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The use of operant instrumental conditioning with positive reinforcement for collection of saliva from marmosets (Callithrix jacchus) for research purposes

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Para que haja uma melhora no bem-estar de animais silvestres utilizados em pesquisa é necessário o emprego de técnicas que reduzam os efeitos estressantes, aos quais os animais são constantemente submetidos. Sabemos que o estresse pode ocasionar alterações fisiológicas podendo alterar os resultados de um experimento e que a cooperação voluntária obtida através do aprendizado pode minimizar sensações de ansiedade vivida por animais mantidos em laboratório. Sendo assim, a busca de métodos não-invasivos para a coleta de material biológico e exames laboratoriais é de grande importância. O objetivo do presente estudo foi condicionar um grupo de primatas *Callithrix jacchus* para coletas de saliva com finalidade de pesquisa. Para tanto utilizamos a técnica do condicionamento operante com auxílio instrumental e reforço positivo (recompensa) em 24 saguis adultos, de ambos os sexos. Segundo as condições deste estudo, o condicionamento para coleta de saliva foi realizado de forma satisfatória, rápida e eficiente demonstrando que a utilização do método de condicionamento instrumental com recompensa nesta espécie pode ser efetuado com sucesso.

Palavras-chave: *Callithrix jacchus*; estresse; condicionamento; bem-estar; pesquisa.

In order to improve the welfare of captive wild animals used in research, it is necessary to apply methods that minimize the stress to which the animals are constantly subjected. Stress produces physiological changes that may interfere with the results of laboratory experiments, and voluntary cooperation through learning may minimize the anxiety experienced by laboratory animals. It is therefore important to develop non-invasive methods for the collection of biological samples and the performance of laboratory exams. The aim of this study was to train common marmosets (Callithrix jacchus) for the collection of saliva samples. Instrumental operant conditioning with positive reinforcement (reward) was applied to 24 adult marmosets of both genders. All animals were satisfactorily conditioned for the rapid and effective collection of saliva samples.

Keywords: *Callithrix jacchus*; stress; conditioning; welfare; research.

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Introduction

Stress often affects captive wild animals (Pachaly et al., 1993). It is therefore important to consider the life quality of these species during their lifetime in captivity, requiring the adoption of husbandry alternatives to improve their welfare (Costa & Pinto, 2003; Laule, 2003), such as behavioural conditioning (Coe, 2003; Costa & Pinto, 2003; Laule, 2003). Operant conditioning is a behaviour that is associated to its consequences, i.e., it is the process through which an individual modifies the occurrence and form of its own behaviour due to the association of that behaviour with a stimulus. Operant conditioning is produced by a stimulus followed by a reaction; if the behaviour is followed by positive results, it will be repeated more frequently, however if it is followed by negative consequences, the behaviour will tend to be repeated less frequently (Vila et al., 2008).

In operant conditioning, animals are trained to develop voluntary behaviours through reward. Voluntary cooperation reduces the sensations of fear and anxiety that the animals normally experience during the performance of invasive procedures, therefore contributing to reduced stress (Coe, 2003). Positive reinforcement conditioning is performed by providing rewards to the animal when it presents the desired behavioural response. Instrumental conditioning is the technique that depends on the response the animal towards what has been thought. In this type of training, a clicker or another sonorous device is activated whenever the animal correctly performs the desired behaviour, acting as a bridge between that moment and the delivery of the reward (Cipreste & Costa, 2002).

Saliva collection may be performed more easily and is less invasive than blood collection, raising great interest from researchers (Dawes, 1993; Moura et al., 2007). Experiments on wild animals using saliva for the determination of circulating hormone concentrations have been developed with increasing frequency in the past years, as this method is non-invasive and reduces interference from stressful situations (Cross & Rogers, 2004).

The aim of this study was to train common marmosets (Callithrix jacchus) for the collection of saliva samples.

Methods

The study was performed at Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Botucatu, Brazil. Twenty-four adult Callithrix jacchus were used, 12 males and 12 females. Animals were housed in individual 60 x 120 x 60 cm cages, exposed to natural light and weather conditions. Animals received daily meals of fruit and eggs in the morning and protein mash in the afternoon; only vitamin supplements and mealworms (Tenebrio molitor) were offered weekly.

During an initial experimental phase of two months, several food items were offered to determine which was preferred by all the animals, for example: Danoninho® petit-suisse cheese (Danone™), several flavours of industrialized baby porridge, and variations of porridge prepared with fresh fruits and honey. Danoninho® petit-suisse cheese was the item preferred by all animals in the group, and was thus chosen as a reward.

On a second experimental phase of five months the animals were subjected to conditioning sessions twice a week. To facilitate learning during the initial learning period, cotton swabs were soaked in petit-suisse cheese and offered to the animals; at the moment the animals had the swabs in their mouths, the clicker was activated as an instrumental reinforcement (Figure 1), and then the reward was given, this being a teaspoon of petit-suisse cheese. If the animal refused to perform the desired behaviour, the maximum time spent trying was kept under ten minutes; if the attempts were excessively prolonged the animals could become stressed, associating our presence negatively (Mckinley et al., 2003).

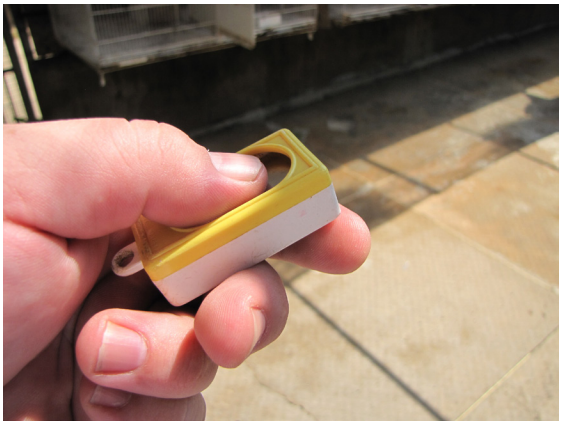

Figure 1. "Clicker" Instrumental reinforcement.

Ethics Note

The experiment was performed with authorization by the Animal Ethics Committee (Comissão de Ética no Uso de Animais – CEUA) of the Universidade Estadual Paulista "Júlio de Mesquita Filho", authorization nr. 126/2010.

Results and Discussion

After five months, all animals were satisfactorily conditioned and accepted the swabs without the need for them to be soaked with the reward cheese, providing adequate saliva samples without physical or chemical restraint (Figures 2 and 3).

Laboratory animal science considers animal welfare one of the most relevant factors affecting experimental results, valuing the ethical use of animals and underlining the three Rs principle: replacement, reduction and refinement (Russel & Burch, 1959). Non-human primates kept in laboratory environments are frequently handled and subjected to a variety of invasive procedures, requiring specific husbandry approaches. It is therefore critical to assure these procedures are the least stressful possible, ensuring reliable research results and improving animal welfare. In this context, operant conditioning with positive reinforcement may be used to produce the voluntary cooperation of non-human primates for a variety of research procedures.

Benefits of this study include stress reduction, greater flexibility and reliability in sample and data collection, and reduction in the use of anaesthetic agents. Training may also provide the opportunity to reduce agonistic behaviour, improve behavioural enrichment programs, and increase the safety of the staff handling these animals (Laule et al., 2003).

Figure 2. Adequate saliva samples without physical or chemical restraint.

Figure 3. Adequate saliva samples without physical or chemical restraint.

Mckinley et al. (2003) conditioned twelve laboratory-captive common marmosets to evaluate the feasibility of positive reinforcement as a method for the handling of these primates. Through ten-minutes training sessions, the authors conditioned the animals to be weighed and to provide urine samples without the need for restraint. That study revealed the conditioned animals became confident towards the researchers, and data collection using trained animals was considerably faster than when the animals were subjected to more invasive approaches, suggesting that positive reinforcement training is a practical option for the handling of laboratory-housed common marmosets. Savastano et al. (2003) developed an operant conditioning program for 17 species of New World primates at the Bronx Zoo, New York, USA; eighty-six callitrichids and cebids in 26 social groups were studied. Individual responses to training were highly

variable; however the authors considered that marmosets were more attentive to the training sessions than other callitrichids. In that study, conditioning produced considerable changes in individual behaviour such as the reduction of aggressive behaviour towards the zoo staff, indicating a reduction of stress and improvement of animal welfare.

Conclusions

The use of instrumental conditioning for the collection of saliva samples from common marmosets was a bridge that connected the performance of the desired behaviour and the delivery of the positive reinforcement reward. Saliva collection for scientific purposes was conducted with ease and success after the training, with no need for physical or chemical restraint, reducing stress, improving welfare, and contributing to a better reliability of the research data.

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