UNIVERSITY OF TRÁS-OS-MONTES E ALTO DOURO

"Operant conditioning training with positive reinforcement in Zoo animals for cooperation in management and medical procedures"

- Final Version –

Master Thesis in Veterinary Medicine

Inês de Figueiredo Pires

Supervisor: Maria de Lurdes Ribeiro Pinto, DVM, MSc, PhD

Vila Real, July of 2019

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Examination Committee

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To my Mom

“It is the human earthling who tends to dominate the earth, oftentimes treating other fellow earthlings and living beings as mere objects. This is what is meant by 'speciesism'. By analogy with racism and sexism, speciesism is a prejudice or attitude or bias in favor of the interests of the members of one's own species and against those of members of other species.”

— Earthlings

Acknowledgments

To my family, especially my mom and brother for being my biggest supporters throughout my life, for never doubting my abilities and always keeping my fears away. To my grandparents for sparing no efforts in my education journey and allowing me to have new experiences abroad. To my boyfriend for being so kind and patient at times of distress and for always joining me in personal and professional adventures. I would not be able to make it without your love and support.

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Resumo

No passado, os parques zoológicos e aquários, tinham como único propósito a exibição de animais para o entretenimento humano. No entanto, a crescente preocupação com os direitos e bem-estar animal, físico e psicológico, alteraram a perceção da sociedade em relação às suas necessidades. Esta alteração de mentalidade, que advém de uma alteração de paradigma na forma como o Homem se relaciona com o ambiente e os animais impeliu os zoos e outras coleções de animais a investir os seus recursos na melhoria do bem-estar animal, na conservação de espécies e na educação ambiental. Contudo, a vida em cativeiro carece habitualmente de estímulos físicos e mentais suficientes e impõe práticas de maneio que podem ser fonte de desconforto e stress para os animais envolvidos. Assim, nestas instituições, o refinamento de procedimentos de maneio geral e veterinário, bem como a avaliação do comportamento e bem-estar animal são imperativos diários aos quais urge responder.

Neste trabalho, é descrito um plano de treino e um estudo comportamental num Rinoceronte Branco do Sul (*Ceratotherium simum*) elaborado de forma a proporcionar-lhe um maior estímulo mental e a facilitar procedimentos veterinários futuros, sem a necessidade de o imobilizar por métodos físicos ou químicos, minimizando o stress para o animal e diminuindo o risco de lesão da equipa veterinária. Os trabalhos foram realizados no Zoo de Santo Inácio, entre janeiro e março de 2019. Neste estudo, o plano de treino foi dividido em três fases: fase I - Dessensibilização, fase II - Treino de "clicker" e fase III - Treino de "alvo". Simultaneamente foi efetuado um estudo comportamental, baseado no método de observação contínuo focal.

Embora sem significado estatístico, os resultados demonstraram uma progressão positiva nas sucessivas fases de treino consideradas. Os comportamentos exploratórios demonstraram igualmente um aumento progressivo da fase I à fase III. As interações agonistas e o tempo despendido na alimentação sofreram também alterações. Os resultados obtidos demonstram a exequibilidade e os benefícios de um plano de treino dirigido à melhoria dos procedimentos de maneio geral e veterinário em animais de cativeiro.

Palavras-chave: Condicionamento operante, Comportamento, *Ceratotherium simum*, Maneio, Medicina Veterinária

Abstract

In the past, zoological parks and aquariums, had as their only purpose the exhibition of animals for human entertainment. However, the increasing concern about animal rights, and their physical and psychological welfare, have changed society's perception regarding their needs. This change in mentality, which arises from a paradigm shift in the way humans relate to animals and the environment, has impelled zoos and other animal collections to invest their resources in the improvement of animal welfare, conservation of species and environmental education.

Nevertheless, it is common for animals in captivity to have limited access to physical and mental stimuli. Moreover, life in captivity also bears management practices that may be a source of discomfort and stress for the animals involved. Thus, the refinement of general and veterinary management procedures, as well as the assessment of animal behaviour and welfare, are daily imperatives that must be addressed by these institutions.

In the present work, a training plan and a behavioural study conducted on a Southern White Rhino (*Ceratotherium simum*) are described; these were elaborated in order to provide an opportunity for mental stimulation and to facilitate future veterinary procedures, without the need of physical or chemical immobilization, minimizing stress for the animal and decreasing the risk of accidental lesions for the veterinary team. The work was conducted at Santo Inácio's Zoo, between January and March 2019. In this study, the training plan was divided in three stages: stage I – Desensitization, stage II - Clicker training and stage III – Target training. A behavioural study was conducted simultaneously, based on the continuous focal sampling method.

Even though a statistical significance was not achieved, the results have demonstrated a positive progression on the successive training stages. Exploratory behaviour also demonstrated an increasing progression from stage I to stage III. Agonistic interactions and the time spent on Feeding also varied during the different training stages. These, the results demonstrate the feasibility and benefits of a training plan directed to improve general husbandry and veterinary procedures for animals in captivity.

Keywords: Operant conditioning, Management, Behaviour, *Ceratotherium simum*, Veterinary Medicine

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List of Acronyms, Abbreviations and Symbols

am	*Ante Meridiem*
BCS	Body Condition Score
BIAZA	British & Irish Association of Zoos and Aquaria
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
EAZA	European Association of Zoos and Aquaria
EEP	European Endangered Species Programmes
ESB	European Studbooks
IUCN	International Union for Conservation of Nature
Kg	Kilogram
Km	Kilometre
Km^2	Square Kilometre
pm	*Post Meridiem*
ZIMS	Zoological Information Management System

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1. Introduction

Social movements arising from organized critics and the media, as well as from visitors, members and donors, led to the necessary change of paradigm regarding the conditions of animals in captivity. Nowadays, zoo's and aquariums have as their main focus the health and well-being of the animals under their care (1). Two of the main areas by which welfare can be monitored and improved in captivity is through behavioural research and training. Behavioural research of numerous species in zoological parks and aquariums has provided information on how animals are affected by the captive environment, the individual's or species' requirements for resources (such as food or social partners), their preferences, (e.g. food choice), cognitive capacity, and ways of coping and adapting to challenges or changes in the environment, amongst others.

Insights from these studies have been fundamental to develop breeding programs, assure excellent husbandry and welfare and to insure good health care (2). Training based on operant conditioning can not only facilitate the daily husbandry routine, but also provide a complex and stimulating environment, improving animal welfare. Through training it is possible to minimize the number of captures and handlings, which otherwise would put at risk the safety of both animals and caretakers. When it comes to veterinary care, training sessions provide an opportunity to examine the individual closely, creating conditions that facilitate particular care. Additionally, the animals can be trained to participate voluntarily in their own medical procedures (3) (4).

The present work describes a study performed in a Southern White Rhino *(Ceratotherium simum)*, with the following structure and goals:

- Design and implementation of an operant conditioning training program, as a mean to facilitate husbandry and veterinary procedures;
- Monitor and investigate the effects of an operant conditioning training program on the animal's behaviour, through a behavioural observation plan;
- Provide guidelines for training plans for other individuals and species in captivity.

2. Literature Review

2.1. Veterinary and Husbandry Training

Some of the arguments against keeping animals in captivity include the lack of sources for mental and physical stimulation that inherently come with limited size and space, which can create difficulties to meet the physical, behavioural and psychological needs of animals (5). Furthermore, in captivity food is plentiful and predators are non-existent, which may generate a lack of daily occupation that in turn can result in behavioural disorders such as stereotypic behaviour, social aggression, self-injurious behaviour, and coprophagia (1) (5).

Animals in captivity are constantly learning through signals presented by keepers and their routines: they can predict when food will be offered by listening to a certain sound that is associated with that event; they may even try to control caregivers actions by displaying a certain behaviour they know they'll be rewarded for (with food or attention, for example) (6). This ability demonstrates that animal's behaviour can be modified by applying classical and/or operant conditioning techniques (further detailed in latter paragraphs), presenting the animal with new challenges, allowing it to accomplish deliberate goals, to learn new skills and providing it with freedom of choice. These provide mental stimulation and increased activity, which can be considered as important as supplying optimal nutrition, a complex habitat or an appropriate social structure, mitigating behavioural disorders and enhancing welfare (3) (5) (6).

Through a well-planned training regime, compliance to participate in husbandry practices (regular care) and veterinary care can be obtained, without punishment or chemical/physical immobilisation. The advantages of training include reduced risk of injury (both to humans and animals) and better control of the animal with less manpower, time invested and cost, making it an important tool in veterinary care. (5) (7) (6).

Medical procedures may be often chaotic, forceful and neglected in poorly trained animals, in particular when it comes to dangerous species (8) (5) (9). On the other hand, examination and treatment of zoo animals can be especially challenging, particularly when clever restraint devices (squeezes or crushes) are used, since it can be very difficult to differentiate between actual pain form injury or simple fear and struggle (5) (10). Operant conditioning, through positive reinforcement training, can help an animal acclimate to new situations through desensitization, providing a less-invasive protocol of medical monitoring and intervention (8) (5) (7) (9).

Animals of all types have now demonstrated to be able to be trained to cooperate in every aspect of their care, namely in voluntary blood samples, x-rays, inoculations, ultrasound, wound suturing, tooth removal, biopsy and anaesthesia without restraint (1) (5) (6).

Even though this type of training is necessarily time-consuming the development of better training techniques for medical procedures is a foundational key worth examining. In the long term it can save time, money, and even patients' lives by reducing anaesthetic risk. The adverse effects of physical and chemical restraint that can be accumulative on the physical and psychological well-being of the individuals concerned are also avoided by training (11) (10) (6).

However, it is very important that training is conducted by professionals with basic knowledge on animals' learning and to assure that they do not use punishment, which could result in failure in the training process, decrease in welfare and risk of attack. Another key factor to assure success is to always keep in mind that training often involves contact between the animal and the trainer, therefore, risks regarding the species, training facility and training protocol should be carefully assessed (6).

2.2. Learning and memory

Generally speaking, an alteration in behaviour as an outcome of practice or experience is called learning, or training in case it occurs through influence of a person (12) (4). Hediger (1950, 1969) acknowledged that training was a form of occupational therapy for animals as it engaged the individuals at a cognitive level, providing mental and physical stimulation, considering it "disciplined play". In animals, it is generally accepted that there are four main types of learning: habituation, classical conditioning, operant (or instrumental) conditioning and complex learning (4).

In order to create a behaviour through operant conditioning, the learning process occurs through the relation between three components: a stimulus (or antecedent stimulus), a response and an outcome (or stimulus/consequence), as represented in Figure 1. The outcome can either be a positive reinforcement (the delivery of something the animal desires), a negative reinforcement (the removal of something the animal finds aversive) or an aversive consequence (punishment). The process is called the ABCs – for *Antecedent, Behaviour and Consequence* (4) (5) (13) (14).

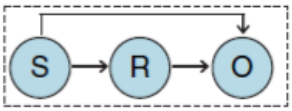

Figure 1 - Theoretical associative structure underlying instrumental conditioning. S=stimulus representation, R=response representation, O=outcome representation. Arrows indicate hypothetical unidirectional excitatory associations between representations. The dashed-line box envelopes the contents of learning. Associations are hypothesized to form between S and R, between S and O, and between R and O. Adapted from Byrne,2008

The likelihood of a behaviour being repeated is determined by the nature of the outcome: a reinforcement will strengthen the behaviour and a punishment will diminish it, as the response-outcome association allows the outcome to motivate the response (4) (13) (14). Furthermore, the motivation to perform a certain behaviour is reinforcer-specific, which means the animal evaluates de amount of effort needed to expend to receive a reward (the cost of action). It will be more likely for the individual to perform a certain behaviour if the reinforcer is naturally linked to a reinforcer in the repertoire of the species (15) (16).

To explain behaviour on the light of body functions, we must consider the brain as the most important piece of this equation. Learning through habituation, in mammals is processed in several structures of the nervous system, such as the spinal cord, basal ganglia and the striatum, whereas response-outcome learning is processed by cortical structures: prelimbic area and the insular cortex. When it comes to endogenous neurotransmitters it has been successfully demonstrated that dopamine is capable of mediating reinforcement, since brain areas that give positive reinforcement coincide with areas rich in dopaminergic tracts or cell bodies and dopamine levels increase after stimulation of these areas (17) (16) (13).

2.2.1. Operant conditioning

Classical conditioning and operant conditioning differ from each other in that classical conditioning is used to re-train reflect behaviours that are already a part of the animal's repertoire in a certain situation (unconditioned stimulus – not previously learned) to appear in the presence of a new situation (conditioned stimulus – involves learning), as in Pavlov's experience (18) (19) (14). Operant conditioning, on the other hand, teaches the individual to act upon the consequences of the action, this means the animal learns to associate consequences to its own behaviour. The operant conditioning learning process acts upon reversible behaviours

maintained by reinforcement schedules (a reinforcement is delivered under a well-defined schedule) (4) (20) (14).

Operant conditioning is a learning mechanism by which most behaviours in animal training are created, based on Edward's Thorndike Law of Effect (1898, 1911) and later described by B. F. Skinner (1937) (4) (14). Thorndike stated that behaviour is determined by its consequences, which can either be reinforcing, neutral or punishing (5). Voluntary behaviours that produce a rewarding outcome are more likely to be repeated since the strength of the behaviour is increased by the reward, whereas a behaviour that results in punishment is more likely to become extinct (17) (14). Assuming those premises, the reward will increase the frequency of the behaviour, whether it is positive – the delivery of something the animal desires, or negative – the removal of something undesired (10) (17) (19). A neutral consequence will not change the predictability of the future behaviour (5).

Skinner demonstrated, through his work with rats, that operant behaviour reflects the adaptation of the individual to the outcomes of his actions during life, proving that the probability of expression of a certain behaviour will be altered according to the following consequence (17) (16) (18) (14). Conditioning means that learning happens beyond the animal's control, depending on the outcomes of its experience (4) (14).

Species-specific knowledge is key to a successful training program as it is an integrating part of the operant conditioning principles. Also, in order to guarantee successful training results, several weeks of practice are required and regular reinforcement needs to be maintained (10) (21). However, the schedules of reinforcement can vary between continuous (every behaviour is followed by a reward) or partial (only some responses are followed by a reward). Partial schedules can then either be of fixed ratio (the reinforcement is delivered after a predetermined number of responses) or of interval ratio (the reinforcement is delivered after a predetermined time period). Partial schedules can also be classified as fixed if the ratio or interval is constant, or variable if the ratio or interval varies. Eventually, within the later, the ratio can also be labelled as progressive if the number of responses required to obtain reinforcement increases progressively. High response rates are kept more easily and are less likely to become extinct with partial schedules of reinforcement, as the animal on a continuous schedule won't respond in the absence of a reward. Progressive ratios have been useful to study the motivation of each individual, as well as to assess how animals choose between effort and reward, making it possible to establish a breaking point (the maximum amount of effort an animal is willing or capable of making for a certain reward) (15) (19).

2.2.1.1. Types of operant conditioning techniques

There are six basic techniques of operant conditioning for animal behaviour modification: scan and capture, mimicry, negative reinforcement, manipulation, baiting, and target training (5).

2.2.1.1.1. Scan and capture technique

"Scan and capture" is an operant conditioning technique based on the capture of a spontaneous behaviour and the timely reinforcement of it as soon as possible. The trainer "searches" or "scans" for the wished behaviour, and once the animal performs it, the behaviour is "captured", which means the trainer will timely reinforce the individual (4) (5). According to the Thorndike's law of effect, the reinforcement will then increase the frequency of the behaviour, which is therefore considered as "captured" (5) (14).

This technique is particularly useful in situations where the behaviour that is being trained already exists in the natural repertoire of the individual, or to "refine" an existing behaviour (5). Non the less, this technique may present a downside: as it is not explained to the animal what is being requested, except for the timing of when the correct behaviour is performed, the sessions can turn into a frustrating "guessing game", leading to a less specific behaviour with artefacts. Frustration can also lead to aggressiveness and deterioration of the relationship with the trainer; to avoid these issues, training sessions should be short and small approximations to the desired behaviour reinforced (5). Another disadvantage of this technique is that since it doesn't involve any tools, if not trained for a while, the animal may forget the behaviour, and the trainer won't have anything to bring it back to the animal's memory (5).

2.2.1.1.2. Target technique

Target training is a process that uses contact points, or targets, to define where the animal should touch, allowing the trainer to place the individual where he desires, for as long as it is needed to stay (5) (22). The animal can be conditioned to touch the target with any part of his body and, later, these points can be moved in a sequence to create a specific physical behaviour or simply to "station" the animal, for extend stationing (5) (23). However, the most usual training points are the nose (for most mammals) and the hands for primates (23). When

trained to move a foot, a paw, arm, leg, chest, or any other part of the body to the target, physical examination can be a lot safer and easier for the veterinary team and can even be used to train other behaviours such as injections' acceptance and other treatments without restraint (6) (23).

Because animals are curious, the majority will spontaneously move towards it in order to touch or sniff it. However, some may display some apprehension and fear to make contact right away and, in these cases, rewards should be offered every time the animal moves towards the target, until he makes contact with it (23).

The scanning phase is usually trained with aid from other operant conditioning techniques such as "scan and capture" or "baiting". With "scan and capture", the trainer will reinforce the approximations the animal makes towards the target until he finally touches it; when using the "bating" technique, the animal his lured by the trainer towards the target until it incidentally touches it when moving in the bait direction. (5)

To facilitate the process, and provide information regarding timing, a bridge stimulus should be concurrently used, which will give highly specific references to the animal about the exact time the correct behaviour is being performed; ideally, the animal should keep the contact until the bridge tells it otherwise (5).

One of the most common fails when using a target is the tendency of animals to try to grab hold of the target; if this happens, it should be ignored for 60 seconds (put in "time-out") before asking the animal to perform the behaviour again (6). Despite of this, it is considered the most specific operant conditioning technique, as the animal does not need to "guess" what is being asked (5).

2.2.1.1.3. Clicker training technique

Clicker training is a technique used to condition the clicker sound as a reinforcer or as the bridge, helping with timing, as it is emitted immediately after the animal performs the desired behaviour, and only then, presenting the reward (24) (25). It makes use of a tool called a "clicker" that emits a quick and brief "click" sound when pressed, working as a secondary reinforcement when paired up consistently with a reward (10) (25). Working with wild animals can increase the risk of incorrect timing, leading to confusion and impairing the learning process, as it can be particularly hard to deliver the primary reinforcer in the right time (such as offering food directly into the animal's mouth) (10) (26).

2.2.2. Behavioural observations program planning

Animal behaviour has been proven essential in many different science domains, such as biology, behavioural ecology and conservation; therefore, it is important to study this discipline with as much rigor as possible (27).

When conducting a behavioural research project, the experimenter should acquire the fundamental information about the species (or of a similar species if information is scarce about the one being studied) and the individual, by consulting keepers and zoo reports, before starting to develop skills of measuring and interpreting behaviour (2) (22).

Behavioural studies are hypothesis-driven and systematic; to collect accurate and reliable data, the researcher must spend a lot of hours doing observations, that can be presential or recorded with a video camera, demanding concentration, an objective mind and attention to details (2).

Before starting to collect the "usable data", there should be a previous pilot study, in which the researcher will practice the chosen method, learn to identify the individual being studied and acclimatize the animals to one's presence (2).

2.2.2.1. Recording methods

There are several different ways by which information regarding behaviour can be recorded. The observer can either study one individual (focal sampling) or a group of subjects that will be rapidly scanned at regular intervals (scan sampling) (28).

When it comes to time, the researcher can choose to record all-occurrences (continuous recording) or to sample the behaviour periodically (time sampling), as summarized in the scheme below (Figure 2) (28). Because the continuous recording method provides information about the exact time at which the behaviour started and finished and, therefore, of the duration of each behaviour, it is considered a more exact and trustful record of behaviour (28). In contrast, the time sampling method preserves less accurate information; however, it is a way of condensing information (28).

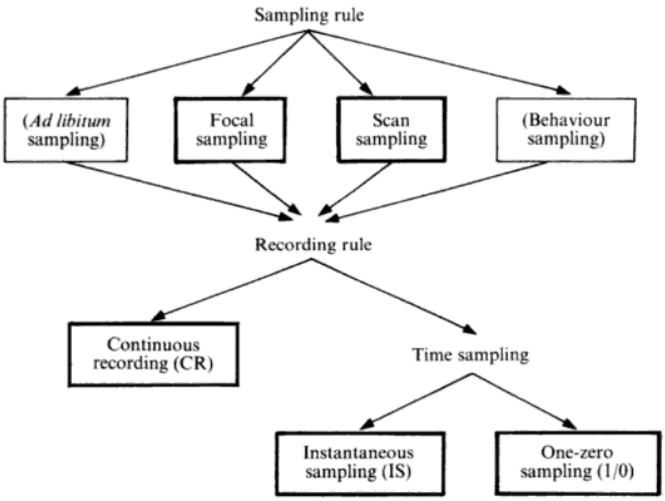

Fig. 6.1. The hierarchy of *sampling rules* (determining who is watched and when) and *recording rules* (determining how their behaviour is recorded).

Figure 2 - The hierarchy of sampling rules (determining who is watched and when) and recording rules (determining how their behaviour is recorded). Adapted from Martin, 1993

2.3. White Rhinoceros, *Ceratotherium simum*

Comprising five different species (*Ceratotherium simum* and *Diceros bicornis* in Africa and *Dicerorhinus sumatrensis*, *Rhinoceros unicornis* and *Rhinoceros sondaicus* in Asia), rhinoceros are one of the most primitive large mammals of the planet (29).

Despite of their long existence, they are an incredibly vulnerable species thanks to their most capacious predator of all – the human – that hunts this species for poaching, to create products used in traditional medicines, and to manufacture ceremonial dagger handles or decorative carvings. Human actions other than hunting also have an impact on this species, by destroying their habitat, either for human settlement or for logging and agricultural purposes (30) (29) (31).

2.3.1. Taxonomy

The taxonomic classification of the white rhinoceros is displayed in Figure 4. The scientific name *Ceratotherium simum* takes upon this large mammal morphology traits (Figure 3), as it derives from the Greek word “cerato” that means horn, the word “thorium” that stands for wild beast, and the word “simus” that means flat nosed (32).

Figure 3 - Draft of the Southern White Rhino, adapted from du Toit, 1998

Kingdom	Phylum	Class	Order	Family
Animalia	Chordata	Mammalia	Perissodactyla	Rhinocerotidae

Taxon Name: *Ceratotherium simum* (Burchell, 1817)

Kingdom: Animalia (Animals)
Phylum: Chordata (Chordates)
Sub phylum: Vertebrata (Vertebrates)
Class: Mammalia (Mammals)
Order: Perissodactyla (Odd-toed ungulates)
Family: Rhinocerotidae (Rhinoceros)
Genus: Ceratotherium (White rhinoceros)
Species: Ceratotherium simum (White rhinoceros)
Ceratotherium simum simum (Southern white rhinoceros) (32)

Figure 4 - Taxonomy of Ceratotherium simum, adapted from Emslie, 2012

2.3.2. Population Status and Conservation

The white rhinoceros is in the "near threatened" category according to the IUCN red list (Figure 5) (32). According to the latest data, collected on December 2015 and in July 2018 respectively, there are around 20.378 white rhinos in the wild and 654 in captivity (33) (32).

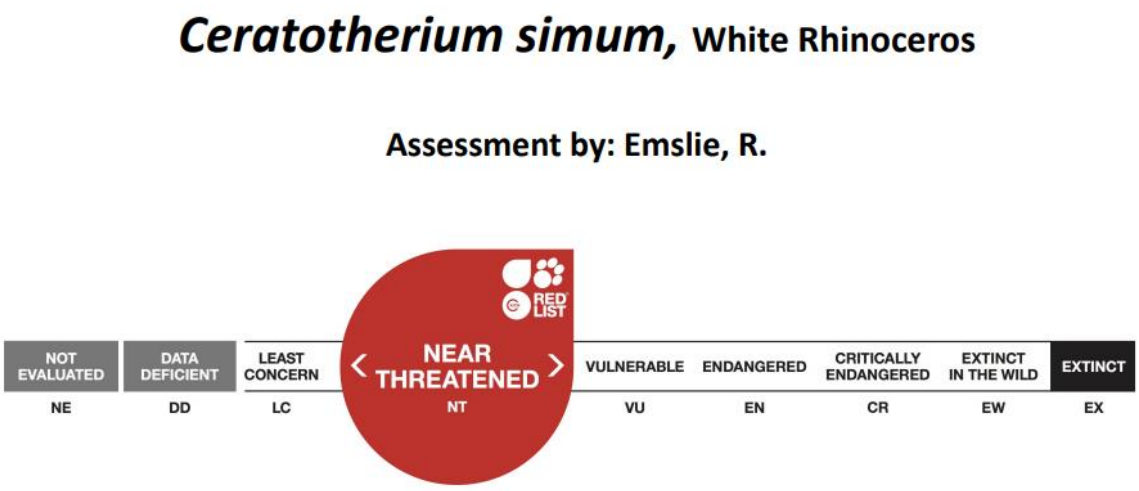

Figure 5 - IUCN Red List: Ceratotherium simum, adapted from Emslie, 2012

Due to anthropogenic causes, such as horn poaching and habitat destruction, the number of African rhinos as significantly decreased in the past years, which has urged for conservation measures such as: horn removal and creation of protected reserves and sanctuaries (30) (34). If protected against habitat loss and poaching, the species reproduces successfully in the wild, with populations growing up to 5-10% each year, meaning, there is also a quick increase within small reserves, that lead to high density within the reserve and restricted dispersal on demography (34) (35). As a result, surplus animals have been translocated to form new populations within and outside their former range (36).

However, despite of the conservation efforts and population growth, in the last years, the level of poaching as greatly increased which as threatened the survival of the specimens (33) (32). Furthermore, life export and limited export of hunting trophies are legal, according to the CITES listing (37) (32).

To effectively protect this species law enforcement measures are required, local communities should be integrated into conservation efforts and a careful monitoring plan,

conducted by specialists, should be implemented, to provide information to guide biological management (36).

Although the monitoring plan for biological management needs to include a variety of disciplines that are key for conservation, behavioural knowledge is crucial to develop conservation strategies, provide animal care and specific management and environment. Behavioural studies provide the fundamental basis that enable to breed and maintain the animals in captivity, encourage welfare, reintroduce animals into the wild or to conserve wild populations in situ (30). Thus, decisions regarding management or conservation should always consider the behaviour of the species (30).

2.3.3. Distribution

Most specimens of White rhinos are distributed between four countries: South Africa, Namibia, Zimbabwe and Kenya (36) (32), as depicted in Figure 6. With the southern white rhino being the most numerous among the rhinos, having sizeable populations in South Africa at Kruger national park and at Hluhluwe-Imfolozi (32).

There have been populations reintroduced in Namibia, Botswana, Zimbabwe and Swaziland (historical range), but also outside the former range of the species, in Kenya, Uganda and Zambia; a small population still survives in Mozambique (32)

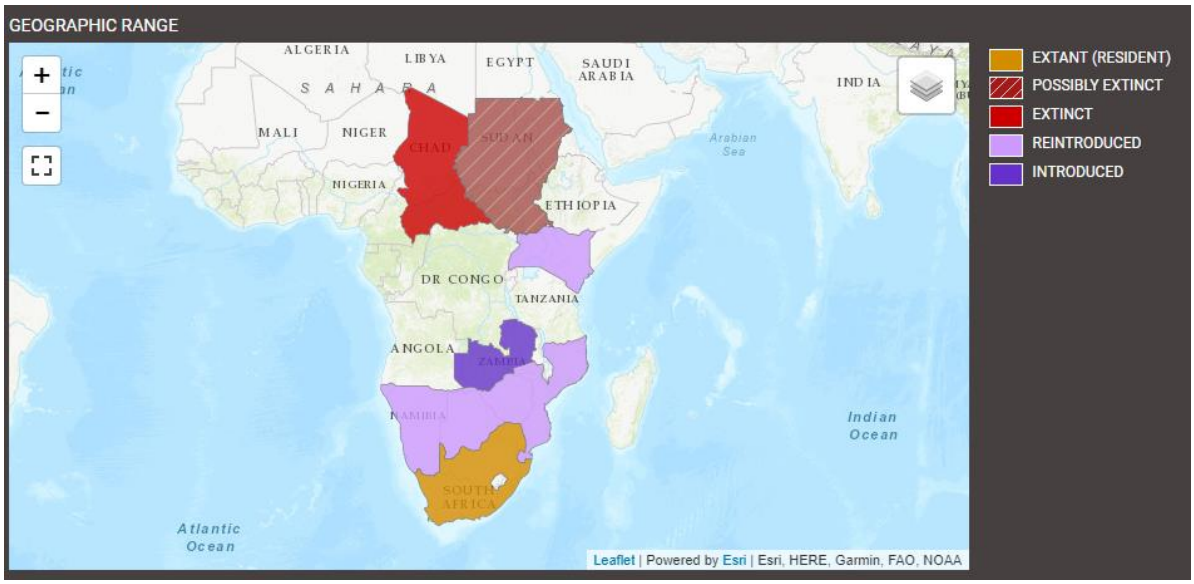

Figure 6 - IUCN Geographic Range of Ceratotherium simum, adapted from Emslie, 2012

2.3.4. Habitat and Diet

White rhinoceros are terrestrial grazing mega herbivores that inhabit the flat short-grassed savannah, with access to trees/bush covers for shade, mud holes for baths and water pounds for wallowing and drinking (29) (36) (32) (38). Their habitat provides their dietary requirements: large amounts short, sweet, palatable grasses (30) (29) (32) (38).

As monogastric animals, that do not ruminate and eat low-quality food, their digestion process is very inefficient and, therefore, they must spend a considerable amount of their time (at least 50%) foraging to allow bulk feeding (31) (30) (39) (32) (38). To cope with the low/moderate protein and high fibre feeding pattern, they evolved to possess high crowned cement covered teeth, lengthened skull and wide lips (31) (32).

Even though they can live up to 4 to 5 days without water, rhinos require regular access to water, drinking on average 12 litres per day (40) (29) (38). Because of their fast gut transit, they should drink daily or every other day to allow water resorption from the faeces (29). When water is abundant drinking usually occurs twice a day, however, in the dry season, when availability of the resource decreases, journeys to water sources occur every 2-4 days (31).

In captivity, it is very hard to provide natural grass, therefore the natural requirements must be well understood before formulating diets; these shouldn't exceed 10% in supplementation from dry lucerne and horse cubes (32) (38).

2.3.5. Behaviour

White rhinoceros are diurnal with most activity taking place between 05.00am to 09.00am and 03pm.00 to 06.30pm; in order to avoid the hot, dry weather their activity is low on the hottest time of the day and becomes higher as the weather gets cooler, bulk feeding during the morning and late afternoon, which helps with thermoregulation (30) (38) (41). The low activity hours of the day are spent resting and wallowing in the mud; wallowing in mud pools helps to cool off during the hottest part of the day and acts as an anti-parasitic by repelling insects and external parasites, and as a sunscreen protecting the skin from dehydration and sun (30) (29) (32) (38) (41). If mud pools are not available, rhinos will roll in dust (32).

Despite being sedentary, semi-social and territorial, this species is well tempered and usually move away from humans instead of attacking them (32) (38). White rhinoceros usually roam around 4-5km per day and can achieve a speed of 40 km/h (38). When it comes to

intraspecies relationships, cows are usually accompanied by one calf, while bulls are usually solitary as they are aggressive towards each other and to calves; however, if other adult male rhinos are submissive they may be tolerated by the bull (30) (38) (42). Herds can be constituted of up to six rhinos; however, larger groups only result from lack of resources, like food, water and resting spots (32).

Bulls can be territorial or non-territorial; territorial bulls separate themselves by delineating their ranges with marking behaviours, while females and nonterritorial adult males do not mark their ranges (30) (34). Rhinos tend to defecate near other rhinoceros dung, usually located at territory boundaries, behaviour that serves both as communication and marking purposes (30) (32). The territories are usually around 1-3km², depending on resources availability (32).

Marking behaviours through visual signs include scrapes and broken vegetation while olfactory signs include a ritualised scattering of dung and urine spraying; scattering is performed by kicking the rear legs before and after defecation and spray-urination consists of 2-5 pulses of urine sprayed backwards (30) (31) (34) (42). Former literature demonstrated a relation between faecal testosterone and territoriality which links physiology with the behaviour of males (34). If a female rhino goes into the territory of a territorial bull, he will try to figure out if she is in oestrus and ready to mate (38).

Non-territorial rhinos, or subordinate rhinos, may coinhabit the same space as territorial bulls, however, they neither scatter dung nor spray urine, urinating in a conventional stream instead (31) (42). When faced with a territorial bull, the subordinate gives a threat display by lifting his head, roaring and making rushes at the territorial bull (42).

This well-structured social system of territoriality, together with their large size, non-seasonal reproduction and low predation, as allowed for the survival of the species (31).

White rhinoceros use different vocalization sounds to communicate between them (table1) (32). Usually males are louder than females and vocalize in various situations such as following a cow in oestrus, fighting another bull, and when rejected by a female; the calves usually squeal when away from their mothers (41).

Table 1 - White Rhino Calls, adapted from Versteege, 2018

Sounds	**Call**	**Situation / meaning**
Tonal sounds	Whine*	Begging for food
	Squeaks*	Separation
Puffing sounds	Snort*	No obvious
	Threat	First warning
	Puff	No obvious
Growling sounds	Snarl*	Aggressive
	Grunt*	Powerful warning
	Grouch	Foraging and other activities in proximity of other members of the herd
	Groan	Moan, body discomfort
Repetitive sounds	Pant*	Greeting, contact call
	Hoarse	Feeding, approach to female

* = calls recorded also in Southern white rhinoceroses (Owen-Smith, 1973). Other calls of the Southern white rhino ("shriek, squeak, squeal, gruff squeal and gasp-puff") were not recorded in this study of Northern white rhinoceroses.

As mentioned previously in this work, one of the downsides of captivity is the lack of mental and physical stimulation that can result in stress, aggression and stereotypic behaviour (5). Some of the stereotypic behaviours exhibited by rhinos include exaggerated horn rubbing, pacing, bar biting, licking metal barriers, rubbing and lethargy (these stress indicators are summarized in table 2) (32). This can be prevented by enrichment to avoid boredom, an appropriately sized enclosure, escape margin from other members and from public view, freedom to display natural behaviours, company from other animals and/or people during feeding and training (amongst other displayed in table 3) (32).

Table 2 - Stress indicators, adapted from Versteege, 2018

Stress indicators
Pacing
Change in normal behaviour
Running or increased locomotion
Spending time away from the heard
Aggression
Increased vocalisation and snorting
Loss of appetite
Reduced laying time
Unresponsive or quite
Loose faeces and more frequent defecating

Table 3 - How to minimize stress, adapted from Versteege, 2018

Minimize stress by
Enrichment
Providing enough space
Separate feedings
Individual pens

2.3.6. Management and husbandry in captivity

Keeping rhinoceros in captivity, while avoiding stress, boredom or frustration, demands having in consideration aspects like their behaviour, social organization, number, age and sex (30). Their size, territoriality and the fact that they are a semi-social species implies that not to many adults can be kept together and that the exhibits need to have the adequate size (30) (32).

Even though it is unlikely for a Zoo to be able to provide the home range of the species in nature, it still has the obligation to ensure facilities that meet their behavioural needs (30). Such needs can be met by having an enclosure designed in a way that allows for the rhinoceros to hide or escape from human view, while replicating features of their natural habitat, with wallows, pools, sand pits or rubbing posts (30). To ensure adequate mental and physical stimulation, environmental enrichment must be performed, in order to increase the diversity of

behaviours, to keep the animals occupied, and to avoid high chronic stress levels (increased cortisol) that are directly related with disease and mortality (30). With human presence being one possible source of stress, as rhinoceros might fear them, habituating the rhinos to our presence can transform this relationship to a neutral or positive stimulus; keepers can accomplish this through regular touch (a lot of rhinos enjoy being scratched and petted) and through training (30).

2.3.7. Training

One particularity of the rhinoceros species is that, in captivity, most husbandry procedures can be carried out without a planned conditioning programme because these animals react well to tactile touch and are usually quite tameable (3). Despite of this characteristic, before starting a training program, the temperament and behaviour of the individual must be carefully assessed prior to any contact being made (3).

Literature regarding operant conditioning in rhinoceros is still scarce though. The book "Rhinoceros Husbandry Resource Manual" by Fouraker and Wagener (1996), is one of the few available sources, in which an interval between three to five months of 10-30 min sessions/day is indicated as the required to train for blood withdrawal and up to nine weeks for stationary standing (43).

A thriving training process will depend on the task, the sessions intensity, proper incentive and preparedness of the people executing the sessions (43). Given their knowledge regarding the animals, a multi-disciplinary team, constituted of keepers and veterinary staff should design the training plan. In it, different inputs regarding the specific animal and its behaviour should be accounted for. Aspects like the behaviour required from the animal, when, where and how training will happen should be well defined among the people involved (3). Other factors that can influence the animal's performance, are external variables such as low-frequency noises and stage of reproductive cycle (43).

In order to assess the progress in training, every aspect of it needs to be recorded: the procedure, the behaviour, time, place and other details that might vary each day. With these records it is possible to verify if progress is being made and to provide guidelines for training plans for other individuals and species (3).

3. Materials and Methods

3.1. Material

3.1.1. Location

The study described in the present work was performed at Santo Inácio's Zoo, in Porto, Portugal.

Santo Inácio's Zoo, which opened its doors in 2000, is a private collection that belongs to the Aveleda group and that has recently integrated the Thoiry group, making a positive move towards conservation goals. One of the Zoo's most important missions is to increase awareness for protection of wildlife and animal welfare. The entity cooperates in more than 40 relevant breeding programmes, such as the European Endangered Species Programmes (EEP) and the European Studbooks (ESB) and maintains healthy populations of great genetic variety. The park houses several endangered species, including three Southern White African Rhino's (Figure 7) (44) (45).

Figure 7 - Santo Inácio's Zoo Map: the area pertaining to the Rhinos park and enclosure is circled in red

3.1.2. Subjects

Santo Inácio's Zoo houses three adult male Rhinoceros – Sam, Tabu and Nono – that are part of the "Savanna" exhibit. Sam was the individual chosen to take part in the study because he is the rhino that responds the best to inputs, according to the veterinary team. General information on his identity, history and past experiences were collected from zoo records (ZIMS - Zoological Information Management System) and from enquiring the veterinary team.

Sam is a male Southern White Rhinoceros (Figure 8), born in captivity, on the 15th of November 2011 (7 years old), at the Safaripark Beekse Bergen, in the Netherlands. He was reared by his parents. Currently his weight is unknown and has got a BCS (body condition score) of 3/5. Sam has anatomical particularities that enable the distinction between him and the other two rhinos: a curved frontal horn and hair on the tip of his ears and on the end of his tail.

Figure 8 - Sam at the indoors enclosure

3.1.3. Recording and Training Material

The **clicker** (Figure 9) used for the training sessions was a regular clicker bought in a pet shop. It is a small yellow plastic box with a metal piece in the centre that snaps when pushed down and released. To make it easier for the person performing the training session, it was attached to the umbrella that worked as the target with a rubber band.

In this project, the handle of an old umbrella served the purpose of the **target** (Figure 9) for the training sessions. The handle was a metal stick with a blue ball on the tip that the animal was supposed to touch during the training. The **camera** used to record the training sessions was a "Sony Cyber-Shot DSC-RX100 II", and the **tripod** was a "hama STAR61" (Figure 10).

Figure 9 - Clicker and target used in the training sessions

Figure 10 - Camera and tripod used to record the training sessions

3.2. Methods

3.2.1. Housing and husbandry procedures

At the Savanna park (outdoors) Sam shares the space with the other two male rhinoceros, one zebra, two ostriches and one eland. Indoors, there are separate enclosures for each rhinoceros. Although they can see each other through the bars used for separation, they cannot have any physical contact. The animals are let outside between 10.30/11am and placed indoors between 16.15/16.30pm. In the morning (opening time), the rhinos, are fed with 1Kg of "Grazer" (concentrate pallets), and in the afternoon (closing time) with 1Kg of "Grazer" (concentrate pallets) and 200g of carrots. They have simple *ad libitum* hay during the entire day. Also, a salt rock is always available indoors and outdoors. At the time the study was carried out, they were not receiving any supplementation.

In the past, they rarely received any type environmental enrichment, but objects for maintenance behaviours, like scratching and wasting their horns (wood trunks and a trunk-like object) were available.

During his stay at Santo Inácio's Zoo, Sam's medical record indicated that he had never been submitted to any type of veterinary procedure.

3.2.2. Experimental design

3.2.2.1. Study definition

The study was conducted at Santo Inácio's Zoo from January until March 2019. It consisted of three training stages (Stage I – Desensitization, Stage II – Clicker training and Stage III – Target training), during which a behavioural study was also conducted (detailed further on this work). Prior to the design and implementation of the training plan, random *ad libitum* observations of Sam's behaviour throughout the day in the outdoor park were performed during the first week of February (from the 4th until the 8th). These observations served different purposes: they allowed the animal to get undisturbed by the presence of the trainer, while at the same time enable the later to ascertain the animal's behaviour repertoire in order to build the ethogram used in behavioural study. It was also during the *ad libitum* observation period that

the schedule and duration of the training sessions was established and the intervals for behaviour recording were stipulated.

3.2.2.2. Training Plan

Sam's behavioural background (personality traits, aggression issues, security norms or any other information that affects the management) was collected from the Zoo's staff, who described him as being calm around most people, despite of presenting agonistic behaviours towards the other two rhinos sharing the enclosure. Prior to the training performed on this project Sam had learned the commands: "go" to move forward, "back" to move backwards and "come" to move closer to the person giving the command. This train was conducted in a non-systematic manner by the zookeepers and veterinary team.

The training plan was designed to teach Sam to perform the behaviour target touching, which enables the trainer to place the animal where it is need, for as long as it is necessary. In terms veterinary management, this behaviour allows an easier physical examination and can also be a step in forming new behaviours such as blood withdrawal or acceptance of an ultrasound.

The training was conducted between the 8th of February and the 29th of March (23 days of training sessions) with a total of 23 sessions, one per day. The duration of each session was around 15 minutes, as studies show that learning becomes inefficiently when the sessions are long and infrequent (46) (6). As mentioned before, the training process was conducted in several training phases, each one having a specific goal: stage I - desensitization, stage II - clicker training, and stage III - target training. Desensitization had a total of 5 training sessions, clicker training 4 sessions and target training 14 sessions. The decision to move to the next stage was made based on the assessment of the previous training session. All the sessions were recorded with a video camera and a written report was made for each one, describing exactly what was performed and assigning a quantitative score (termed "Progress to goal") to the session. The value was obtained by applying the following formula:

Progress to goal:

The value is calculated with the following formula:

$$\frac{2 \times Behaviour + Cooperation + Aggression}{4}$$

This formula, based on the method used at Bristol Zoo Gardens (47), reflects the animal's progress to a pre-established goal within a particular training session, which is assessed based on the following criteria: rating of subject's behaviour, rating of subject's co-operation and display of aggressive behaviours. Based on a Likert-type scale, comprising values ranging from 0 to 10, all of these criteria were scored subjectively by the trainer. The subject's behaviour reflected Sam's temperament on that session, in respect to calmness, alertness, anxiousness, and so on, with a score of 0 attributed if he was having a negative temperament and 10 corresponding to a calm and relaxed state. The co-operation assessment was made according to the animal's response to the commands given during the training session, with 0 corresponding to a non-co-operative response and 10 corresponding to Sam responding to all the commands. Finally, the display of aggressive behaviours, such as growling or trying to hurt the trainer with its horn, is assessed with a score below 10, whereas not displaying aggressive behaviours is assessed with a score of 10.

According to the veterinary's team belief, the parameter that gives the objective information regarding the progress of the training itself is the rating of subject's behaviour, which is why its value is multiplied by two.

The training sessions were performed in the morning as soon as the keeper of the day started the management of the "Savanna" sector; this would happen between 10am and 11am. The space in which the sessions occurred was the sleeve, which is a corridor delimited by vertical steel pillars, that connects the indoor enclosure to an exterior patio. This sleeve has a sliding door at the front and another at the back, then when closed, provides a room to work safely with the animal. This room was chosen for safety reasons and because the food reward can be offered relatively fast, however, ideally, the space should be a little bit bigger so that the animal could move more freely (6).

In every session there were at least two people present, one performing the training and the other one observing and recording, which is very helpful in assessing the animal's behaviour.

For safety reasons, specific procedures must be followed when managing this species. During the training sessions these included: never stand in front of the rhino's horn; apart from the sleeve, never stand close or share the same area with the animal; when by his side in the sleeve, a position that allows easily sliding through a space between bars without the risk of getting an arm or another part of the body crushed between the animal and the bar should always be adopted.

Stage I - Desensitization

Desensitization is a process in which an aversive event, that usually causes an undesirable response, stops being perceived as negative by the animal (4) (10). It can be achieved either by exposing the animal to the stimulus at a low degree of intensity, and increasing that intensity gradually (with the undesirable response never being triggered), or by pairing the stimulus with a positive reinforcement so that it loses the aversive character (4) (10).

In this case, the goal was to desensitize Sam so that his attitude towards the trainers' presence and touch would be neutral, in order to facilitate the following training steps (Figure 14).

The desensitization process was performed according to the following steps:

1. The food (concentrate) was placed in the enclosure's sleeve;
2. The door that separates the enclosure from the sleeve was opened and the command "go" was given. Once the animal moved forward, he was rewarded with "good boy";
3. When his body was completely inside the sleeve, the door behind him was closed and the reward "good boy" was given if the animal did not try to move backwards. If the animal didn't cooperate and tried to move backwards, he was ignored for 10 seconds after which the command "go" would be repeated for him to move forward.
4. Once the animal was in the sleeve with both doors (back door and front door) closed, the animal's entire body was touched (by petting), including sensitive body parts (horns, periorbital area, lips) in one side of the sleeve. Every time the animal fed while being touched, he was reward with "good boy".
5. With the session finalized, the back door was opened, and the command "back" was given. When Sam started walking backwards, he was rewarded with "good boy".

Regarding this particular stage, ignoring the animal when he does not cooperate is a training technique called "type II punishment" or "time out", and it is performed by withholding the positive reinforcement (6).

In a more advanced phase at this stage, the training steps were slightly modified by offering edible rewards different from the concentrate (fresh fruit or vegetable) once desensitization was achieved in a particular body part. While continuing the desensitization process, this procedure allowed to find out the animal's preference regarding the fresh food being offered, in order to define a primary reinforcer for the following training sessions. The primary reinforcer must be something the animal highly desires, it has to be species specific and specific to the taste of the individual, so that it is highly motivating (10). Food is the most used reinforcer, as it is usually the most desired; it can be of just one type, but a variety of foods

can also be used (10). Food can be completely restricted prior to the training session to make the reward more appealing. However, if the animal is too hungry, it might become frustrated, aggressive and unresponsive (6). In this case, Sam was offered the usual first meal of the day before the session and, as a reinforcement, a different food item, which included carrot, apple and sweet potato. The food item for which he displayed more interest was the apple slices, which was selected for the following stages. Another effective reward in rhinos is scratching or petting, however, during training it is harder to deliver it promptly after the correct behaviour is performed. In a more advanced phase Sam also started being petted on both sides of the sleeve and not just one, to ensure desensitization of his entire body.

Figure 11 - Trainer petting Sam in order to desensitize him from trainer's touch

Stage II - Clicker Training

The goal of this stage was to introduce the clicker to Sam. For that goal to be accomplished it was necessary to desensitize the animal to the clicker sound and to create a positive association with it (Figure 15).

The training steps were performed as follows:

1. The food (concentrate) was placed in the enclosure's sleeve;
2. The door that separates the enclosure from the sleeve was opened and the command "go" was given. Once the animal moved forward, he was rewarded with "good boy";
3. When his body was completely inside the sleeve, the door behind him was closed and the reward "good boy" was given if the animal did not try to move backwards. If the animal didn't cooperate and tried to move backwards, he was ignored for 10 seconds after which the command "go" would be repeated for him to move forward;
4. Once the animal was in the sleeve with both doors (back door and front door) closed, the animal's entire body was touched (by petting), including sensitive body parts (horns, periorbital area, lips) on both sides of the sleeve. Every time the animal fed while being touched, he was reward with "good boy";
5. After desensitization (touching his body), Sam was given another edible reward, and the clicker was used to create a positive association on the left side of the sleeve;
6. With the session finalized, the back door was opened, and the command "back" was given. When Sam started walking backwards, he was rewarded with "good boy".

In this case, the clicker sound will later act as a secondary or conditioned reinforcer when associated with the primary reinforcer (the apple slices). This will allow the animal to predict the arrival of the primary reward, bridging the gap between displaying the wanted behaviour and the primary reinforcer, thus helping with timing (6).

Figure 12 - Trainer offering food reward after using the clicker

Stage III – Target Training

This training stage had, as a first goal, the introduction of the target and the desensitization to its presence, and secondly the actual target training. The later allows the positioning of the animal at a specific location (Figure 16).

This stage was performed as follows:

1. The food (concentrate) was placed in the enclosure's sleeve;
2. The door that separates the enclosure from the sleeve was opened and the command "go" was given. Once the animal moved forward, he was rewarded with "good boy";
3. When his body was completely inside the sleeve, the door behind him was closed and the reward "good boy" was given if the animal did not try to move backwards. If the animal didn't cooperate and tried to move backwards, he was ignored for 10 seconds after which the command "go" would be repeated for him to move forward;
4. Once the animal was in the sleeve with both doors (back door and front door) closed, the animal's entire body was touched (by petting), including sensitive body parts (horns, periorbital area, lips) on both sides of the sleeve. Every time the animal fed while being touched, he was reward with "good boy";
5. After desensitization (touching his body), Sam was given another edible reward, and the clicker was used to create a positive association on both sides of the sleeve;
6. The target was introduced by being placed near Sam while he ate the apple and the clicker was used;
7. His response was observed and assessed to see whether he displayed signs of fear or got nervous/anxious in its presence. The target was moved further from him when he was not comfortable, and it was approximated slowly if he was relaxed. In the first training sessions, the target was placed at ground level and, in the following sessions, placed in a higher position;
8. With the session finalized, the back door was opened, and the command "back" was given. When Sam started walking backwards, he was rewarded with "good boy".

As mentioned previously, the final step of target training had as its goal the positioning of the rhino. To accomplish this, the target is placed at the location where the trainer wants the rhino positioned, and when the rhino approaches the target it will touch the target with its upper lip.

In order to get Sam to display this behaviour, the training was conducted according to the following steps:

1. The food (concentrate) was placed in the enclosure's sleeve;
2. The door that separates the enclosure from the sleeve was opened and the command "go" was given. Once the animal moved forward, he was rewarded with "good boy";

3. When his body was completely inside the sleeve, the door behind him was closed and the reward “good boy” was given if the animal did not try to move backwards. If the animal didn’t cooperate and tried to move backwards, he was ignored for 10 seconds after which the command “go” would be repeated for him to move forward;
4. Once the animal was in the sleeve he was petted occasionally until he finished eating the pallets;
5. The clicker sound was introduced by performing the sequence: clicker – apple slice, that was repeated a couple of times;
6. The target was introduced by placing it against one of the bars in the front part of the sleeve at a height between 75 and 100 cm the sleeve and the clicker used when he moved towards/touched it (using the clicker to bridge the reward);
7. Once Sam understood what was being requested the cue “target” was used, every time he touched the target, before the bridge (clicker);
8. The target was moved between different bars that the animal could reach with his upper lip.
9. With the session finalized, the back door was opened, and the command “back” was given. When Sam started walking backwards, he was rewarded with “good boy”.

Only in this stage a cue was added to the training. The cue’s purpose is to serve as a verbal command that the animal will always be able to associate with a specific behaviour and, therefore, understand what is being asked, in this case, the target touching. Cues should be constituted of one or two words and should be new to the animal (6).

Figure 13 - Sam performing the requested behaviour "target touch"

3.2.2.3. Behavioural sampling methods

The method chosen to record the behaviour was the continuous focal sampling, in which only one individual is observed, and the behaviours are recorded continuously. This method has been proven to be the one that preserves the most reliable and highest amount of information within a given behavioural category, by allowing the record of the frequency and duration of a specific behaviour (28) (Appendix I).

The observations occurred between 11am and 4.30pm so to span the entire workday period and were divided in two intervals of 30 minutes each. Within this schedule, the time at which the observations took place each day was chosen randomly and according to the veterinary's team work schedule for the day. Only occasionally the observation was limited to a single observation period due to constraints related to weather conditions and the zoo's logistic.

The observations were used for the construction of an ethogram and a behavioural observation sheet, in which each behaviour and the time spent displaying it were registered (activity time-budget). Variable factors such as weather, noises in the surroundings and number of visitors were also recorded, as well as the occurrence of a training session on the day of the observation.

3.2.2.4. Ethogram

After the *ad libitum* observations, it was possible to list a repertoire of behaviours, that when combined with ethograms collected from scientific literature, provided enough information to build a new ethogram for the species. Based on a detailed Sumatran rhino cow-calf behavioural ethogram (48) , on an Indian rhinoceros ethogram (9) and having as guidelines the book "The Ethogram and Animal Behavior Research" (49) an ethogram for the Southern White Rhinoceros under study was created (Table 4).

The behaviours observed were divided into six different categories according to their finality and purpose (Table 4). To facilitate the registration of the behaviours on the behavioural observation sheet an abbreviation was linked to each behaviour. Also, a detailed description of each behaviour was included to clarify exactly what they depicted. A few examples of the mentioned behaviours are depicted in the Figures 11, 12 and 13.

Table 4 - Ethogram of Santo Inácio's Zoo Southern White Rhinoceros, Sam

Category	**Behaviour**	**Abbreviation**	**Description of the behaviour**
Feeding	Eating	E	Animal feeds in a stationary position.
	Grazing	G	Animal walks while feeding from plants that grow in the soil of the enclosure.
	Drinking	D	Animal drinks water.
Maintenance	Urinating	U	Animal urinates in a stationary position.
	Defecating	DF	Animal defecates in a stationary position.
	Wasting Horn	WH	Animal rubs horn against something to waste it.
	Scratching	SC	Animal rubs his body against a tree or another object from the enclosure.
Resting	Sleeping	S	Animal lays with eyes closed, completely relaxed.
	Laying	L	Animal lays down while being awake
Exploratory behaviour	Wandering	W	Animal walks, exploring the surroundings.
	Smelling	SM	Animal lowers its nose and inspires near the ground or object, then lifts his head and trembles his upper lip for a few seconds.
	Stationary standing	ST	Animal stands still bearing in all four limbs, usually to stay alert to a different noise/smell/person/animal/etc.
Agonistic interactions	Running	R	Animal runs towards something/someone in an aggressive way.
	Vocalizing	V	Animal vocalizes in an agonistic manner, towards another animal.
	Interaction/social behaviour	I	Animal interacts with its partner in an agonistic way.
Others	Others	O	Behaviours not described in this list. Sporadic and non-species-specific behaviours, such as kicking his belly with rear limbs, jumping back if spooked (by another animal for e.g.), grabbing a stick with his mouth from the ground.

Figure 14 - Sam laying next to the other two rhinos

Figure 15 - Sam "wasting horn" against a dead tree trunk

Figure 16 – Sam (on the left) displaying an agonist interaction with another rhino

4. Data analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (IMB SPSS Statistics 22.0). The non-parametric Friedman's test (non-parametric ANOVA) was used to analyse the score ("progress to goal") of the training sessions. In order to assess if the operant conditioning training had a significant statistical effect on the behavioural categories under study, the non-parametric Friedman's test was also performed, since the conditions of applicability of the parametric test did not occur, namely, the condition of homogeneity of variance between groups ($p<0.05$). Whenever it was possible, multiple comparison of mean ranks, was performed. A probability of error type I (α) of 0.05 was used.

5. Results

Results regarding the evaluation of the training sessions, as well as the mean scores obtained during the three stages of operant conditioning training (Stage I – Desensitization; Stage II – Clicker training and Stage III – Target training) are detailed in the following paragraphs.

Regarding the behavioural study, the results are described further in this section. Data pertaining the time Sam spent in each behavioural category during the different stages of training is expressed in percentage.

5.1. Operant Conditioning Training

During the **first training stage (Desensitization)** (Figure 17), Sam never showed any signs of aggressiveness towards the trainer and responded well to most commands, despite being hesitant to go in and out of the sleeve on certain occasions. His individual behaviour during this stage, varied from showing some signs of apprehension and alertness, such as keeping his ears up, not going inside the sleeve as soon as the door was opened and not leaning towards the side of the sleeve where the trainers were standing, at the beginning, to feeding almost completely relaxed while being touched by the trainer, by the end of this stage. However, on the last session, there was some regression in the progress made, with Sam showing alertness and impatience, perhaps due to management changes on that specific day (he was kept closed for longer than usual).

Sam was always more responsive to being touched on the right side of the sleeve, which is the side where the keepers stand when performing their management routines. The training score mean of this stage, displaying the "progress to goal", was 7.9.

On the **clicker training stage (Stage II)**, Sam was still more responsive to being touched on the right side of the sleeve on the first training sessions, however, towards the end of this stage, he got comfortable being touched on both sides.

When the clicker was firstly introduced, he reacted with signs of fear to the sound produced by stepping back, putting his ears up, lifting his head and staring at the trainer. However, in the following sessions, the trainer managed to use the clicker in a way that did not produce such a loud noise and Sam continued to eat calmly and relaxed. Once again, Sam did

not show any signs of aggressiveness towards the trainer and responded well to most of the given commands. The average training score for "progress to goal" at this stage was 8.3.

On the **final training stage (Stage III – target training)**, Sam responded well to most of the given commands, except on one occasion, and never showed aggressiveness towards the trainer. During the first training sessions of this stage, that had as its goal the desensitization of the target, Sam showed a lot of apprehension towards the object during the first three sessions, in which he would stop eating if the object was brought close to him and stared and the observer with his ears up. It wasn't until the seventh session that he started to touch it voluntarily and only after this behaviour was performed, did the actual target training started. The average score of the "progress to goal" in this final training stage was 8.75.

In summary, the mean ranks of the training score (Figure 17) showed an evolution between the stage I (Me=1.5) and stage II (Me=2.25), but the value remained the same on stage III (Me=2.25), demonstrating no significant changes between the three training stages ($\chi 2_{(2)}$= 2.455; p =.293)

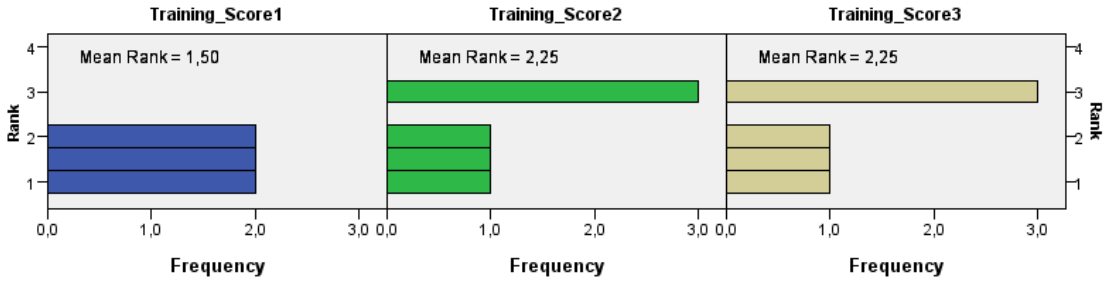

Figure 17 - Distribution of the Training score variable (progress to goal) on the three training stages

5.2. Behavioural study

The results regarding the behavioural focal sampling study are displayed in Figures 18 to 20. The mean ranks of the Feeding, Agonistic and Exploratory behavioural categories are depicted in Figures 21 to 23. The mean ranks of the remaining categories (Maintenance, Resting and Others) are not displayed since their values are close to 0 in all stages under study. Overall, Feeding behaviours, are predominant during stages I and II, but suffer a significant decrease on

stage III (Figures 18, 19, 20 and 21). Contrary to this, the Exploratory category of behaviours showed a progressive increase from stage I (Me=1.5) to stage III (Me=2.67) as demonstrated by their respective mean ranks (Figures 18, 19, 20 and 22). Agonistic interactions displayed by Sam were less frequent in stage II (Figures 18, 19, 20 and 23).

During **stage I of training** (Figure 18), Sam spent most of his time budget performing Feeding behaviours (66%). Most of the time spent on this category was used for eating, even though drinking was also recorded for brief moments in the observations. Agonistic interactions were the second most performed category (22%) and Exploratory behaviour the third (11%). The latter was divided equally between standing and smelling behaviours, with the remaining of the time spent on wondering. Finally, the category Others represented 1% of the total time observed.

On the **clicker stage** of the training process (**stage II**) the most performed category was Feeding (Figure 19), as in the previous stage, even though on this occasion it represented up to 78% of the time observed. Time spent on the Exploratory behaviour category during this stage was similar to the desensitization stage, with a slight increase of 2% in comparison to the previous stage, being the second most performed category once again. It was mainly composed by smelling and wondering behaviours. The maintenance category percentage was mostly represented with the behaviour wasting horn, having occupied 7% of the total time budget. Despite without statistical significance ($\chi^2_{(2)} = 4.261$; $p = .119$), a noticeable change between desensitization and clicker training stages, was the decrease in time spent performing Agonistic interactions, dropping from 22% to 2%.

During the **target training stage (stage III)**, the most performed category by Sam was Feeding (Figure 20), as in the previous stages, but only with a span of 40% of the total amount of time, reaching a statistical significant difference when compared to the previous training stage ($\chi^2_{(2)} = 7$; $p = .028$). The remaining 60% of the time budget was divided as follows: 27% for Agonistic interactions, 22% spent on Exploratory behaviour, 7% Resting, 2% on Maintenance behaviours and finally, the remaining 2% displaying behaviours categorized as Others. At this stage Agonistic behaviours showed the highest percentage, when compared to the previous stages, being 5% higher than those observed at the desensitization stage, and 25% higher than the ones exhibited on the clicker training stage, however this difference was not significant ($\chi^2_{(2)} = 4.261$; $p = .119$). As for the Exploratory behaviours, they increased to 22% at this stage, although without reaching statistical significance ($\chi^2_{(2)} = 4.333$; $p = .115$). Within this category, the most frequent behaviours displayed by Sam were the wondering and

stationary behaviours. It is also noteworthy that for the first time during the study, Sam was recorded displaying Resting behaviours.

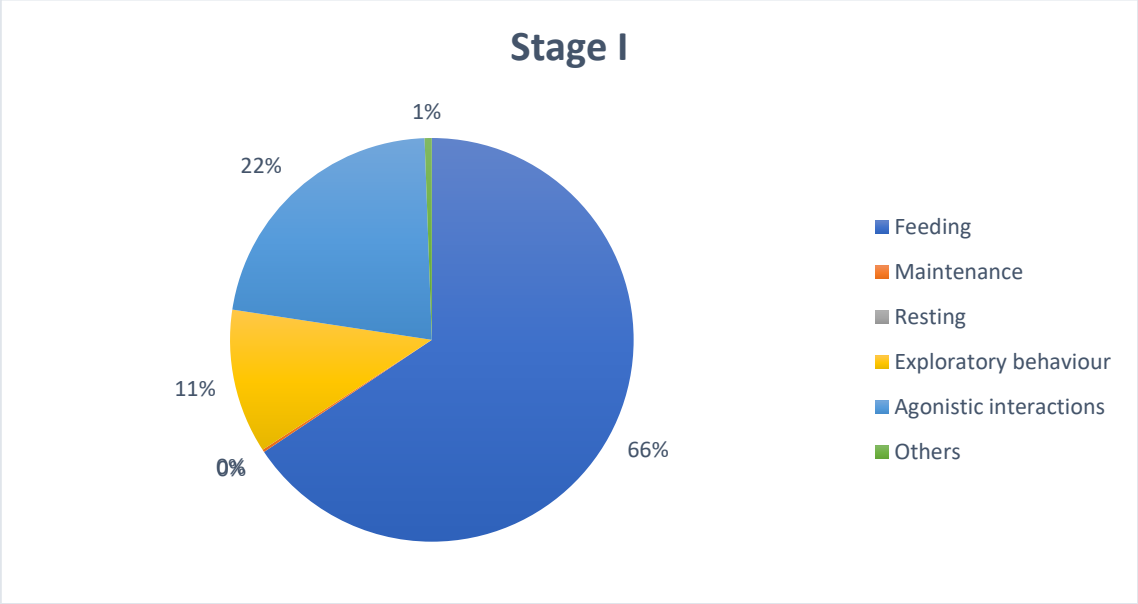

Figure 18 - Sam's behaviour, distributed according to the different behavioural categories during stage I - Desensitization

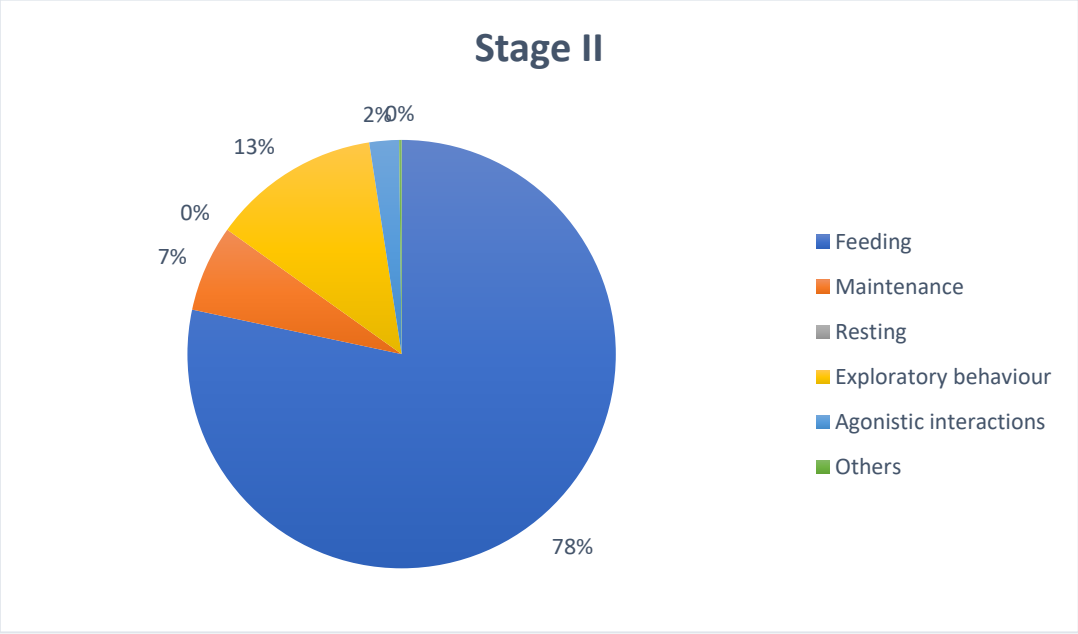

Figure 19 - Sam's behaviour, distributed according to the different behavioural categories during stage II – Clicker training

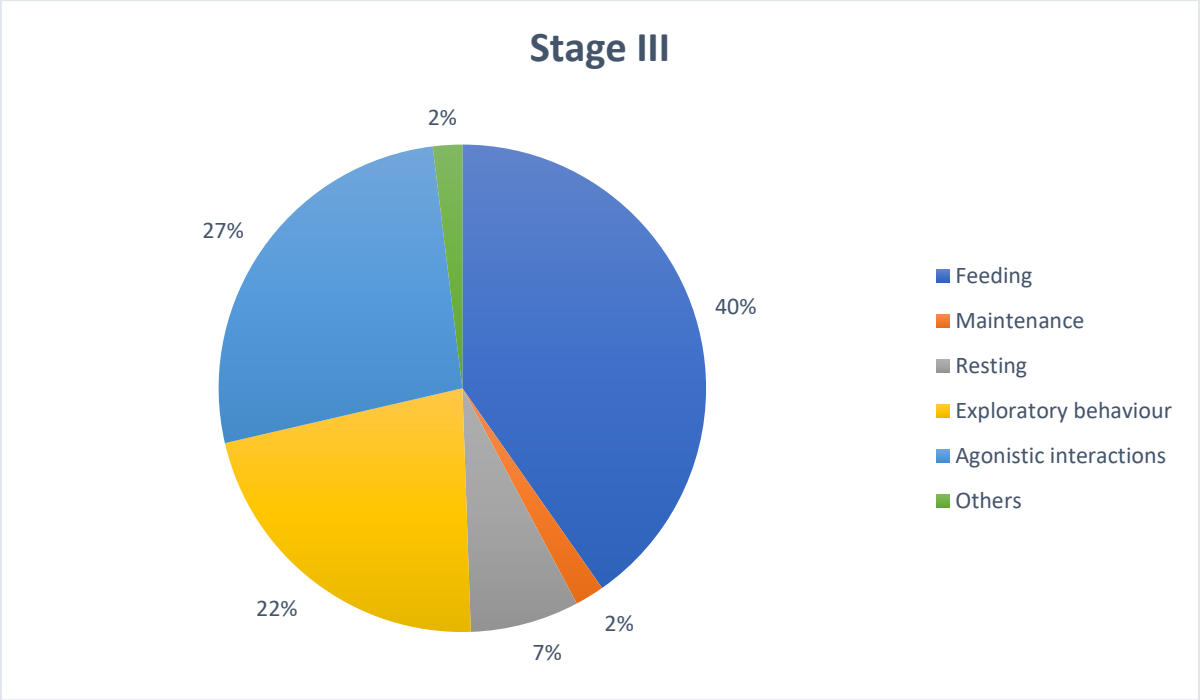

Figure 20 - Sam's behaviour, distributed according to the different behavioural categories during stage III – Target training

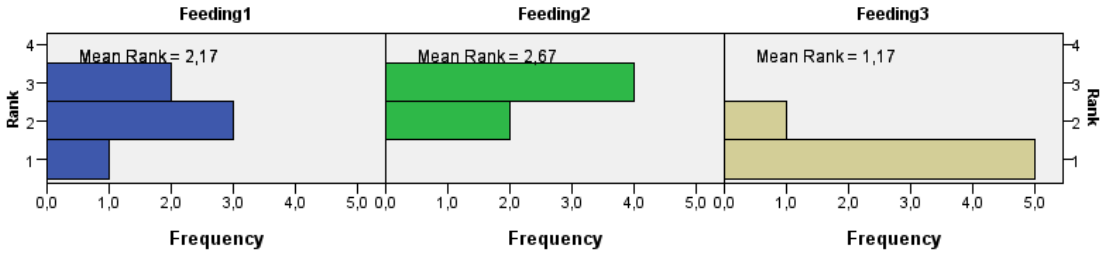

Figure 21 - Distribution of the Feeding category variable on the three training stages

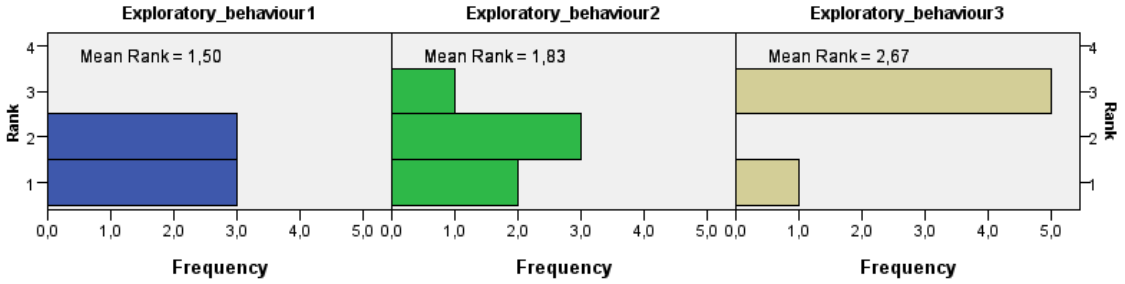

Figure 22 - Distribution of the Exploratory category variable on the three training stages

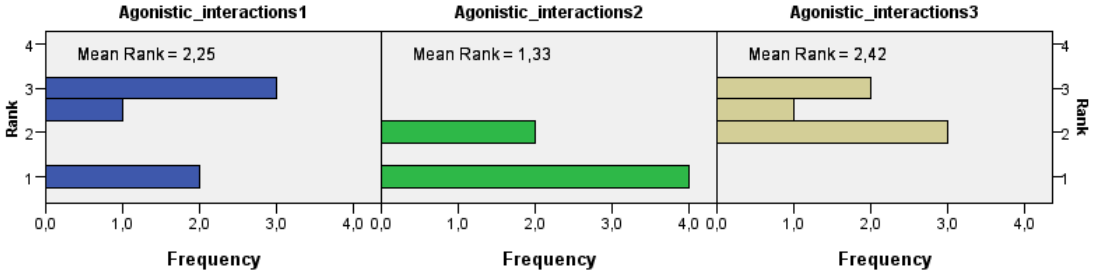

Figure 23 - Distribution of the Agonistic interactions category variable on the three training stages

6. Discussion

In the past, rhinoceros were very diverse and widespread throughout Eurasia and Africa, however, only five species survive today: White rhino, Black rhino, Greater one-horned rhino, Sumatran rhino and the Javan rhino, with all of them being under threat of extinction. Between 2018 and 2019, the last males from two of the mentioned species – Northern White rhino and Sumatran rhino – have died, proving how critically important conservation programs are (50) (51). At the present time, most of the living rhinos do not survive outside reserves and national parks, due to human activities, such as poaching and habitat destruction, that deprive them from their life requisites (51) (52).

This work describes a study on operant conditioning training and a behavioural observation program conducted on Sam, a Southern White Rhino, which is a "near threatened" species, according to the IUCN red list, at Santo Inácio's Zoo (50). The study aimed to design and execute an operant conditioning training program, as a mean to facilitate husbandry and veterinary procedures; to monitor and investigate the effects of an operant conditioning training program on the animal's behaviour, and finally, to provide guidelines for training plans for other individuals and species.

According to our knowledge, this has been the first time a training program and a behavioural observation study were conducted simultaneously, in order to assess the effects of operant conditioning training in the rhinoceros' behaviour.

During this study, Sam demonstrated interest and willingness to participate voluntarily in the training sessions and, even though a statistical significance was not achieved, the results showed a positive progression in the successive training stages, which suggests that these activities have an enriching potential (53). This aspect is reinforced by the fact that the category of Exploratory behaviours also demonstrated a progressive increase from stage I to stage III, which may be related to the positive effect of training in mental stimulation, improving the subjects awareness to his environment and encouraging Sam to investigate the surroundings. Although very different at the cognitive level, research in zoo-housed chimpanzees has also demonstrated that training programs can be considered enriching activities that engage animals in a positive manner, with clear benefits on subjects' welfare (53). The literature on rhinoceros training and behaviour is rather scarce, and even lesser that relating both aspects, but a study conducted by Capiro et al. (2014), on "operant conditioning effects in Indian Rhinoceros (*Rhinoceros unicornis*) showed that glucocorticoids levels and individuals' behaviour were not

affected by positive reinforcement operant conditioning techniques. Another study on rhinoceros operant conditioning training concluded that the management and monitoring became less stressful and presented lesser risks for the animals and keepers. Furthermore, the relationship between animals and the veterinary team was improved, as there was no need for immobilization when performing medical procedures (3).

During this study, agonistic interactions and time spent on Feeding behaviours also suffered alterations. However, these differences observed in Sam's behaviour may be justified by exogenous factors, other than the operant conditioning program. In fact, some of the challenges faced during this work were related to the particular zoo settings, like husbandry procedures and weather conditions that cannot be controlled. Opening time schedules and the time in which training was conducted, varied according to the keeper performing the routine on that specific day. This may explain Sam's impatience on some of the training sessions when the opening time was delayed, as well as the agonistic interactions between the rhinos. Another occurrence that may have had a negative impact on his behaviour and on his interaction with the other rhinos, was when the gate dividing Sam's and Nono's enclosure was sent down during the night by one or both of them; this event also prevented some of the training sessions to occur until it was fixed.

On the other hand, training sessions could have been facilitated, if the place where they occurred was slightly wider so that Sam could promptly reach the target and the rewards. Another variable that may have had influence on Sam's performance were the noises in the surroundings, that seemed to make Sam more distracted (with his ears constantly up) and less focused on the training.

When conducting the observational study, difficulties were related to the weather conditions that impaired some of the sessions as there was no shelter for the observer from the rain and stormy weather.

Despite all these challenges, the results of this study are in accordance to the ones from the existent literature regarding the influence of operant conditioning training in the animal's behaviour and welfare (9) (54).

In the future, training performed on this individual, will be facilitated since the described systematic training program has desensitized Sam from the presence and touch of the trainer and it may ease the introduction of new people. It also provided the zoo staff with information regarding his own particularities: the primary reinforcement that motivates him the most, what makes him display signs of fear, what constitutes a distraction during a session, how new

training materials should be introduced and at what pace a new command is expected to be learned.

Even though, more extensive evaluations of operant conditioning programs on welfare and behavioural patterns, need to be performed for longer periods and on a wider variety of species, this work's results, along with the results from existent literature, can be used as a base for future studies in which the goal is to offer animals a greater mental stimulation and to facilitate management and medical procedures, without compromising the individual's welfare.

Training for medical procedures minimizes stress and decreases the risk of injury for the veterinary team and the animal, which can be helpful, not only in routine physical examinations but also to perform complex exams and treatments such as reproductive examinations with a transrectal ultrasound with no need for immobilization (9) (54).

This highlights the importance of operant conditioning training techniques, as a tool that can play an essential role in conservation, when used for reproductive purposes.

Additionally, the structure of this work can be easily adapted for similar projects in other species, after assessment of each individual's needs is carried out. It can be particularly useful for large herbivores that are likely to benefit from this type of training and that might have a similar enclosure structure. Even though individual cognitive differences exist, it can also be used as a structure to predict and assess the progress in rhinoceros learning.

However, confronting wildlife kept in captivity for conservation with ethical obligations and animal welfare, can be very conflicting. Some of the concerns with conservation programs include the human intervention in populations and ecosystems and the role of the receiving institutions (zoological parks and aquariums) in research (55).

Therefore, a pragmatic approach to ethical analysis and an examination of the institutional character, practice and goals are essential to prevent further species decline and extinction, without compromising welfare and ecological integrity (55) (56).

7. Conclusion

Zoological parks and aquariums provide unique conditions to study species that have been proven difficult to study in their natural habitat, generating fundamental knowledge that latter can be translated to conservation programs, both *in situ* and *ex situ* (2) (57).

In the present study, an operant conditioning training program was elaborated for a Southern White Rhino, a near-threatened species, designed to facilitate husbandry and veterinary procedures. The animal subjected to the training plan, demonstrated great cognitive and learning abilities. Sam was able to learn a new behaviour, target touching, in a total of 23 training sessions, without having had any previous systematic training. When analysing the evolution of his training score throughout the different training stages, the results demonstrated a positive progression, which suggests the animals' interest and willingness to participate in the performed activities. The assessment of Sam's behaviour, through a behavioural observation study, showed that the Exploratory category of behaviours also had a positive progression from training stage I till stage III, and alteration on the animals' routine which suggests an enhanced perception and curiosity to its surroundings. Agonistic interactions and time spent on Feeding behaviours also suffered alterations during the study. However, these differences observed in Sam's behaviour may be justified by exogenous factors, other than the operant conditioning program.

Taken together, these results point out the positive effects in mental stimulation and enriching potential of operant conditioning training activities, as well as, demonstrate the feasibility and benefits of a training plan directed to improve general husbandry and veterinary procedures for animals in captivity.

Additionally, this study can be helpful in providing guidelines for training programs in other species and individuals, after a careful and planed evaluation of its needs and particularities is carried out, to ensure a positive outcome and the welfare of the animals involved. By accomplishing this, we stand in the privilege position to contribute to what Martin Luther King Jr once stated: "One day the absurdity of the almost universal human belief in the slavery of other animals will be palpable. We shall then have discovered our souls and become worthier of sharing this planet with them."

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9. APPENDIX

APPENDIX I

Behavioural Observations Sheets

Day	**Hour**	**Observations**		**Training (yes/no)**
		Level of activity; Number of visitors; Weather conditions.	Description of the behaviours performed and their respective duration.	