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A Pilot Study on Behavioural Responses of Shelter Dogs to Olfactory Enrichment

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ABSTRACT

The influence of essential oils (EOs) on emotions has been widely described among humans and animals. Several studies have investigated the effects and the actions of EOs on behaviour, mood and perception. In this study, shelter dogs (n=23) were exposed to olfactory stimulation through diffusion of 9 anxiolytic essential oils in one blend (olfactory enrichment) for 8 weeks in order to check long-term effects on behaviour. First, dog's postures have been evaluated in both groups before and after exposure. Secondly, in order to collect the preliminary results on the distance necessary to obtain an effect of EOs, dogs were divided in 2 groups according to the distance from the diffuser.

Our results indicate that olfactory enrichment with *this blend of EOs* is related to less time spent by dogs in high posture. More research is needed to investigate a potential gradual effect of distance and concentration of EOs on dog's welfare.

1. Introduction

Essential oils (EOs), which are obtained through distillation from aromatic plants, have been widely used for bactericidal, viricidal, fungicidal, antiparasitic, insecticidal, medicinal and cosmetic applications [1].

The influence of EOs on emotions has extensively been described among humans [2-6]. Several studies have investigated the effects and the physiological pathways of EOs on behaviour, emotions and perception [7]. In the last few years, scientific research has focused on very specific effects of different EOs among humans (e.g., the anti-anxiety effects of *Lavandula angustifolia* or the improved alertness effect of *Mentha piperita*).

Also, in animals, the use of aromatherapy as environmental enrichment has been studied in e.g. zoo animals [8-10], kenneled dogs [11-13], kenneled cats [14], and horses [15-16].

Shelter dogs often live in stressful situations. In some of these cases, specific anxiolytic essential oils may be helpful for these dogs in order to decrease their stress.

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Graham and colleagues [11] exposed 55 dogs of mixed breed housed in an animal rescue shelter to four types of olfactory stimulation: the diffused essential oils of lavender, chamomile, rosemary and peppermint. Animals were also studied in their normal kennel environment minus the introduction of any artificial odours (control). The dogs received each condition of stimulation for 4 hours a day for 5 days, with an intervening period of 2 days between conditions. Certain aspects of the dogs' behaviour were found to be influenced by the odours. Specifically, dogs spent significantly more time resting and less time moving upon exposure to lavender and chamomile than any of the other olfactory stimuli. These odours also encouraged less vocalization than other types of scent. The diffusion of rosemary and peppermint into the dogs' environment encouraged significantly more standing, moving and vocalizing than other types of odour.

The Cognitive Bias Test is another approach that can be used to understand the effect of Eos. Olfactory enrichment with a blend of 9 anxiolytic essential oils (*Cananga odorata, Cistus ladaniferus, Citrus aurantium, Cupressus sempervirens, Juniperus communis var. montana, Lavandula angustifolia, Laurus nobilis, Litsea citrata, Pelargonium graveolens*) resulted in a reduced latency to the ambiguous cue (cognitive bias test), indicating a more optimistic bias [13]. A cognitive bias test in this context refers to the propensity of a subject to show behaviour indicating the anticipation of either relatively positive or relatively negative outcomes in response to affectively ambiguous stimuli [17]. Changes in cognitive bias reflect an individual's experience of positive and negative events and thus its affective valence and welfare [18]. This recent and innovative approach utilizes the influence of affective states on the interpretation of current experience. The resulting affect-induced cognitive biases can be measured [17] through cognitive bias tests as indicators of the animal's psychological well-being [17, 19].

Observation of dog's posture is often used as a valid instrument to interpret emotions. In a study where researchers checked potential welfare effects of two different housing conditions through behavioural and physiological parameters, shelter dog's postures were evaluated as behavioural parameters [20]. In the SAB Test (Socially Acceptable Behaviour Test) most dogs that threaten or bite other dogs will have a high posture or a behavioural state of high arousal [21].

We tested the same blend of Eos applied in [13]. The aim of this study was to evaluate dog's postures before and after EOs diffusion. Dogs have been exposed to the blend at two different distances in order to explore the distance to induce an effect (diffuser in the same corridor and diffuser at 10 m distance from the corridor). We hypothesized that the blend could reduce the dogs' reactivity demonstrated by a reduction in time spent in high posture due to the anxiolytic effect of the EOs-blend. However, the group that was directly exposed to the diffusion is expected to display bigger changes in posture then the group at a 10 m distance of the diffuser.

2. Material and Methods

2.1 Subjects and housing

This study was carried out during a period of 8 weeks at a rescue shelter (Het Blauwe Kruis, Zinnialaan 2, Oostende, Belgium). A total of 23 shelter dogs participated: Group 1 (G1) included 7 males and 4 females, Group 2 (G2) included 10 males and 2 females. All dogs were declared to be in good health at the onset of the study.

The dogs were between 1 and 9 years old and arrived at the shelter within 1 month up to 5 years of the start of the study (mean for G1 and G2: 1.1 year). All dogs were spayed.

The sample of dogs was comprised of several breeds (G1: 1 Belgium shepherd, 2 Staffordshire terriers, 1 Bull terrier, 2 Labradors, 2 crossed Rottweilers, 1 Red nose pitbull, 1 Husky, 1 Mixed breed; G2: 1 Belgium shepherd, 2 Staffordshire terriers, 1 Shar-Pei, 1 Pincher, 1 Teckel, 2 Jack Russell terriers, 1 Yorkshire terrier, 1 Akita Inu, 1 French bulldog, and 1 Cross poodle).

The dogs of G1 and G2 were individually housed in typical indoor (G1: 2 x 2 x 2.5 m; G2: 1.5 x 2 x 2.5 m) – outdoor (G1 and G2: 2 x 4 x 2.5 m) pens (Figure 1). The diffuser was located in the G1 corridor, meanwhile the G2 was at 10 meters far from the diffuser. All the dogs remained in their own shelters throughout the study.
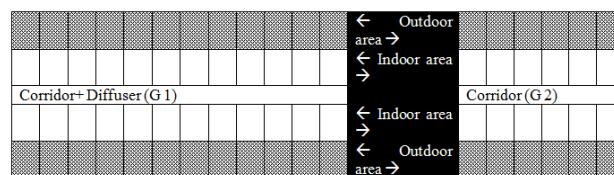

Figure 1. Shelter

2.2 Experimental Design of the Olfactory Enrichment Procedure

The dogs were exposed to olfactory enrichment through a blend of essential oils (*Litsea citrata, Cupressus sempervirens, Citrus aurantium, Pelargonium graveolens, Lavandula angustifolia, Cananga odorata, Juniperus communis var. Montana, Cistus ladaniferus and Laurus nobilis*). The

blend was diffused by a specific instrument (diffuser) manufactured by Voith©, able to diffuse up to 300m². This diffuser was placed in the central corridor of G1 and was activated from day 3 until day 56 from 5PM until 2PM the following day (21 hours per day). A concentration of 3 ml was gradually diffused each day over 21 hours. This concentration was chosen according to preliminary data collected by authors. The diffuser was silent and there was no unfamiliar auditory stimulation during the experiment.

The dogs of G2 were not directly exposed to this olfactory enrichment, though, but were expected to perceive these odours. The dogs of G1 and G2 were used to the normal odors from the shelter, and those odors were considered to be the neutral control odors. Dogs of G1 and G2 were all studied at the same time within the same kennel environment.

A camera filmed each dog during a session of 20 minutes each day: (1) throughout the control period (day 1 until day 3; no diffusion of EOs), (2) during week 1 (day 4 until day 10) and (3) again during week 8 (day 57 until day 63) to check long-term effect. Filming occurred after feeding the dogs at 9AM. According to [22], the first 5 minutes and the last 5 minutes were not used to avoid human interference due to installing and removing the camera. However, dogs ignored the camera in all occasion. Additionally, no human disturbance was allowed during testing.

The postures of the dogs were recorded on videotape using a surveillance camera (Digital Video Camera Recorder, DCR-TRV27E, Sony®). The camera was placed in front of the cage. Videotapes were scored by one of the authors, and independently by one student. This student received training by the authors to score the behaviours. Intra-observer reliability exceeded 95%. Postures were evaluated according to Table 1.

Table 1. Postures description according to [22] and [23]

Postures	
High	The breed specific posture as shown by dogs under neutral conditions, but in addition the tail is positioned higher or the position of the head is elevated, and the ears are pointed forwards, or the animal is standing extremely erect
Neutral	The breed posture shown by dogs under neutral conditions
Half low	Two or more of the following three features are displayed: a lowered position of the tail (compared to the neutral posture), a backward position of the ears and bent legs
Low	The position of the tail is lowered, the ears are positioned backwards, and the legs are bent
Very low	Low posture, but now the tail is curled forward between the hind legs
Not seen	Unable to determine the behaviour of the dog owing to darkness or the position of the dog

3. Data analysis

The effect of period (control, week 1, week 8) on the percentage of high posture was modelled. However, since the observations belonging to the same dog are correlated, as well as the observations taken on the same day, two random effects were included in the model to capture these correlations. This implies the estimation of the following mixed linear model:

$$Y_{ij} = \beta_0 + \beta_1 x_{ij} + \delta_i + \gamma_j + \varepsilon_{ij}$$

Where Y_{ij} is the measured percentage of high posture of dog_i on day_j, x_{ij} represents the period (control, week 1, week 8) for dog_i on day_j, δ_i is the random effect of dog_i, γ_j is the random effect of day_j, and ε_{ij} is the random error of the individual observation. If hypothesis testing showed that there was a significant effect of "period" on the percentage of high posture, multiple comparison testing with Tukey correction was performed to identify the significant differences between the three periods considered.

The statistical analysis was performed using JMP pro 13. The significance level was set at 0.05.

4. Results

4.1 Results for Group 1 (G1)

The average percentages of high posture suggest a decrease over experimental period in G1 (Table 2). The standard deviations are however quite high.

Table 2. Average percentage of high posture for each period as well as the standard deviation for G1

Period	Mean	Std dev
Control	86.75	23.12
Week 1	84.31	28.96
Week 8	57.53	36.74

The estimation of the mixed linear model reveals that G1 shows a significant effect of "period" on the percentage of high posture (p-value<0.0001, Table 3).

Table 3. Results fixed effects test of mixed linear model

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Period	2	2	161.4	21.507886	<.0001*

This significant effect is the result of a significant difference in average percentage of high posture between control period and week 8 (p-value <0.0001), and between week 1 and week 8 (p-value <0.0001). There is no significant difference between control period and week 1 for G1 (Table 4).

Table 4. Subsequent pairwise comparison testing for G1

Period	-period	Difference	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Control	week_1	0.90607	6.033051	0.15	0.9876	-13.3656	15.17774
Control	week_8	27.65306	6.053850	4.57	<.0001*	13.3322	41.97394
week_1	week_8	26.74699	4.413390	6.06	<.0001*	16.3068	37.18723

4.2 Results for Group 2 (G2)

G2 shows similar results than G1. The results for G2 show a decline in average percentage of high posture over the experimental period, suggesting that period has an effect on the percentage of high posture. Also here, the standard deviations are quite high (Table 5).

Table 5. Average percentage of high posture for each period as well as the standard deviation for G2

Period	Mean	Std dev
Control	87.91	25.89
Week 1	77.45	34.41
Week 8	56.59	37.98

The estimation of the mixed linear model reveals that G2 shows a significant effect of "period" on the percentage of high posture (Table 6).

Table 6. Results fixed effects test of mixed linear model

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Period	2	2	143.4	7.2587544	0.0010*

Subsequent pairwise comparison testing shows that there is a significant difference in average percentage of high posture between the control period and week 8 (p-value = 0.0007), as well as between week 1 and week 8 (p-value = 0.0368) for G2. There is no significant difference between control period and week 1 (p-value = 0.1403) for G2 (Table 7).

Table 7. Subsequent pairwise comparison testing for G2

Period	-period	Difference	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Control	week_1	11.05006	5.793235	1.91	0.1403	-2.67027	24.77040
Control	week_8	24.34497	6.463416	3.77	0.0007*	9.03742	39.65252
week_1	week_8	13.29490	5.338127	2.49	0.0368*	0.65242	25.93739

5. Discussion

The results of the present study indicate that olfactory enrichment with this blend may be helpful for dogs in shelter in order to spend less time in high posture. When dogs are decreasing their high posture, they become more relaxed [23]. In a daily routine at the shelter, when no stimuli are present, dogs are supposed to stay in a neutral posture that could be considered as an energy-saving posture.

The diffusion of the EOs blend can be helpful in relaxing dogs during long-term confinement in shelter. Effects have been reported both in G1 and G2: dogs spend significantly less time in high postures at the end of the study (week 8) than during the control period. Although we still do not define precisely the maximum distance that still has an effect, we observed that time spent in "high posture" decreased after exposure to EOs blend, even in dogs at 10m distance from the diffuser. Canine high posture has been categorized among agonistic behaviours [20-21]. In dogs' encounters with other conspecifics, body size and body posture are the first visual signals perceived, providing the very first information about other individuals' intentions. Dogs can communicate confidence, but also arousal, alertness, or threat by increasing their body size, pulling themselves up to their full height, and increasing the tension of the body muscles [24].

Living in a shelter has been correlated with some behavioural and welfare problems: abnormal social behaviour, enhanced or abnormal reactions in threatening situations, and retarded development of independence [25]. Abnormal reactions are quite common in routine shelter life. Vigilance and alertness without any apparent stimulus are often reported. According to [11], the use of EOs blend as olfactory enrichment provides new possibilities to enhance animal welfare and decrease behavioural problems due to stress (e.g., barking, whining, and high activity) among shelter dogs. Moreover, a combination of EOs seems more effective than the application of the EOs separately [13]. These results also build further on [26], who found that olfactory enrichment provides a sense of safety for animals.

Our results can be useful in order to understand how exactly EOs can be applied. However, this study shows that there are still several unsolved research questions. The minimal concentration and the minimal distance to get an effect has not been tested yet. The bibliography mentions various methods for analyzing the effects of EOs on behaviour in the lab and under field conditions. There is however a lack of consistency between these methods, and the authors of this study favor a standardized method for EO olfactory exposure, exposure time, observations (filming) and exposure to external stimuli like e.g. shelter stimuli.

The authors are convinced that olfactory enrichment can become part of routine management not only for animal shelters but also for veterinarians who are seeking to prevent the development of behavioural problems and to stimulate a positive relationship between owners and their animals.

6. Conclusion

Our results indicate that olfactory enrichment with this blend of EOs is related to less time spent by dogs in high posture. More research is needed to investigate a potential gradual effect of distance and concentration of EOs on dog's welfare.

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Authorship

The idea for the paper was conceived by Dr. Anouck Haverbeke. The experiments were designed by Dr. Anouck Haverbeke and Dr. Stefania Uccheddu. The experiments were performed by Dr. Anouck Haverbeke, Dr. Stefania Uccheddu and Mathilde Debel. The data were analyzed by Dr. Heidi Arnouts Arnouts and Dr. Adinda Sannen. The paper was written by all the authors.

Conflicts of Interest

There is no conflict of interest.

Approval of the ethical treatment of animals, including the identification of the institutional committee that approved the experiments, was not required in the present study.

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