Adapted Primary Literature for Teaching

Positive Reinforcement Training Affects Blood Chemistry Values in Captive Chimpanzees

Original research report: Lambeth, S. P. et al. 2006. Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (*Pan troglodyes*). Amer. J. Primatology. 68: 245-256

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Abstract

Non-human primates in captivity in research facilities and zoos may be stressed by the limited space and activity available in their environment. Other studies have shown that this stress might be reduced by training that involves positive reinforcement (that is, giving animals rewards when they show the kind of behavior or body function that the trainer wants).

In this study, trainers wanted chimpanzees in a research lab environment to present a leg for an intramuscular (IM) injection, a procedure which the animal would not normally do because it would be regarded as unpleasant and stressful. Researchers took blood samples once a year for seven years as part of the annual physical examination, and measured those features of blood that are known to indicate the degree of stress. They compared the measures in chimps that had been given positive reinforcement training and those that had not. Chimps that voluntarily presented a leg for an injection had significantly lower blood indicators of stress.

Introduction

Nonhuman primates are often used in biomedical research because they are the species that are most closely related to humans. Thus, experimental data from these animals is most likely to reflect what would happen to humans under similar test conditions. Previous researchers (cited) have suggested that the laboratory environment is stressful to a wild animal and could affect the quality of the data collected. Anything that can be done to reduce the stress should improve the quality of data.

Other investigators (cited) had shown that stress can be reduced, in both animals and humans, by providing positive reinforcement training. In such training, rewards are given when the animal does the "right thing" under conditions of stress. For example, training animals to voluntarily cooperate under stressful conditions should be useful in management, husbandry, and veterinary procedures in both farm and laboratory animals. Such cooperation should be associated with less stress and reduce the risk of injury (for both animals and humans).

A great deal of previous research (cited) has identified certain properties of blood cells and blood chemistry that are reliable indicators of stress.[1] For example, one such indicator is the blood level of the (stress) hormone, cortisol, which comes from the adrenal gland. Several studies (cited) compared the effects on cortisol under positive or negative reinforcement training when blood samples were drawn from the veins of monkeys, and lower cortisol levels were demonstrated in the trained monkeys. In another study (cited) positive reinforcement was used to train marmosets to urinate on command (to provide a urine sample). They showed fewer behavioral signs of stress than untrained animals. However, another similar study with marmosets (cited) in which the measure of stress came from blood samples rather than observing behavior showed no benefit of training on the amount of cortisol in the urine. Another cited study compared three species of macaques and found that the effects of

[1] "Stress" is a word with many meanings. Most simply stated, stress is caused by conditions that cause something bad to happen, which may occur in many forms of being emotionally upsetting and/or physically damaging..Stress often affects both mind and body.

training of cage-confinement stress, as measured by blood cortisol, depended on the species.

So these present authors felt there is some confusion in the literature that needs to be clarified. They pointed out that reducing animal stress is important to do because it is humane. But there are also practical reasons. The quality and usefulness of an animal as a biomedical research model depends on its health and well-being. An animal under great stress is not a normal animal. Therefore, results from experimental procedures may well be misleading. The most obvious illustration would be experiments involving blood cells or blood chemistry, which are known to be affected by the level of stress as well as by any experimental manipulation. (Scientists would call these "confounding variables").

The present study tested whether positive reinforcement techniques (PRT) to train chimps to voluntarily participate in husbandry, veterinary, and research procedures would enhance the welfare of the animals, as determined by physiological measures that are known to reflect the level of stress.

Methods

Subjects and Housing. Subjects were 128 chimps (55 males, 73 females, 3-41 years old) that were housed in social groups in indoor/outdoor runs, similar to the environment that others have used (cited). Research facilities were located at the University of Texas M.D. Anderson Cancer Center in Bastrop, Texas. The social groups ranged in size from two to fifteen. The routine management program was designed to ensure the animal mental health and well-being, including daily environmental enrichment procedures, compatible social groupings, and PRT (details cited from prior study).

All animals participated in a veterinary management program to maintain their physical health. Complete physical examinations were performed annually under light anesthesia.[2] To ensure safety of the animals, the procedure was to temporarily separate an animal from its group and not fed for 12 hours (because anesthetics tend to cause vomiting and inhalation of food from the stomach could cause irreversible lung damage). Animals were motioned to come to the front of the cage to present a leg for intramuscular injection (IM) of the anesthetic. PRT animals that presented their thigh for injection were given a secondary reinforcer, such as a click or a whistle. Chimps that did not cooperate were shown a dart gun (a negative reinforcer) and given another chance to present their leg. If the chimp still refused, one of two methods were pursued. One was to shoot the anesthetic into them with a dart gun or use a wire stick to move the chimp to the front of the cage, while hiding the syringe, and injecting the anesthetic without any warning.

PRT program. PRT has worked so well at this research facility that it is routinely used in management of the colony. The methods used in this study were adapted from much previous research (cited). These procedures have proven useful in collecting urine samples, moving animals to different housing quarters, presenting the anal area for parasite monitoring, or presenting a leg for IM injection.

The PRT procedure in this present study was not explained, other than to say that it was done in a small series of steps involving traditional "operant conditioning" principles. Appropriate references were cited, but details were not provided here because those techniques are well known (to experts at least) and because the purpose of this study was to examine the ***effects*** of PRT, not PRT itself.

Data Analysis. The data used came from samples taken between 1996 and 2003. The data were serum[3] chemistry and blood cell information as well as the method used to deliver anes-

[2] The drug used to produce anesthesia was known as Telazol, a trade name for a nonnarcotic, nonbarbiturate mixture of two chemicals. It is not a true anesthetic, but does immobilize an animal. It is normally used in dogs and cats.

[3] Look up the difference between whole blood, serum and plasma.

thesia. The mean number of physical examinations (and blood sampling) was 4.5 (range 1-7).

Data from any animals that were pregnant, wounded or ill were not used.

Data were segregated into four groups, depending on how the anesthesia was administered, when the animal: 1) had PRT and voluntarily presented, 2) had to be warned by seeing the dart gun, 3) had to be shot with a dart fun, and 4) were tricked.

Blood samples collected from the leg vein of the anesthetized animals were analyzed in conventional ways. These included counting and evaluating the characteristics of white blood cells and measuring various chemicals in blood. The data used as indices of stress were: 1) total white blood cell count (CBC), 2) number of neutrophils (a type of white cell) that had a fragmented nucleus, 3) blood glucose level, and 4) the packed volume of red blood cells after they had been spun down in a centrifuge ("hematocrit"). After blood was centrifuged, the fluid at the top (serum) was used to perform blood chemistry measurements. Cited literature indicates that all of these are reliable indicators of stress, although the evidence for hematocrit as an indicator is tentative.

Within Subjects. In 79 subjects (34 male, 45 female) the data could be compared in the same animal under all test conditions (voluntary and involuntary leg presentation).

Statistical Analysis. The investigators expected there would be less indication of stress in blood taken from PRT chimps that had voluntarily presented their leg than from the animals that had to be forced or tricked.

One test procedure was Analysis of Variance (ANOVA) which basically compared the means and variation around the means of each of the four groups of chimps (PRT group and the three forced or tricked groups). This was an "among subjects" analysis. The measures differed significantly greater than chance across all groups. Since there was no significant difference among the three involuntary groups, those data were then combined into one group to allow another comparison between the voluntary PRT group and all the involuntary groups.

Another statistical test, called the "Students' t-test" was used to compare the four blood measures to see if any were more sensitive indicators or stress.

For the 79 chimps that had both PRT and involuntary data, a variation of the t-test, known as a "paired t-test" was used to compare PRT and involuntary results.

No citations are given for performing these statistical tests, because they are standard and a routine feature in the training of all scientists.

Results

Voluntary vs. Involuntary Methods of Giving Anesthesia. Chimps that voluntarily presented their leg for injection had statistically significant lower WBC and segmented WBC than the chimps that had to be forced or tricked (Table 1.) The forced group also showed higher blood glucose.

TABLE I. Comparison of Relevant Dependent Mean values.

Measure	Voluntary n = 222	Involuntary n = 353	P < [4]
WBC[a]	10.2	11.6	0.05
seg. WBC[a]	6.54	8.1	0.05
glucose[c]	82.5	91.4	0.05
hematocrit	42.5	42.1	--

[a] ($\times 10^3/\mu l$) [b](mg/dl), [c](%)

Comparison of Voluntary vs. Darting (Table II). Chimps in the voluntary group had significantly lower WBC and segmented WBC, and glucose. A marginal decrease was seen in hematocrit.

TABLE II. Comparison of Relevant Dependent Mean values.

Measure	Voluntary n = 222	Darted n = 268	P <
WBC[a]	10.2	11.49	0.002
seg. WBC[a]	6.54	8.08	0.001

[4] P values are the probability, based on analysis the variation in the data, that the observed effect could be due to chance. In this case, there is less than a 5% chance of such error. 5% is the standard cut-off point for concluding that an effect was not likely to be due to chance.

glucose[c]	82.5	90.96	0.001
hematocrit	42.5	41.86	006

[a] (x 10^3/µl) [b](mg/dl), [c](%)

<u>Within-subjects Comparison (Table III).</u> Significant decreases were seen in WBC and blood glucose in the voluntary group.

TABLE I. Comparison of Relevant Dependent Mean values.

Measure	Voluntary n = 79	Involuntary n = 79	P <[5]
WBC[a]	9.71	10.76	0.043
seg. WBC[a]	6.38	7.31	0.093
glucose[c]	79.2	92.24	0.001
hematocrit	42.65	42.53	--

[a] (x 10^3/µl) [b](mg/dl), [c](%)

Discussion

The authors concluded that exposure of research animals to involuntary participation in management and medical procedures, such as traditional administration of anesthetic, may stressful and potentially reduce the validity of research findings. They said that the results showed that PRT changed the physiological response to the stress of giving an IM injection. This was said to be reflected in the number of WBC and segmented WBC, and blood glucose.

The hematocrit data were said to be less likely to be a reliable measure of stress, because not much change was noted in those values.

The within-subject data were considered the most noteworthy, because the subjects "served as their own controls." Specifically, other studies (cited) had shown that the measures of stress used in this present study are affected by age and sex.

The authors also stated that WBC and blood glucose seem to be the most useful indicators of stress.

The authors then claimed from cited papers that the values they obtained from the untrained, involuntary group were similar to those reported by other investigators under other typical housing conditions.

They further concluded that "trained subjects will yield better (i.e. potentially less variable) data than untrained subjects."

They state that PRT training increases opportunities for animals to express choice and to voluntarily cooperate (perhaps also in other procedures). The advantages of such training are said to include increased ability to gain access to animals quickly and safely. In their colony, they now have 69% of the animals PRT trained to voluntarily present for injection. They cite papers from several other labs that routinely use PRT procedures for their research animals.

The authors finally say that stress is an important factor that could distort research findings. Therefore, researchers should try to minimize stress in their experimental animals to improve the quality of research data (as well as to improve animal welfare).

[5] P values are the probability, based on analysis the variation in the data, that the observed effect could be due to chance. In this case, there is less than a 5% chance of such error.