Restraint Methods of Laboratory Non-Human Primates: A Critical Review

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ABSTRACT

Published information provides scientific evidence that traditional, involuntary restraint techniques of research non-human primates are intrinsically a source of distress resulting from fear. It has been documented that common methods of enforced restraint result in significantly increased adrenal activity as well as significant changes in a variety of other physiological parameters. There is no scientific evidence that the animals adequately habituate to involuntary restraint. Numerous reports have been published demonstrating that non-human primates can readily be trained to cooperate rather than resist during common handling procedures such as capture, venipuncture, injection and veterinary examination. Cooperative animals fail to show behavioural and physiological signs of distress. It was concluded that the advantages of training techniques over traditional restraint techniques will have to be explored more extensively in the future for the sake of research subjects and scientific methodology.

Keywords: animal welfare, handling, non-human primates, psychological well-being, restraint, social support, stress, training, unfamiliar environment

Published in Animal Welfare 4(3), 221-238 (1995)

INTRODUCTION

One of the major drawbacks to the use of non-human primates is that they can be very difficult and even dangerous to handle; restraint is therefore necessary and desirable to protect both the investigator and the animal (Robbins et al 1986). Restraint of laboratory non-human primates is tacitly accepted by many investigators, veterinarians and colony managers as a given methodological characteristic of research protocols and husbandry practices. Many authors of scientific publications are unaware of how their research animals are handled (Arluke 1988) and consequently do not mention the conditions under which their research data were collected, even if such data are stress-sensitive (Reinhardt 1991). Some primatologists, however, have long recognized that traditional restraint procedures may constitute uncontrolled methodological variables (eg Ives & Dack 1956; Cope & Polis 1959; Mason et al 1968). The animals often vigorously resist being restrained because of adverse conditioning (Robbins et al 1986). Scientific data collected from such fearful subjects must be evaluated with particular reservation, taking into account possible stress-related deviations from expected normal physiological functions and behavioural reactions.

The present review evaluates traditional restraint techniques of laboratory non-human primates and discusses possible methods of refinement.

COMMON RESTRAINT METHODS

Squeeze-back cages

Squeeze-back cages can be stressful for the animal but are safe for the handler (Sainsbury et all 989). Such cages are equipped with a special back-panel that can be moved in such a way that the animal is forced to come to the front of the cage and tolerate being partially or completely immobilized (see Figure 1 in Reinhardt et al 1991b). Squeeze-back cages are commonly used to facilitate venipuncture, injection, topical application of drugs, close-up examination, capture and other procedures.

Pun et al (1981) restrained nine adult male rhesus macaques (*Macaca mulatta*) in their home-cages and obtained blood samples at 20 minute intervals. While testosterone levels significantly declined, serum cortisol concentrations significantly increased over a one-hour sampling period. Fuller et al (1984) analysed sets of two blood samples taken from 10 squeeze-back restrained female rhesus macaques. Serum cortisol concentrations significantly increased from the initial bleeding to the second bleeding after 30 minutes.

Manual restraint

During manual restraint non-human primates are usually held by two people (see Figure 10.1 in Reinhardt 1992a) to allow sample collection, drug administration or physical examination.

Bush et al (1977) analysed blood samples taken during routine manual restraint episodes of 56 subjects of the primate families Callithricidae, Cebidae, Cercopithecidae and Pongidae. Restraint resulted in severe alterations of the animals' acid-base balance, which led to serious metabolic acidosis. Kissinger and Landi (1989) subjected eight long-tailed macaques (*M. fascicularis*) to brief manual restraint and observed significant increases of two enzymes commonly monitored during toxicological and pharmacokinetic studies: aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The authors underlined that these enzymatic responses are important and should be taken into consideration when differentiating between drug-related and experimental effects. Reinhardt (1992a) assessed serum cortisol responses to manual restraint in 10 adult female rhesus macaques. The monkeys were habituated to being held in a supine position on a table while a blood sample was collected from the femoral vein. The procedure resulted in a significant increase in serum cortisol concentrations within 15 minutes.

Restraint boards

Rather than holding an animal down with the hands, some investigators strap its extremities or its waist on specially designed restraint boards (see Figure 2 in Schultea & Stein 1981) or restraint tubes (see Figure 2 in Heam 1977) to allow sample collection, electrocardiographic or ultrasonographic recording.

Swan (1970) noted that after 1-1.5 hours of board-restraint, bonnet macaques (*M radiata*) would exhibit signs of impaired mobility as a result of the restraint and consequent struggling. Berendt and Williams (1971) observed marked changes in tidal volume and respiration rate in 18 rhesus and 24 long-tailed macaques restrained for five minutes or longer on such a board. The authors warned that restraint may produce results that are difficult or impossible to interpret. Landi et al (1990)

reported significant increases in aspartate and alanine aminotransferase levels in eight long-tailed macaques restrained for two hours on restraint boards. Goncharov et al (1979) strapped five male and five female baboons (*Papio hamadryas*) to a table for two hours. In the males, cortisol levels increased significantly while testosterone levels exhibited a significant, rapid decrease which persisted for two days after the immobilization. In the females, a significant decrease in progesterone was observed during the luteal phase and there was a significant decrease in oestradiol during the follicular phase. Cortisol was significantly elevated both in the follicular and luteal phase. Quadri et al (1978) strapped four female rhesus macaques on restraint crosses. Immobilization induced a significant progressive increase in prolactin concentration in all animals throughout a 60 minute immobilization period. Removal from the cross was followed by a decline in prolactin.

Restraint chairs

Restraint chairs maintain an animal in a sitting position with restraint being effected by pillory type attachments at the neck and waist (see Figure 1 in Mason 1958). Such chairs are commonly used (National Institutes of Health 1991) for procedures requiring free access to the subject's body, head and extremities as well as for psychological and metabolic experiments. Restraint chairs have been qualified as comfortable (Milhaud et al 1980; Lennox & Taylor 1983) though they inherently cause monkeys physical and emotional stress (Nakamura et al 1982). Despite attention to details of conditioning and daily assessments of the animal's health status, chronic chair restraint is accompanied by inherent problems (Morton et al 1987) such as decubital ulcers (McNamee et al 1984), skin abrasions, necrosis of the ischial callosities, position-dependent oedema, inguinal hernia, rectal prolapse and laryngeal air sacculitis (Morton et al 1987).

Bouyer et al (1978) habituated eight young baboons one at a time to an experimental room. When placed into a restraint chair and left alone in the same experimental room as before, subjects soon closed their eyes and became motionless. The electrocorticogram showed 'drowsiness rhythms', which typically appear during transition from wakefulness to sleep. Administration of an anxiolytical drug (diazepam) caused both the behaviour and the electrocorticogram of the restrained animal to return to normal. The authors inferred that the unusual responses underline a reaction to the 'stress' conditions brought on by restraint. Drowsiness has also been noted as a typical state in chair restraint rhesus macaques (Golub & Anderson 1986).

Morrow-Tesch et al (1993) studied immune responses in eight chair-naive adult male rhesus macaques. White blood cell counts, the percentage of neutrophils and plasma cortisol levels showed a significant increase; the percent lymphocytes and monocytes as well as the natural killer cell activity, showed a significant decrease during a three-hour chair restraint period. Goosen et al (1984) analysed haemograms of three adult male and one adult female chair-experienced baboons, during 90 minute chair restraint sessions. Blood samples were collected at 30 minute intervals. Total white blood cell count more than doubled over a 90 minute test period. The authors inferred from this that the animals were acutely stressed.

Kling and Orbach (1963) placed 13 stump-tailed (*M. arctoides*), 10 long-tailed and 10 rhesus macaques in restraint chairs for 24 hours and analysed corticosteroid concentrations in blood samples obtained shortly before and at the end of the restraint session. Under the severe stress of restraint in the primate chair for 24 hours, the values for all three species rose significantly with little or no difference between species (Kling & Orbach 1963). Hayashi and Moberg (1987) observed in six adult male rhesus macaques a *significant, transient increase* of luteinizing hormone (LH) immediately following the initial stress of being restrained in a chair, and significantly elevated serum corticosteroid levels throughout a subsequent seven-hour restraint period. Norman and Smith (1992) and Norman et al (1994) confined four adult male and 10 adult female rhesus macaques for six hours in restraint chairs. In the males (Norman & Smith 1992), adrenocorticotrophic hormone

(ACTH) and cortisol significantly increased within 15 minutes after initiation of restraint and remained elevated for most of a six-hour restraint period. Conversely, luteinizing hormone (LH) and testosterone levels significantly decreased immediately after restraint and remained suppressed for several hours after the animals were returned to their hone-cage. In the females (Norman et al 1994), ACTH and cortisol showed a significant increase with the initiation of restraint, and although ACTH had generally returned to baseline by the end of the restraint, cortisol levels remained elevated after the animals were returned to their cages. In animals sampled in the follicular phase, mean plasma LH levels were significantly lower during restraint and remained suppressed for several hours after the animals were removed from the chair. LH levels were not notably inhibited by restraint in the luteal phase. The authors concluded that chair restraint stress inhibits fertility in both male and female primates. Mason and Mougey (1972) assessed thyroid response, as measured in plasma butanol extractable iodine (BEI) levels, to chair restraint in 14 adult male rhesus macaques. Levels were significantly elevated throughout the first three weeks of chair restraint. During the second month BEI levels did not differ significantly from those observed as a response to net-catching from the home-cage.

Mason (1972) and Mason et al (1973) examined urinary corticosteroid and catecholamine excretion in eight adult male rhesus macaques that were chair restrained. Both metabolites showed a greater than threefold increase during the first three days of chair restraint, remaining significantly elevated through the first week. The authors advised that patience in allowing at least two to four weeks of restraint adaptation to occur is rewarding because of the increased reproducibility and interpretability of experiments. Fleischman and Chez (1974), however, warned that prolonged restraint may be stressful enough to prematurely terminate pregnancy. Seven out of ten baboons experienced premature labour and delivery within ten days of continuous chair restraint. Golub and Anderson (1986) measured three physiological indicators of stress in 11 pregnant rhesus macaques during 23 two-hour chair restraint sessions distributed over a period of eight weeks. Initially elevated heart rate, blood pressure and plasma cortisol declined from the first to the last session. The reduction in these parameters was most marked during the first three sessions, but continued in some animals over the entire eight-week period. Cortisol concentration consistently rose throughout each chair restraint session.

Tether

Chronic cannulation of blood vessels or other organs allows remote sample collection via a tether system. The subject has relative freedom of movement in an experimental cage and is capable of lying down (see Figure 1 in McNamee et al 1984). A major clinical concern associated with chronic vascular catheter implantation is the development of septicaemia (Morton et al 1987; cf Crockett et al 1993).

Kaplan et al (1983) assessed cardiac response to tethering in 30 long-tailed macaques. In over 80 per cent of cases, heart rate measured eight days following attachment to the tethering system was significantly elevated. Of four monkeys tethered for a month or longer, three failed to show heart rate decline to pre-tether levels. Beta-adrenergic blockade tended to reduce heart rates, indicating that the animals were sympathetically aroused while tethered. The authors inferred that some amount of cardiovascular (and perhaps hormonal) disturbance may persist in tethered animals, even if several weeks are allowed for 'habituation'. Repetition of this experiment led to the same findings and conclusion (Adams et al 1988). Crockett et al (1993) did a similar study, measuring urinary cortisol as a stress indicator in ten female and ten male adult long-tailed macaques. Levels increased after the animals were attached to the catheter-tether system and remained significantly elevated during a three-week follow-up period.

Restraint chutes

Restraint chutes are equipped with adjustable space restrictors and/or guillotine panels (see Figure 18 in Fielder & Casmer 1966) allowing partial or complete immobilization of a primate away from its home-cage. They make the animal uncomfortable and require much technician-animal contact to perform many research procedures (Sullivan & Crary 1991) such as venipuncture, injection, nasogastric intubation or topical application of drugs.

Landi et al (1990) found significantly elevated enzyme levels (asparate and alanine aminotransferase) in eight long-tailed macaques who were placed for two hours in a restraining box. Elvidgeet al (1976) reported significantly elevated plasma cortisol concentrations in six adult female rhesus macaques that were venipunctured in such a box. Reinhardt et al (1990, 1991 a) took two blood samples in 15 minute intervals from ten adult female and six adult male rhesus macaques in their home-cages, and on another day in a routinely used restraint chute. It was not necessary to immobilize the animals in either situation since they readily presented a leg for venipuncture. Both sexes showed a significant cortisol increase when venipunctured in the restraint apparatus but not when venipunctured in their home-cage.

The authors inferred that removal from the familiar home-cage rather than venipuncture per se was a distressing event for the animals. This assumption is supported by Phoenix and Chambers (1984) who transferred adult rhesus macaques from their home-cages to comparable cages in a different room. The animals were subjected to the same venipuncture technique in both conditions, but their serum cortisol levels were significantly higher when blood was collected shortly after they were moved to the unfamiliar environment, than when blood was collected only days before in the familiar environment. Mason (1972) and Mason et al (1973) reported less-pronounced stress responses (as measured in urinary corticosteroid and catecholamine excretion) in rhesus macaques that were chair restrained in familiar surroundings than in males chair restrained in an unfamiliar environment. Mitchell and Gomber (1976) videoed rhesus macaques in their individual home-cages and immediately after they were moved in transfer boxes to identical but unfamiliar cages. The subjects exhibited behavioural signs of distress, ie significant increases in barks, screeches and in stereotyped pacing and somersaulting in the unfamiliar cages. Line et al (1987) found significantly elevated serum cortisol concentrations in rhesus macaques placed for 5 minutes in transfer boxes. The authors found no evidence of habituation.

Nets etc

The removal of laboratory non-human primates can be achieved not only with transfer boxes or modified restraint boxes (Sullivan & Crary 1991), but also with the help of heavy gloves (see Figure 1 in Sainsbury et all 989), poles (see Figure 1 in Nahon 1968), leashes (see Figure 1 in Schmidt et al 1989) or nets (see Figure 3 in Gay 1960). These devices, similar to the transfer box, teach the subject fear (Nahon 1968) through association and are likely to trigger apprehensive distress responses (cf Manuck et al 1983). At the same time, however, some of these capture-transfer devices may be a source of distress in themselves. Being captured in a net, for example, is probably a distressing experience on its own. Caught monkeys often become entangled, requiring forceful removal (Fielder & Casmer 1966). Luttrell et al (1994) described a conventional catching procedure of a troop of rhesus macaques using a net, and noted that incidents of acute diarrhoea, rectal prolapses and lacerations were common.

ALTERNATIVES

Alternatives to compulsory restraint have been developed in an attempt to minimize the research subject's stress responses.

Chemical restraint

Chemical restraint allows undisturbed handling of the physically free, yet unconscious research subject.

Wickings and Nieschlag (1980) noted significantly lower serum cortisol responses to venipuncture in four adult male rhesus macaques when anaesthetized with ketamine than when left conscious during the procedure. However, levels of prolactin increased significantly when the animals were anaesthetized but not when they were conscious during venipuncture. A similar effect has been described by Pun et al (1981), in three adult male rhesus macaques showing an immediate prolactin response to ketamine injection but not to injection of distilled water. Pun et al (1981) also observed that the three males responded with progressively increasing cortisol concentrations to multiple venipunctures when anaesthetized with ketamine. This finding has been confirmed by Fuller et al (1984) who noted a progressive, significant increase in serum cortisol in four adult female rhesus anaesthetized with ketamine, in response to multiple venipuncture. The authors concluded that ketamine does not modify the stress-induced increase of cortisol. Goosen et al (1984) recorded progressively increasing, total white blood cell counts under similar conditions in four adult baboons and interpreted this as an adrenalin effect resulting from acute stress. Line et al (1991) demonstrated in six adult female rhesus macaques that a single ketamine injection resulted in significantly elevated heart rates which did not return to normal levels for three hours. Crockett et al (1993) injected ten female and ten male long-tailed macaques with ketamine and observed a significantly increased urinary cortisol excretion during the day and subsequent night of injection.

Avoiding the possible disturbance associated with the injection of the drug, Castro et al (1981) examined ten adult male long-tailed macaques anaesthetized and blood sampled via chronic venous cannulas. The data clearly showed that ketamine per se did not affect plasma levels of cortisol nor arterial blood pressure.

Psychological support

Fleischman and Chez (1974) proposed to keep chair restrained baboons as pairs in order to minimize stress and anxiety. At the Wisconsin Regional Primate Research Center all headcapimplanted rhesus macaques assigned to research involving chair restraint are housed in compatible pairs. If one of the two is chair restrained during an experiment, the other one is kept close by as psychological support (see Figure 2 in Reinhardt et al 1989). The assumption that the presence of another conspecific has a protective effect under stress (Bovard 1959) is supported by numerous psychological studies (Mason 1960; Epley 1974; Gunnar et al 1980; Coe et al 1982; Gonzalez et al 1982; Hennessy 1984).

Coelho et al (1991) assessed the physiological effect of companionship in four adult male baboons during the potentially distressing restraint condition of being tethered. The animals were tested under individual housing conditions and under an experimental social condition which implied that subjects had visual, tactile, and auditory contact with compatible males. Being tethered in company with social partners ameliorated stress responses to the restraint condition, as measured in significantly lower blood pressure and lower heart rate than when being tethered alone.

Training

Training non-human primates to cooperate rather than resist during manipulations circumvents the need for physical and chemical restraint. The subject has considerable control over the situation, by retaining relative freedom of movement.

It has been reported that non-human primates may cooperate during various procedures such as: capture from home-cage (Nahon 1968; Clarke et al 1988; Heath 1989; Reinhardt 1992b), capture from group (Smith 1981; Bunyak et al 1982; Reinhardt 1990; Phillippi-Falkenstein & Clarke 1992; Welker 1993), venipuncture (Michael et al 1974; Elvidge et al 1976; Rosenblum & Clouston 1981; Bunyak et al 1982; Walker et al 1982; Herndon et al 1984; Wall et al 1985; Hem et al 1989; Scallet et al 1989; Vertein & Reinhardt 1989; Clarke et al 1990; Priest 1991; Reinhardt & Cowley 1992; Eaton et al 1994), systemic drug administration (Levison et al 1964; Priest 1991; Reinhardt 1992a), oral drug administration (Turkkan et al 1989), topical drug administration (Reinhardt & Cowley 1990), urine collection (Kelley & Bramblett 1981; Bloomsmith 1992; Anzenberger & Grossweiler 1993; Rice 1994), vaginal swabbing (Bunyak et al 1982), and veterinary examination (Turkkan 1990; Laule 1993).

The actual training procedure and exact time investment in its successful execution have been reported in only a few cases. Reinhardt (1990) outlined a training technique that allows one person to catch all or selected members of group-housed rhesus macaques. The technique is based on positive reinforcement, and animals exit voluntarily or on hand-clapping command into transport boxes without apparent signs of excitation (Reinhardt 1 992c). Luttrell et al (1994) tested this method in a zoo-group of 45 rhesus macaques. Six hours, distributed over 18 training sessions lasting 1 O~35 minutes each, were invested to condition the animals to voluntarily enter a chute system one by one and enter a transport cage attached to the chute's exit. Catching all troop members in succession took less than 15 minutes. None of the animals showed signs of distress during the catching procedure. Reinhardt and Cowley (1990) described a training technique that ensures cooperation of stump-tailed macaques during experimental topical drug application on their forehead (see Figure 1 in Reinhardt & Cowley 1990). One to 14 sessions, each lasting for 1-5 minutes, were required to successfully condition 17 adult males and 3 adult females to voluntarily present their foreheads and allow treatment while reaching for food treats offered by the handling personnel. Reinhardt (1991), and Reinhardt and Cowley (1992) delineated a safe, positive-reinforcement training technique for ensuring the active cooperation of adult macaques during in-home-cage venipuncture. Average total training time invested until a subject voluntarily presents a leg in a specially designed opening of the door and displays no resistance during subsequent venipuncture, was 40 minutes (1~74 minutes) for 15 adult male rhesus macaques, and 35 minutes (15~5 minutes) for six adult female stump-tailed macaques (see Figures 4 and 5 in Reinhardt 1991; see Figure 2 in Reinhardt & Cowley 1992).

Elvidge et al (1976) compared plasma cortisol concentrations of six adult female rhesus macaques that were trained to cooperate during venipuncture with those of six untrained females and 12 anaesthetized females. Cortisol response to venipuncture was significantly lower in the trained than in the untrained or in the anaesthetized subjects. These findings are in line with those of Reinhardt et al (1991a) and Reinhardt and Cowley (1992), who were unable to detect significant cortisol responses to venipuncture in 15 male rhesus and 6 female stump-tailed macaques that had been trained to actively cooperate during the procedure. Reinhardt (1991) compared mean white blood cell counts of six adult female rhesus macaques that were trained to cooperate during blood collection, with those of six untrained females and found significantly lower counts in the trained than in the untrained animals. Untrained subjects showed the typical leukocytosis alarm reaction (Ives & Dack 1956) to involuntary physical restraint (cf Loomis et al 1980).

REGULATORY RECOMMENDATIONS

Restraint methods of laboratory non-human primates are regulated in a flexible manner. Improvement of traditional restraint techniques are recommended but not prescribed.

The *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health 1985) stipulates that brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices such as squeeze-backs. Prolonged restraint should be avoided unless essential to research objectives. Less restrictive systems should be used when compatible with research objectives. Animals to be placed in restraint equipment should be conditioned to such equipment prior to initiation of the research. Restraint devices must not be used simply as a convenience to investigators in handling animals. Attention must be paid to the possible development of lesions or illnesses associated with restraint, including contusions, decubital ulcers, dependent oedema and weight loss. *The Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care 1993) recommends that restraint procedures should only be invoked after all other less stressful procedures have been rejected. Investigators should consider how physiological, biochemical and hormonal changes occurring in any restrained animal will influence their proposed experiments. The guide underlines that isolation from conspecifics, and the degree of immobilization have a strong influence on the degree of stress experienced by a restrained animal. The *US Federal Animal Welfare Act* (US Department of Agriculture 1991) leaves it to the Committee at research facilities (Institutional Animal Care and Use Committee) to decide which type of research justifies the maintenance of non-human primates in restraint devices. Non-human primates must not be maintained in restraint devices unless required by a research proposal approved by the Committee. Maintenance under such restraint must be for the shortest period possible. In instances where long-term (more than 12 hours) restraint is required, the non-human primate must be provided the opportunity daily for unrestrained activity for at least one continuous hour during the period of restraint, unless continuous restraint is required by the research proposal previously approved by the Committee. The British *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures* (Home Office 1989) does not specifically address the scientific implications of non-human primate restraint, but it recommends that advantage should be taken of the animals' ability to learn and therefore emphasizes that the least distressing method of handling is to train the animal to cooperate.

Traditional restraint techniques of non-human primates not only raise scientific but also ethical concerns (Prentice et al 1986). These concerns are based on the assumption that procedures that cause pain or distress in human beings may cause pain or distress in other animals (US Government Principles 1985). Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain is therefore imperative (US Government Principles 1985). Widely accepted ethical standards require scientists to subject animals to as little pain or distress as is necessary to accomplish the objectives of procedures (Office of Technology Assessment 1986). All experiments should be designed to avoid distress and unnecessary pain and suffering to the experimental animal (European Economic Council Directive 1986). To reduce stress and pain, non-traumatic restraining techniques should be taught (Schwindaman 1991).

CONCLUSIONS

The scientific information available suggests that traditional restraint techniques of non-human primates assigned to research need to be refined both for scientific and ethical reasons.

Common restraint methods are intrinsically a source of distress resulting from fear, discomfort and possible pain. Being involuntarily and forcefully restrained and subsequently subjected to uncomfortable handling or painful treatment is a distressing experience not only for a human but also for a non-human primate (cf US Government Principles 1985). Significantly increased adrenal activity, a classical indicator of distress, has been observed in non-human primates subjected to all common methods of restraint (Table 1). Other possible physiological responses to conventional restraint techniques include statistically significant changes in the secretion of testosterone, progesterone, oestradiol, prolactin and

luteinizing hormone, as well as significant changes in thyroid function, commonly monitored enzymes, heart rate, respiration rate and blood pressure, severe alterations in acid-base balance, electrocorticographic abnormalities, leukocytosis, immune suppression and abortion (Table1).

Significant physiological changes continue in animals subjected to routine restraint, or to long-term restraint. This persistency of the distress response, suggests that non-human primates do not become fully habituated to involuntary restraint. The partial return to quasi-normal conditions after prolonged restraint stress should be regarded as a sign of gradual physiological exhaustion rather than of habituation (cf Burton et al 1981). Prolonged restraint is likely to exacerbate the subject's discomfort and pain resulting from hypokinesia, decubital wounds and other clinical problems.

Table 1: Traditional restraint of laboratory primates and techniques of refinement.

Traditional Restraint Technique *response*	**selected reference**	***Refinement Technique*** *procedure*	**selected reference**
Squeeze-back *cortisol increase* *testosterone decrease*	Fuller et al 1984 Puri et al 1981	***Training*** *capture* *venipuncture* *injection*	Reinhardt 1992 b Priest 1991 Priest 1991
Manual restraint *cortisol increase* *AST and ALT increase* *metabolic acidosis*	Reinhardt 1992a Kissinger & Landi 1989 Bush et al 1977	***Training*** *examination* *venipuncture*	Turkkan 1990 Reinhardt 1991
Restraint board *cortisol increase* *AST and ALT increase* *testosterone decrease* *progesterone decrease* *oestradiol decrease* *prolactin increase* *respirat. rate increase*	Goncharov et al 1979 Landi et al 1990 Goncharov et al 1979 Goncharov et al 1979 Goncharov et al 1979 Quadri et al 1978 Berendt & Williams 1971	***Chemical restraint*** *ketamine anaesthesia*	Castro et al 1981

Traditional Restraint Technique *response*	selected reference	*Refinement Technique* *procedure*	selected reference
Restraint chair *cortisol increase* *ACTH increase* *catecholamine increase* *LH decrease* *testosterone decrease* *thyroxin increase* *heart rate increase* *blood pressure increase* *leukocytosis* *immune suppression* *abortion* *drowsiness*	Kling & Orbach 1963 Norman et al 1994 Norman & Smith 1992 Norman & Smith 1992 Mason & Mougey 1971 Golub & anderson 1986 Golub & Anderson 1986 Goosen et al 1984 Morrow-Tesch et al 1993 Fleishcman & Chez 1974 Bouyer et al 1978	***Psychological support*** *companionship*	Fleischman & Chez 1974
Tether *cortisol increase* *heart rate increase*	Crockett et al 1993 Adams et al 1988	***Psychological support*** *companionship*	Coelho et al 1991
Restraint chute *cortisol increase* *AST and ALT increase*	Elvidge et al 1976 Landi et al 1990	***Training*** *venipuncture* *topical drug application* *systemic drug application* *oral drug application* *examination*	Vertein & Reinhardt 1989 Reinhardt & Cowley 1990 Reinhardt 1992 Turkkan et al 1989 Laule 1993
Nets *acute diarrhoea*	Luttrell et al 1994	***Training*** *capture* *venipuncture* *urine collection*	Smith 1981 Bunyalk et al 1982 Kelley & Bramblett 1981

The inability of non-human primates to adequately adjust indicates that any research, testing or husbandry procedure involving involuntary restraint is a direct cause of distress to the restrained subject. This endorses the regulatory recommendations of avoiding restraint altogether (Canadian Council on Animal Care 1993) or limiting restraint to no longer than 12 hours (US Department of Agriculture 1991). Exemptions should be granted only under the condition that the restrained animal is not left alone but has visual and auditory contact with at least one compatible conspecific acting as a source of comfort (Kiesler 1966) and buffer against stress.

Most handling and experimental procedures imply that the subject not only is restrained, but simultaneously also removed from its cage. The conspicuous cortisol response and behavioural fear responses to being removed from the home-cage suggest that the situation is a distressing experience on its own, and affects subsequently collected scientific data even *before* the subject is exposed to the extra stress associated with the restraint. Non-human primates can readily be conditioned to cooperate during common handling procedures in the familiar home-cage environment. This methodological refinement eliminates both sources of uncontrolled variables: the distress associated with being removed from the home-cage, and the distress associated with being involuntarily restrained.

Primatological investigators underestimate their research subjects' level of intelligence when they hesitate to train rather than restrain them. The time investment in properly designed and gently/firmly executed training protocols is not high, and quickly pays off in research data that are not unduly confounded by avoidable distress reactions of the research subject. The initial time investment becomes insignificant when animals are trained for routine procedures. Training facilitates sample collection and drug application in the subject 5 home enclosure. This not only eliminates the time expenditure for moving individuals to a special handling area but at the same time avoids serious lower-back health risks, because heavy animals (eg a 15kg stump-tailed macaque) no longer have to be lifted and carried in transfer boxes (Cowley et al 1993). Working 'with' (cooperative) rather than 'against' (fearfully resisting) experimental animals also increases the safety of the handler because the interaction with the animal subject is based on trust rather than fear.

Anaesthesia seems to be an optimal alternative to restraint both from the investigator's and from the subject's standpoint. Unlike training, however, chemical restraint traditionally includes some form of involuntary restraint to allow the administration of the drug. This in turn triggers the above outlined uncontrolled physiological responses, affecting data subsequently collected from the anaesthetized research subject. Even during anaesthesia, nonhuman primates show a notable, albeit diminished stress response to injection. Training the subject beforehand to cooperate during the injection of the anaesthetic would eliminate this unnecessarily data-biasing circumstance.

The empirical evidence strongly suggests that training rather than restraining non-human primates during procedures is better for all parties: animals, handlers and investigators. The advantages of training techniques deserve more extensive exploration in the future for the sake of the research subjects and of the quality of the scientific experiments conducted on them.

Animal welfare implications

Training non-human primates to cooperate rather than resist during handling procedures offers a simple refinement to traditional, involuntary restraint techniques. Working with a cooperative rather than against a fearfully resisting experimental subject is a safeguard against unnecessary distress.

ACKNOWLEDGEMENTS

We are thankful to Klan Fajzi and two anonymous referees of Animal Welfare. The manuscript greatly benefited from their critical but constructive comments. The senior author very much appreciates the assistance of Doug Cowley and Russel Vertein who were instrumental during the development and implementation of various training techniques at the Wisconsin Regional Primate Research Center.

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Published in *Animal Welfare 4(3),* 221-238 (1995)