REVISTA CIENTÍFICA DE MEDICINA VETERINÁRIA - ISSN 1679-7353 Ano XV - Número 31 – JULHO de 2018 – Periódico Semestral

EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE BEHAVIOR AND CONCENTRATIONS OF CORTISOL IN DOGS USED IN ANIMAL RESEARCH

Letícia Vinhas RAMPIM[1]

Juliana Tessália WAGATSUMA[2]

Valéria Nobre Leal de Souza OLIVA[1]

ABSTRACT

Environmental enrichment consists of techniques used in the modification of the physical or social environment of wild captivity animals but isn't extensively used for domestic animals. The lack of challenges in an animal's domestic life tends to create behavioral disorders. This research's objective is to evaluate the welfare of research dogs through environmental enrichment techniques, evaluating behavior and the concentration of serum cortisol. It was possible to observe a decrease in behaviors such as anxiety and vocalization and in cortisol concentrations. Environmental enrichment has shown a technique able to promote the welfare of research dogs.

KEYWORDS

Welfare; Ethology; Laboratories

1. Departamento de anestesiologia, Faculdade de Medicina Veterinária, Universidade Estadual Paulista - UNESP – Araçatuba/São Paulo - Brasil
2. Departamento de anestesiologia, Universidade Federal do Piauí – UFPI – Teresina/Piauí - Brasil

INTRODUCTION

Many areas of scientific knowledge recurrently use animals for research (BAUMANS; VANLOO, 2013). The effects of the environment and handling may affect the results in those researches and decrease the reproducibility of the results (Faith and Huerkamp, 2009).

Taylor et al. (2008) estimated that in 2005, 115,3 million vertebrates have been raised for experimentation. Environmental enrichment increases animal life quality; however, those techniques are most commonly used in neuroscience studies and not related to the animal's welfare. (FISCHER et al., 2016).

In lab animals, stress is caused mainly due to inadequate handling, discomfort, fear stimuli and the incapability of maintaining normal standards of behavior. However, the biggest cause of stress in those animals is pain. When feeling pain, the animal tries to adapt by means of posture of stereotypical behavior (ANDRADE et al., 2002).

The concern with the welfare of experiment animals has made Russel and Burch, in 1959, idealize the principle of the 3Rs (Replacement, Reduction and Refinement), which defends the replacement of animals with tissue culture and computer models, reduction of animals used in and the usage of less invasive techniques. Even nowadays this principle is still discussed and stimulated. (CRISSIUMA; ALMEIDA, 2006).

The authors have also considered environmental enrichment as an ethical necessity in the environment of laboratory animals. The benefits of environmental enrichment can be obtained without compromising research data. Decreasing stress levels and contributing to the natural behavior expression, experimental data is shown more reliable and reproducible (MEDINA, 2012).

However, laboratory systems have been created, frequently, based on economic and ergonomic aspects with little or no consideration for animal welfare. Maintenance and care techniques traditionally used in laboratory animals, normally do not include the specific needs of a specie in relation to their environment (BAUMANS, 2005).

Dogs have been used in research for a long time. Recently, it has been noted that there has been an increasing pressure from the population who consider these researches cruel or that dogs should not be held in laboratories for their entire life (GRAEBIN, 2014).

METHODS

Five dogs from the Beagle breed have been destined to the research and education in veterinary anesthesiology at the veterinary medicine's college from Universidade Estadual Paulista.

The dogs – two males and three females -, all adults, were acquired from a commercial kennel for experimentation. They arrived at the college six months old and remained in these dependencies for three years, being used in some anesthetics tests and in the conduction of practical classes for the students at Veterinary Medicine's college.

All animals were kept in a kennel at night, in separate stalls. During the day, from 8am until 6pm they are kept in collective solariums with access to sunlight and shades.

The feeding routine consisted of good quality dry commercial dog food (super premium), in fixed quantities in accordance with the animal's size, twice a day (6am and 6pm) with unlimited access to fresh and clean water.

The vaccination plan used is the one recommended by the college's medical clinic of small animals, which includes: anti-rabies, polyvalent V10 (parvovirus, coronavirus, distemper, infectious hepatitis, laryngotracheitis, parainfluenza and leptospirosis) and Canine Leishmaniasis. Before this research was started, the dogs were evaluated by veterinary doctors at the college's veterinary hospital, attesting their health.

The behavioral evaluation of the dogs was analyzed based in ethograms from Palma et al. (2005) (Chart 1), by use of Focal Sampling. The animals were observed by cameras (Canon Powershot SX400IS and DVR HD) during the weekend, when they have no activities and are kept in the solariums all day long. Five 30 minutes long observations were made, always during daytime, for two moments: before and after environmental enrichment.

Chart 1 – Ethogram (PALMA et al. 2005) used for behavioral analysis

Behavioral Category	**Behavior**
Activity	Walking, jogging, digging, playing.
Agressiveness	Growling, raised hackles, biting.
Anxiety	Itching, walking in circles, shaking, whimpering, self-mutilation, licking objects, yawning.
Attention	Raised ears, looking outside, looking at the environment, looking at other dogs, lifting front paw, sniffing environment, sniffing other dogs, sniffing objects.
Dominance	Mounting, urinating with a lifted leg.
Fear	Tail between legs, shyness, tense, salivating, trying to escape.
Playfulness	Playing, calling to play.
Idleness	Laying down, sitting, sleeping.
Sociability	Wagging tail, licking and/or sniffing genital area of other dogs.
Vocalization	Barking.

After performing five evaluations without environmental enrichment, five types of environmental enrichment were included: food (frozen fruits), social (walks to socialize with campus students), cognitive (puzzles, hidden treats and training classes), sensorial (grass, burlap and toys hung up by sisal) and physical (running water, tunnel house, ball pool) (Images 1 and 2), and another five evaluations were performed, in the same way as before.

As for hormonal evaluation, blood samples were collected once every week for the period of five weeks in both evaluation moments. The samples were collected from the jugular (5ml/sample), being immediately centrifuged for plasma extraction and frozen at -80ºC for laboratory analysis in a single phase, at the end of each collecting phase.

The dosage method used was the competitive immunoassay of solid phase by chemical enzymes (alkaline phosphatase). 10µl from the sample was used for the dosage with the Immulite Cortisol KIT (Lot number 0419 from catalog LKC01). For the standard curve construction, canine plasma treated with activated charcoal was used. The dosage was done at the Laboratory of Endocrinology of the veterinary medicine's college from Universidade Estadual Paulista. Cortisol concentration results were compared before and after environmental enrichment methods by use of the paired t-test.

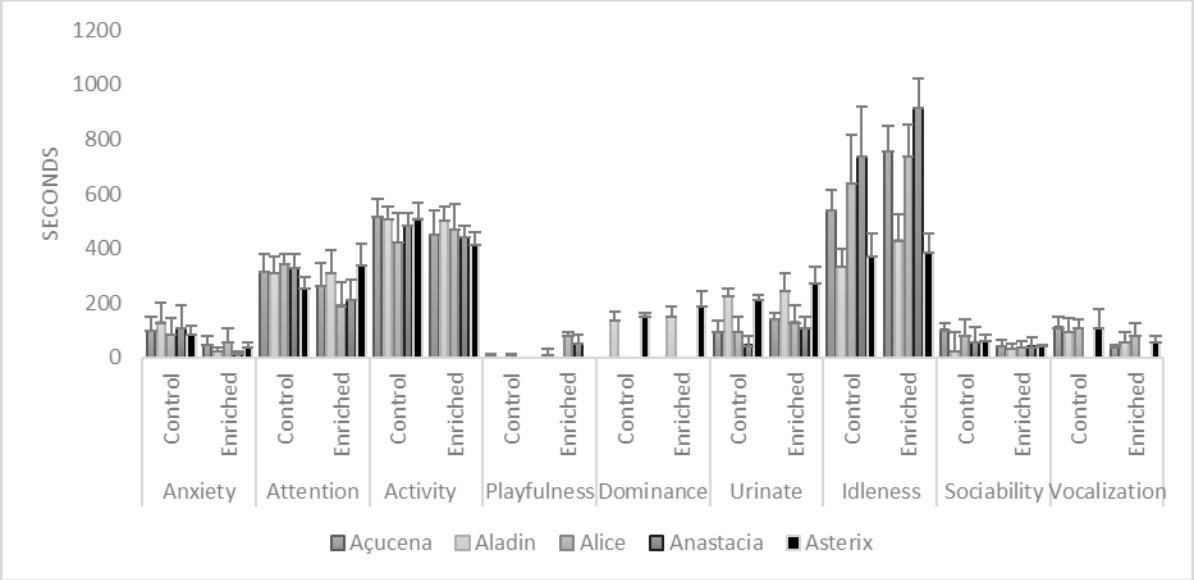
The videos obtained were analyzed using the AVITRICKS software, allowing the use of slow motion and close-ups, in accordance to the Focal Sampling methodology. The behaviors were analyzed by duration (seconds) and registered into files. Each behavior was compared against results obtained before and after environmental enrichment methods, using paired t-test.

After Shapiro-Wilk test, all statistical analyzes were performed using the BIOESTAT 5.0 software, considering the 95% of confidence level.

RESULTS

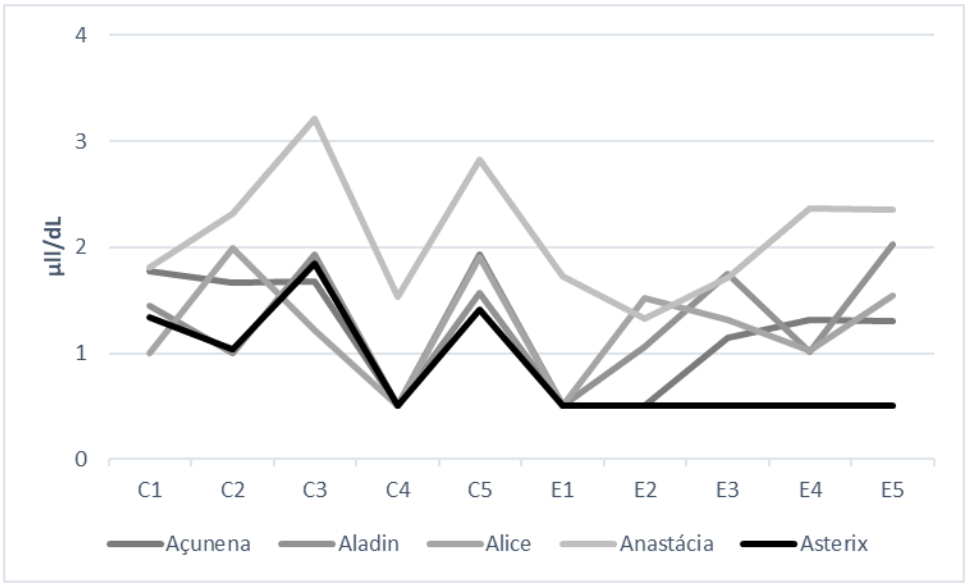
Only two items were destroyed by the dogs (balls), with no consequence to the experimentation. Aggression, fear and subordination behaviors weren't observed in any occasions. Anxiety behaviors (p=0,011) and vocalization did decrease significantly (p=0,034). Urination behavior raised significantly (p=0,005). However, one of the objectives of environmental enrichment, the reduction of idleness was not verified. On the contrary, a significant increase (p=0,027) was observed. There wasn't a significant difference in playfulness (p=0,084) (Figure 1).

Figure 1 - Graphical representation of the durations of individual behavioral categories (dogs), in control (C) and enriched (E) with standard deviation.



There was a significant decrease in serum cortisol concentrations (p=0,031) (Figure 2).

Figure 2 - Graphical representation of cortisol concentrations in control (C1 to C5) and enriched (E1 to E5) (dogs).



DISCUSSION

According to Landsberg et al. (2013), destructive behaviors can be explained by the presence of phobias, Separation Anxiety Syndrome, stimuli poor environments and other possible anxiety disorders. 30 items were added to the environment; therefore, the low frequency of destructive behaviors corroborates with the reduction of anxious behaviors.

In a case study of Veeder and Taylor (2009), a pregnant seven-year-old Beagle was submitted to euthanasia after presenting symptoms of lethargy, dehydration, vomiting and abortion. During necropsy, plastic pieces were found inside the stomach's pylorus. These pieces belonged to a bed that was part of the animal's environmental enrichment program. The study emphasizes that enrichment should be carefully evaluated before implementation.

No behavior related to fear was noted during observations, however, the dogs showed fear during blood collection and during interaction with unknown individuals (mainly males). According to Foyer et al. (2014), the experiences that dogs goes through at the beginning of their lives, influence their behavioral development. Adolescence can also be an important period, and their experiences may cause long term effects. Showed fear can be explained by the dog's history, for example, experimentations and practical lessons where they were used. Furthermore, these animals, when acquired, aging six months old, presented withdrawn, fear and submissive behaviors toward any person. The frequency of these behaviors diminished, without completely disappearing.

This significant decrease in anxiety and vocalization is explained by environmental adaptation and the existence of stimuli during the environmental enrichment phase. McCrave (1991) characterized canine anxiety behavior such as: excessive vocalization, destructive behavior and physiologically relieving themselves in inappropriate places.

Before the implementation of environmental enrichment, dogs expressed behaviors such as walking in circles and whimpering. The kennel is located next to the Veterinary Hospital and they would respond to any sound with anxiety and vocalization.

Significant increase in urination was likely due to the implementation of a water fountain. According to Feldman el at. (2010), running water stimulates water consumption in domestic animals.

The increase in idleness has not appeared to cause damage to the dog's welfare, taking in consideration the decrease in anxiety and the non-significant difference observed in the behaviors of activity. According to Arhant's (2010), dogs are incapable of resting while in a state of alert, stress or anxiety.

There was little playful behavior during the two experiment phases. Dog interactions with toys were mostly investigative (sniffing objects). According to Kelley et al. (1989), Pierce and Courshesne (2011) and Siwak et al. (2001) this behavior requires a high level of sensorial processing. A new environment or new objects offer opportunities to learn and explore, responsible for cognitive development in mammals. Exploration depends on intact precordial circuits, such as the cerebellar function.

Therefore, even when not playing (explained by the condition in which the animals grew in, without playful stimuli in their first infancy), the interest in investigating was considered positive and a producer of animal welfare.

In a study by Hubrecht (1993), the author compared social and physical enrichment in the welfare of beagles that were kept in laboratories and proved that environmental enrichment can increase the complexity in a dog's behavior, stimulating natural behaviors while helping prevent undesirable ones. Physical enrichment is the most recommended, but the author also defends that laboratory employees should be motivated to socialize with the dogs.

The significant reduction in cortisol serum concentrations corroborates with the behavioral parameters, thus, environmental enrichment was efficient in promoting welfare in the observed dogs.

Cortisol concentration reduction, through environmental and/or social enrichment in dogs, was also observed by Willen et al (2016). The same was observed in fish, wild and farm animals (XU et al. 2015; Casal et al. 2016; Giacomini et al. 2016).

CONCLUSION

This study shows that environmental enrichment is efficient in promoting welfare in dogs kept in laboratories, reducing serum cortisol concentrations and anxiety behavior, while increasing desirable behavior.

REFERENCES

ANDRADE, A.; PINTO, S.; OLIVEIRA, R. Animais de laboratório: criação e experimentação. 1. ed. Rio de Janeiro: Fiocruz, 2002.

ARHANT, C. et al. Behaviour of smaller and larger dogs: Effects of training methods, inconsistency of owner behaviour and level of engagement in activities with the dog. Applied Animal Behaviour Science, v. 123, n. 3-4, p. 131-142, 2010.

BAUMANS, V. Science-based assessment of animal welfare: laboratory animals. Rev Sci Tech., v. 24, n. 2, p. 503-513, 2005.

BAUMANS, V.; VAN LOO, P. How to improve housing conditions of laboratory animals: The possibilities of environmental refinement. The Veterinary Journal, v. 195, n. 1, p. 24-32, 2013.

CASAL, N. et al. Effect of environmental enrichment and herbal compound supplementation on physiological stress indicators (chromogranin A, cortisol and tumour necrosis factor-α) in growing pigs. animal, v. 11, n. 07, p. 1228-1236, 2016.

CRISSIUMA, A.; ALMEIDA, C. Experimentação e bem-estar animal – artigo de revisão. Saúde e Ambiente em Revista, v. 1, p. 1-10, 2006.

FAITH, R.; HUERKAMP, M. Environmental considerations for research animals. Traducao . 1. ed. London: Elsevir, 2009. p. 50-85

FELDMAN, E.; NELSON, R. Canine & Feline Endocrinology. Traducao . 3. ed. Saunders, 1987.

FISCHER, M. et al. Enriquecimento ambiental como princípio ético nas pesquisas com animais. Revista Bioética, v. 24, n. 3, 2016.

FOYER, P. et al. Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test. Applied Animal Behaviour Science, v. 155, p. 93-100, 2014.

GIACOMINI, A. et al. Environmental and Pharmacological Manipulations Blunt the Stress Response of Zebrafish in a Similar Manner. Scientific Reports, v. 6, n. 1, 2016.

GRAEBIN, C. Os movimentos sociais 2013: A invasão do Instituto Royal e a efetivação dos direitos de proteção aos animais não humanos. Revista Eletrônica do Mestrado em Direito da UFAL, v. 5, p. 138-155, 2014.

HUBRECHT, R. Dog housing and welfare. Universitites Federation for Animal Welfare, 1993.

KELLEY, A.; CADOR, M.; STINUS, L. Exploration and Its Measurement: A Psychopharmacological Perspective. Psychopharmacology, p. 95-144, 1989.

LANDSBERG, G.; HUNTHAUSEN, W.; ACKERMAN, L. Behavioural problems of the dog and cat. Traducao . Edinburgh: Saunders, 2013.

MCCRAVE, E. Diagnostic Criteria for Separation Anxiety in the Dog. Veterinary Clinics of North America: Small Animal Practice, v. 21, n. 2, p. 247-255, 1991.

MEDINA, M.; ORLANDINI, L.; CASSIMIRI, A. Efeitos do enriquecimento ambiental no comportamento e bem-estar de animais de laboratório convencionais. Msc.— Universidade Federal do Rio Grande do Sul, 2012.

NEVES, S.; FILHO, J.; MENEZES, E. Manual de cuidados e procedimentos com animais de laboratório do biotério de produção e experimentação da FCF-IQ/USP. Traducao . 1. ed. São Paulo: FCF-IQ/USP, 2018.

DE PALMA, C. et al. Evaluating the temperament in shelter dogs. Behaviour, v. 142, n. 9, p. 1307-1328, 2005.

PIERCE, K.; COURCHESNE, E. Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. Biological Psychiatry, v. 49, n. 8, p. 655-664, 2001.

SHEPHERDSON, D. et al. The influence of food presentation on the behavior of small cats in confined environments. Zoo Biology, v. 12, n. 2, p. 203-216, 1993.

SIWAK, C. Effect of Age and Level of Cognitive Function on Spontaneous and Exploratory Behaviors in the Beagle Dog. Learning & Memory, v. 8, n. 6, p. 317-325, 2001.

TAYLOR, K. et al. Estimates for Worldwide Laboratory Animal Use in 2005. Altern Lab Anim., v. 36, n. 3, p. 327-342, 2008.

VEEDER, C.; TAYLOR, D. Injury Related to Environmental Enrichment in a Dog (Canis familiaris): Gastric Foreign Body. J Am Assoc Lab Anim Sci., v. 48, n. 1, p. 76-78, 2009.

WILLEN, R. et al. Factors determining the effects of human interaction on the cortisol levels of shelter dogs. Applied Animal Behaviour Science, v. 186, p. 41-48, 2017.

HU, X. et al. Effects of environmental enrichment on behaviors and fecal cortisol levels in captive golden snub-nosed monkeys (Rhinopithecus roxellana). ACTA Theriologica Sinica, v. 35, n. 3, p. 304-311, 2015.