UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIA

DEPARTAMENTO DE MEDICINA VETERINÁRIA

Cognitive Bias and Welfare in Shelter Cats

Viés Cognitivo e Bem-estar em Gatos de Gatil

Ana Ribeiro Pereira

Orientação:

Doutor Alfredo Manuel Franco Pereira

Mestre Sara Raquel Fragoso Sousa

Mestrado Integrado em Medicina Veterinária

Dissertação

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…to my beautiful daughter, Camila. She gives me the strength to carry on.

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Abstract

Cognitive Bias and Welfare in Shelter Cats

Welfare has traditionally focused on assessing physiological parameters, but over the last decades there has been growing interest in finding scientific and objective methods to evaluate emotional states and mental health of animals. Cognitive bias measures have emerged as tools to assess animal emotion. This preliminary study was undertaken at the Municipal Animal Shelter (MAS) of Sintra and aimed at evaluating if cats subject to environmental enrichment showed more optimistic responses towards ambiguous stimuli in a cognitive bias test. Of an initial group of twenty-four cats, divided into three groups (Enrichment using Training (EuT), Enrichment using Play (EuP) and not Enriched (nE)), eight completed the test (three EuT, two EuP and three nE) as the other were excluded primarily because they were adopted (nine). Latency to reach the unrewarded-near position was similar in the three groups. More differences were found in the latency to reach rewarded-near position, where trained cats showed a shorter latency, which could be indicative of more optimism.

Key-words: Shelter, Cat, Cognitive bias, Welfare, Enrichment

Resumo

Viés Cognitivo e Bem-estar em Gatos de Gatil

Tradicionalmente a avaliação de bem-estar tem-se focado em parâmetros fisiológicos, mas ao longo das últimas décadas tem surgido interesse crescente em encontrar métodos científicos e objetivos para avaliar estados emocionais e saúde mental animal. Medidas de viés cognitivo têm se assumido como ferramentas de avaliação de emoções animais. Este estudo preliminar foi efetuado no Centro de Recolha Oficial (CRO) de Sintra e teve como objetivo avaliar se gatos sujeitos a enriquecimento ambiental teriam respostas mais otimistas perante estímulos ambíguos num teste de viés cognitivo. De um grupo inicial de vinte e quatro gatos, divididos em três grupos (Enriquecidos com Treino (EuT), Enriquecidos com Brincadeira (EuP) e Não Enriquecidos (nE)), oito completaram o teste (três EuT, dois EuP e três nE) tendo os restantes sido excluidos predominantemente devido a adopção (nove). A latência de chegada à posição próxima da não-recompensada foi semelhante nos três grupos. Na latência de chegada à posição próxima da recompensada foram encontradas mais diferenças, com os gatos treinados a mostrarem latências menores o que poderá ser indicativo de maior otimismo.

Palavras Chave: Abrigo, Gato, Viés cognitivo, Bem-estar, Enriquecimento

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List of abbreviations and symbols

APPA - American Pet Products Association

B – Box

c – Video Camera

CRO – Centro de Recolha Oficial

e. g. – Latin “Exempli gratia”, it stands for “for example”

EuP – Enriched using Play

EuT – Enriched using Training

F – Female

HPA – Hypothalamus-Pituitary-Adrenal

M – Male

Mnn test – Mann Whitney u test

MAS – Municipal Animal Shelter

nE – Not enriched

NR – Negative reinforcement

pCPA – Chlorophenylalamine

PR – Positive reinforcement

QoL – Quality of Life

R – Rewarded Position

R-n – near Rewarded Position

R/U – Rewarded/Unrewarded, equidistant position

SS – Stress Score

U – Unrewarded Position

U-n – near Unrewarded Position

Chapter 1 – Introduction

In our modern, often city-based lives that include long hours away from home, cats have assumed themselves as the companion animal *par excellence*, even surpassing dogs in numbers and popularity. According to the 2015-2016 American Pet Products Association (APPA) National Pet Owners Survey, 85.8 million pet cats live in the United States, in comparison to 77.8 million pet dogs (APPA National Pet Owners Survey, 2016). While millions of people realize that a cat could be the companion animal best adapted to their lifestyle, requiring less space and attention, no walks, and probably less costly veterinary care (Stephens & Yamazaki, 2001; Overall, 2013), there are still many myths to be dispelled: they are not solitary animals whose behaviour can't be moulded, and they most certainly do not fare well in impoverished, barren environments (Gourkow & Fraser, 2006). More studies are needed relating environmental conditions to welfare, allowing us to get deeper insight into the fascinating world of cats.

1.1.The Myth about Solitude

Although historically cats were considered solitary species, and some members of the felidae family, such as tigers, are mostly solitary animals which need large territories (Gour et al., 2013) our domestic cat, *Felis catus*, is in fact a social animal (Kessler & Turner, 1997). They build social groups whose core is the queen and her litter, and which usually only disperse if the food resources are sparse. The formation of groups of related and familiar individuals around food resources is the first step in the development and organization of social behaviour, and larger colonies are composed of several queens, often related, who cooperate in ways to facilitate the survival of their young (Cromwell-Davis, 2006). Individual members of a colony recognize each other, and acceptance and integration of strangers is gradual and likely to be resisted (Alger & Alger, 2002). Within a colony, affiliative and antagonistic relationships are formed. Affiliated cats greet each other by rubbing heads and bodies and sometimes entwining tails. They also groom each other what may contribute to a general group odour which may help with the identification of members. These associated cats will often be found sleeping together and sharing space and food. Antagonistic encounters are preferably avoided within a group, using time sharing to access common areas (Alger & Alger, 2002; Curtis et al., 2003). Integration of kittens abandoned near a colony seems to be easier than the integration of adult cats which might cause great disruption of the social order (Cromwell-Davis, 2006).

1.2. Developmental Stages

The individual development of the kitten can be influenced by various factors, such as genetic factors, maternal factors, environmental factors and sexual differences (Landsberg et al., 2013). Kittens are born after around 63 days but even before birth the health conditions of the queen (physically as well as mentally) play an important role: e.g. maternal malnutrition may lead to deficits in brain development (Vilanova, 2002; Landsberg et al., 2013), reduced social attachment capacities and fearful and aggressive behaviour, effects which may be permanent (Cromwell-Davis, 2006). Stress affecting the mother during pregnancy besides increasing the rate of newborn deaths also has been known to lead to learning impaired adults, with memory problems in delayed alternation task in rats (Lordi et al., 1997). The neonatal period extends from birth until around one week of age, and is a period of sleep and nursing during which the kitten is exclusively dependent on the mother. Sensory development and locomotion emerge around the second week, and characterize the so called transitional period. This transitional period is then followed by the socialization period, in the third week, which is the beginning of a very important phase that extends up to seven to nine weeks of age and will assume great weight in the modelling of the adult cat's behaviour. The juvenile stage ends with sexual maturity, between six to twelve months, even though social maturity is not reached before two and a half years (Landsberg et al., 2013).

1.3. Human and Parental Effect on Socialization

As early as 1961, studies found that early handling has important effects on the social capacities of kittens, influencing the physical and central nervous system development. Kittens that were held and gently stroked daily during the first weeks opened their eyes earlier, began to explore earlier and were less fearful of humans (Meier, 1961). Kittens handled during five minutes daily for the first 45 days of their life are less fearful, approach strange humans and strange toys more readily and are slower to learn avoidance (Wilson et al., 1965). Besides early pleasant interactions with humans, which predispose to friendlier adult cats, kittens may also be influenced by the behaviour of the mother: if the queen is shy, reserved or fearful, her offspring may learn these behaviours. Ideally, the mother should at least not be overly fearful of humans and ideally the sire should be outgoing and confident (Landsberg et al., 2013). According to some studies, paternity has an adding effect to socialization, influencing the tendency a kitten shows to approach and explore new stimuli (Vilanova, 2002; McCune, 2016), similar findings were found in an experiment which evaluated the response of a cat to a novel box, and which showed that cats with friendly-fathers were quicker to approach, investigate and enter the box (McCune, 2016) . This father-based response suggested boldness in approaching people or objects might be inherited (Cromwell-Davis, 2006; McCune, 2016). Despite their socialization, adult cats will still show a great variability in their friendliness towards other animals, according to their personality types (Overall, 2013).

1.4. Welfare in Shelter Cats

1.4.1. Definitions and Historical Overview of Welfare

Broadly speaking, defining animal welfare is not an easy task, as the concept can be understood differently depending on whether it is explained by producers, consumers, veterinarians or politicians (Velde et al., 2002). Up to recent years, veterinarians and farmers have seen animal welfare chiefly in terms of the body and the physical environment (shelter, feed, among others) focusing on the premise that if an animal is in good physical health and producing (or reproducing) well, his welfare must be adequate (Broom, 1991; Hewson, 2003). The most utilized tools for welfare evaluation focused on the body, using physiological measures to examine how the animal is coping with his environment (Broom, 1991). Nowadays the limitations of this physical approach to welfare are being challenged, based on the argument that genetics and the environment can produce desirable physical outcomes, even though the animal's mental state is compromised. Studies have traditionally focused on plasma, salivary and urinary cortisol as a measure of stress (Beerda et al., 1998), but the quantification of these parameters is considered increasingly non-specific, and may not reflect an emotional state (Gourkow et al., 2014), as it is unclear if eventual reduction in glucocorticoid levels represents actual adjustment by the animal to his new environment or a dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis to the continued stress (Hennessy, 2013). Some behavioural patterns have also been used, correlating them to poor welfare, such as excessive auto-grooming and persistent vocalisations e.g. in dogs (Dalla Villa et al., 2013). When matching behaviour with physiological parameters, results are confusing and not consistent (Hiby et al., 2006), which may be due to the fact that cortisol is dependent on temporal context, being used as an acute stress indicator (Hennessy, 2013). Measuring oxidative stress has been suggested as a physiological indicator for chronic stress (Passantino et al., 2014), even though there is scarce information relating behaviour and oxidative status. Caffazo et al. (2014) related however, the improvement of shelter dog's welfare through daily interaction with humans and daily walks, including lower frequencies of displacement behaviours and stereotyped behaviour.

Conversely to the all-physical approach is another one that proposes that animal welfare consists entirely of feelings and that these serve the purpose of protecting the animal's primary needs and that consequently an animal that feels well is living in good welfare (Duncan, 2002). The feeling-based approach to welfare research cannot quantify physiological parameters but resorts to measuring behavioural outcomes, such as willingness to perform specific tasks to gain access to food, and behavioural signs of fear and/or frustration. Research on this subject led to the conclusion that there are fundamental behavioural needs that must be satisfied if welfare is to be assured (Petherick & Rushen, 1997). But even if the definition varies, common elements in it are, transversally, the physical and psychological well-being of animals (Hewson, 2003). Other terms have been suggested, such as Quality of life (QoL), happiness or well-being, but they all can be simplified to the concept that *"the animals feels good and enjoys life*" (McMillan, 2002).

Measuring welfare and QoL is a great challenge and even though there has been a lot of research describing welfare indicators in the last years, mainly based on physiological and behavioural parameters (Dalla Villa et al., 2013), but more studies are needed so formerly subjective parameters such as "mental state" can also be evaluated scientifically. A list of nine signs has been suggested as a welfare assessment for shelter animals, assessing the QoL through the evaluation of: alertness; range of the specie's appropriate context and age behaviour; low range or absence of unpleasant or abnormal behaviour; sleeping and eating patterns and physical health (McMillan, 2013).

Another different approach has been the assessment of positive emotions in animals to improve their welfare (Mendl et al., 2009; Hotzel & Martendal, 2010). It is suggested that by promoting positive experiences, such, as physical, social and cognitive enrichment, animal health and QoL can be improved (Boissy et al., 2007; Matheson et al., 2008; Brydges et al., 2011; Richter et al., 2012; Douglas et al., 2012).

1.4.2. Stress and its Effect on the Welfare of Shelter Cats

The interplay between behavioural and physical health becomes very clear when looking at the role that stress plays on every aspect of health. Stress can be defined as a change to homeostasis or psychological well-being which will lead to physiological and behavioural responses to re-achieve this homeostasis (Kessler & Turner, 1997; Hennessy, 2013). It can be adaptative (eustress), leading to a re-establishment of homeostasis, or mal-adaptative (distress) if homeostasis is not re-established (Zawistowski, 2015). If homeostasis is not achieved, acute stress may turn into distress and chronic stress (Moberg, 2000).

Stress responses are mediated by an individual's behaviour, the sympathetic nervous system, the HPA axis, and the immune system: when faced with an unfamiliar and/or startling situation, the sympathetic nervous system is activated to facilitate the flight-or-fight response. Epinephrine is secreted into the bloodstream as the nervous system stimulates the adrenal cortex, this leading to an increase of blood glucose and increased blood flow to the voluntary muscles to prepare for high activity. The adrenal cortex will also secrete cortisol (hydrocortisone) to increase the presence of glucose and the metabolism of fats to produce energy (Zawistowski, 2015). The heart rate increases and the pupils dilate so the animal is in full alert mode (Rodan, 2010). These physiological responses get the animal ready for either flight (which a cat, provided the opportunity, will most often choose) or fight to defend itself. If either of these mechanisms are successful in eliminating the perceived threat and thus resolving the situation, the cat will go back to homeostasis. If not, the stress becomes chronic, and a continued release of cortisol will suppress the immune system which leads to a significant reduction in the cat's welfare and even physical health (Zawistowski, 2015).

Thus, in the long term, the stress response can be maladaptive and if the stress response continues, for whatever reason, cardiovascular, metabolic, reproductive, digestive, immune, and anabolic processes can all be pathologically affected (McEwen, 2000;

Tynes et al., 2015). The results can include myopathy, fatigue, hypertension, decreased growth rates, gastrointestinal distress, and suppressed immune function with subsequent impaired disease resistance. Chronic stress can even lead to structural and functional changes in the brain, and when extreme conditions persist, permanent damage can result (McEwen, 2000). Stress can arise from a variety of different sources, both physiological and psychological. Physical stress can be caused by hunger, thirst, pain, exposure to extreme temperatures, illness, and sleep deprivation and is more easily quantifiable and fixable. Psychological stressors can assume various forms, from exposure to novelty over unpredictable environments, social conflict, constant exposure to fear or anxiety provoking stimuli and situations leading to frustration or conflict (Tynes et al., 2015). A lack or loss of control is another important psychological stressor which assumes paramount importance in animal husbandry in general, as we shall see further on (McEwen, 2000; Tynes et al., 2015).

According to McEwan (2000), the most powerful stressors seem to be: 1) novelty, 2) withholding of anticipated rewards and 3) anticipation of punishment. The response to stress is also highly variable between individuals, depending on such factors as genetics, temperament, experience, environment and learning. For example, cats not socialized to people have been shown to be more likely to experience high levels of stress when exposed to people in a shelter setting (Kessler & Turner, 1997). Studies have shown that the experiences during the first weeks have a great impact on the future ability to cope with stress (Foyer et al., 2013).

As the individual's perception of stress is largely based on experience, this is the area where we can do the most prevention work through socialization and promoting positive experiences in order to achieve more confident, calmer, less stress-prone adults (Overall, 2013). Cats have shown to react with stress when they were subject to unpredictable handling and husbandry routines (Carlstead et al., 1993). Increased density of group-housed cats has been shown to be positively correlated with stress levels (Kessler & Turner, 1997). Stressed shelter cats have shown to be at higher risk of upper respiratory tract infections, besides eating less than their unstressed counterparts and consequently loosing weight (Tanaka et al., 2012). When stressed, cats substitute normal exploratory and play behaviours for more time alert but hiding or attempting to hide – if hiding is not possible, stress increases (Carlstead et al., 1993). Confined, stressed cats also tend to resort to apathy or escape behaviours (often alternate, depending on the presence or not of people), vocalization and aggressive behaviours (Kessler & Turner, 1997).

It has been suggested that both humans and stressed animals require greater amounts of sleep, which leads us to conclude that even cats which appear relaxed and "sleepy", may indeed be suffering from high levels of stress, so it should be taken into consideration that decrease in activity and increase in hiding and sleeping may be indicative of stress (Rushen, 2000). Overgrooming, decreased grooming, panting and excessive drooling may also be signs of distress, as can failure to eat or use the litter box during daytime hours. Some distressed cats refuse to eat or drink, while others will urinate or defecate where they lay rather than move from their hiding spot or bed to use the litter box (Miller & Watts, 2015).

In a shelter environment without adequate enrichment young cats and very active cats are the two classes that most rapidly suffer the effects of under stimulation, resorting to their own enrichment using cat litter, bedding, the food bowls or whatever may be available to play, paw and tear, besides quite often extending these behaviours to people who are passing by. Playful at first, reaching their paws through the bars in boredom can escalate to biting or scratching caretakers' hands or legs as though toys or prey, making it difficult or even dangerous to clean the cage or handle the cat (McCune, 1994; Arhant et al., 2015; Miller & Watts, 2015). The study of Kessler et al. (1997) gave a two week stretch of time for notable stress reduction in 2/3 of kennelled cats, but also showed that 4% of the cats never reduced their stress scores during the study period, which suggests that some cats may in fact be unfit for shelter housing during an extended period. When choosing a pet adopters are most likely looking for a friendly, sociable, playful animal and therefore those cats displaying fearful, avoidant, defensive, destructive, or aggressive play behaviours are probably less adoptable (Gourkow & Fraser, 2006; Weiss et al., 2012). Therefore, effects of enrichment extends as far as the chances of adoptability, as studies have shown that adopters show a preference for more active cats and cats housed in more interesting environments (Fantuzzi et al., 2010). In addition to this more appellative presentation of the animal, enrichment can facilitate positive interactions between cats and adopters (e.g., through play with interactive toys), helping adopters to bond with a cat while encouraging the cats to approach, important factors in the choice to adopt (Weiss et al., 2012).

So how can we use enrichment to improve the welfare of cats in general and shelter cats in specific?

1.5. Environmental Enrichment

1.5.1. Definitions and Goals

Having mentioned the term "enrichment" several times throughout this study, it seems important to define what is meant by it, in the specific context of welfare or QoL improvement. Environmental enrichment may be defined as the provision of a captive animal with the ability to maintain or improve his physical, behavioural and psychological functioning via modifications to the housing environment (Young, 2003) or as a "concept which describes how the environment of captive animals can be changed in order to benefit its inhabitants" (Carlstead & Shepherdson, 1994). The focus of environmental enrichment must always lie on what it wants to achieve, otherwise it does not deserve to be called enrichment at all: if a toy or other element is added but it does not alter the animal's QoL in a positive way, it has failed its purpose (McMillan, 2013). Furthermore, even though effective enrichment can alleviate the effects of current (and even future) stressors, it should not be implemented only when the animal displays problematic behaviour, but it should be a mandatory part of the animals daily care plan (Miller & Watts, 2015). But let us summarize the goals of a successful environmental enrichment, to begin with. They are, according to Young (2003):

1- Increase behavioural diversity;

2- Reduce the frequency of abnormal behaviours;

3- Increase the range or frequency of normal behaviours;

4- Increase a positive utilization of the environment;

5- Increase the ability to cope with challenges in a normal way.

These challenges can be of a broad range, and for a cat entering a shelter, many are unavoidable stressors. Morgan & Tromborg (2007) proposed the following comprehensive list:

- Confinement in unfamiliar, small, often uncomfortable surroundings;

- Change of daily routine;
- Disruption of social bonds and isolation;
- Reduced positive social contact with people;
- Increased negative social contact with people (restraint, medical procedures, e.g.);
- Reduced physical and mental exercise;
- Aversive, inescapable thermal or sensory stimulation including drafts, loud and sudden noise, and unfamiliar and aversive odours;
- Exposure to conspecifics especially if not previously socialized with other cats;
- Exposure to unfriendly conspecifics and unfamiliar humans;
- Reduced ability to retreat or hide;
- Boredom;
- Unpredictability;
- Lack of choices and control over interactions with the environment.

1.5.2. Types of Environmental Enrichment

While most cats have similar basic behavioural needs, effective enrichment programs should permit for individualization, monitoring and flexibility. Broadly speaking, Bloomsmith et al. (1991) have divided environmental enrichment into five major types:

1- Social

2- Occupational

3- Physical

4- Sensory

5- Nutritional

Those categories can be further subdivided and although most environmental enrichment can be organized into these types, of course these categories are not mutually exclusive, but quite often an item of enrichment can assume various functions at the same time.

The social type of environmental enrichment can be divided into a) No Contact, when the social counterpart is not in direct contact with the cat, but present in an adjacent space that allows for visual and or auditory contact, or if there is the odour of another cat or other animal/human; and b) Contact, when the social counterpart is in direct contact with the cat, either temporarily (playgroups, training/play session) or permanently. Both can be inter- or intraspecific.

Not only is human contact important, but also the quality of this contact: it should be positive, consistent, and avoid excessive restraint. This contact is especially important for kittens in the socialization phase, around two to seven weeks of age (Meier, 1961; Wilson et al., 1965). Consistent and gentle handling by a familiar person, particularly with slow petting and soothing tone of voice, can help cats to become more accepting of unfamiliar people, such as adopters, and it is useful for the shelter staff to include small treats into their day-to-day routines (Gourkow & Fraser, 2006).

Cats have a flexible social structure, whereby they can live independently or in groups, depending on availability of food and other resources (Cromwell-Davis, 2006) and group-housing can have benefits in terms of social companionship and motivation to move and play while allowing monitoring of the health and behaviour of individuals (Kessler & Turner, 1997). Groups should not exceed four to eight individuals and care should be taken in order to avoid incompatible matchings, otherwise stress becomes a problem (Alger & Alger, 2002). Well socialized juveniles and kittens may adapt most quickly to new social groupings and can greatly benefit from the socialization and exercise that cohousing provides (Landsberg et al., 2013; Miller & Watts, 2015). Care has to be taken to assure two exits from perches and hiding areas and a sufficient number of soft beds, food bowls, water bowls and litter boxes dispersed in space to minimize fighting and monopolization (Newbury et al., 2010).

Occupational enrichment includes psychological enrichments such as puzzles or elements that promote control over the environment, but also exercise which can be motivated by giving the animal access to a run or engaging it in other activity stimulation behaviours such as training or play (also referred to in the point on social and physical enrichment) (Bloomsmith et al., 1991; Young, 2003). Control is a factor in occupational enrichment of the psychological kind and may be the single most important factor in maintaining quality of life. An animal has control when it can help itself by expressing a behaviour that satisfies a need. Animals without control develop unresponsiveness termed learned helplessness (Isaacowitz & Seligman, 2007). If enrichment is done adequately, it allows cats to control exposure to humans, animals, light, temperature, and drafts; the view; and expression of locomotory behaviour, overlapping once more with physical enrichment.

Physical enrichment includes the size of the enclosure but also its complexity, the accessories used, the furniture, toys, among others.

All cats should be provided with enough space to stand and sit fully upright, lie down, turn around, walk, stretch out, and retreat to a hiding area. The available space should make separation of functional areas (sleeping, eating/drinking and elimination) possible, as many animals refrain from using the area designated as a toilet should it be too close to the food area (and vice-versa). Studies suggest areas of at least 1,70m^2 per cat and enough space to allow for an inter-cat distance of 1-3m (Barry & Crowell-Davis, 1999; Gouveia et al., 2011). If possible, "Office fostering" or a "real-life room" are systems that work very well for cats that are either very used to human contact, or are not and need to become used to a "home like" environment, as it uses furnished rooms (that may even be used otherwise by staff) at the facility to home selected cats or groups of cats. It is important to point out that it is the quality of the space, not simply the quantity, which is important for mitigating stress, so a mere increase of cage size would not have the desired effect (Miller & Watts, 2015).

The way in which a cage or enclosure is furnished and decorated can be motivating for the animal to move around and engage in exploratory behaviour. It should include retreat areas, high vantage points and separate functional areas. An area which can be visually assessed in its whole from just one point is an area that leaves little to the imagination and does not encourage exploration or allow for retreat and hiding. A retreat area that is partially or even fully hidden from view is a very important element when it comes to behaviourally cope with a stressor, as it gives the animal a choice of either facing the stressor, or not facing it that moment (McCune, 1994). Creating visual separations and distinct areas within the cage is not difficult neither does it need to be expensive: curtains, partial cage dividers, interconnected cages, hiding boxes and draping towels or blankets over parts of a cage will give animals the option of retreating to a more protected area. A plastic carrier that stays with the cat during his entire stay and can go home with the cat is ideal as it provides hiding and perching place at the same time and is easily cleanable (Miller & Watts, 2015).

Elevated perches are all time favourites, as any cat owner can relate to: besides providing a vantage point for observation while stimulating the cat to engage in further activities such as stretching, jumping, and climbing. To build these areas inside the enclosure, besides plastic carriers sturdy cardboard or plastic boxes can be used, but also shelves, hammocks or small tables (Miller & Watts, 2015).

Soft bedding is an important addition as cats have longer periods of deep sleep on soft bedding and seem to prefer sleeping on materials that are not subject to great temperature changes (McCune, 1994)

As scratching is a natural and necessary behaviour for cats, scratch post is an indispensable requisite. Besides stretching all leg and foot muscles, the outer sheath of the nail is removed and scent marks are left behind, which are communicative elements. If the space does not allow for standard scratchers, cardboard scratchers can be adapted, as well as bricks, blocks of rough wood or logs, carpet remnants, or sisal rope wound around a block of wood (Miller & Watts, 2015).

Toys are commonly provided in shelters, but they are not necessarily enriching. Whether the animal plays and with which toys depends on various factors, such as age of the animal and previous experience with toys. Toys should be matched to the specific animal intended to use them and rotated often to maintain interest (Young, 2003).

Sensory enrichment includes visual enrichment, such as the presence of windows or at least the opportunity to observe the environment; auditory enrichment such as music or, in a shelter environment the reduction of noise which is omnipresent; olfactive enrichment can be achieved through various scent-impregnated objects or the use of catnip; and tactile enrichment includes different materials used in the enclosure and various textures in toys and furniture (Newbury et al., 2010; Miller & Watts, 2015).

In the wild, animals spend a great amount of their time foraging and hunting, not only to find the food they need to survive, but also as a way of gathering information about the environment (Cromwell-Davis, 2006). Confined animals, such as shelter cats, cannot engage in these activities naturally, and an always full food bowl leads to frustration, lethargy, and weight gain. Nutritional enrichment, whether in the way the food is delivered or in the type of food used, can offer opportunities for mental and physical activity and can be part of an enhanced activity program for overweight cats (Miller & Watts, 2015). The cats natural feeding strategy would include a sequence of preying, hunting, chasing, grabbing and killing to obtain various small meals along the day (Young, 2003). To mimic this kind of feeding behaviour it is best to substitute regular food bowls for food dispensing devices or by self-made means such as scattering food in bedding or shredded paper, hiding it in nooks and crannies, creating a scent trail with tuna juice to a hidden meal, tucking food into wads of paper or empty paper towel rolls, or freezing canned food inside empty plastic bottle caps or halves of plastic Easter eggs (Miller & Watts, 2015).

1.6 Training Cats as Environmental Enrichment

1.6.1. Are Cats Trainable?

While there is a substantial history and industry associated with training dogs, there has been much less attention devoted to training cats (Zawistowski, 2015). Probably this has its roots in the domestication form of the cat, which has developed a rather unique relationship with humans, a commensal living together, which did not require much modification of feline behaviour, or even in any other traits (e.g. size and coat colour), especially when compared to dogs (Price, 2002). Dogs have always fulfilled functions and met goals in human cohabitation, being selected for specific tasks in which training probably came naturally as some degree of control was needed (Bradley, 2011; Bradshaw, 2011). Much of we expected cats to do, since earliest days (if we ever expected anything) was already patent in the natural behavioural repertoire of this species: predation of vermin, and even more specific skills such as cleanliness and the use of a litterbox (Driscoll et al., 2009; Zawistowski, 2015). As a result of this peaceful cohabitation without formal training, their different response to the highly aversive training dogs have been subject to for millennia, cats have earned a reputation of being "untrainable", which is most untrue (Hiby et al., 2004; Zawistowski, 2015). In reality, as early as 1913 scientists such as Edward Thorndike had used cats in the early development of the learning theory, discovering that they could rapidly learn how to pull looped cords or press paddled to escape from a cage. The behaviours leading to success and escape (which was also food rewarded) would be shown more and more rapidly over successive trials and those behaviours that did not lead to escape reduced in frequency. Thorndike called this the "Law of Effect" and it established the fundamental basis for the development of reinforcement theory (Thorndike, 1913). In the years since, cats have been subjects in a wide range of studies that have addressed the neurophysiology of learning, memory, sensory systems, and sleep. More recently, greater attention has been paid to the concept of training cats, and Karen Pryor and others have since demonstrated that clicker training can be used successfully with cats and this has been incorporated into a range of enrichment programs for cats in laboratories and animal shelters (Seksel, 2001; Pryor, 2003; Case, 2010; Overall, 2013).

As training is inherently a communicational exercise between two species, the way an animal communicates naturally needs to be taken into account when attempting to train it. As most other species, cats rely on four modes of communication: auditory signals, olfactory signals, tactile signals, and most importantly for communication with humans, visual signals (Overall, 2013). Auditory signals include vocalizations and are longer distance signals, working best in the present. According to Landsberg (2013) more than 23 vocalizations have been described, and the most important ones from a practical perspective are meowing, purring, growling and hissing. Olfactory signals are passible of being used beyond the present and even if the animals are separated by distance. Cats use the flehmen response to conduct pheromones to their well-developed vomero-nasal organ (Salazar et al., 2011). They also give up scent to their environment through sebaceous glands in tail, lips, chin and pads, as, for example, during hunting. Feces and urine are also used for communication purposes in different ways (Overall, 2013). Tactile signals are an essential part of social signalling and may serve as an assay for risk or comfort. Hairs on the plantar side of the carpus connected to receptors and vibration detecting corpuscles allow for sensory acuity important for predatory behaviour and play (Overall, 2013). Also, the somatosensory area of the cat is larger than that of the dog and sensory hairs (vibrissae) are found on cheeks, above the eyes and on the side of the face. All vibrissae are supplied with mechanoreceptors allowing for very precise adjustments of position which is extremely important in predation (Bradshaw, 1992; Overall, 2013) Visual signals are used when the intervenient parties are at a close distance and usually is acted upon immediately, in domestic cats they involve the use of eyes, ears, mouth, tail and coat in an interplay of expressions, body postures and movements (Landsberg et al., 2013; Overall, 2013). The facial signs are the swiftest in changing giving the most up to date information about the state of the animal. The ears are fluid and move very quickly, and pupillary changes can be very informative (Overall, 2013). A detailed analysis would exceed the scope of this study, but figure 1 gives a comprehensive overview of the various expressions. Body posture also communicates a lot of information, with special attention to overall resting body posture, head carriage, back position (arched or level), leg and tail positioning and activity.

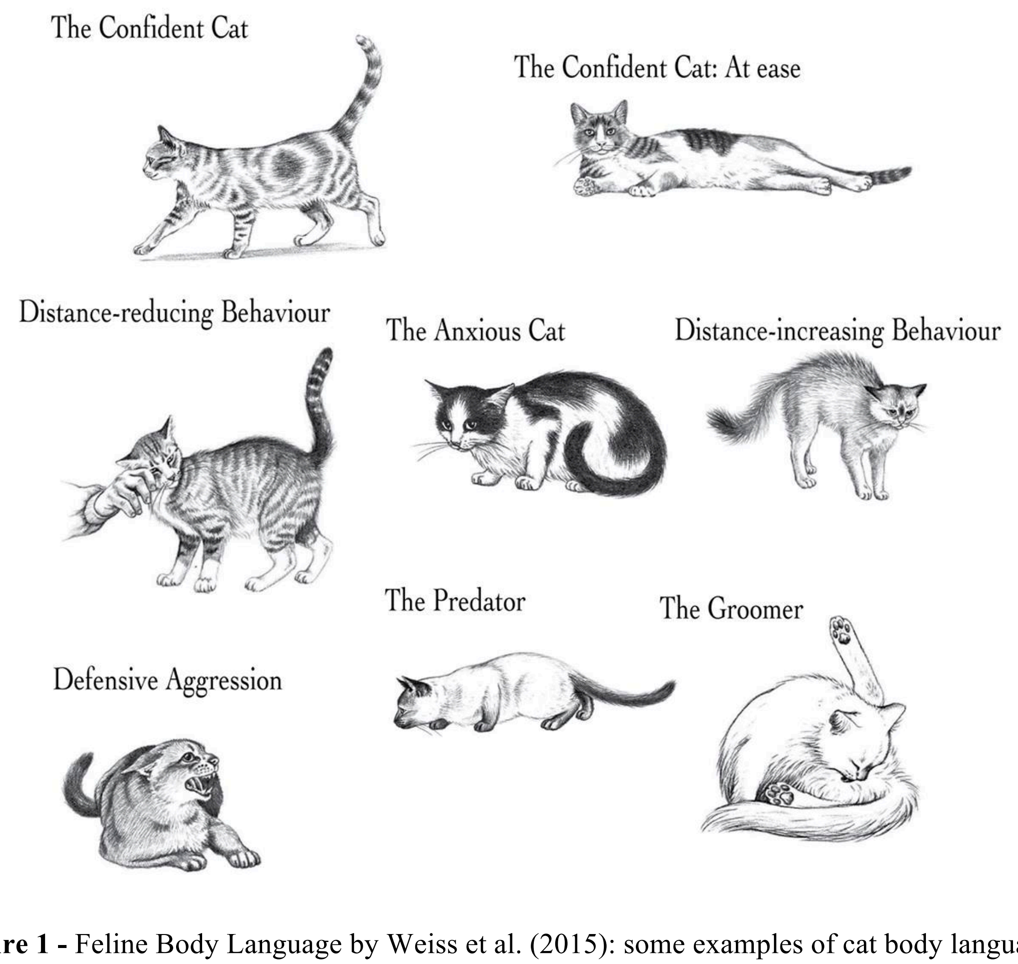


Figure 1 - Feline Body Language by Weiss et al. (2015): some examples of cat body language.

A thorough understanding of feline body language is very important in a shelter environment in order to aid in assessing the animal's state of mind as best possible, and, most importantly, to recognize abnormal behaviours which could be indicative of illness or injury and acute and/or chronic stress which impairs welfare (Arhant et al., 2015; Nibblett et al., 2015; Arhant & Troxler, 2016). To make these evaluations easier and less subjective, usually predefined scores are used, such as the Adapted Scale for Use in Homes, Shelters, or Free-Ranging Social Situations (henceforward referred to as Stress Score (SS)) of Overall (2013) (Figure 2) based on the Cat Stress Score of Kessler et al (1997), which in its turn is based on the Cat Assessment Score of McCune (1994).

Adapted Scale for Use in Homes, Shelters, or Free-Ranging Social Situations

Score	Behavior
0	• Content and quiet when unattended • Comfortable when resting • Interested in or curious about surroundings
1	• Signs often subtle and not easily detected; more likely to be detected by owner(s) at home • Earliest signs at home may be withdrawal from surroundings or change in normal routine • May seem unsettled • Less interested in surroundings but will look around to see what is going on
2	• Decreased responsiveness, seeks solitude • Quiet, loss of brightness in eyes • Lays curled up or sits tucked up (all four feet under body, shoulders hunched, head held slightly lower than shoulders, tail curled tightly around body) with eyes partially or mostly closed • Hair coat appears rough or fluffed up • May intensively groom an area that is painful or irritating • Decreased appetite, not interested in food
3	• Constantly yowling, growling, or hissing when unattended • Unlikely to move if left alone

Note: This scale sorts cats into those who are within their comfort zone and those who are not, regardless of the cause.

Figure 2 - Adapted Scale for use in Homes, Shelters, or Free-Ranging Social Situations. Allows sorting cats according to their comfort or stress level, regardless of the cause (Overall, 2013).

1.6.2 How do Cats Learn?

Learning is a complex process which can be divided in several types. This is not the primordial focus of this study, therefore the enumeration that follows aims only at a summary description of those learning types, processes and tools which assume greater importance and applicability in animal training.

Learning, per definition, is basically the long-term acquisition of information or of a behaviour in response to a repeated exposure to a stimulus (Overall, 2013). Even though the biological structures for learning are defined in the animal's genes, his individual aptitude for it is the result not only of genetic make-up but also of early experiences (Immelmann et al., 1996). The ability of altering behaviour and adapting to ever changing and new environments has great advantage over the hard-wired heritable traits that are much less flexible: through learning new information the animal does get better chances of survival and reproductive success, and this is why it is a survival tool of higher organisms (Schöning, 2006).

1.6.2.1 Associative Learning

Classical Conditioning – The most typical example of this way of learning is the Pavlovian reflex, which is the association of a neutral stimulus like the ringing of a bell to the presence of food – which naturally cues a given response - for as many times as it took to get the natural response (in this case salivation) at the mere sound of the neutral stimulus (bell), even in the absence of food (Pavlov, 1927). Animals learn through this mechanism in various day-to-day situations, some associations being positive, such as the sound of the can opener standing for food, or the fridge door signifying the possibility of treats, but others are uncomfortable or frightening such as the sight of a small bottle signifying the use of eye or ear medication or the presence of a specific carrier signifying a hated trip to the vet. The occurrence of classical conditioning depends on different factors, such as: a) contiguity – both stimuli, the neutral one and the non-conditioned one must closely follow each other, in space and time; b) continuity – this sequence of stimuli must occur unconditionally; c) relevancy – the value of the non-conditioned stimuli to the animal influences the rate of conditioning: the more important it is, the faster it happens (Case, 2010).

Operant Conditioning – This form of conditioned learning is based on the consequences of exhibited behaviour. It can also be called “instrumental learning” or “trial and error learning” and includes three events – the stimulus, the behavioural response and the consequence. The probability of repeating the behaviour is dependent on the perceived consequences of this behaviour (Laser, 2008; Case, 2010). So basically, behaviour is determined by its consequences (Laser, 2008) and therefore we have to manipulate the consequences of the animal's action if we want it to learn what we want to teach him.

Behaviour is always to be seen as any action a living being might perform, as opposed to a non-living being, and not only as activity – e.g. sleeping or standing motionless are also behaviours, even though they do not include motion (Laser, 2008). Also, it is important to recall that learning occurs permanently, and not only when we define to be "training" (Laser, 2008).

The consequences of a behaviour can be divided into:

1 – Reinforcement: which lead to an increased behaviour, in frequency as well as in intensity and duration, and which can be:

a) Positive – the addition of something pleasant to the animal, such as a food treat every time the animal approaches a stranger; or

b) Negative – the cessation of something unpleasant to the animal, such as the frightening stimulus (e.g. person) taking some steps back when the cat moves to the front of the cage instead of crouching and hiding.

2 – Punishment: which lead to a decreased behaviour, in frequency as well as in intensity and duration, and which can be:

a) Positive – the addition of something unpleasant to the animal, such as spraying the cat with water when it is scratching the couch; or

b) Negative – the cessation of something pleasant to the animal, such as closing the door that gives access to the tinned cat food which the animal likes, when he is meowing, or removing the toys when the cat is careless with his claws.

These are the four quadrants of operant conditioning, and they cannot be seen as independent and impervious, because negative reinforcement/positive punishment and positive reinforcement/negative punishment are closely related, as every reinforcement also involves a punishment and vice-versa: when the positive punishment ceases it is at the same time a negative reinforcement (Bowen & Heath, 2005; Case, 2010). Learning is a continuous and cumulative process and operant conditioning occurs as a result of all experiences in the animal's life, not only those the owner/trainer/staff is trying to manipulate consciously and this has to be brought into awareness to heighten the chances of success in using these tools.

When talking about associative learning there are three techniques that cannot go unmentioned when speaking about how to train an animal to perform a specific behaviour: we can employ to train —luring, shaping, and capturing (Pryor, 2003; Laser, 2008; Bollen, 2015).

Luring involves using something the cat wants and will follow to get the cat into a desired position which we can then reward. For example, if we want the cat to turn in a circle, we have her follow a food treat or a toy that you move around her body and then reinforce that action (Bollen, 2015).

Shaping involves reinforcing successive approximations of the behaviour until you get the final desired response. To shape a cat to turn in a circle, you would reinforce the cat for turning her body slightly to the right and then you would require her to turn her body farther and farther to the right each trial in order to earn the reinforcement until you have shaped her to turn all the way around in a circle (Bollen, 2015).

Capturing consists in reinforcing a behaviour that the animal is casually performing in that moment. If the cat should happen to turn her body in a circle in agitated expectancy of the food bowl, we could capture this behaviour when it is occurring by reinforcing it, and later put it on cue (Bollen, 2015).

1.6.2.2 Non-Associative Learning

In the category of non-associative learning we have habituation, flooding and desensitization. Habituation is the reduction of the response to a given stimulus after a continuous and prolonged exposition to this said stimulus, with no adverse or pleasurable consequences for the animal (Leussis & Bolivar, 2006; Overall, 2013) and it is the normal way for a kitten to integrate diverse non-threatening environmental stimuli into his repertoire of normality (Landsberg et al., 2013). Flooding is one of the subcategories of habituation, and it consists in exposing the animal to the full force stimulus in the hope that it might get used to the stimulus when noting that no physical harm follows – this most often does not work and may lead to increased problems (Wright & Rozier, 2008;

Yin, 2009). The other subcategory is desensitization, and in this approach the animal is exposed to the fear evoking stimulus in a weakened form (lower intensity, farther away) in a manner that does not elicit fear, the intensity or proximity slowly increasing while the animal gets used to it (Yin, 2009).

Though more efficient than flooding, desensitization is a rather weak form of behaviour modification and works best when the stimulus or object only induces a weak fear response, but it can and should be coupled with more powerful behaviour modification tools: the classical and operant counterconditioning (Wright & Rozier, 2008; Yin, 2009).

1.6.3. A Short Word on Clicker Training

Having briefly summarized learning theory, and the basics of training and behaviour modification, a short mention must be made of the powerful tool that is clicker training. Clicker training is a type of so called marker-based training which involves using a clicking signal to indicate the exact behaviour that has earned reinforcement.

This training methodology is the most effective way to train animals to perform behaviour because it involves precise and fast communication to the animal. It is based on both classical conditioning and operant conditioning and is therefore presented at this point of the text. The method involves first pairing the sound of the clicker with the delivery of a reinforcement (classical conditioning), and once the animal learns that the sound of the clicker predicts the reinforcement, the click sound is used to "mark" the exact behaviour to be reinforced, thus the clicker becomes what is called a "conditioned reinforcer." Essentially, the clicker marks the behaviour that earns the reinforcement and tells the animal that the reinforcement is on its way, even if it takes a few seconds to produce it (Pryor, 2003; Laser, 2008; Bollen, 2015).

1.7. How to Assess Affective State in Animals

Traditionally, as has been mentioned earlier, when introducing welfare and stress, the focus of welfare evaluations has been on assessing more objective parameters such as stressors that cause pain, fear, anxiety and frustration which we know to induce poor welfare, assuming that the absence of stressors inducing these negative states will presume good welfare. Recently, it has been suggested that the focus should not only lie on avoiding harm and discomfort, but also on place value on positive experiences, resources and pleasurable activities (Starling et al., 2014). Therefore, over the last decades there has been a growing interest in finding scientific and objective methods to evaluate emotional states and mental health of animals, which is not easy, as animals do not use spoken language to communicate their sensations to humans and therefore we have to rely on other types of measures to quantify and characterize feelings and sensations and thus evaluate welfare (Hotzel & Martendal, 2010). Cognitive bias measures such as the judgement bias task have recently emerged as promising tools to assess animal emotion, but before going into further detail, some terms need clarification, here as defined by Bethell (2015) (Table 1).

Table 1 - Glossary of relevant terms and concepts when referring to animal emotion and his assessment, according to Bethell (2015).

Affect	A general term that covers short-lived emotions and longer-lasting moods. Affect and mood are diffuse states. An emotion is elicited by a stimulus.
Cognition	Information processing in the brain. In its broadest sense, this includes attention, interpretation, cognitive action selection, and storage and retrieval (memory) processes.
Cognitive bias	In the field of animal cognition and welfare, this term describes the influence of affect on cognition. Positive emotions are coupled with positive cognitions, negative emotions with negative cognitions.
Emotion	A short-lived response to a stimulus that guides animals toward rewards (e.g., food and mates) and away from danger (e.g., freeze, flight, and fight).
Feeling	The subjective experience of an underlying affective state, including (conscious) awareness of (preconscious) emotion.
Judgment bias	The influence of affect on the interpretation of, and response to, ambiguous stimuli. The model presumes that positive emotion or

	mood is reflected in more positive judgments about ambiguous stimuli (“glass half full”), whereas negative emotion or mood is reflected in more negative judgments about ambiguous stimuli (“glass half empty”).
Judgment bias task	A task in which an animal is trained to discriminate between two stimuli that differ along one dimension (e.g., size, color, and location). One cue (e.g., “left location”) is rewarded, and the other cue (e.g., “right” location) is not rewarded. During a judgment bias task, an ambiguous cue is presented (i.e., an intermediate location). Speed and frequency of response reflect whether the animal judges the intermediate cue to be more positive or negative.

1.7.1. Evaluation of Affective State through Cognitive Bias Tests

Per definition, “cognitive bias” is a term that has been used in human health related literature to describe how an affective state can influence a range of cognitive processes such as the way decisions are made and how information is processed (Ehrlinger et al., 2016).

In non-human animals it has also been tested with the conclusion that the current affective state of the animal influences the way in which it judges how to interpret ambiguous signals (Harding et al., 2004). This interpretation can be more inclined to expecting positive outcomes in case of doubt, or, on the other hand, more inclined to expect negative outcomes. This specific form of cognitive bias is called judgement bias (Harding et al., 2004). A negative affective or emotional state leads to a higher expectation of negative outcomes such as negative bias when faced with an ambiguous signal, which has been referred to as “pessimism” in animal literature (Bateson & Matheson, 2007; Burman et al., 2009). Conversely, a positive affective or emotional state leads to higher expectation of positive outcomes, a positive bias, when faced with the same ambiguous signal, therefore representing “optimism” (Matheson et al., 2008; Brydges et al., 2011).

The evaluation of this emotional state is essential when evaluating welfare (Harding et al., 2004) and studies on cognitive bias can contribute in several areas of animal husbandry, such as shelters, farm animal or even privately owned pets because it can give us the necessary tools to assess if we are in the presence of optimistic ("content") animals, or pessimistic ("depressed") ones.

Therefore it becomes possible to evaluate when, if and how stress, an empoverished environment and the impossibility of adapting to the environment or changing their living conditions can affect the cognitive state of an animal (Harding et al., 2004; Paul et al., 2005; Bateson & Matheson, 2007) and if altering these factors leads to differences in the way these animals perceive their environment.

Chronic stress can be described as a long-term negative affective state induced by an accumulation of negative emotional experiences that alters an individual's interactions with the environment. In humans, chronic stress induces both persistent judgment biases and learning deficits (Destrez et al., 2013). As mentioned above, depressed or anxious animals show an increase in the probability of showing pessimist responses when confronted with a novel situation (Bateson & Matheson, 2007), in opposition to content animals in good welfare conditions which tend to overestimate their chance of success (Matheson et al., 2008).

By changing the environmental conditions the animals are subject to, in a way that is thought to induce either a positive or negative emotional state and then evaluating if the judgmental bias changes accordingly, this concept can be tested, as has been done with several different species (Bethell, 2015).

1.7.2. Advantages of Cognitive Bias Tests and the use of Judgement Bias Tasks

There are several advantages of this method over physiological measures, such as the possibility of distinguishing emotion from arousal. Physiological measures such as corticosteroids measure arousal, but they do not distinguish arousal associated with positive emotions (e.g., excitement and exploratory behaviour associated with foraging and mating activity or an enriched environment) (Mendl et al., 2010) from arousal associated with negative emotions (e.g., distress in fight or flight) (Hemsworth et al., 2015). As cognitive bias tasks are tasks in which the animal or person has to choose between response that are grouped positive and negative, and these choices should be independent of the level of arousal (Bethell, 2015).

Another advantage is that the measurements are dependent on the underlying emotions, which in the case of measurements relying on observable behaviours only, is not always representative. Some behaviours are coincident in distinct affective states: e.g. it would be difficult to distinguish an animal that is contented and sleeping from one who is depressed, feigning sleep as some stressed cats do, or suffering from learned helplessness. Judgment bias may detect more subtle shifts in emotions that lack distinguishable behavioural indexes.

The measurements are also useful when it comes to test predictions about responses following affect manipulation, which could provide a useful means of assessing the effectiveness of husbandry interventions to improve mood state. (For example, as is the focus of this study, improved environmental enrichment through training should lead to more optimistic judgment bias). A positive shift in judgment bias, confirmed by these tests (i.e., more positive responses to one or more ambiguous probes) would allow the assessment, in a non-subjective way, of improvements (or, by contrast, deterioration) in emotional or psychological state (Bethell, 2015).

1.7.3. Types of Judgement Bias Tasks

Several studies have been made over the last couple of decades to assess the importance of the mental state of animals, using different species and different methods to measure judgment bias.

Well-designed operant tasks can be used to measure an animal's judgment bias by "asking" them whether they expect positive or negative outcomes following certain behaviours. Three types of judgment bias tasks have been developed:

a) The Go/No-Go task, using tone cues has been used in dogs (Starling et al., 2014) and pigs (Douglas et al., 2012). The Go/No-Go task, using spatial cues has been more widely adopted. Animals are trained to approach one location for food and to avoid approaching another location that has no food, unpalatable food, or some other mildly aversive reinforcer. Tendency and speed to approach the intermediate probe locations are then tested. The Go/No-Go task with spatial cues has been developed for use with goats, pigs, sheep, horses, dogs, cats, mice, hamsters, rats and chickens (Bethell, 2015) and in some cases location cues were combined with colour cues or substituted by visual cues. The main criticism of this task is that a "no go" must not necessarily represent a pessimistic judgement, but may also simply stand for lack of motivation, arousal, distraction or confusion (Mendl et al., 2009).

b) The second type of judgment bias task, the active choice task with positive reinforcement, was developed to address problems of interpretation in the Go/No-Go task. In this case, the animal does not have a go-no go option, but is rather trained to distinguish between a cue that signifies a high value reward and a second cue that signifies a low value reward. Then they are tested on their responses to intermediate cues. Variables like lack of motivation, arousal, between others, are annulled because both responses imply an equivalently active performance of the animal, the third option being non-compliance with the task. Potential problems with the active choice task with positive reinforcement are that it may be more difficult to train animals to discriminate between the cues as both are rewarded, and although the test may be suitable for detecting shifts in judgments about possible future rewards, it may not be suitable for detecting shifts in judgments about possible future neutral or negative events (Mendl et al., 2009).

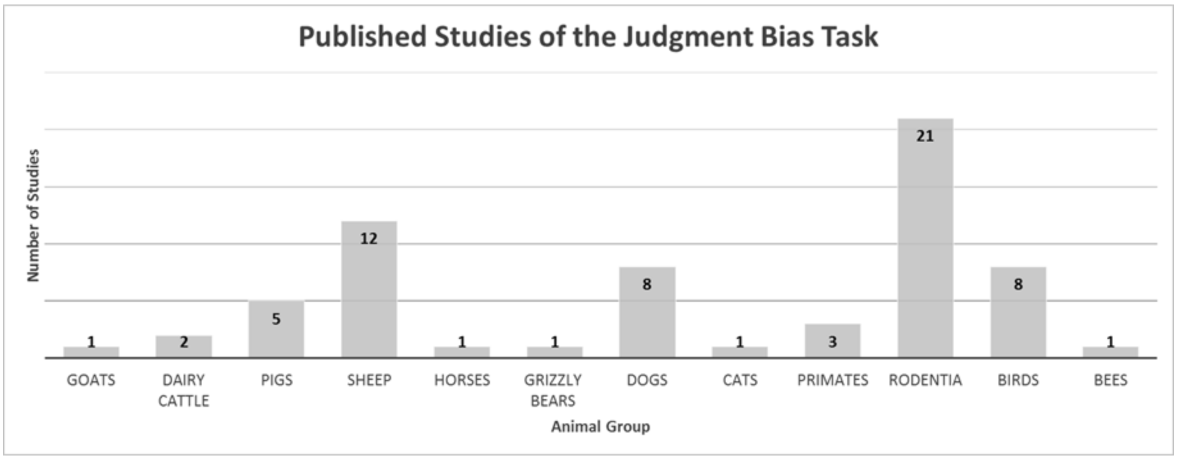
c) A third type of judgment bias task is the active choice task with negative reinforcement (e.g., electric shock) in which the animals are rewarded for responses to the positively conditioned stimulus, but responses to the negatively conditioned stimulus are now negatively reinforced so that animals "go" to the location to stop the onset of a negative reinforcer. Negative reinforcement is generally not suitable for welfare studies, but the results from this third approach, so far conducted with rats undergoing pharmacological manipulations concur with data from the Go/No-Go and active choice (reward–reward) tasks (Bethell, 2015).

1.7.4. Other Cognitive Bias Methods

The judgment bias task is not the only cognitive bias method, but it is the most explored one in terms of scientific studies (Bethell, 2015). Besides judgement bias, other cognitive bias methods that should not go unmentioned are a) attention biases, or innate biases in attention to biologically relevant stimuli (Brilot et al., 2009; Bethell et al., 2012; Cussen & Mench, 2014), b) expectancy biases, akin to optimism and pessimism regarding future events (van der Harst et al., 2003) and c) reward sensitivity, or susceptibility to despondency following loss or failure (Burman et al., 2008). These processes are closely related to, judgment biases, probably even influencing them, and they represent interesting areas of further study (Bethell, 2015).

1.7.5. Published Studies on Judgement Bias Tasks

The aim of this study is not to attempt an exhaustive analysis of all published studies on this subject, but in this section we aim to summarize important scientific information that has already been yielded by studies in this area of cognition in order to frame the undertaken experiment with the according academic overview. A review by Bethell (2015) counted 64 published studies on the subject, distributed over various animals as can be seen in Graphic 1:



Graphic 1 - Published Studies of Judgment Bias Tasks on various species, available for download in April 2015, according to Bethell (2015).

Harding (2004) did a sequence of cognitive bias tests using rats training them to respond by pressing a lever when they heard a tone associated with a positive event (food reward) and to refrain from pressing the lever as a way to avoid a negative event (loud noise) when they heard another tone. Once they had been effectively conditioned to this pattern, in a second phase, the animals were housed in two groups, one group in a so called "predictable" housing, with no negative interventions, and one group in "unpredictable" housing which included negative interventions throughout the day, inducing some stress/mild depression. Both groups of rats were then subject to an ambiguous sound which was neither clearly positive nor clearly negative, to evaluate if they would assume positive or negative consequences and thus push or refrain from pushing the lever. The choice of the rats varied according to the welfare conditions of their housing: when they were poor the rats tended to show a reduction of the anticipation of positive events, this is, they more frequently assumed that following an ambiguous sound, a negative experience might follow, not pushing the lever. This is a result similar to studies made with humans diagnosed with depression or anxiety.

Bateson and Matheson (2007), aiming to extend the work of Harding et al. (2004) developed a different cognitive bias task that is quicker to train than the operant task used with rats, and applied it to a new species, the European starling (*Sturnus vulgaris*). The idea was to find out whether birds deprived of environmental enrichment show biases in their classification of ambiguous signals, and the hypothesis was that starlings in enriched cages should be more likely to classify ambiguous signals as being associated with a positive outcome than starlings housed in standard, unenriched cages. Starlings were trained to discriminate between two visual stimuli (either white or dark grey lids) which signified distinct values (palatable and unpalatable mealworms hidden underneath). Next, the responses to unreinforced, intermediate stimuli (chromatic in-between shades) were examined while each bird was housed sequentially in both standard and enriched cages. The probability of a bird classifying an ambiguous pale grey lid as "positive" (hiding a palatable mealworm) was lower for those housed in standard cages than for those housed in enriched cages, but this difference was found only in birds that went from enriched cages to standard ones. So the pessimistic bias in birds was shown when the animals had recently experienced a decline in their environmental quality. In a similar experiment with pigs, it was also found that the animals were more likely to execute the task (approach the hatch) in response to the ambiguous cue when currently housed in the enriched environment, including an interaction between current and past environment: pigs that started in the enriched environment were less likely and slower to approach the hatch when moved to a barren environment than pigs initially housed in the barren environment

(Douglas et al., 2012). These results show that pigs, as well as starlings, have more optimistic judgement biases in enriched environments indicative of a more positive affective state (Bateson & Matheson, 2007; Douglas et al., 2012). Also, pigs that have spent time in an enriched environment react more negatively to being subsequently housed in a barren environment (Douglas et al., 2012).

These experiments, the one by Bateson & Matheson (2007) as well as the one by Harding (2004) were criticized with the argument that depressed animals as well as depressed people are less active and also less motivated by food or other generally pleasant activities (anhedonia). Therefore, rather than indicative of pessimistic bias in judgment of ambiguous stimuli, the reduced pressing of the lever and the reduced tendency of obtaining meal-worms could be due to a simple reduced motivation for food.

In 2008 the same team of scientists followed this experiment with a similar one, testing the cognitive bias of wild-caught, captive starlings housed in enriched, large cages promoting better welfare, and housed in standard laboratory cages. In opposition to the previously described studies, in this one the cognitive bias task was based on a choice procedure instead of a Go/No Go procedure. This means that the subject would have to respond in both cases (positive and negative stimuli) reducing the possible confusion between pessimism and lack of motivation previously criticized. The differential value of the reinforcements, in this case was the delay to reward – immediate in case of positive stimuli, and delayed in case of negative stimulus (Matheson et al., 2008).

In 2010 another study by Brilot et al., on starlings, produced results which were not entirely consistent with the ones described up to this moment, with this species. Contrary to predictions, changes in the level of cage enrichment had no effect on "pessimism". The cognitive bias test task was another choice procedure, this time choosing between a light colour (small reward) and a dark colour (large reward). However, the time it took the birds to choose and the probability of choosing at all suggested a rapid learning effect, as trials with ambiguous stimuli were unreinforced. An interesting second hypothesis explored in this study was if differences in stereotypic behaviour (repetitive somersaulting) were indicative of "pessimism", which was found to be true, as birds that somersaulted were more likely to choose the dish associated with the smaller food reward in the presence of the most ambiguous discriminative stimulus. Therefore it was concluded that somersaulting is indicative of a stress response to captive conditions that is symptomatic of a negative affective state (Brilot et al., 2010). Abnormal stereotypic behaviour is widespread among captive animals and largely associated jeopardized well-being (Brilot et al., 2010; Pomerantz et al., 2012). However, some behaviours may be better indicators of stress than others and perhaps not all of these repetitive, unvarying and apparently functionless behaviours assume the same significance: in a study with capuchins (*Cebus apella*) it was found that monkeys with higher levels of stereotypic head twirls exhibited a negative bias while judging ambiguous stimuli and had higher levels of faecal corticoids compared to those with lower levels of head twirls. Levels of stereotypic pacing, however, were not correlated with the monkeys' emotional state (Pomerantz et al., 2012).

As welfare evaluations are a paramount concern in the production of livestock, some years after the first cognitive bias tests on laboratory species (rats and starlings), the studies to measure emotional states via judgement bias on livestock species started to appear. The first was a spacial location task requiring a go/no go response to detect pessimistic-like or optimistic-like evaluations of the environment by sheep released from stressful situations. It showed that the recently released sheep had a more positive emotional state, even though they were stressed, or a lesser perception of risk, than that exhibited by control sheep (Doyle et al., 2010). Similar findings were recorded in a study that reports a more positive judgement bias in sheep after they were released from an acute stressor, shearing (Sanger et al., 2011). So the removal of an acute stressor such as confinement or being immobilized for an intervention such as shearing could reflect a more optimistic affective state (Doyle et al., 2010; Sanger et al., 2011). In goats the same seemed to apply, to an extent, as a study from 2013 found that after several years of good care, rescued goats displayed optimistic moods (females) or similar moods as controls (males) triggered by release from long-term conditions of poor welfare (> two years). This suggests that goats probably recover from neglect, and that sex differences in mood potentially exist (Briefer & McElligott, 2013). In a study with captive rhesus macaques (*Macaca mulatta*) touchscreen responses to ambiguous stimuli were measured to see if and how they were affected by husbandry procedures (environmental enrichment, and a health check involving restraint and ketamine hydrochloride injection) (Bethell, et al., 2012). In this case monkeys made fewer responses to ambiguous stimuli after having been contained for the health check compared to those individuals in enriched cages (Bethell et al., 2012). This suggests that, differently from the findings with sheep (Sanger et al., 2011), the greater expectation of negative outcomes following the health check was more powerful than the release from an acute stressor. Shifts in affective state following standard husbandry procedures may therefore be associated with changes in cognitive bias (Bethell et al., 2012).

Some studies also experimented pharmacologically to improve knowledge on the relation between emotions and the judgment of the environment. Diazepam can be used to manipulate the affective state as it is known to reduce fearfulness, so a study was devised to assess whether it would induce more optimistic judgements in sheep. Treated lambs apparently display a more positive judgment of an ambiguous event than control lambs. Reduction of fearfulness may thus induce a more positive affective state (Destrez et al., 2016). To test the involvement of the serotonergic system in judgmental biases, a study by Doyle et al. (2016) in which sheep were administered a serotonin inhibitor (Chlorophenylalanine (pCPA)) to simulate a depletion of brain serotonin and assess if this would induce negative judgement biases in sheep. The treated sheep did approach the most positive ambiguous location significantly less than the untreated ones, which suggests pessimism, which supports that judgement bias is a cognitive measure of affective state, and that the serotonergic pathway may be involved (Doyle et al., 2016). A study from 2014 investigated whether the opioid system is involved in the formation of judgement biases in sheep. The sheep's affective state was altered by the presentation of either palatable food (inducing a more optimistic bias), and unpalatable food (less optimistic bias). Then, to one group of sheep, morphine was administered which supposedly would further enhance the optimistic judgement bias in a spacial location task requiring a go/no go response after consumption of a food reward and reduce a pessimistic bias after receiving unpalatable food. The results show that consumption of a food reward induced an optimistic judgement bias and suggest that morphine administration further enhanced this optimistic judgement bias (Verbeek et al., 2014-a). Oxytocin, which is also involved in the modulation of human optimism and emotional processing, influences how dogs judge ambivalent situations: In dogs, the administration of this hormone seemed to induce a positive cognitive bias (Kis et al., 2015). The fact that a pharmacological treatment of a negative affective state and associated behaviours in a non-human species can produce a shift in cognitive bias, demonstrating that not only behaviour improves, but also psychological state (welfare) has also been studied: In a study by Karagiannis et al, in 2015, dogs showing signs of stress and separation anxiety (vocalizing, destruction of property, and toileting) were treated with fluoxetine and set on a standard behaviour modification plan for two months. They were then evaluated using a spatial cognitive bias test to evaluate changes of affective state. Prior to treatment, the dogs reacted negatively to ambiguous positions in the cognitive bias test negatively compared to control dogs, while on weeks two and six of treatment they displayed similar responses in the cognitive bias test to control dogs (Karagiannis et al., 2015). Another study on dogs had already indicated that dogs suffering of separation anxiety might show pessimistic cognitive biases (Mendl et al., 2010).

A study on effects of food restriction on cognitive bias and the involvement of ghrelin in its regulation found that ghrelin administration tended to induce a pessimistic judgement bias and increase plasma cortisol concentrations (Verbeek et al., 2014-b). Besides, chronic food restriction tended to induce an optimistic judgement bias (Verbeek et al., 2014-b) although the study design was of the go/no go type, which arises the same issues criticized in the works of Harding (2004) and Bateson (2007): how motivation, in this case hunger caused by food deprivation is different from a positive or negative cognitive bias (Matheson et al., 2008). In a test with horses, trained by either Negative Reinforcement (NR) or Positive Reinforcement (PR), NR mares showed a more optimistic mood compared to PR mares, although they were experiencing more negative emotions during training. NR mares, just as mentioned above, could have been more motivated to obtain a food reward than PR mares, which had been rewarded throughout the treatment phase, or the optimistic bias could be triggered by release from stressor of NR during training. Similar results were found within a group of beagles, also subject to a cognitive bias task after being offered food rewards (Burman et al., 2011).

Several studies analysed the effect of chronic stress on judgement biases in sheep, reaching the conclusion that those sheep subject to chronic stress took longer to approach the ambiguous locations of the bucket, which is equivalent to a more pessimistic judgement bias, besides also exhibiting learning deficits (Destrez et al., 2013). More importantly, for welfare, this seems to be reversible, as another study came to prove, repeatedly exposing chronically stressed sheep to positive events, which induced optimistic-like judgment (Destrez et al., 2016).

The relation of pain and cognitive processes had not been explored until recently, the first evidence of cognitive bias in response to pain in a non-human species being a study from 2013 in which dairy calves were tested in terms of judgement bias before and after the routine practice of hot-iron disbudding. After disbudding calves were more likely to judge ambiguous stimuli as negative (Neave et al., 2013). This 'pessimistic' bias indicates that post-operative pain following hot-iron disbudding results in a negative change in emotional state, similar to that shown by calves after separation from the dam in a subsequent study by the same group of scientists, which provided the first evidence of pessimistic judgement bias in animals following maternal separation (Daros et al., 2014).

1.8. Aim and Hypothesis of the Study

After the period of the author's traineeship at the Municipal Animal Shelter (MAS) in Sintra, and having had the opportunity of visiting several other shelter-like facilities in the metropolitan area of Lisbon, we have reached the conclusion that tools such as environmental enrichment in housing of shelter cats need to be further explored in Portugal. Cats housed under empoverished/barren conditions do in fact show negative changes in their behaviour and cats in sub-optimal welfare conditions will also be cats that are less outgoing, less sociable, and therefore less attractive for potential adopters – besides more susceptible to health problems (Boissy et al., 2007; Richter et al., 2012; Tanaka et al., 2012; Arhant & Troxler, 2016). In a shelter environment, of course there are always some economic constraints so studies are needed to assess the importance of the various factors of welfare and QoL so it becomes easier to objectively prioritize the allocation of resources. In these evaluations of welfare there is still a clear tendency of focusing mainly on the physical and medically assessable health in detriment of somewhat less easily assessable behavioural health and affective state. Our idea was to bring palpable proof of the importance of environmental enrichment, specifically in terms of social enrichment, to the shelter reality (Table 2), for the second most numerous animal admitted to governmental shelters and probably also in private facilities.

Table 2 - Data regarding animals housed in MAS and respective outcomes. (With kind permission of Professor Yolanda Vaz, Portuguese National Autority for Animal Health)

Year	Cats housed in MAS	Cats returned to owners	Adopted cats	Euthanized cats
2013	6.138	340	2.136	2.821
2014	6.947	401	3.466	2.302
2015	6.486	184	3.289	1.987

The aim of this study was to assess the impact of training, as an environmental enrichment tool, on the welfare of shelter cats.

Our hypothesis was that training, being a specific type of social enrichment would positively influence the affective state of the animals, and therefore improve their welfare. To evaluate this, a study was conceived which assessed the effect of environmental enrichment (training) using a spatial judgment task. Cats submitted to training sessions were expected to have a more positive affective state and thus to respond more optimistically to ambiguous stimuli when compared to other cats.

Chapter 2 - Material and Methods

2.1. Subjects

The MAS of Sintra does not yet have an established socialization program but the cats are exposed to varying levels of human contact during day-to-day activities at the shelter. Accordingly, the cats showed various levels of sociability and handleability.

From their arrival the cats were housed in one of the two interior rooms designated to housing cats, in wire-meshed indoor cages (1m × 1m × 1m; length × width × height) with front openings, that each contained one litter tray with shredded newspaper or sand, and some had an uncovered tray as a bed, others a covered one. Water and dry food were available ad libitum. Lighting was dependent mainly on external conditions but temperature was controlled. Environmental noise was usually loud, not only due to the presence of various vocalizing cats but mainly due to the presence of dogs in the same facility and day-to-day shelter activity. There was a constant mixture of scents originating from various sources: cats, people, dogs and from outside the facility. Figure 3 and Figure 4 show an example of the cages used.

Figure 3 - One of the cages used at the MAS in Sintra, including food and water bowl, bed and litter tray (original).

Figure 4 - The same cage with the door open, for better notion of size (original).

2.2. Inclusion and Exclusion Criteria

From all those cats housed in the MAS of Sintra, the choice of cats for this study was random, but to achieve some uniformity and avoid certain biases in the study group, inclusion and exclusion criteria were defined.

Only those animals which complied with all inclusion criteria were admitted to the study, and they could be excluded before or during the study if this ceased to be the case, or if any of the exclusion criteria were fulfilled.

Inclusion criteria:

- age > six months;
- no signs of fear or aggression towards people;
- no signs of illness;
- > one week shelter;
- If housed in pairs or small groups, only one cat per group*.

Exclusion criteria:

- signs of Illness**;

- fearful or aggressive behaviour;
- adoption or death;
- unable to adapt to the protocol.

**In this case we chose the cat most comfortable with human presence, or the one quickest to adapt to the carrier.*

***When considering absence of illness, we are referring to cats whose health showed no evidence of pathology capable of causing pain or altering the behavior of the cat.*

2.3. Study Design

Aiming to test our hypothesis, this study was projected and applied, using as a guiding protocol the one used by Tami et al (2011), also with cats. This is a judgement bias task of the type Go/No Go, with spacial cues. The study design is presented below.

When applying the study latency times for each ambiguous position are recorded and shorter times, possibly more similar to those measured in the previous phase for the R (rewarded) position, indicate that the cat expects to find a reward in this ambiguous position too, even though it is not the usual position for rewarded bowls. This in its turn indicates higher levels of optimism in this cat. Conversely, longer times, possibly similar to those measured for the U (unrewarded) position indicate that the cat does not expect to find food in an unusual position of the bowl. This "lack of hope" is related to pessimism and consequently a more negative emotional state.

The study comprises five phases:

- first phase: habituation to carrier and to experimental room;
- pre-test phase: according to the random group assignment;
- second phase: habituation to the test apparatus;
- third phase: training for the test;
- forth phase: test.

Before going through all the phases in detail, as we will, further ahead, for better comprehension of the reader the process is explained by a flowchart which gives a schematic overview (**Figure 5**: Flowchart).

1st phase: Habituation to carrier and to experimental room (blind)

- Cat: _________

- **day 1**: __/__/____

- 20 min

- Stress level (SS): ___

- Cat: _________

- **day 2**: __/__/____

- 20 min

- Stress level (SS): ___

If stress level (SS) **day 2** = 2 or 3

- Cat: _________

- **day 3**: __/__/____

- 20 min

- Stress level (SS): ___

If stress level (SS) **day 3** = 2 or 3

Cat EXCLUDED

If stress level (SS) **day 3** = 0 or 1

If stress level (SS) **day 2** = 0 or 1

RANDOM GROUP ASSIGNMENT

Pre-test phase (training):

- 2 weeks;

- 30 minute periods of training (daily);

- week 1:____________, ___number of acquired behaviours;

- week 2:____________, ___number of acquired behaviours;

- NOTE: 0 acquired behaviours in 1 week => EXCLUDED

OR

Pre-test phase (play):

- 2 weeks;

- 30 minute periods of play (daily);

- week 1:____________

- week 2:____________

OR

Pre-test phase (not enriched):

- 2 weeks;

-30 minute periods of presence in test room (daily);

- absence of interaction;

- weeks 1:____________

- weeks 2:____________

Resource motivation test (same day or following day):

- day: __/__/____, morning/afternoon

- max 10 min

→

2nd phase: Habituation to test apparatus

Alternate with pre-test phase (morning/afternoon or every other day)

- 3 days minimum and 5 days maximum;

- **day 1**: __/__/____

- **day 2**: __/__/____

- **day 3**: __/__/____

- 20 min/day;

- relase cat 1 min and then put it back in the carrier between 15 to 30 seconds to manipulate the test apparatus, as a simulation. No food given.

- repeat procedure 12 times

After 3rd day before putting cat away –> evaluate stress level (SS): ____

If stress level (SS) on **day 3** = 2 ou 3

Repeat + 1 day:

- **day 4**:__/__/____

- stress level :____

If stress level (SS) on **days 3, 4 or 5** is = 0 or 1

If stress level (SS) on **day 4** = 2 ou 3

Repeat + 1 day:

- **day 5**:__/__/____

- stress level :____

If stress level (SS) on **day 5** = 2 ou 3

Cat EXCLUDED

Resource motivation test (same day or following day):

- day: __/__/____, morning/afternoon

- max 10 min

→

3rd phase: Training for the test

Alternate with pre-test phase (morning/afternoon or every other day)

- 3 days until max. 9 days; R LEFT or RIGHT

- **day 1**: __/__/____ sequence: _________________

- **day 2**: __/__/____ sequence: _________________

- **day 3**: __/__/____ sequence: _________________

- **day 4**: __/__/____ sequence: _________________

- **day 5**: __/__/____ sequence: _________________

- **day 6**: __/__/____ sequence: _________________

- **day 7**: __/__/____ sequence: _________________

- **day 8**: __/__/____ sequence: _________________

- **day 9**: __/__/____ sequence: _________________

- Mnn test + -> one group takes longer than the other (U takes longer) (reject H0).

Discrimination:

- If 2 days (one after the other) Mnn test + => success, continue to TEST

- If 0 or only 1 day Mnn test +, or 2 days but alternate => add one more day, up to 9 days

- 9 days without 2 days Mnn test + => cat EXCLUDED

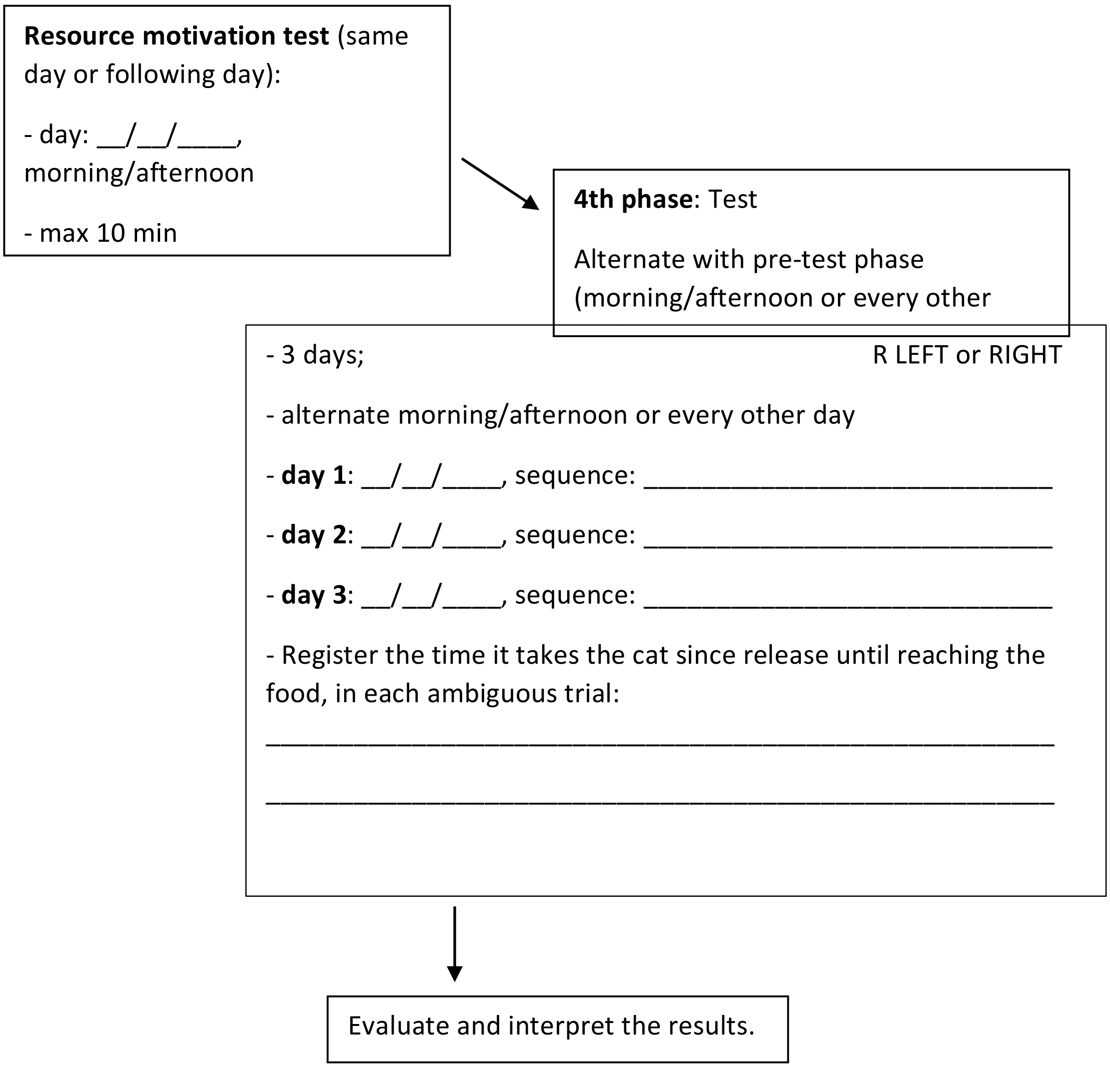


Figure 5 - Flowchart.

2.3.1. First Phase – Habituation to the Carrier and to the Experimental Room

This phase focuses on training the cat to accept the carrier and enter it. The training started with a habituation to the presence of the carrier, only then the cat entrance in it, and finally to the motion of the carrier while the cat is inside. As it is a "blind phase", i.e. a phase that would occur independently of the group the cat would later be part of, clicker training was avoided. Habituation, classical and/or operant conditioning, and also some food treats were used in the process (tinned cat food).

This first phase should not extend for longer than two or three days, but as it sometimes took longer and the number of cats available was limited we chose not to limit the number of days, provided it did not prejudice the study.

When the cats were already trained to enter the carrier, and before randomly grouping the cats, all animals were subject to two or three sessions, one per day, during which the animals were habituated to the experimental room. The animals were put in the carrier and taken to the room, where they were released and left to explore the room for 20 minutes. The stress levels were evaluated according to the SS (Overall, 2013) (refer back to figure 2) and animals with persistently high scores (>2, which implies that they are out of their comfort zone) were excluded from the study.

At the end of this phase the group to which the cat would belong was randomly determined.

2.3.2. Pre-Test Phase

In this phase the animals were divided into three groups, and each of these groups subjected to differential treatment for two consecutive weeks. After those two weeks, and while the cat was taking part in the study, this differential treatment continued, but intercalated with the other phases either morning/afternoon or on alternate days.

The three, randomly attributed groups with differential treatment were:

- enriched using training (EuT);
- enriched using play (EuP);
- not enriched (nE).

Independently from the attributed group, in this phase sessions were daily, on weekdays, over 30 minutes. Depending on the group, these sessions comprised:

EuT: clicker training – after first conditioning the cat to the sound of the clicker (classical conditioning) (“charge” the clicker) it was then proceeded to teach the animal various behaviours using small food rewards as positive reinforcement. The behaviours taught were:

- ✓ Sit (Figure 6)
- ✓ Look (Figure 7)
- ✓ Enter carrier
- ✓ Stay in carrier

- ✓ Jump (over obstacle)
- ✓ Get in box
- ✓ Sit pretty (Figure 8)
- ✓ Wave paw
- ✓ Heel
- ✓ Target
- ✓ Paw touch (object)
- ✓ Meow
- ✓ Roll over
- ✓ Turn around
- ✓ Slalom through legs (Figure 9)
- ✓ Coming when called (cue = clicking fingers two times)

At least two behaviours had to be acquired over the time of two weeks for the cat to be considered apt and within normal learning speed.

Figure 6 - Training "sit" (original).

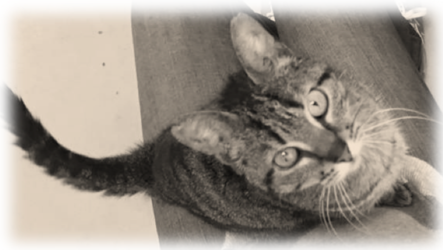


Figure 7 - Training “look” (original).

Figure 8 - Training “sit pretty” (original).

Figure 9 - Training “slalom through legs” (original).

EuP: play – with human and using various toys. Feathers, balls and other cat toys were used, but no food and no prompting for specific tasks (Figure 10).

Figure 10 - Cat playing (original).

nE: minimum levels of interaction. In the same room as the other cats, but without play or training. In these cats handling was kept to the minimum necessary, besides carrier training. Toys were removed from the room.

2.3.3. Second Phase – Habituation to the Test Apparatus

Before the training for the test, in the third phase, as we shall see further ahead, the cats were habituated to the experimental arena and the manipulation protocol. This phase serves the purpose of eliminating biases and sources of stress in the subsequent phases, as cats will not encounter the test apparatus for the first time once they reach phase three.

The experimental arena was the training room, a rectangular space of 3,97m × 2,27m. As this room was very close to the other infrastructures of the MAS, care was taken to reduce external stimuli as much as possible, isolating door and windows with foam applications and removing all physical stimuli from the room, leaving just the author's desk and the objects necessary to the completion of the test. The outlay of the arena can be seen in Figure 11. The start point (B) was made of a cat carrier to which a manually operated guillotine door, made of opaque acrylic, had been adapted (Figure 12). A video camera (GoPro Hero2®) (c) set up in one of the walls recorded the experiment.

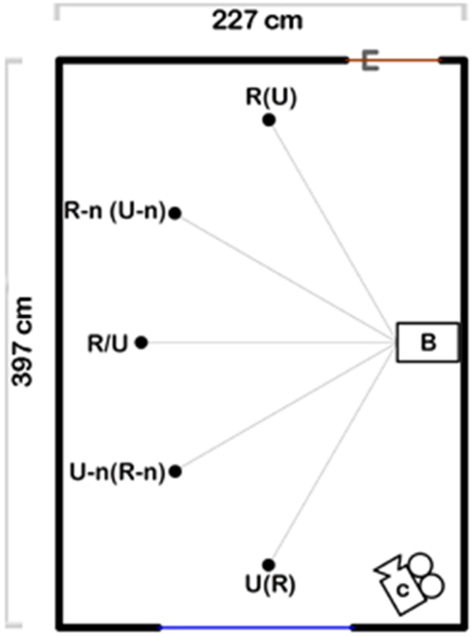


Figure 11 - Experimental Arena. Label: B = start point; U = unrewarded position; R = rewarded position; U-n/ U, R-n = intermediate positions; c = video camera; U-R: 297cm; B-all positions: 160cm; 100cm between contiguous positions. (R and U, and the intermediate positions consequently, were alternated between right and left).

Figure 12 - Carrier to which a manually operated guillotine door, made of opaque acrylic, had been adapted (original).

This phase takes place during three to five days, with daily 20 minutes sessions. The cat, in the carrier, was put on B, covered with a towel so the cat cannot see what is going on in the room. Next, the protocol to be used in the following phases is simulated: the cat is released, using the guillotine door (the researcher manipulates the door of an impartial position), and one minute after is conducted to the carrier again, where it waits about 20 to 30 seconds, only to be released again. This is repeated 12 times, including simulations of the noises the cat might encounter during the real test (e.g. cans opening, bowls scraping).

Cats were considered to be habituated to the experimental arena and to the procedure when they could be categorised as having a score of zero or one in the Overall (2013) Stress Score (refer to Figure 2). Cats that score lower than two (zero or one) after three, four or five days, proceeded to the next phase, while cats that maintain higher Stress Scores after five days were excluded from the study.

2.3.4. Resource Motivation Test

This test serves the purpose of assessing if the animal has motivation for a given resource and will work to obtain it. It does NOT establish food preferences among various types of food. It preceeds the second, third and fourth phase. The first time it is applied serves the purpose of familiarizing the cat with the procedure, the second and third times it is then used to determine if the resource (food) is valued.

The test included three trials, in each one: were presented three bowls (Figure 13), one with dry food for kittens and two others with canned food of two different flavours; always presented in a different order; all at the same distance from de carrier and between each other. The bowls were not interchanged. When released from the carrier, without having seen the manipulation of the bowls, it was recorded which food the cat preferred (which would be the one it chooses to eat) in each trial, and the resource chosen more than one time was the one used for this cat in posterior steps. If the cat chose a different type of food in each one of the three trials, a fourth trial was added.

Latency to approach the bowls wasn’t measured but the trials only count when cats go to the bowls as soon as they see them, and within one minute maximum time.

Figure 13 - The three bowls for the resource motivation test (original).

2.3.5. Third Phase – Training for the Test

The training for the test took a minimum of three days and included one cat at a time, and an opaque plastic bowl into which a transparent silicone lid fixed half-height, invisible from the outside, which while allowing scent exchange. When fitted, the lid does not allow the cat to reach the food, when not fitted the food is accessible, but this cannot be inferred from the outside/the cat's perspective. The bowl served as a goal object: a cat introducing his head into it indicated the animal's decision to access the food contained within it.

During the training period, each cat was exposed to 12 trial sessions for the number of days needed, a minimum of three, to discriminate between the rewarded (R) and the unrewarded locations (U). In each trial, the bowl was either in R, containing half a spoonful of accessible palatable cat food, or in U, containing half a spoonful of inaccessible canned cat food. The food used was the one previously chosen by the cat in Resource Motivation Test (referred on 2.3.4.). For one half of the cats, R was on the left side of arena and for the other it was on the right. The location was attributed alternately. This serves the purpose of reducing biases related to the disposition of the room such as the presence of the window on one side, or the location of the door. In each session, half the trials were rewarded and half unrewarded, the sequence being pseudo-random with no more than two consecutive presentations of the bowl in the same location and equal numbers of both locations in trials 1–6 and trials 7–12, e. g. U-R-R-U-R-U-U-R-U-U-R-R (an excel sheet was used to create sequences which respected these criteria).

In each trial, we registered cats' latency (in seconds) to introduce their head into the bowl since they leave B. Once this occurred, we waited until the cat had eaten the food (rewarded location) or simply waited 20 s after the head had been introduced in the bowl and then returned the animal to the carrier with the guillotine door at B for the time necessary to prepare the following trial (around 30 s). There was a one min cut-off point, and if the cat failed to put his head into the bowl within this time, the animal was returned to B and the next trial was prepared. In these cases the latency registered was of 60 s.

This phase was filmed to allow an easier measuring or confirmation of the latency times.

Cats were considered to be able to discriminate between reference locations when they showed a significant difference in their latency to approach the rewarded and unrewarded locations for two consecutive days (discrimination criteria), which was assessed using a Mann Whitney Test. When the test rejects H0 this indicates that one approach takes longer than the other, and if this happens on two subsequent days, we can consider our criterion achieved. A maximum of nine days was defined for the cats to reach this goal.

2.3.6. Forth Phase – The Test

The test comprises three sessions of 13 trials each, one per day. In each session cats were exposed to three ambiguous locations (R/U, R-n, U-n, refer to figure 10), interspersed within a sequence of R and U's. The sequence consisted of 13 trials: five rewarded, five unrewarded, and three unrewarded ambiguous trials, respecting the same rules used in the sequences of the previous phase. In such a sequence, the ambiguous trials were five, nine and thirteen and their order was counterbalanced over the three days (to create these sequences an excel sheet was again used).

Black tape was used to mark the five locations on the floor.

Latency to approach the bowls in the ambiguous locations was recorded similarly to the previous phase, including video recording.

Once more, the food used was the one previously chosen by the cat in the Resource Motivation Test (referred to on 2.3.4.).

The protocol used in this study is an adaptation of the one Burman et al. (2008) used with rats and Tami et al. (2011) used with cats.

3. Results

The following table (Table 3) summarizes the cats that were tested up to some phase of the study, and are, therefore, the initial group from which our n=8 was cast.

Table 3 - Cats that participated in the study. Animals organized by numbers in chronologic order; gender; phase of study reached; group attributed; reason of exclusion if excluded.

Label: M – male; F – female; EuP – enriched using play; EuT – enriched using training; nE – not enriched.

Cat	Gender	Study phase	Group	Status progress
1	M	1st phase/3rd day (Stress Score =0) -> ok to next phase	?	Included -> adopted -> excluded
2	M	Carrier training	?	Included -> identified gingivitis -> excluded
3	F	Pre-test phase, 8th day	EuP	Included -> adopted -> excluded
4	F	Done	EuP	Included
5	F	Carrier training	?	Included -> adopted -> excluded
6	M	Done	EuP	Included
7	M	Lengthy carrier training in his own crate + 1st phase/3rd day (Stress Score=2)	?	Included -> unable to adapt to the protocol -> excluded
8	M	Done	EuT	Included
9	F	Done	EuT	Included
10	M	2nd phase/3rd day (Stress Score = 0) -> ok to next phase	EuT	Included -> adopted -> excluded
11	M	3rd phase/1st day	EuT	Included -> adopted -> excluded

12	M	1st phase/2nd day	nE	Included -> aggressiveness -> excluded
13	M	Carrier training	?	Included -> adopted -> excluded
14	F	Carrier training	?	Included -> adopted -> excluded
15	F	Lots of carrier training in his own crate + 1st phase/3rd day (Stress Score=2)	EuT	Included -> unable to adapt to the protocol -> excluded
16	M	Pre-test phase 1st day	EuT	Included -> worsening of the injury -> excluded
17	F	Pre-test phase 11th day -> training does not seem to be effective	EuT	Included -> unable to adapt to the protocol -> excluded
18	F	Done	nE	Included
19	F	Done	EuT	Included
20	M	3rd phase/2nd day + pre-test phase 18th day	EuT	Included -> adopted -> excluded
21	F	Done	nE	Included
22	F	Pre-test phase 4th day	EuT	Included -> adopted -> excluded
23	M	Done	nE	Included
24	F	Pre-test phase 7th day	EuT	included -> sterilized -> sick -> death

From this initial group of twenty-four cats considered for the study, a total of eight (three EuT, two EuP and three nE) completed the test, as nine were excluded because they were adopted, three because they were unable to adapt to the protocol, not fulfilling the criteria established for passing through to the next phase, three because of poor health (in one case death) and one because of behavioural issues (ongoing aggression). The phase in which the cats were excluded was variable. Therefore, eight domestic short-hair cats, three males and five females, all neutered, all adult, were the subjects of the complete study. Precise ages were difficult to determine as the animals were all rescue cats at the MAS of Sintra.

Analysing the data of the eight cats that completed the study, we realized that cat number 9 probably did not comply with the admission criterion for phase four. When reviewing the video recordings it became clear that although the passing criterion was complied with, in the three days of testing she did not seem to make a difference between R and U, not maintaining the previously established criterion. This animal was excluded from the test, as will be discussed with further detail in point 4.

3.1. Data Collection and Statistical Analysis

Only descriptive statistics have been used to summarize data as the final n=7 (two groups of two cats and one of three) was too small to allow for further analysis. The data was analyzed using Microsoft Excel®.

Although we have data on how many days it took the cats to achieve the distinction criterion between R and U, this is not very relevant in terms of the study's aim. Data on the latency (in seconds) of each cat to reach ambiguous locations is more relevant, and will be discussed.

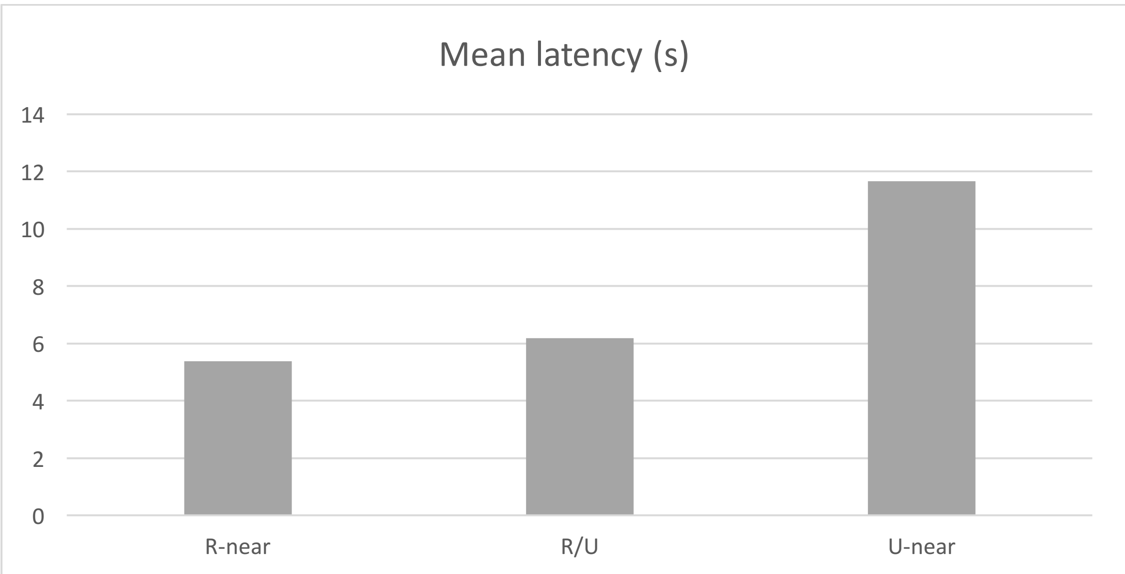
The time (in days) that it took each cat to distinguish R and U is presented in the following table (table 4).

Table 4 - Number of days it took each cat to distinguish between R and U. Organized by group the cat belongs to.

Group	Cat	Days
EuT	8	6
	19	4
EuP	4	3
	6	3
nE	18	3
	21	7
	23	6

The average number of days needed to distinguish R and U was of 4.75, with minimums and maximums of three and seven days, respectively. We can also highlight the fact that this average was of four for the EuT and EuP cats grouped together, and slightly above five (5.33) for the cats of the nE group.

As happened between groups, differences were also found between the three ambiguous positions. Graph 2 allows a comparison of average latency (in seconds) until cats reach the various positions, independently from the group they belong to.



Graphic 2 - Average latency (in seconds) for each position.

The mean latency to reach an ambiguous position was much smaller for R-near (=5.38s) than for U-near (=11.67s).

On the other hand, average latency times (in seconds) until cats reach the various ambiguous positions for each group are summarized in table 5.

Table 5 - Average latency times for each group and each positions.

	EuT	EuP	nE
R-near	3.17	8.67	4.67
R/U	8.67	5.00	5.33
U-near	11.33	12.33	11.44

Latency to reach U-near was similar in the three groups. In contrast, more differences were found in the latency to reach R-near position, where trained cats showed a shorter latency. Those cats belonging to the EuT group took, in fact, longer to reach this position R/U than those belonging to the other groups, which we analysed and attempt to explain in point 4.

4. Discussion

When analysing the results regarding the mean latency to reach the ambiguous location, transversally to all groups, it was much smaller for R-near ($\bar{x}$=5.38s) than for U-near ($\bar{x}$=11.67s), which indicates that the animals not only assimilated the concept of rewarded and unrewarded location but they also assume that closeness to the rewarded location may indicate a higher probability of reward. Latency to reach U-near was similar in the three groups. In contrast, more differences were found in the latency to reach R-near location, where trained cats showed a shorter latency as expected, which may be indicative of a more positive cognitive bias. Even though this is a promising result in terms of improving mental state through social environmental enrichment, a larger sample would have to be obtained in order to undertake conclusive statistical analyses. However, these preliminary findings show that cats undergoing training enriched sessions seem to have a more optimistic response bias towards ambiguous stimuli which reinforces the importance of the use of environmental enrichment in shelters. Although all of the above is true and wanted, the results for the position R/U were unexpected as the trained cats were, interestingly, slower to reach the position to look for food, which may be explained by the fact that they were the most motivated to get out of the carrier to run to the rewarded position R that as soon as they got out of B they always went to R first and took a moment to start and look for another option if the food bowl was not there.

Retrospectively we understood that it would have been interesting and profitable to have the different time-spans it took the cats to understand the difference between the R and the U position, as this could have been used as a further tool of comparison among groups. This should be taken into account in subsequent studies.

Initially this study was designed to be an independent work similar to those of Burman et al. (2008) and Tami et al. (2011), but along the time dedicated to the study it became apparent that due to time constraints, the limited duration of the study and the number of available cats this would not be possible, which is why we assume it to be a preliminary study. Hopefully it will be followed by similar studies yielding more statistically robust results. One advantage of the existence of such a preliminary study is, in fact, the opportunity of detecting flaws or biases to avoid in the future. Some of the most relevant flaws identified were: Disparity between the elements of the sample (age, previous history, mental status, health); difficulty of controlling variables besides the ones evaluated by us (training, play or no interaction) which could eventually bias the study; spatial and logistic constraints; limited human and material resources; dependence of our sample on the fluctuations of the MAS population.

As for the fluctuation of the cat population, the study was meant to use a sample of 30 animals, with three groups of ten cats each, which should have been established before beginning the study but the practical reality of the shelter did not allow us to follow this through. Cats were admitted to the shelter, introduced to already existing groups and adopted out regardless of their inclusion in the study, which made it difficult to maintain a stable group of test animals for as long as necessary to complete the whole protocol. Furthermore, we had to deal with such problems as health-issues and even the decease of one cat during the study, for reasons not related to it.

Our sample depended on the cats present for the total time of the study and as the study did not, at any point, assume supremacy over the opportunity for adoption, this meant that elements of our sample could suddenly leave the study, independently from which point of it they reached. This happened to nine cats, and is one of the main reasons for the reduction of the sample from the initial 24 to the final eight.

Of course, being a MAS, there rarely were any previous histories of the cats, so the mental status of the animals due to variable experiences in the past were not homogenous throughout the sample.

If time had allowed it, it would have been interesting to project a study which would evaluate not different groups of cats subject to distinct treatment, - as was the case in this preliminary study -, but rather the same group of cats in two distinct chronologic moments, before the implementation of the enrichment, and after it. Using the same group of cats would, of course, eliminate many of these biases as the changes in mental state would be seen in the same animal. Obviously this sort of study would need a control group and the best way of putting it in practice without ethical dilemmas would have to be carefully thought through. Time constraints were also relevant to the study design as it was, considering that freshly admitted cats although being passible of being included would need to begin the protocol from the beginning, which put them behind other cats which has already started earlier, therefore delaying the analysis of combined results. The spatial, logistic and material constraints included the existence of only one small room for the test procedure, which was difficult to access and isolate from sound and other environmental stimuli. There was no extra storage room for those materials that could or should not be kept in the test room, and in order to reach the room the cats had to be subject to a panoply of stressing stimuli.

Another problem appears related to scarce human resources: the fact that it was always the same person responsible for the several phases, which meant that this person knew exactly to which group each animal belonged, which compromises impartiality both in manipulation and registration of the data. We try to kept this bias to a minimum through the use of video recordings in the third and fourth phase, but ideally they should be supervised by a different person which would not know to which group each animal belonged. Video recording would still be a useful tool in any case.

Besides these specific issues arising during the study protocol, there are some other problems more related to the conceptual discussion of judgement bias tests:

A study from 2010, by Doyle, focused on a methodologic issue, namely the effect of repeated testing using unreinforced, ambiguous cues in a judgement bias test with sheep. The conclusion was that over time the animals leant that the ambiguous locations were unrewarded, which represents a limitation with the potential to provide misleading results. This undesirable effect needs to be kept to a minimum by reducing the number of effective trials. In this study, none of the animals seemed to have assimilated the fact that ambiguous cues were always unreinforced because the number of ambiguous trials was limited and diluted in the remaining ones.

Another criticism appears related to the type of task: when it is a Go/No Go type of task, the “no go” must not necessarily represent a pessimistic judgement, but may also simply stand for lack of motivation, arousal, distraction or confusion (Mendl et al., 2009), which is why similar tests have been developed using active choice tasks with positive reinforcement (Mendl et al., 2009). In the case of this study, the only behaviour noted that might indicate lack of motivation, distraction or confusion was that of cat number 9, a female which after passing to the test phase (phase four) seemed to make no difference between R and U. After re-watching the video recordings and analysing the results, it became clear that the latency of reaching the food bowls in the different locations, as analysed in the previous phase, was no longer in compliance with the pre-defined criteria of passing on to phase four (positive Mann Whitney u test (Mnn test), i.e. one side takes longer than the other -> H0 is rejected). We considered an error of interpretation, or accidental compliance with the criterion, which would invalidate the criterion. In this case, we should perhaps extend the compliance with it to the test phase, requiring that the Mnn test maintained itself in positive values throughout the three days of testing. Considering this result, the animal was excluded from the test.

One of the main problems of this study is, in the author’s opinion, the fact that the very variable that is to be tested in the cognitive bias test is at the same time, with the present study design, the solution for a major problem arising in the completion of the test. Given that the shelter environment is extremely stressful to cats and their behaviour is affected by that stress, this often leads to freeze reactions (Overall, 2013) incompatible with further testing. In our study no freeze reactions were recorded in the more advanced phases, just in the first, and, if maintained, this led to the exclusion of the cat. Training cats in the shelter does provide mental and physical stimulation, facilitates positive associations with humans, and can build confidence in shy or fearful cats which could then be subject to the test and might show improved affective state reflecting itself in more positive (optimistic) judgement bias, but we would lose the term of comparison from the pre-training phase (Bollen, 2015). Obviously, the aim of these studies being the evaluation of welfare in order to improve it, it would be contradictory to, within the study, purposefully diminish said welfare. Different strategies, maintaining welfare, such as the use of active choice tasks should be developed and focused upon.

When dividing the cats into the three groups, establishing that only one of the groups would be subject to environmental enrichment through training, the other two being subject to only environmental enrichment through play or as little enrichment as possible, respectively, it was not as apparent to the author that the protocol adopted in this study would require such extensive manipulation of the animals. Each cat was returned to the start box 12 times per session, and as many of the cats were poorly socialized with people they rose to very high levels of stress which made it impossible to complete the test. Part of the animals would not even come out of the carriers to execute the test procedure, and although some degree of training was inevitable, care had to be taken to keep it at a minimum not to influence the variable "enrichment and/or training". The implementation of the test protocol for non-socialized, nearly feral cats may require a long period of habituation that may affect animals' perceived level of stress and possibly alter his welfare status. Tami et al. (2011) suggested a simplified or shortened version of the protocol which may be easier for cats to tolerate, which the author did not attempt, but we did attempt the second solution mentioned by this study, which was training the cats to enter the start box in order to avoid manipulation. But, as mentioned above, the fact that several animals resorted to hiding and freezing as a coping mechanism for stress, was a rather inconvenient limitation to the selection of a study-group of the size we had planned.

The hypothesis tested in this study, which needs to be confirmed in further studies would serve as the scientific background to advocate the importance of social environmental enrichment in shelters as its advantages promote medium/long term cost reductions by heightened adoptability rates and considerable benefits to the animals QoL.

The value of cognitive bias measures for welfare in shelter animals could probably lie in two areas: the development of individual profiles, or, with due intervals, the assessment of changes in judgement bias over time; and the establishment of guidelines of welfare to be adapted to shelter animals (or privately owned ones) which have been verified via this way of measuring.

Even if positive or negative emotional states cannot be categorically identified, the assessment of change over time would be useful to detect animals that are emotionally worsening or improving, both useful tendencies to keep track of, and relate to changes in the environment, in order to optimize conditions and QoL.

5. Conclusion

The preliminary findings of this pilot study suggest that those cats subject to training as a type of social environmental enrichment showed more optimistic responses towards ambiguous stimuli in the subsequent cognitive bias test, which reflects a more positive affective state. This is an important finding as scientific evidence is needed to reinforce the importance of environmental enrichment in shelters, which in many cases is still optional and considered accessory. A larger sample of animals would have to be studied in order to undertake conclusive statistical analyses, but as a preliminary study, this work was important to identify some flaws and discuss alternatives which can now be used to draw a follow-up study which further validates the effect of enrichment in general and training in particular as valuable tools for an improved welfare and heightened adoptability of shelter cats. Considering all that was said, with the knowledge gained throughout the study, if it were to be repeated the most relevant changes would be: consequent filming of all phases and the use of at least two different people – one for the random assignment of the cats into groups and the pre-phase and one for the remaining phases (this one not having any knowledge of the group each cat belonged to).

Implementing behaviour modification programs to reduce stress and fear is imperative to ensure feline welfare, as is improving the cat's emotional response to procedures that it must endure while housed in a shelter through systematic desensitization and counterconditioning (Yin, 2009; Bollen, 2015) – which would easily work for the test procedure as well. Another important consequence of this type of behavioural training is giving the cats a sense of control as they learn that their behaviour can produce reinforcement which should have a positive effect on the affective state. Training sessions can help abate boredom and frustration as well as give the cat an outlet for their energy and desire to engage in active behaviour instead of exhibiting undesirable behaviour resulting of the frustration and stress they feel from captivity (Bollen, 2015).

Training (and behaviour modification) besides being tools to keep shelter cats behaviourally healthy also increase their adoptability. Research has shown that the way a cat behaves is the basis for being chosen by an adopter, even more than his physical appearance (Gourkow & Fraser, 2006; Fantuzzi et al., 2010). If we can use behaviour modification to help cats to become more outgoing and friendly and in addition train them to perform behaviours that attract attention of potential adopters (Bollen, 2015), all the while promoting positive emotional state, this would be a win-win situation. As many people do not realize that cats can be trained, a cat that has been trained to offer a cute behaviour will appear very smart in the eyes of the average adopter (Laser, 2008; Miller & Watts, 2015).

Incorporating simple training procedures into daily caretaking is a simple way to increase positive human social interaction, desirable animal behaviours, and mental stimulation, e.g staff can provide a small treat each time they interact with or pass an animal in his cage, increasing positive human interaction (Miller & Watts, 2015).

It is our hope that even though the present study is but a pilot study which needs redesigning before being further applied, it can be, to some extent, a beacon in the right direction and some proof of the importance of environmental enrichment – especially of that environmental enrichment that involves the relationship between the animals and the caretakers. This enrichment, and all kinds of training can be powerful tools at the shelter, improving not only the welfare of the animals but also of human staff.

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