dDenotes statistical significance (P≤.05).

**TABLE V. Comparison of Relevant Dependent Measures for Subjects (n = 79) Receiving at Least One Voluntary and at Least One Involuntary Injection**

<table>
<thead>
<tr>
<th>Physiological variables</th>
<th>Present</th>
<th>for injection</th>
<th>Involuntary injection(Present after+dart+wire stick)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>WBCa</td>
<td>.043d</td>
<td>9.71</td>
<td>± 3.3</td>
<td>10.76</td>
</tr>
<tr>
<td>SEGa</td>
<td>.093d</td>
<td>6.38</td>
<td>± 3.6</td>
<td>7.31</td>
</tr>
<tr>
<td>GLUb</td>
<td>.001d</td>
<td>79.20</td>
<td>± 19.0</td>
<td>92.24</td>
</tr>
<tr>
<td>HCTc</td>
<td>.738</td>
<td>42.65</td>
<td>± 3.6</td>
<td>42.53</td>
</tr>
</tbody>
</table>

*a(× 10^9/μl). b(mg/dl). c(ⅹ%). dDenotes statistical significance (P≤.05).
GLU level may be among the most valuable and consistent physiological indicators of acute stress in chimpanzees.

The values recorded for the animals that received an injection involuntarily were not outside published normal ranges for chimpanzees [Herndon & Tigges, 2001; Howell et al., 2003; Ihrig et al., 2001], yet they differed significantly from the values of animals that presented voluntarily. The values obtained after voluntary injections were also within the normal ranges for chimpanzees. It is important to note that the “normal ranges” of values presented by the aforementioned authors were derived from samples obtained from chimpanzees that were involuntarily anesthetized (typically by a dart gun or a wire stick).

Although the biological significance of the statistically significant differences we observed is still somewhat unclear, it is our premise that trained subjects will yield better (i.e., potentially less variable) data than untrained subjects.

In addition to increasing the reliability and validity of the data, PRT also increases opportunities for the animals to express choice and to voluntarily cooperate with procedures. As a function of PRT, the performance of desired behaviors becomes more reliable, which in turn reduces stress for the caregiving staff and improves their work environment. The specific benefits of the training discussed in this paper include an increased ability to gain access to animals rapidly and safely, as well as a reduction in the need for involuntary injections of anesthesia. Currently, over 69% of our chimpanzee population reliably presents voluntarily for an anesthetic injection. The rest of the animals are in training, but have yet to meet the stringent criteria for reliable performance [Schapiro et al., in press]. Although each chimpanzee is typically trained to present by a single trainer, other dedicated trainers have readily learned how to teach the animals this behavior, and veterinary technicians are also learning the training process.

Extremely similar PRT techniques have also been used to train chimpanzees to voluntarily participate in a variety of physical examination and diagnostic procedures [Lambeth et al., 2000; Laule et al., 1996; Perlman et al., 2001, 2003, 2004; Schapiro et al., 2003, 2005].

The role of extraneous stress-related variables must be considered in the development of research methodologies to improve the reliability and validity of scientific results. This study provides a preliminary foundation of physiological evidence that suggests that PRT techniques can reduce the stress associated with anesthetic injection, and improve the welfare of the chimpanzees, the quality of the scientific data collected, and the utility of the chimpanzee as a model for biomedical research. It is essential to continue research in this arena to further determine not only the physiological but also the behavioral effects of providing nonhuman primates with opportunities to voluntarily participate in husbandry procedures and research protocols. We are currently conducting prospective studies to further investigate these issues [Lambeth et al., in press].

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