


Olfactory stimulus as environmental enrichment for shelter dogs: a pilot study

Estímulo olfativo como enriquecimento ambiental para cães de abrigo: um estudo piloto

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ABSTRACT

Environmental enrichment techniques include olfactory stimuli for improving animal welfare. This study aimed to analyze the reactions of 41 shelter dogs exposed to odorous stimuli, such as the method used in another study on wild canids. The focal animal method analyzed the dogs' reactions, with all behaviors recorded. Behavioral responses were classified as positive (P+), negative (N-), or other (Ot). Independent variables were all dogs and the size of the packs. The behavior between the basal (without stimulus), exposure, and after-stimulus withdrawal was analyzed. For all dogs, olfactory stimuli significantly increased P+ ($P=0.001$) and N- ($P=0.004$), contrasting with the decrement of Ot behaviors ($P=0.001$) from the basal to the exposure phase. After the withdrawal of the stimuli, P+, N-, and Ot behaviors returned to basal levels ($P>0.05$). There were no significant differences ($P>0.05$) in the conduct of small or large packs exposed to stimuli. Dogs are sensitive to olfactory stimuli, but arousal is generalized to P+ and N-. It is undesirable to an N- increase for improvement of animal welfare. Contrary to what was observed in a study with wild canids, the method failed in shelter dogs because N- was increased. The introduction of sudden novelty (olfactory stimulus) in an impoverished shelter environment may have caused excitement in the dogs. It is suggested that changes in the method, such as stimuli exposition to each dog in an isolated room, are necessary to increase sheltered dog well-being.

Keywords: *Canis lupus familiaris*. Environmental enrichment. Olfactory stimulus. Scents. Shelter.

RESUMO

As técnicas de enriquecimento ambiental incluem estímulos olfativos para aumentar o bem-estar animal. O objetivo deste estudo foi analisar as reações de 41 cães de abrigo expostos a estímulos odoríferos, como o método utilizado em outro estudo com canídeos selvagens. As reações dos cães foram analisadas pelo método animal focal, com todos os comportamentos registrados. As respostas comportamentais foram classificadas como positivas (P+), negativas (N-) ou outras (Ot). As variáveis independentes foram todos os cães e o tamanho das matilhas. Foi analisado o comportamento entre o basal (sem estímulo), exposição e após a retirada do estímulo. Para todos os cães, os estímulos olfativos aumentaram significativamente P+ ($P=0,001$) e N- ($P=0,004$), contrastando com a diminuição dos comportamentos Ot ($P=0,001$) da fase basal para a de exposição. Após a retirada dos estímulos, os comportamentos P+, N- e Ot retornaram aos níveis basais ($P>0,05$). Não houve diferenças significativas ($P>0,05$) no comportamento de matilhas pequenas ou grandes expostas a estímulos. Os cães são sensíveis a estímulos olfativos, mas a excitação parece ser generalizada para ambos, P+ e N-. É indesejável um aumento de N- para melhoria do bem-estar animal. Ao contrário do que foi observado em um estudo com canídeos selvagens, o método falhou em abrigar cães porque o N- foi aumentado. A introdução de uma novidade repentina (estímulo olfativo) em um ambiente de abrigo empobrecido, pode ter causado excitação exagerada nos cães. Sugere-se alterações no método, como a exposição de estímulos a cada cão em uma sala isolada necessária para aumentar o bem-estar do cão abrigado.

Palavras-chave: *Canis lupus familiaris*. Enriquecimento ambiental. Estímulo olfativo. Aromas. Abrigo.

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Introduction

Confinement can modify and compromise the behavior and well-being of domestic dogs (*Canis lupus familiaris*) that live in shelters and institutional kennels (Wells & Hepper, 2006). The lack of stimuli in confinement can trigger behavioral problems such as stereotypes, self-mutilation, and psychological distress, which is unacceptable according to most countries' moral, ethical, and legal values (Newbury, 2010). Animal welfare is an animal's physical and mental state of the conditions in which it lives and dies. In many countries, ethical and legal guidelines determine that caretakers should aim to provide better animal welfare to captive animals (De Briyne et al., 2020). Therefore, it is necessary to develop methods to mitigate institutionalized dogs' suffering, often in highly precarious conditions.

Environmental enrichment (EE) is a set of techniques and methods applied to captive animals, which can reduce boredom, and mitigate many of the problems of captivity, resulting in an improvement of the individual's welfare (Young, 2003). EE has become essential in managing animal welfare because it is a proven strategy to improve individuals' physical, immune, emotional, and cognitive health (Young, 2003). However, implementing an environmental enrichment program for shelter dogs faces some theoretical and practical obstacles. First, it is necessary to understand a species' behavioral needs and adaptation mechanisms without compromising individuals' physical and psychological health (Newberry, 1995). Second, the modified behavior should not cause health problems or distress to the animals. Third, the changes should be safe for both animals and attendants. Lastly, the changes should be below cost. Therefore, preliminary studies must be conducted to avoid mismanagement and the risk of harming the animals' welfare (Newberry, 1995).

Many of these studies on animal welfare are conducted using sensory environmental enrichment (Figueira et al., 2021; Graham et al., 2005; Wells & Hepper, 2006). The olfactory organ of domestic dogs is well developed, with neural pathways that connect directly to the central nervous system, which involves as many limbic areas related to emotions as cognitive areas (Green et al., 2012). Recent studies have shown that animal use of smell can profoundly affect cognition and emotions. Olfactory stimulus is linked to neurogenesis in the olfactory bulb, influencing the memory and behavior of captive animals over the long term (Kouremenou et al., 2020).

Humans, non-human primates, and rats have continuous neurogenesis of neurons that originate in the ventricular-subventricular zone and project their axons into the olfactory bulb (Ming & Song, 2011). In dogs, neurogenesis also occurs in undifferentiated cells that migrate to the hippocampus, an anatomical structure highly related to the olfactory bulb (Lowe et al., 2015). Experimental evidence suggests that dogs' olfactory exploration behavior enhances "optimistic bias" behavior (Duranton & Horowitz, 2019). Indeed, a literature review indicates that olfaction may play a vital role in dog cognition and welfare (Horowitz & Franks, 2020). For these reasons, the olfactory stimulus as an environmental enrichment would bring many advantages, such as the well-being of dogs in the impoverished environment of many shelters (Tuber et al., 1999).

Some studies on EE for domestic dogs exposed individuals to various odors such as aromatic herbs, spices, the scent of prey, and stool (Graham et al., 2005; Wells, 2009; Wells & Hepper, 2006). In some of these olfactory stimulation studies, dogs are challenged to look for hidden food, which requires keepers to enter the stalls and directly contact individuals, spending time and money from the shelter budget (Wells, 2009; Wells & Hepper, 2006).

Recently, Figueira and collaborators (2021) developed a method using olfactory stimuli with minimal invasiveness to increase the well-being of crab-eating foxes (*Cerdocyon thous*) in captivity. Briefly, those authors exposed 22 foxes to four types of odors, externally to the environment, for 5 min. The results were promising since there was an increase in behaviors considered positive and a decrease in negative behaviors. Commonly, dogs in shelters live in environments like fox captivity, with restricted space and stimuli. The study on crab-eating foxes seems advantageous since it was a non-invasive, low-cost, and practical method (Figueira et al., 2021). Therefore, this article analyzes the application of the technique developed by Figueira and collaborators (2021) in sheltered dogs.

Materials and Methods

The study was carried out with 41 dogs between the ages of two and 10 years, of different sizes, mixed-breed and clinically healthy, in a shelter at the Federal University of Viçosa (Viçosa-MG). All the dogs were destined for adoption and arrived at the shelter as puppies (< 6 months old), spending a large part of their lives in a restricted environment.

The shelter has 10 enclosures that can accommodate one to 10 dogs each, depending on the degree of social compatibility, determined through evaluation by the shelter staff due to lesser mutual aggression between the animals. All rooms measure 6 m² with a covered area at the bottom, 2.3 m high sidewalls, and a screened gate that gives access to the corridor (Figure 1a). There are five enclosures arranged on each side of a central access corridor. The dogs had eye contact with only the stall directly across from them, but not those to the sides (Figure 1a).

The present study methods were based on an olfactory enrichment experiment on 22 crab-eating foxes (Figueira et al., 2021). Thus, the dogs were exposed to four olfactory stimuli: 100 g of fresh, ground beef; 100 g of chopped parmesan cheese; 100 g of sawdust soaked in rodent urine, obtained from boxes with rodents from a vivarium; and two chicken eggs, boiled and chopped. The stimuli were put inside permeable cotton cloth bags, which allowed the animals to sense the odor of the contents without being able to see it (Figure 1b). All cloth bags were the same color and size and were washed with unscented soap and water after each observational session. The bags with a stimulus were positioned in front of and outside each wire mesh door enclosure at a distance that prevented the dogs from reaching it with their paws or mouth.

The test sessions were filmed and then analyzed using a behavioral observation program. A compact camera (Samsung® ST77, South Korea) was used for filming, supported on a tripod and positioned in front of the enclosure. Filming took place between 8 am and 10 am, always before the animals' feeding time. In each filmed session, the behavior of everyone inside the stall was observed, with the total time for each dog's behavior recorded. During filming, the stimulus exposure site was surrounded by a curtain to prevent the other dogs in the front stall from having visual contact with the procedure.

After the camera was positioned, filming began in an initial simulate session to rehearse and prepare for the behavior sampling session. The simulating session after that, called "the warm-up session," is a movement sequence identical to placing the olfactory stimulus but without exposing the stimuli. This observation session was performed to prevent individuals from establishing relationships between the researcher's movements and the stimulus. After shooting the footage, the researcher left the animal's view for 5 min. After that, the researcher re-entered the corridor, placed the stimulus, and left again without pausing the recording. After 5 min, the researcher entered and removed the stimulus, leaving again without pausing. After another 5 min, the researcher entered again, and the camera was restarted to film the next EE session. At the end of each session, the next one began until all four stimuli had been presented to each enclosure. For each stimulus, the observational session lasted 23 min, including a "warm-up session," Basal, exposure to the stimulus (Exp), and post-stimulus phase (Pos) (Figure 2). However, the behavior was recorded only in the Basal, Exp, and Pos sessions. Filming took place on one day for each enclosure, with four EE sessions presented,



Figure 1 – (a) arrangement of the stalls at the shelter; (b) the cotton bag used to hide the visual stimulus but not the smell for dogs in the shelter.

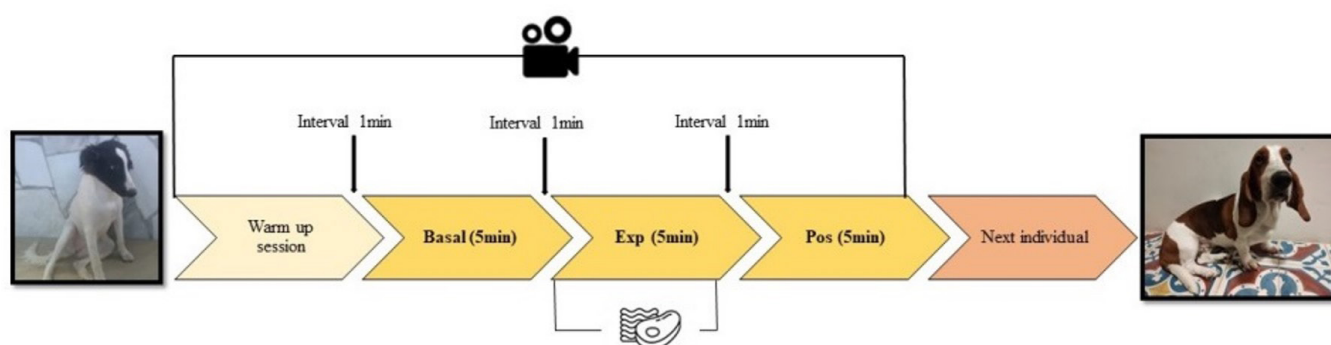


Figure 2 – Flowchart of olfactory stimuli tests for shelter dogs as part of a survey to create a sensorial enrichment program.

Table 1 – An ethogram for the olfactory stimuli (OE) study of 41 domestic dogs (*Canis lupus familiaris*) in a shelter

Behavior	Behavior description	Behavioral category
Play	Individual interacts with the environment or with another animal in a playful, relaxed way	P+
Non-agonistic social interaction	Individual shows friendly behavior towards another animal such as licking or grooming	P+
Attempting to reach the OE	Individual tries to reach the OE with its paw through the cage	P+
Self-maintenance	Individual bites or licks, slowly and calmly, parts of its own body	P+
Sniffing	Individual moves its nostrils, pointing towards objects or regions of the enclosure	P+
Sniffing or pointing OE	Animal points its snout in the direction where the OE is or was placed	P+
Agonistic behavior	Individual shows signs of aggression such as growls, baring of teeth, scratching, or biting another animal	N-
Biting the cage	Individual bites or pulls at the cage with its teeth	N-
Yawning	Self-defined	N-
Scratching itself	Individual rubs one leg or its mouth vigorously on its skin or hair	N-
Stereotypy	Individual perform repeated movements, more than three times for no apparent reason	N-
Climbing the railing or wall	Individual stands up and supports its front limbs on the railings or walls of the enclosure	N-
Sneezing	Self-defined	N-
Other	This means any activity not listed in the behaviors described as P+ or N-	Ot
Out of sight	Focal animal is out of sight of the observer	Ot

P+, positive behavior; N-, negative behavior; and Ot, other behavior.

one for each stimulus. The sessions were on different and consecutive days. The order of presentation of the EE was by chance.

Another author's descriptions were adapted to elaborate an ethogram showing all the behaviors (Table 1) (Figueira et al., 2021). Each dog's behavior was observed in a single session per enclosure, but a "warm-up" session was not analyzed. The camera simultaneously recorded the images of the reactions of all the dogs that were inside the enclosure.

Behavioral responses were separated into three categories: positive behaviors (P+), negative behaviors (N-), and other behaviors (Ot) (Table 1). The averages were calculated from the recorded time for each set of behavioral responses (P+, N-, and Ot). The dogs' behavioral responses were analyzed using the focal animal method with a personal

computer, with all behaviors recorded (Teixeira et al., 2018). The total duration for each behavior was recorded with the behavioral analysis program PROSTCOM (Conde et al., 1999). During the first minute of each phase (Basal, Exp, and Pos), while the researcher entered and was in the dogs' field of view, behaviors were not recorded. The estimated duration of the behavioral categories was the average time for all dogs in the shelter.

All enclosures had 2-6 dogs, but one section had a lone dog. The analysis looked at whether the group size per pen, from now on called a pack, influenced the response to stimuli, using the average of 4.1 dogs per pen as the size classification criterion. Small packs were those with up to four individuals, and large packs were those with more than four individuals per enclosure. For the comparison

Table 2 – Results of the Wilcoxon test (*Z*) and significance value (*P*) when comparing the average time spent on different behavioral responses with olfactory stimuli in the Baal and Exp phases in institutional shelter dogs

Behavioral category	Phase	Mean ± Standard error (s)	Z	P
P+	Basal	23.39 ± 3.91	- 4.38	0.001*
	Exp	39.24 ± 4.04		
N-	Basal	3.63 ± 0.86	- 2.87	0.004*
	Exp	11.68 ± 2.24		
Ot	Basal	273.17 ± 3.96	- 4.73	0.001*
	Exp	249.10 ± 5.07		

P+, positive behavior; N-, negative behavior; Ot, other behavior; *Statistically significant.

Table 3 – Result of the Wilcoxon test (*Z*) and significance value (*P*) when comparing the average time spent on different behavioral responses with olfactory stimuli in the Basal and Pos phases in institutional shelter dogs

Behavioral category	Phase	Mean ± Standard error (s)	Z	P
P+	Basal	23.39 ± 3.91	- 0.82	0.41
	Pos	23.32 ± 3.53		
N-	Basal	3.63 ± 0.86	-1.51	0.13
	Pos	1.99 ± 0.43		
Ot	Basal	273.17 ± 3.96	- 0.67	0.50
	Pos	272.29 ± 3.72		

P+, positive behavior; N-, negative behavior; Ot, other behavior.

between large and small packs, the estimate of each behavioral category was the result of the weighted average of the behavior of the total individuals in each enclosure.

The comparisons verified whether there was a change in the P+, N-, or Ot behaviors in the Exp phase compared to the Basal phase; and in the Pos phase compared to the Basal phase. The direction (increased or decreased) in which changes in behavior occurred, when present, was also verified. It was recorded whether these changes (if any) remained after the withdrawal of the stimulus. These comparisons, within the scope of inferential statistical analysis, were performed using non-parametric analyses, applying the Wilcoxon test for paired samples. For comparing dogs kept in small packs or large packs, the Mann-Whitney non-parametric statistical test was used. All statistical tests used a two-tailed distribution, with an $\alpha \leq 5\%$. The study was approved by the Animal Use Ethics Committee of the Federal University of Viçosa, Protocol No. 09/2013.

Results

The duration of both P+ behaviors and N- behaviors increased significantly from the Basal to the Exp phase (P+, $P = 0.001$; N-, $P = 0.004$). On the contrary, Ot behaviors decreased significantly ($P = 0.001$) from the Basal phase to the Exp phase (Table 2). Comparing the Basal and Pos phases, no significant differences were detected in P+ ($P = 0.41$), N- ($P = 0.13$), and Ot behaviors ($P = 0.50$) (Table 3). There were no statistically significant differences

Table 4 – Average time spent on different behavioral responses comparing small groups (up to four dogs per pen) or large groups (more than four dogs per pen) during the Basal, Exp, and Pos phases in dogs in an institutional shelter

Group size	Behavioral category	Phase	Mean ± Standard Error (s)
Small ($n \leq 4$)	P+	Basal	19.57 ± 5.63
		Exp	36.77 ± 5.39
		Pos	16.63 ± 4.98
	N-	Basal	25.36 ± 5.18
		Exp	40.51 ± 5.47
		Pos	26.78 ± 4.67
	Ot	Basal	3.33 ± 1.60
		Exp	15.18 ± 4.11
		Pos	2.63 ± 0.98
Large ($n > 4$)	P+	Basal	3.79 ± 1.02
		Exp	9.87 ± 2.65
		Pos	1.66 ± 0.41
	N-	Basal	277.11 ± 5.89
		Exp	248.07 ± 8.13
		Pos	273.68 ± 5.95
	Ot	Basal	271.13 ± 5.19
		Exp	249.10 ± 6.46
		Pos	271.29 ± 4.75

P+, positive behavior; N-, negative behavior; Ot, other behavior.

between the mean duration of by behavioral categories (P+, N- and Ot) of large and small packs (Table 4), (Basal phase vs. Exp phase, $P > 0.05$; Basal phase and the Pos phase, $P > 0.05$) (Table 5).

Table 5 – Result of the Mann-Whitney test (*U*) and the significance value (*P*) when comparing small groups (up to four dogs per pen) and large groups (more than four dogs per pen) during the Basal, Exp, and Pos phases in dogs in an institutional shelter

Behavioral category	Phase	<i>U</i>	<i>P</i>
P+	Basal	2980.00	0.860
	Exp	2689.00	0.233
	Pos	2779.00	0.365
N-	Basal	2845.50	0.424
	Exp	2632.00	0.124
	Pos	2723.00	0.166
Ot	Basal	2772.00	0.370
	Exp	2668.00	0.209
	Pos	2931.00	0.741

P+, positive behavior; N-, negative behavior; Ot, other behavior.

Discussion

During the olfactory exposure, the behavioral responses of captive dogs changed, with an increase in both positive and negative behaviors and a decrease in other behaviors. However, the behavior change was not long-lasting, and after removing the stimuli, the dogs' behavior returned to the mean duration like the Basal phase. Among the unfavorable behavioral responses were those related to anxiety or dispute over hierarchy with aggressive behaviors. Traditional dominance is characterized by ritualized communication signals, which are stable over time, particularly in captive canids (van der Borg et al., 2015). Dogs that have been in captivity for a long time and grouped with the same individuals (as in the case of the dogs in this study) have a stable hierarchy and coexistence compared to newly formed packs (van der Borg et al., 2015). Therefore, the olfactory stimulus does not seem to stimulate the agonist behavior in the dogs. New stimuli can increase the level of arousal in a group of dogs, leading them to increase many behaviors recognized as distress (Breuer & Elson, 2017). The general excitement can come from the circumstances of the experiment, such as the frustration of not being able to reach the stimulus.

In a restricted environment, where management must aim at the animal's welfare within the structural and functional conditions of a shelter, the prevention of distress of individuals is essential to minimize the occurrence of suffering (Young, 2003). However, when analyzing the behavioral responses by group size (small and large packs), no difference was found in P+, N-, and Ot behavioral categories in each phase (Basal vs. Exp; Basal vs. Pos). Given that, in larger groups, there is a greater probability of agonistic interactions than in smaller groups, this result suggests that the increase in agonistic behaviors is not due to instability or reinforcement

of the hierarchy of subordination and dominance within the packs. Dogs in shelters have a more fluid, complex, non-linear structure of social relationships, depending on the context and the individuals involved (van der Borg et al., 2015). This seems more plausible that the presence of olfactory stimuli increased the general arousal of the dogs, increasing both P + and N- simultaneously but decreasing Ot. Therefore, olfactory stimuli increased excitability but did not directly affect animal welfare, which would increase P + while decreasing N-.

The Frustration-Aggression Theory (Breuer & Elson, 2017) postulates that: "all acts of aggression are the result of previous frustration"; and "all frustration leads to aggression." Frustration is an act of blocking an individual from gaining expected gratification. Many studies confirm the theory (Breuer & Elson, 2017). The increase in N- could be interpreted as the dogs' frustration in not obtaining the object hidden in the bag. Frustration would be expected to increase negative behaviors and trigger a decrease or stability in the duration of positive behaviors. There seems to be an ambiguous reaction of the dogs in this experiment, as they show excitement from exposure to the stimulus but frustration due to the impossibility of reaching the stimulus.

The effect of the stimulus was short-lived, not continuing after the withdrawal of the stimulus, which frustrated one of the objectives of the olfactory enrichment method to increase well-being. Dogs are naturally curious animals and use smell as one of their principal means of exploring the environment in which they live (Teixeira et al., 2018). The olfactory stimuli in this study seem to have aroused the dogs' exploratory interest, but the stimulus's withdrawal reduced P + and N-. The P+ must have long-lasting effects to be an EE, which did not happen in this experiment. Therefore, the lack of permanence of the increased P + thwarted the success of this olfactory enrichment for the dogs.

The division of dogs into large or small groups in captivity did not alter the responses to the stimulus. Dogs are highly sociable animals and form packs (Serpell). However, in captivity, the animals are forced to live solitarily or in groups in small spaces. In this study, the olfactory stimulus, for both small and large packs, was ineffective as environmental enrichment, suggesting that the group size did not influence the response of the dogs.

The experimental schedule, with the observer entering the corridor, the brief exposure of the stimulus, and the movement of the dogs near the focal animal, increased the general excitement of the dogs in the observed enclosure. Therefore, it is suggested that the behavioral response of

the dogs in each observation session was due not only to the stimulus but also to the whole procedure, including the excitation of dogs in other enclosures. This general increase in excitation may have been an intervening variable that altered the behavior of the groups focused on the stimulus. The increase in N- behaviors may result from this general arousal, which interfered with P+ responses.

The results of this study with dogs contrast with the results obtained in crab-eating foxes (Figueira et al., 2021). In the study with foxes, there was an increase in positive behaviors during exposure, which remained after the stimulus was withdrawn. The foxes' negative behaviors decreased after the stimulus was removed. We suspect that the difference in results between dogs and foxes may be due to two main variables: the enclosure and the motivation of each species to explore odors. However, the lack of a clear result showing an increase in positive behaviors and a contrasting decrease in negative behavior was not achieved. As used, the method showed limitations that should be addressed. The arrangement of many individuals in the same enclosure, and many enclosures nearby within auditory and olfactory contact, limit the method's effectiveness.

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Conclusion

This method for environmental enrichment of dogs in an institutional kennel failed, as there was an increase in positive behaviors. Still, there was also an increase in negative behaviors, which are undesirable and do not improve animal welfare.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

The study was approved by the Animal Use Ethics Committee of the Federal University of Viçosa, Protocol No. 09/2013.

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