

RESTRAINT METHODS OF LABORATORY NON-HUMAN PRIMATES: A CRITICAL REVIEW

V Reinhardt[†], C Liss and C Stevens

Animal Welfare Institute, PO Box 3650, Washington, DC 20007, USA

[†] Contact for correspondence and requests for reprints: 4605 Crescent Road, Madison, WI 5371, USA

Abstract

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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*

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 al* 1989). 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.

Puri *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 toxicologic 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 Schulte & Stein 1981) or restraint tubes (see Figure 2 in Hearn 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 anxiolytic 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 home-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 (aspartate and alanine aminotransferase) in eight long-tailed macaques who were placed for two hours in a restraining box. Elvidge *et 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, 1991a) 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 al* 1989), 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 Puri *et al* (1981), in three adult male rhesus macaques showing an immediate prolactin response to ketamine injection but not to injection of distilled water. Puri *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 headcap-implanted 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; Hein *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 1992c). Luttrell *et al* (1994) tested this method in a zoo-group of 45 rhesus macaques. Six hours, distributed over 18 training sessions lasting 10–35 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 (16–74 minutes) for 15 adult male rhesus macaques, and 35 minutes (15–45 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 (Table 1).

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 <i>et al</i> 1984 Puri <i>et al</i> 1981	Training capture venipuncture injection	Reinhardt 1992b Priest 1991 Priest 1991
Manual restraint cortisol increase AST and ALT increase metabolic acidosis	Reinhardt 1992a Kissinger & Landi 1989 Bush <i>et al</i> 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 <i>et al</i> 1979 Landi <i>et al</i> 1990 Goncharov <i>et al</i> 1979 Goncharov <i>et al</i> 1979 Goncharov <i>et al</i> 1979 Quadri <i>et al</i> 1978 Berendt & Williams 1971	Chemical restraint ketamine anaesthesia	Castro <i>et al</i> 1981
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 <i>et al</i> 1994 Mason <i>et al</i> 1973 Norman & Smith 1992 Norman & Smith 1992 Mason & Mougey 1972 Golub & Anderson 1986 Golub & Anderson 1986 Goosen <i>et al</i> 1984 Morrow-Tesch <i>et al</i> 1993 Fleischman & Chez 1974 Bouyer <i>et al</i> 1978	Psychological support companionship	Fleischman & Chez 1974
Tether cortisol increase heart rate increase	Crockett <i>et al</i> 1993 Adams <i>et al</i> 1988	Psychological support companionship	Coelho <i>et al</i> 1991
Restraint chute cortisol increase AST and ALT increase	Elvidge <i>et al</i> 1976 Landi <i>et al</i> 1990	Training venipuncture topical drug application systemic drug application oral drug application examination	Vertein & Reinhardt 1989 Reinhardt & Cowley 1990 Reinhardt 1992a Turkkan <i>et al</i> 1989 Laule 1993
Nets acute diarrhoea	Luttrell <i>et al</i> 1994	Training capture venipuncture urine collection	Smith 1981 Bunyak <i>et al</i> 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's 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, non-human 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.

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References

- Adams M R, Kaplan J R, Manuck S B, Uberseder B and Larkin K T** 1988 Persistent sympathetic nervous system arousal associated with tethering in cynomolgus macaques. *Laboratory Animal Science* 38: 279-282
- Anzenberger G and Gossweiler H** 1993 How to obtain individual urine samples from undisturbed marmoset families. *American Journal of Primatology* 31: 223-230
- Arluke A B** 1988 Sacrificial symbolism in animal experimentation: Object or pet? *Anthrozoos* 2: 98-117
- Berendt R F and Williams T D** 1971 The effect of restraint and position upon selected respiratory parameters of two species of *Macaca*. *Laboratory Animal Science* 21: 502-509
- Bloomsmith M** 1992 Chimpanzee training and behavioral research: A symbiotic relationship. American Association of Zoological Parks and Aquariums (AAZPA)/Canadian Association of Zoological Parks and Aquariums (CAZPA). *Annual Conference Proceedings*: 403-410
- Bouyer J J, Dedet L, Debray O and Rougenl A** 1978 Restraint in primate chair may cause unusual behavior in baboons; Electroencephalographic correlates and corrective effects of diazepam. *Electroencephalic Clinical Neurophysiology* 44: 562-567
- Bovard E W** 1959 The effects of social stimuli on response to stress. *The Psychological Review* 66: 267-277
- Bunyak S C, Harvey N C, Rhine R J and Wilson M I** 1982 Venipuncture and vaginal swabbing in an enclosure occupied by a mixed-sex group of stump-tailed macaques (*Macaca arctoides*). *American Journal of Primatology* 2: 201-204
- Burton R R, Burns J W and Smith A H** 1981 Restraint of animals in space research. *Physiologist* 24 (Supplement 6): S41-S44
- Bush M, Custer R, Smeller J and Bush L M** 1977 Physiological measures of non-human primates during physical restraint and chemical immobilization. *Journal of the American Veterinary Medicine Association* 171: 866-869
- Canadian Council on Animal Care (CCAC)** 1993 *Guide to the Care and Use of Experimental Animals, Volume 1, 2nd edition*. CCAC: Ottawa, Canada

- Castro M I, Rose J, Green N, Petersen D and Taub D** 1981 Ketamine-HCl as a suitable anaesthetic for endocrine, metabolic, and cardiovascular studies in *Macaca fascicularis*. *Proceedings of the Society for Experimental Biology and Medicine* 168: 389-394
- Clarke A S, Mason W A and Moberg G P** 1988 Interspecific contrasts in responses of macaques to transport cage training. *Laboratory Animal Science* 38: 305-309
- Clarke M R, Phillippi K M, Falkenstein J A, Moran E A and Suomi S J** 1990 Training corral-living rhesus monkeys for fecal and blood sample collection. *American Journal of Primatology* 20: 181 (Abstract)
- Coe C L, Franklin D, Smith E R and Levine S** 1982 Hormonal responses accompanying fear and agitation in the squirrel monkey. *Physiology and Behavior* 29: 1051-1057
- Coelho A M, Carey K D and Shade R E** 1991 Assessing the effects of social environment on blood pressure and heart rates of baboon. *American Journal of Primatology* 23: 257-267
- Cope F W and Polis B D** 1959 Increased plasma glutamic-oxalacetic transaminase activity in monkeys due to nonspecific stress effect. *Journal of Aviation Medicine* 30: 90-94
- Cowley D, Vertein R, Pape H and Reinhardt V** 1993 Improving the work environment for animal care personnel of laboratory primates. *Animal Technology* 44: 129-135
- Crockett C M, Bowers C L, Sackett G P and Bowden D M** 1993 Urinary cortisol responses of longtailed macaques to five cage sizes, tethering, sedation, and room change. *American Journal of Primatology* 30: 55-74
- Eaton G G, Kelley S T, Axthelm M K, Iliff-Sizemore S A and Shiigi S M** 1994 Psychological well-being in paired adult female rhesus (*Macaca mulatta*). *American Journal of Primatology* 33: 89-99
- Elvidge H, Challis J R G, Robinson J S, Roper C and Thorburn G D** 1976 Influence of handling and sedation on plasma cortisol in rhesus monkeys (*Macaca mulatta*). *Journal of Endocrinology* 70: 325-326
- Epley S W** 1974 Reduction of the behavioral effects of aversive stimulation by the presence of companions. *Psychological Bulletin* 81: 271-283
- European Economic Community Council Directive** 1986 *On the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes 86/609*. Journal Officiel des Communautés Européennes, L 358, December 18
- Fielder F G and Casmer C J** 1966 A device to facilitate the restraint and handling of monkeys with minimal human contact. *Laboratory Animal Care* 16: 395-402
- Fleischman A R and Chez R A** 1974 A chair for the chronic study of the pregnant baboon. *Journal of Medical Primatology* 3: 259-264
- Fuller G B, Hobson W C, Reyes F I, Winter J S D and Faiman C** 1984 Influence of restraint and ketamine anaesthesia on adrenal steroids, progesterone, and gonadotrophins in rhesus monkeys. *Proceedings of the Society for Experimental Biology and Medicine* 175: 487-490
- Gay W I** 1960 A net designed for capturing caged monkeys. *Proceedings of the Animal Care Panel* 10: 75-78

- Golub M S and Anderson J H** 1986 Adaptation of pregnant rhesus monkeys to short-term chair restraint. *Laboratory Animal Science* 36: 507-511
- Goncharov N P, Taranov A G, Antonichev A V, Gorlushkin V M, Aso T, Ckan S Z and Diczfalusy E** 1979 Effects of stress on the profile of plasma steroids in baboons (*Papio hamadryas*). *Acta Endocrinologica* 90: 372-384
- Gonzalez C A, Coe C L and Levine S** 1982 Cortisol responses under different housing conditions in female squirrel monkeys. *Psychoneuroendocrinology* 7: 209-216
- Goosen D J, Davies J H, Maree M and Dormehl I C** 1984 The influence of physical and chemical restraint on the physiology of the chacma baboon (*Papio ursinus*). *Journal of Medical Primatology* 13: 339-351
- Gunnar M R, Gonzales C A and Levine S** 1980 The role of peers on modifying behavioral distress and pituitary-adrenal response to a novel environment in year-old rhesus monkeys. *Physiology and Behavior* 25: 795-798
- Hayashi K T and Moberg G P** 1987 Influence of acute stress and the adrenal axis on regulation of LH and testosterone in the male rhesus monkey (*Macaca mulatta*). *American Journal of Primatology* 12: 263-273
- Hearn J P** 1977 A device for restraining small monkeys. *Laboratory Animals* 11: 261-262
- Heath M** 1989 The training of cynomolgus monkeys and how the human/animal relationship improves with environmental and mental enrichment. *Animal Technology* 40: 11-22
- Hein P R, Schatorje J S, Frencken H J, Segers M F and Thomas C M** 1989 Serum hormone levels in pregnant cynomolgus monkeys. *Journal of Medical Primatology* 18: 133-142
- Hennessy M B** 1984 Presence of companion moderates arousal of monkeys with restricted social experience. *Physiology and Behavior* 33: 693-698
- Herndon J G, Turner J J, Perachio A A, Blank M S and Collins D C** 1984 Endocrine changes induced by venipuncture in rhesus monkeys. *Physiology and Behavior* 32: 673-676
- Home Office** 1989 *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures*. HMSO: London, UK
- Ives M and Dack G M** 1956 "Alarm reaction" and normal blood picture in *Macaca mulatta*. *Journal of Laboratory Clinical Medicine* 47: 723-729
- Kaplan J R, Adams M R and Bumsted P** 1983 Heart rate changes associated with tethering of cynomolgus monkeys. *Laboratory Animal Science* 38: 493 (Abstract)
- Kelley T M and Bramblett C A** 1981 Urine collection from vervet monkeys by instrumental conditioning. *American Journal of Primatology* 1: 95-97
- Kiesler S B** 1966 Stress, affiliation and performance. *Journal of Experimental Research in Personality* 1: 27-235
- Kissinger J T and Landi M S** 1989 The effect of four types of restraint on serum ALT and AST in cynomolgus monkeys. *Laboratory Animal Science* 39: 496 (Abstract)
- Kling A and Orbach J** 1963 Plasma 17-hydroxycorticosteroid levels in the stump-tailed monkey and two other macaques. *Psychological Reports* 13: 863-865

- Landi M S, Kissinger J T, Campbell S A, Kenney C A and Jenkins E L** 1990 The effects of four types of restraint on serum alanine aminotransferase and aspartate aminotransferase in the *Macaca fascicularis*. *Journal of the American College of Toxicology* 9: 517-523
- Laule G** 1993 Using training to enhance animal care and welfare. *Animal Welfare Information Center Newsletter* 4(1): 2,8,9
- Lennox M S and Taylor R G** 1983 A restraint chair for primates. *Laboratory Animals* 17: 225-226
- Levison P K, Fester C B, Nieman W H and Findley J D** 1964 A method for training unrestrained primates to receive drug injection. *Journal of the Experimental Analysis of Behavior* 7: 253-254
- Line S W, Clarke A S and Markowitz H** 1987 Plasma cortisol of female rhesus monkeys in response to acute restraint. *Laboratory Primate Newsletter* 26(4): 1-4
- Line S W, Markowitz H, Morgan K N and Strong S** 1991 Effect of cage size and environmental enrichment on behavioral and physiological responses of rhesus macaques to the stress of daily events. In: Novak M A and Petto A J (eds) *Through the Looking Glass: Issues of Psychological Well-Being in Captive and Laboratory Primates* pp 160-179. American Psychological Association: Washington, USA
- Loomis M R, Henrickson R V and Anderson J H** 1980 Effects of ketamine hydrochloride on the haemogram of rhesus monkeys (*Macaca mulatta*). *Laboratory Animal Science* 30: 851-853
- Luttrell L, Acker L, Urben M and Reinhardt V** 1994 Training a large troop of rhesus macaques to co-operate during catching: analysis of the time investment. *Animal Welfare* 3: 135-140]
- McNamee G A, Wannemacher R W, Dinterman R E, Rozmiarek H and Montrey D** 1984 A surgical procedure and tethering system for chronic blood sampling, infusion, and temperature monitoring in caged nonhuman primates. *Laboratory Animal Science* 34: 303-307
- Manuck S B, Kaplan J R, Thomas B and Clarkson T B** 1983 Behaviorally induced heart rate reactivity and atherosclerosis in cynomolgus monkeys. *Psychosomatic Medicine* 45: 95-108
- Mason J W** 1958 Restraining chair for the experimental study of primates. *Journal of Applied Physiology* 12: 130-133
- Mason J W** 1972 Corticosteroid response to chair restraint in the monkey. *American Journal of Physiology* 222: 1291-1294
- Mason J W and Mougey E H** 1972 Thyroid (plasma BEI) response to chair restraint in the monkey. *Psychosomatic Medicine* 34: 441-448
- Mason J W, Mougey E H and Kenion C C** 1973 Urinary epinephrine and norepinephrine responses to chair restraint in the monkey. *Physiology and Behavior* 10: 801-803
- Mason J W, Wool M S, Wherry F E, Pennington L L, Brady J V and Beer B** 1968 Plasma growth hormone response to avoidance in the monkey. *Psychosomatic Medicine* 30: 760-773

- Mason W A** 1960 Socially mediated reduction in emotional responses of young rhesus monkeys. *Journal of Abnormal and Social Psychology* 60: 100-104
- Michael R P, Setchell K D R and Plant T M** 1974 Diurnal changes in plasma testosterone and studies on plasma corticosteroids in non-anaesthetized male rhesus monkeys (*Macaca mulatta*). *Journal of Endocrinology* 63: 325-335
- Milhaud C L, Klein M J and Merkel M C** 1980 A new restraining chair for rhesus monkeys (*Macaca mulatta*). *Journal of Medical Primatology* 9: 62-70
- Mitchell G and Gomber J** 1976 Moving laboratory rhesus monkeys (*Macaca mulatta*) to unfamiliar home-cages. *Primates* 17: 543-547
- Morrow-Tesch J L, McGlone J J and Norman R L** 1993 Consequences of restraint stress on natural killer cell activity, behavior, and hormone levels in rhesus macaques (*Macaca mulatta*). *Psychoneuroendocrinology* 18: 383-395
- Morton W R, Knitter G H, Smith P M, Susor T G and Schmit K** 1987 Alternatives to chronic restraint of nonhuman primates. *Journal of the American Veterinary Medical Association* 191: 1282-1286
- Nahon N S** 1968 A device and techniques for the atraumatic handling of the sub-human primate. *Laboratory Animal Care* 18: 486-487
- Nakamura R K, Coates R, Crawford H and Friedman D** 1982 A flexible restraint chair for the cynomolgus monkey (*Macaca fascicularis*). *Journal of Primatology* 11: 178-185
- National Institutes of Health (NIH)** 1985 *Guide for the Care and Use of Laboratory Animals*. NIH: Bethesda, USA
- National Institutes of Health (NIH)** 1991 *Nonhuman Primate Management Plan*. Office of Animal Care and Use. NIH: Bethesda, USA
- Norman R L, McGlone J and Smith C J** 1994 Restraint inhibits luteinizing hormone secretion in the follicular phase of the menstrual cycle in rhesus macaques. *Biology of Reproduction* 50: 16-26
- Norman R L and Smith C J** 1992 Restraint inhibits luteinizing hormone and testosterone secretion in intact male rhesus macaques: Effects of concurrent naloxone administration. *Neuroendocrinology* 55: 405-415
- Office of Technology Assessment** 1986 *Alternatives to Animal Use in Research, Testing, and Education*. US Government Printing Office: Washington, USA
- Phillippi-Falkenstein K and Clarke M R** 1992 Procedure for training corral-living rhesus monkeys for fecal and blood-sample collection. *Laboratory Animal Science* 42: 83-85
- Phoenix C H and Chambers K C** 1984 Sexual behavior and serum hormone levels in aging rhesus males: Effects of environmental change. *Hormones and Behavior* 18: 206-215
- Prentice E D, Zucker I H and Jameton A** 1986 Ethics of animal welfare in research: The institution's attempt to achieve appropriate social balance. *The Physiologist* 29: 17, 19, 20
- Priest G M** 1991 Training a diabetic drill (*Mandrillus leucophaeus*) to accept insulin injections and venipuncture. *Laboratory Primate Newsletter* 30(1): 1-4
- Puri C P, Puri V and Anand-Kumar T C** 1981 Serum levels of testosterone, cortisol, prolactin and bioactive luteinizing hormone in adult male rhesus monkeys following cage-restraint or anaesthetizing with ketamine hydrochloride. *Acta Endocrinologica* 97: 118-124

- Quadri S K, Pierson C and Spies H P** 1978 Effects of centrally acting drugs on serum prolactin levels in rhesus monkeys. *Neuroendocrinology* 27: 136-147
- Reinhardt V** 1990 Avoiding undue stress: catching individual animals in groups of rhesus monkeys. *Lab Animal* 19(6): 52-53
- Reinhardt V** 1991 Impact of venipuncture on physiological research conducted in conscious macaques. *Journal of Experimental Animal Science* 34: 212-217
- Reinhardt V** 1992a Improved handling of experimental rhesus monkeys. In: Davis H and Balfour AD (eds) *The Inevitable Bond: Examining Scientist-Animal Interactions* pp 171-177. Cambridge University Press: Cambridge, UK
- Reinhardt V** 1992b Transport-cage training of caged rhesus macaques. *Animal Technology* 43: 57-61
- Reinhardt V** 1992c Voluntary progression order in captive rhesus macaques. *Zoo Biology* 11: 61-66
- Reinhardt V and Cowley D** 1990 Training stump-tailed monkeys to cooperate during in-homecage treatment. *Laboratory Primate Newsletter* 29(4): 9-10
- Reinhardt V and Cowley D** 1992 In-homecage blood collection from conscious stump-tailed macaques. *Animal Welfare* 1: 249-255
- Reinhardt V, Cowley D, Eisele S and Scheffler J** 1991a Avoiding undue cortisol responses to venipuncture in adult male rhesus macaques. *Animal Technology* 42: 83-86
- Reinhardt V, Cowley D, Scheffler, Verlein R and Wegner F** 1990 Cortisol response of female rhesus monkeys to venipuncture in home-cage versus venipuncture in restraint apparatus. *Journal of Medical Primatology* 19: 601-606
- Reinhardt V, Houser D, Cowley D, Eisele S and Verlein R** 1989 Alternatives to single caging of rhesus monkeys (*Macaca mulatta*) used in research. *Zeitschrift fuer Versuchstierkunde* 32: 275-279
- Reinhardt V, Pape R and Zweifel D** 1991b Multifunctional cage for macaques housed in pairs or in small groups. *American Association for Laboratory Animal Science Bulletin* 30(2): 14-15
- Rice J M** 1994 Zoo husbandry and research: An integrated approach. *Humane Innovations and Alternatives* 8: 597-600
- Robbins D O, Zwick H, Leedy M and Stearns G** 1986 Acute restraint device for rhesus monkeys. *Laboratory Animal Science* 36: 68-70
- Rosenblum I Y and Clouston F** 1981 Normal range and longitudinal blood chemistry and hematology values in juvenile and adult rhesus monkeys (*Macaca mulatta*). *Ecotoxicology and Environmental Safety* 5: 401-411
- Sainsbury A W, Eaton B D and Cooper J E** 1989 Restraint and anaesthesia of primates. *Veterinary Record* 125: 640-644
- Scallet A C, McKay D, Bailey J R, Ali S F, Paule M G, Slikker W and Rayford P L** 1989 Meal-induced increase in plasma gastrin immunoreactivity in the rhesus monkey (*Macaca mulatta*). *American Journal of Primatology* 18: 315-319
- Schmidt E M, Dold G M and McIntosh J S** 1989 A simple transfer and chairing technique for nonhuman primates. *Laboratory Animal Science* 39: 258-260

- Schultea T D and Stein F J** 1981 A simple restraining device for small primates. *Lab Animal* 10(5): 67-69
- Schwindaman D** 1991 The 1985 animal welfare act amendments. In: Novak MA and Petto A J (eds) *Through the Looking Glass: Issues of Psychological Well-Being in Captive and Laboratory Primates* pp 26-32. American Psychological Association: Washington, USA
- Smith E O** 1981 Device of capture and restraint of nonhuman primates. *Laboratory Animal Science* 31: 305-306
- Sullivan S M and Crary T L** 1991 A new device for restraining nonhuman primates. *American Association for Laboratory Animal Science Bulletin* 30(2): 18-19
- Swan S M** 1970 An apparatus for the transportation and restraint of nonhuman primates. *Laboratory Animal Care* 20: 1131-1132
- Turkkan J S** 1990 New methodology for measuring blood pressure in awake baboons with use of behavioral training techniques. *Journal of Medical Primatology* 19: 455-466
- Turkkan J S, Ator N A, Brady J V and Craven K A** 1989 Beyond chronic catheterization in laboratory primates. In: Segal E (ed) *Housing, Care and Psychological Well-Being of Captive and Laboratory Primates* pp 305-322. Noyes Publications: Park Ridge, USA
- US Department of Agriculture** 1991 Animal welfare, standards, final rule. *Federal Register* 56: 6499-6500
- US Government Principles** 1985 Principles for the utilization and care of vertebrate animals used in testing, research, and training. In: *Guide for the Care and Use of Laboratory Animals* pp 82-83. US Department of Health and Human Services: Washington, USA
- Vertein R and Reinhardt V** 1989 Training female rhesus monkeys to cooperate during in-homecage venipuncture. *Laboratory Primate Newsletter* 28(2): 1-3
- Walker M L, Gordon T P and Wilson M E** 1982 Reproductive performance in capture-acclimated female rhesus monkeys. *Journal of Medical Primatology* 11: 291-302
- Wall H S, Worthman C and Else J G** 1985 Effects of ketamine anaesthesia, stress and repeated bleeding on the haematology of vervet monkeys. *Laboratory Animals* 19: 138-144
- Welker C** 1993 Ethological research work. On and with primates, and especially the crab-eating monkey, *Macaca fascicularis*. *Journal of the International Association of Zoo Educators* 28: 20-31
- Wickings E J and Nieschlag** 1980 Pituitary response to LRH and TRH stimulation and peripheral steroid hormones in conscious and anaesthetized adult rhesus monkeys (*Macaca mulatta*). *Acta Endocrinologica* 93: 287-293