ENVIRONMENTAL ENRICHMENT OF NEW ZEALAND WHITE RABBITS LIVING IN LABORATORY CAGES

by

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(Under the Direction of Sharon L. Crowell-Davis)

ABSTRACT

Environmental enrichment diminishes abnormal behaviors in laboratory animals such as rodents, lagomorphs, dogs, cats, and nonhuman primates.

Thirteen male single housed New Zealand White rabbits were offered three different toys and the time spent chewing on the toys instead of on the cage was evaluated. Every toy was offered to each rabbit for two separate one week periods. Each rabbit was monitored fifteen minutes four times a week to total one hour of observation every week, including one hour baseline data before toys were offered and two hours observation per rabbit per toy.

Having a toy available had a significant effect on the behavior of NZW rabbits. Rabbits with toys spent significantly more time chewing than rabbits did without toys. They did not develop a preference between the offered toys and did not show stereotypies.

This study highlights the importance of environmental enrichment to improve the welfare of laboratory animals.

INDEX WORDS: New Zealand White Rabbit, Environmental Enrichment, Animal Welfare
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Mamma, papa’, this is dedicated to you.

Thanks for all your love and support: without you I would have never reached so far.

Un mondo di bene,

la vostra Sabrotti
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Environmental enrichment of animals in captivity is a topic that has been discussed for decades (Wolfle, 2005). Several definitions about environmental enrichment have been available since the early 20th century, but the broadest comes from the Behavior and Husbandry Advisory Group of the American Zoo and Aquarium Association. This group defines environmental enrichment as a “dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out their species-appropriate behaviors and abilities, thus enhancing animal welfare” (BHAG, 1999). The goals of environmental enrichment can be summarized as follows: 1) to reduce abnormal behaviors; 2) to increase the normal behavior patterns and allow their normal temporal distribution during the day; and 3) to help animals to cope with challenges faced in captivity in a more normal way (Chamove et al., 1990).

These purposes apply absolutely to laboratory animals (rodents, lagomorphs, dogs, cats and non human primates), that spend their days in confined areas for scientific reasons. In order to achieve the above mentioned goals, it is necessary to have extensive knowledge about the behavioral repertoire of the species the laboratory is dealing with (Hutchinson et al., 2005). For example, providing both mice and rats with nesting material is considered a suitable way to enrich their cages because of their natural need as prey species to hide. Nonhuman primates are
well known for being curious creatures that like to interact with their environment; therefore, offering them toys can keep their explorative behaviors and interest high (Lutz et al., 2005). An appropriately stimulating environment (Institute of Laboratory Animals Resources, 1996; Dean, 1999; Bayne, 2003; Baumans, 2005; Wolfle, 2005) may prevent the development of abnormal behaviors such as stereotypies and increase the animal’s immunological response (Whary et al., 1993; Turner et al., 1997; Hutchinson et al., 2005; Fox et al., 2006).

Stereotypies are repetitive, invariant behavior patterns with no obvious goal or function (Mason, 1991). Although the motivational bases for stereotypic behaviors (Rushen et al., 1993; Petherick et al., 1997; Garner, 2005) are not yet well understood, it is commonly agreed that they arise from normal behaviors that are prevented or blocked from being expressed. Different motivational systems (feeding, locomotory, aversion, sexual, and exploratory) seem to be responsible, but nothing definitive has been proven. Usually, a non challenging environment is associated with increased manifestation of these systems (Petherick et al., 1997).

A well known stereotypy in horses is cribbing, a repetitive behavior during which a horse grabs a solid object with its incisors, arches its neck and pulls backwards (Clegg et al., 2008). Pacing is a stereotypic behavior common among captive animals such as bears kept in zoos (Vickery et al., 2004). Circling and tail chasing are locomotory stereotypies well described in dogs (Luescher, 2004). Sexual behaviors like mounting a pillow with pelvic thrusting or exploratory behaviors in which the animal chases after phantom prey are commonly seen in pets.

A stimulating environment is an environment that offers challenges to the animal and thus prevents or reduces the appearance of abnormal behaviors due to boredom. The concept of animal boredom (Wemelsfelder, 1993; Wemelsfelder et al., 1997) and its relationship to stereotypic behavior was introduced by Françoise Wemelsfelder. According to Wemelsfelder,
animals need to interact with and have control over the environment. Once they start losing this
capacity they will experience different emotional stages, including frustration, boredom,
depression, and anxiety. Boredom means that the anticipatory process is impaired and as a
consequence, the animal will start showing stereotypies due to lack of control over the
environment. Depression and anxiety follow as the animal stops interacting with the
environment. Boredom, depression and anxiety are all adverse emotional states indicating animal
suffering (Broom, 1991b; Dawkins, 2000; 2003; Bateson, 2004; Duncan, 2006; Dawkins, 2008;
Wurbel, 2009b; a). Suffering is a general definition for aversive affective states such as pain,
fear, frustration, deprivation, and boredom. These states have the common attribute of being
unpleasant, and therefore anyone experiencing them would avoid these emotions as much as
possible. Animals show the above mentioned states as well and will, for example, remove
themselves from a dangerous situation if they are suffering from fear. Suffering can be used as
an important parameter to assess animal welfare especially when animals are kept in a barren
environment without any kind of stimuli, such as in a laboratory cage. This is the reason why
environmental enrichment plays a critical role in animal welfare. There are several ways in
which environmental enrichment can be achieved.

**Social Enrichment**

One form of environmental enrichment is through social enrichment in which the animal
socializes by sharing his or her place with conspecifics or interacts with a different species such
as the human beings that attend to them (Baumans, 2005). Sharing space with conspecifics by
creating pairs or groups is highly recommended, especially in gregarious species. A companion
is considered a daily stimulus that promotes behaviors such as alertness and exploration.
However, aggressive behavior may occur in this situation, so to prevent injuries a shelter should
be provided to offer refuge to the victims and extremely aggressive animals should be isolated from the others. Humans should not play a passive role, but instead should interact directly with the animals by touching them, speaking to them or handling them on a daily basis (Boers et al., 2002; Hawkins et al., 2008). This interaction is done in order to prevent fearful responses that could adversely affect future and unavoidable interactions with human personnel. It has been shown that exposing young rabbits to human handling during the first week post partum reduces their fear response later on when they are adults. Podberscheck et al. (1991) exposed both penned and single-caged rabbits to the handling of a familiar person (a worker employed at the facility where the rabbits were kept) and subsequently to the handling of an unfamiliar person (someone with no experience around rabbits). Each trial lasted five days with a two week interval in between the trials. The rabbits from both housing systems showed less fearful responses toward both the handlers after the first day of exposure to them. Bilkó et al. (2000) emphasized the importance of handling rabbits during the first week postpartum because it is considered a sensitive period in preventing the fearful response later on in their life. Csatádi et al. (2005) not only confirmed the existence of such a period, but added that handling the rabbits within 0.5 h after nursing makes them tamer and less fearful than rabbits handled at longer intervals after being nursed.

More recently, Swennes showed that even adult rabbits can be desensitized around human beings by being exposed to them 4 minutes a day for 3 weeks in a row (Swennes et al., 2009).

**Physical Enrichment**

Physical enrichment, a second means of environmental enrichment, is achieved by offering complex enclosures and both sensory and nutritional stimuli. One example would be to
place a wooden box in the enclosure where the rabbit could hide when scared or when not willing to interact. In the wild, pregnant females start digging warrens to offer a nest and a shelter to the kittens (Lockley, 1961). Also, wild rabbits live underground much of their lives to protect themselves from predators. They usually come out in small groups around sunrise and sunset to graze (Southern, 1940; Stodart et al., 1964; Nelissen, 1975; Crowell-Davis, 2007). To mimic grazing, food can be scattered all over the floor rather than placed in a single location. Alternatively, it can be hidden in easily accessible places or inside toys in order to promote foraging behavior. Auditory, olfactory, and tactile stimuli can be secured by the presence of another rabbit in the same cage or, when this is not possible, in cages placed just beside or in front of each rabbit.

The above examples are given to illustrate how it is possible to improve the captive environments of animals by giving them the opportunity to perform natural behaviors. By doing so, it has been shown that environmental enrichment can have both a therapeutic and protective effect against stress. Different studies, carried out mainly on mice and rats, support this concept. A review by Fox (2006) gives more details on the topic. Unfortunately, explorations on rabbits have yet to be undertaken.

*Studying Consequences of Environmental Enrichment in Rabbits*

Rabbits are commonly used in research because of their small size that allows housing them easily and working with a great number of subjects, their docile temperament, and the ease of restraining them. They are considered good animal models because of the physiological similarities they share with human beings. Their bone density and resistance to fractures, for example, is similar to those of human beings; therefore, they have been chosen as good candidates for orthopedic surgery. For similar reasons they are exploited for the development of
vaccines and for better understanding the progression of diseases such as Alzheimer’s, atherosclerosis, and ocular diseases (Blanco et al., 2004; Chiavolini et al., 2008; McCanna et al., 2008; Shiomi et al., 2008; Sparks, 2008; Woodruff-Pak, 2008; Calasans-Maia et al., 2009; Gupta et al., 2009). Because rabbits are so widely used, it is vital to know their basic behaviors and allow them to express those in order to assure their welfare. If their welfare is not taken into account, this factor can affect the results of any research in progress.

**Ethogram**

An ethogram (Martin et al., 1993b) is a detailed list of the known behaviors and activities of a given species. Although the rabbit’s ethogram is complex, chewing is widely recognized as one of its very common and most characteristic behaviors. Since rabbits have been employed in laboratories for a long time, several ethograms have been published and then made available to the scientific community (Lehmann, 1991; Podberscek et al., 1991; Morton et al., 1993; Gunn et al., 1995; Lidfors, 1997; Hansen et al., 2000). Podberscek (1991) arranged behaviors in six different groups: locomotory, maintenance, comfort, marking and investigatory, agonistic, and stereotypic. Lehmann (1991) and Morton (1993) listed and defined twenty and thirty five behaviors respectively. Gunn (1995) gave a list of forty one behaviors with detailed definitions. Lidfors (1997) divided the observed behaviors into three groups: all interactions with the object, abnormal behavior, and normal behavior. Hansen (2000) listed and defined fourteen behaviors.

The welfare of rabbits improves when rabbits are able to express their normal behavioral repertoire because enrichment is provided in their cages (Duncan et al., 1997). In general, welfare can be improved if the environment in which the animal is living in provides opportunities for interaction. Without these opportunities, captive animals that live in predictable and structured environments develop boredom. To prevent that from happening, animals should
be able to explore their environment to collect information that would be necessary for their survival in the wild. Exploration itself is a strong motivator for animals, not just because they can obtain information but also because it permits animals to interact with their environment (Wemelsfelder, 1993; Wemelsfelder et al., 1997). From these interactions animals expand their behavioral repertoire because they learn how to deal with new, unpredictable situations. For example, presenting food in a whiffle ball forces the rabbit to manipulate that object in order to get the treats in it by rolling it until the kibble is released through one of the holes in the ball. This activity not only keeps the rabbit busy, stimulated, and active but affects its welfare positively because boredom is decreased.

Previous studies (Lidfors, 1997; Harris et al., 2001; Johnson et al., 2003) considered the influence of different objects on rabbits’ behavior. Lidfors (1997) offered hay in a water bottle, grass cubes, gnawing sticks, and a box to male New Zealand White (NZW) rabbits to determine if they would interact with those objects and to assess the effects of the objects on normal and abnormal behaviors. Whether or not the objects had some influence on weight gain and water consumption was also assessed. The results showed that rabbits preferred hay over the other objects and that they engaged in fewer abnormal behaviors, e.g. excessive fur-licking, sham chewing and bar-biting. Harris et al. (2001) divided eighteen NZW rabbits (both females and males) into three groups: food enriched (Bunny Stix, Bunny Blocks, Celery), toy enriched (Jingle Ball, Kong Toy, Nylabone), and not enriched. The rabbits were observed 1h/day for fifteen days. Food was preferred over any other kind of enrichment and the Bunny Stix was used by rabbits the most. The authors recommended rotating the enrichment items to prevent boredom. Johnson et al. (2003) offered stainless-steel rabbit rattles on spring clips to individually housed NZW rabbits. The rabbits were observed over an eight week period and all of them interacted with the
device. As in the Harris et al. (2001) study, Johnson highlighted the importance of rotating the items offered to the rabbits at least every two weeks to keep their interest high and therefore prevent boredom.

In this research, the colony was composed of SPF (specific pathogen free) rabbits. Specifically, they were all free from infectious diseases, including Pasteurellosis, Cilia Associated Respiratory Bacillus (CARB), Treponemiasis, Clostridoisis, and oral Papilloma viruses. They were free from parasitic diseases such as mites, pinworms, teniasis, and coccidiosis as well. Hay was not offered to them to avoid the risk of exposing them to pathogens that could have infected them and compromised their SPF status. The goal of this study was to further understand the effects of commonly available, inexpensive toys on the behavior of New Zealand White rabbits.

The toys were offered in order to test the following hypotheses:

1. When toys were available, rabbits would chew on the toy rather than on inappropriate objects such as the cage bars and their feeding dishes;
2. Rabbits would exhibit preferences for certain kinds of toys;
3. Rabbits would be more active, but exhibit less stereotypic behavior with toys rather than without toys.

This study focused on the importance of chewing by offering the rabbits appropriate outlets that would not compromise their health or the results of the studies in progress. Rabbits need to chew to wear down their open rooted and continuously growing teeth. If rabbits do not have the opportunity to chew on appropriate materials, they will chew on any cage component, thus favoring the development of severe dental diseases such as malocclusions or abscesses that could impair vital needs such as feeding, grooming or ingesting the caecotrophs (Lennox, 2008;
Reiter, 2008). Several studies have shown that dental diseases are fairly common among laboratory rodents (Legendre, 2002; Capello, 2004; 2008; Harvey et al., 2009) as well as rabbits. Therefore, offering opportunities to chew or gnaw to wear their teeth down may be additionally beneficial to rats, mice, chinchillas, guinea pigs, and voles.
CHAPTER 2
MATERIALS AND METHODS

Thirteen SPF (specific pathogen free) intact male New Zealand White (NZW) rabbits were caged individually in a windowless room (7.30 m long by 2.9 m wide) at the Life Science Building, the University of Georgia. From 7pm to 7am the lights were off without dim light phases. The room temperature was 66°F (18°C) ± 4°F and the humidity was in the range of 40-60%. The rabbits were fed a commercial pelleted diet once a day and water was available 24/7 through a stainless steel watering system. All the rabbits came from the Myrtle’s Rabbitry (www.myrtles.com) and arrived already weaned (approximately 8 weeks old) at the facility. Upon arrival they weighed approximately one kilogram.

This study was reviewed and approved by the IACUC (The Institutional Animal Care and Use Committee) a self- regulating entity that oversees and evaluates all aspects of the institutions animal care and use program. The laboratory where the rabbits were kept was AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited; this association promotes the humane treatment of animals in science.

Each rabbit was housed singly in plastic cages (internal cage dimensions: 689mm x 689mm x 457mm) (www.allentowninc.com) that were stacked in racks. Once a week, the cages were sanitized and the rabbits were moved to different cages. The rabbits were able to see or sniff each other, but could not touch each other.

In selecting toys, ease of obtaining the same or similar toys by various labs and the economics of using the toys were considered. In this study, three different kinds of toys were
offered to the rabbits: cardboard rolls, cardboard rings and rubber balls with a bell inside (www.leithpetwerks.com). All of the toys were of types commonly available from distributors of rabbit toys and were inexpensive.

The cardboard rolls were in different colors, fruit flavored, 2.5 centimeters across and 7.62 centimeters long. They were given one at a time. The cardboard rings had the same characteristics as the rolls, but were smaller, i.e. 2.54 centimeters across and 1.52 centimeters long. Five rings were offered at a time to each rabbit. The rubber balls were 2 inches in diameter and made of dense rubber with a bell inside. Again, each rabbit received one ball at a time.

For several months before starting the study, New Zealand White rabbits that were not going to participate in the study, but were in the facility where the study took place, were observed in order to develop an ethogram. The recorded behaviors were considered as “states” (Martin et al., 1993a). Behaviors are defined as “states” when they last long enough to determine when they begin and when they stop so that their durations are easily recorded. Behaviors are defined as “events” when their duration is not determined because they are short, and their salient characteristic is their frequency, i.e. how many times they are performed in a unit of time. The states recorded in this study are listed and defined in Tables 1 and 2.

In the week they arrived, the rabbits in the study were observed 15 minutes a day until one hour of baseline data was obtained on each rabbit. Toys were offered starting on the second week after the rabbits’ arrival and were assigned randomly to each rabbit. The toys were not autoclaved but were new and clean and were replaced any time they were shredded into tiny pieces. The toys were continuously available to the rabbits in the study.

The rabbits were observed over a period of 3 months (mid August 2007 – mid November 2007): each rabbit was observed 15 minutes a day until 2 hours observation on each toy was
obtained. All the behaviors were reported on a prepared check list. The rabbits were videotaped as well. Standing approximately a foot away from the cages when recording their behavior, the observer never interacted with them and wore lab coat, mask and gloves.

Data about the ten observed behaviors were analyzed using the General Linear Model for Repeated Measures (SPSS). Since 10 behaviors were analyzed, a Bonferroni correction resulted in defining a $p \leq 0.005$ as significant. A significant effect occurred with sitting and chewing, but there was no significant effect due to the presence or absence of toys on rearing, sniffing, stretching, grooming, eating, drinking, hopping, and laying down. Paired T-tests were run on all possible pairs between the above mentioned behaviors to ascertain exactly where the differences were.
CHAPTER 3

RESULTS

A significant effect occurred with sitting and total chewing, but there was no significant effect due to the presence or absence of toys on rearing, sniffing, stretching, grooming, eating, drinking, hopping, and laying down. When toys were not available in their cages, rabbits spent significantly more time sitting ($p = 0.004$) (Fig. 1). Rabbits spent significantly more time in total chewing when toys were present than when they were absent ($p = 0.001$) (Fig. 2). In addition, when a toy was present, rabbits spent significantly more time chewing the toy than chewing the cage (Cardboard Roll $p = 0.003$; Five Rings $p = 0.001$; Rubber Ball $p = 0.009$). The presence or absence of a toy did not have a significant effect on the amount of time spent chewing on the cage ($p = 0.514$). When a toy was present, there was no significant difference between any of the toys in the amount of time spent chewing on a toy (CR/5R $p = 0.170$; CR/RBB $p = 0.078$; 5R/RBB $p = 0.630$).

The seconds per hour for each behavior, by treatment, are presented in Table 3. Significant differences are indicated with an asterisk. The actual $p$ value for General Linear Model (GLM) for each main behavior is given in Table 4.
CHAPTER 4
DISCUSSION

Social enrichment is achieved by providing interactions with conspecifics, contraspecifics, and human beings. Environmental enrichment is characterized by offering nutritional and sensory (visual, auditory, olfactory, tactile, and taste) stimuli. Toys belong to the latter group because they stimulate all the rabbit’s senses.

When a toy is available, rabbits prefer chewing on that toy rather than chewing on less appropriate objects such as cage components. They also engage in more total chewing when a toy is available. These results are consistent with those obtained by Lidfors (1997), Harris et al. (2001), and Johnson et al. (2003).

When rabbits were offered the toys, the impact of play on their behavior was immediately evident. Even though play is well recognized in mammals, its purposes are still not clear. Different models and hypotheses (Burghardt, 1988; Barber, 1991; Byers, 1998; Thompson, 1998; Špinka, 2001) have been proposed such as the “surplus resource model” (Burghardt, 1988), the “surplus energy hypothesis” (Barber, 1991), the “sensitive or motor training hypothesis” (Byers, 1998), and the “self-assessment hypothesis” (Thompson, 1998). More recently Špinka et al. (2001) suggested another theory in which the main reason why mammals engage in play is to train themselves for unexpected situations. Mammals achieve this by developing locomotory versatility (i.e. self handicapping themselves) and enhancing their capability to cope emotionally with unexpected situations.
The rabbits were involved in object play because they were manipulating toys and did not have any free access to other rabbits, making social play impossible. Play can be used as a welfare indicator, as it has been shown that sick animals usually do not play (Spinka et al., 2001). As mentioned above, play has different purposes and possibly in rabbits one of these is to wear down their open rooted and continuously growing teeth. Chewing on toys increases the total amount of time spent in mastication, thus preventing the appearance of severe health problems such as malocclusion or abscesses that would affect food intake, self grooming, and caecotroph ingestion (Legendre, 2002; Verstraete, 2003; Capello, 2004; 2008; Jekl et al., 2008).

The thirteen rabbits in this study did not exhibit a clear preference for any one of the three toys offered. However, if a larger sample size had been studied and/or a broader variety of toys was tested, a more preferred toy might have been identified.

Alternatively, it may be that offering laboratory rabbits any kind of object suitable for chewing will have the effect of increasing their total chewing. While none of the toys used in this study caused a decrease in the amount of time spent chewing on the cage, there may be one or more toys that does so. In addition, economic considerations play an important role in the choice of toys to be offered to lab rabbits. Other than the price, sturdiness should be considered. As rabbits chew on their toys until they are destroyed, given toys will last a short or long time depending on the material they are made of. Therefore, a durable, more expensive toy may be more cost effective than a cheaper, but less durable toy.

None of the rabbits in this study initially exhibited stereotypic behavior, making it impossible to assess the effect of offering toys on stereotypic behaviors. In this case, chewing on the cage in the absence of non-cage items, i.e. toys, to chew on, was considered a normal behavior that was performed on an abnormal substrate as previous authors have mentioned.
before (Morisse et al., 1997; Verga et al., 2007); the behavior did not take up the large amounts of time considered necessary to define it a stereotypy, and did not disappear or increase when a toy, a more appropriate substrate, was presented to the rabbits.

Podberscek et al. (1991), Gunn and al. (1995), Lidfors (1997), Berthelsen et al. (1999), and Hansen et al. (2000) all dealt with rabbits showing stereotypic/abnormal behaviors such as both cage biting and chewing, hair chewing, head swaying, and pawing at the cage walls. In all of these studies the rabbits benefitted from both social and environmental enrichment. The authors gave the percentage of time the rabbits spent on the different stereotypic behaviors without indicating why these behaviors were considered abnormal. In the case of chewing the bars, for example, they made no mention about the amount of time that rabbits usually spend on chewing in the wild. This makes it difficult to classify the above mentioned behavior as abnormal without a reference range. It is even plausible to think that rabbits were simply showing a normal behavior, such as chewing, but on an abnormal substrate.

While there was no significant statistical effect, rabbits did spend more time, on average, chewing the rubber ball. An experiment with a larger sample size may have identified a significant difference for the following reasons. Since the balls were made of sturdy rubber, they could be chewed on for long periods of time without being destroyed. This material may have helped maintain the rabbits’ interest. The presence of the bell may have made the ball more attractive to the rabbits because of the sound it emitted anytime it was carried around or bounced against the walls.

The rabbits in this study were young adults and were studied over a three month period. It may be the case that studies of longer duration are necessary to adequately induce stereotypic behaviors. Study conditions, in terms of the number of subjects, their age, sex and breed, the
environment, the methods, and the time spent studying the rabbits varied between the current study and the previous studies. This variation may affect the final results in many different ways. This could possibly explain why abnormal behaviors did not occur within the colony.

Podberscek et al. (1991) observed twenty seven rabbits over a three month period: NZW rabbits and their crosses, Dutch breeds, and Lop crosses, both female and males (castrated). The rabbits were split in two separate groups: caged rabbits (from 2 to 3 years of age) and penned rabbits (approximately 5 months old). Gunn et al. (1995) studied 18 NZW rabbits (11 females and 7 males aged 8-13 months) kept in standard laboratory cages over a 24 h period. Lidfors (1997) provided sixty single caged male NZW rabbits with hay in a water bottle, grass-cubes, and two gnawing sticks over a month period. When acquired, the rabbits were approximately 83 days old. Berthelsen et al. (1999) tested 86 rabbits (NZW and French Lop rabbits crossbreeds both female and male; average age 18 months old) by offering them hay as environmental enrichment in two different cage systems (with or without a box inside). The study was conducted over a period of 6 months. Hansen et al. (2000) divided 96 NZW and French Lop crossbreeds rabbits (49 females and 47 males) in two different cage systems (a conventional cage and an enriched cage with a box) over a 6 month period. When observations started, the rabbits were between 11 and 25 months old.

A study conducted on a group of young beagles (Clark et al., 1997) examined the effect of supplemental exercise on their physical and psychological health by measuring their immune, endocrine, and behavioral responses. The dogs were divided in four different groups: exercised individually, exercised with a conspecific, non exercised, and cage control. Over a twelve week period the exercised dogs were brought to a room three times a week for 20 minutes. The authors concluded that the exercise treatment failed to prevent the development of stereotypies.
because there was no difference between any of the groups in the amount of time spent in stereotypic behaviors. By the end of the study all exercised dogs showed more barking than they did at the beginning. All dogs, regardless of treatment, showed a significant increase in stereotypic behaviors as the study progressed; demonstrating that the amount of time an animal lives in a laboratory environment can be relevant to the development of abnormal behavior.

In summary, the current study supports the importance of environmental enrichment for research rabbits to prevent the appearance of stereotypies. Rabbits were offered three different kinds of toys to chew on and they showed a preference for chewing on toys instead of chewing on less appropriate materials such as components of the cage they were housed in. As the Guide (1996) emphasizes, the goal of environmental enrichment is to increase opportunities for the expression of species-typical postures and activities and enhance the animals’ well-being. In order to achieve well-being, an animal should be able to interact with its environment by exploring and manipulating it. If this opportunity does not exist because the environment does not allow the animal’s natural behaviors to be expressed, the animal will not be able to exert control over it. Therefore, its anticipatory processes will be affected. According to Wemelsfelder (1993), the animal will go through different stages beginning with frustration (i.e., the animal is unable to perform its natural behavior patterns) (Dawkins, 2000), boredom, and ending with depression and anxiety, all signs of chronic suffering. Suffering is a general term that encompasses different subjective emotions such as pain, fear, boredom, distress, frustration, thirst, and hunger, just to cite a few. Animals, especially vertebrates, are considered to be sentient beings (Duncan, 2006) capable of experiencing a wide spectrum of emotional states, mainly because they are provided with a nervous system that shares similarities with human beings (Bateson, 1991). Researchers are usually more focused on states considered to be relevant
to suffering because anytime an animal suffers, its welfare will be affected and therefore compromised.

Animal welfare has been defined as the animal’s attempt to cope with its environment and when the animal fails to cope, its welfare is poor and the animal is suffering (Broom, 1988a). Even if suffering is a very subjective emotion, there are different objective parameters, the so-called welfare indicators that allow researchers to measure it (Broom, 1988b; 1991a; b; Dawkins, 2003; 2008). Welfare indicators can be divided into physiological (heart and respiratory rate, adrenal cortex activity), behavioral (activity level, abnormal behavior such as stereotypies), physical (injury, disease), and fitness (mortality rate, reproduction) measurements. Dawkins (2003; 2008) recently introduced another approach to evaluate animal suffering. Dawkins stated that suffering is caused by negative reinforcers that are objective, measurable, and behavioral ways of understanding what matters to animals. To guarantee animal welfare it is important to focus on an animal’s well-being, to ask if the animal is healthy and what the animal wants. This last factor can be achieved through preference tests to determine what an animal likes or dislikes.

To our knowledge there are no studies about how much time rabbits spend on chewing in the wild and this poses a problem because it is very difficult to interpret behaviors such as chewing on a cage. Is it an abnormal behavior or simply a normal behavior performed on an inappropriate substrate? A cage is not a perfect substrate because it is not abrasive enough to wear the rabbits’ teeth down; therefore, molar malocclusions due to molar overgrowth could manifest and affect the animals’ health status. A recent study about this topic in pine voles supports this finding (Harvey et al., 2009). Five adult pine voles living in a research laboratory were presented to the attending veterinarian because of their poor body condition and weight loss. The necropsies performed following euthanasia revealed molar overgrowth in every vole.
Voles are known to chew on the roots and bark of orchard trees; therefore, just adding hardwood sticks in the voles’ cages was enough to prevent molar malocclusions in that colony.

Toys and food items would be more appropriate substrates for rabbits to wear their teeth down on than cages, because they are more abrasive and thus can prevent medical problems that could compromise the animal’s welfare.

**Conclusions**

The results of this study confirm that facilitating rabbits’ natural behaviors such as chewing or playing with a toy improves their welfare as it gives them control over the environment. It may also explain why the animals in the study did not show any abnormal behaviors, since they were allowed to express species-typical behaviors even in a limited space like a cage from an early age.
REFERENCES


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Table 1

Rabbit Ethogram

<table>
<thead>
<tr>
<th>Rabbit Behavior</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chewing</strong></td>
<td>Biting with teeth a non food item such as an object or a toy</td>
</tr>
<tr>
<td><strong>Drinking</strong></td>
<td>Consuming water</td>
</tr>
<tr>
<td><strong>Eating</strong></td>
<td>Biting with teeth and picking up food with mouth and swallowing food</td>
</tr>
<tr>
<td><strong>Grooming</strong></td>
<td>Licking or scratching coat</td>
</tr>
<tr>
<td><strong>Hopping</strong></td>
<td>Moving from one place to another pushing forward with hind limbs followed by forelimbs</td>
</tr>
<tr>
<td><strong>Laying Down</strong></td>
<td>Being in a horizontal, recumbent, or prostrate position on the floor</td>
</tr>
<tr>
<td><strong>Rearing</strong></td>
<td>Standing on hind limbs with torso perpendicular to the floor and forelimbs not touching floor</td>
</tr>
<tr>
<td><strong>Sitting</strong></td>
<td>Fore paws and hind paws, but not torso, touch the floor</td>
</tr>
<tr>
<td><strong>Sniffing</strong></td>
<td>Inhaling air through nose with dilation of nostrils</td>
</tr>
<tr>
<td><strong>Stretching</strong></td>
<td>Extending limbs and muscles to their full extent</td>
</tr>
</tbody>
</table>
Table 2

Mutually exclusive behaviors

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking (D)</td>
<td>LD – S – SN</td>
<td>C – E - G - H – R – ST</td>
</tr>
<tr>
<td>Eating (E)</td>
<td>LD – S – SN</td>
<td>C - D – G – H – R – ST</td>
</tr>
<tr>
<td>Hopping (H)</td>
<td>NONE</td>
<td>ALL</td>
</tr>
<tr>
<td>Rearing (R)</td>
<td>NONE</td>
<td>ALL</td>
</tr>
<tr>
<td>Stretching (ST)</td>
<td>NONE</td>
<td>ALL</td>
</tr>
</tbody>
</table>
Table 3

Total time (sec/h) spent on each behavior

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Baseline</th>
<th>Cardboard Rolls</th>
<th>5 Cardboard Rings</th>
<th>Rubber Balls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chewing object</td>
<td>76 ± 107</td>
<td>52 ± 59</td>
<td>46 ± 39</td>
<td>77 ± 93</td>
</tr>
<tr>
<td>Total Chewing</td>
<td>76 ± 107</td>
<td>248 ± 194*</td>
<td>414 ± 290*</td>
<td>461 ± 402*</td>
</tr>
<tr>
<td>Eating</td>
<td>504 ± 222</td>
<td>559 ± 220</td>
<td>522 ± 257</td>
<td>513 ± 205</td>
</tr>
<tr>
<td>Drinking</td>
<td>81 ± 97</td>
<td>95 ± 59</td>
<td>128 ± 125</td>
<td>87 ± 52</td>
</tr>
<tr>
<td>Grooming</td>
<td>437 ± 225</td>
<td>320 ± 162</td>
<td>302 ± 121</td>
<td>304 ± 168</td>
</tr>
<tr>
<td>Hopping</td>
<td>135 ± 72</td>
<td>166 ± 126</td>
<td>229 ± 161</td>
<td>246 ± 170</td>
</tr>
<tr>
<td>Lying Down</td>
<td>1148 ± 444</td>
<td>1620 ± 486</td>
<td>1465 ± 427</td>
<td>1373 ± 409</td>
</tr>
<tr>
<td>Rearing</td>
<td>20 ± 27</td>
<td>40 ± 38</td>
<td>49 ± 51</td>
<td>56 ± 63</td>
</tr>
<tr>
<td>Sitting</td>
<td>2290 ± 390</td>
<td>1765 ± 428*</td>
<td>1853 ± 380*</td>
<td>1922 ± 407*</td>
</tr>
<tr>
<td>Sniffing</td>
<td>492 ± 270</td>
<td>277 ± 152</td>
<td>314 ± 160</td>
<td>325 ± 152</td>
</tr>
<tr>
<td>Stretching</td>
<td>7 ± 6</td>
<td>9 ± 6</td>
<td>4 ± 4</td>
<td>3 ± 3</td>
</tr>
</tbody>
</table>

* Significant effect from Baseline.
Table 4

P-values for the analyzed behaviors

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Chewing</td>
<td>0.001*</td>
</tr>
<tr>
<td>Drinking</td>
<td>0.285</td>
</tr>
<tr>
<td>Eating</td>
<td>0.921</td>
</tr>
<tr>
<td>Grooming</td>
<td>0.192</td>
</tr>
<tr>
<td>Hopping</td>
<td>0.255</td>
</tr>
<tr>
<td>Lying Down</td>
<td>0.024</td>
</tr>
<tr>
<td>Rearing</td>
<td>0.199</td>
</tr>
<tr>
<td>Sitting</td>
<td>0.004*</td>
</tr>
<tr>
<td>Sniffing</td>
<td>0.028</td>
</tr>
<tr>
<td>Stretching</td>
<td>0.024</td>
</tr>
</tbody>
</table>

* Significant effect
Figure 1

Mean time (± SD) spent on Sitting
Figure 2

Mean time (± SD) spent on Chewing