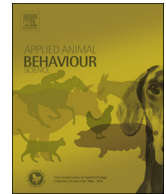




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The effects of green odour on domestic dogs: A pilot study

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ABSTRACT

Green odour (a mixture of cis-3-hexenol and trans-2-hexenal), similar to cut grass, has been demonstrated to appease subjects of various species (rats, cattle, humans etc.) subjected to different stressful stimuli. The aim of this study was to investigate whether green odour has a calming effect also on the domestic dog. Sixteen companion dogs participated in the study with their owners. Each dog was tested through a simplified version of the Ainsworth Strange Situation Test. Dogs acted as their own control, being tested three weeks apart in an experimental room once with the green odour and once without it. Saliva samples were collected, for both conditions, before the test and 20 min after separation in order to measure cortisol levels.

The presence of green odour in the experimental room did not affect dog behaviour during separation from the owner. In addition, deltas of salivary cortisol levels in the two tests did not differ. However, differences in dog behaviour were observed when the owner was present, both before and after separation, indicating that, when tested in the presence of green odour, dogs sat more and spent less time in proximity to the owner. When tested without the green odour, dogs instead sought more contact with the owner. These findings suggest that green odour has a light stress-alleviating effect, which appears in more relaxed behaviour in the presence of the owner.

1. Introduction

The sense of smell provides animals with information for locating food, recognition of conspecifics and warning of predators and danger. Besides, odour cues, both from animals and from plants, are essential stimuli that influence mammals' behaviour (Nakashima et al., 2004).

Some studies have assessed the properties of plant-derived volatile components called 'green odour', a mixture of cis-3-hexenol and trans-2-hexenal, two of the eight compounds that constitute the characteristic odour of fresh green leaves (Hatanaka, 1996). In particular over the last decade, a strong body of evidence has directly linked green odor exposure to stress relief using a variety of stress inducing paradigms and analytical techniques, showing the effects of green odour exposure on the behaviour, neurochemistry, autonomic and endocrine response (Spiers et al., 2015). Exposure to green odors has demonstrated subtle changes in basal behaviors, and more pronounced effects in stress-targeted behavioral testing (Spiers et al., 2015). Therefore, laboratory animals have been tested in many different situations ranging from mildly stressful (e.g. clean cages as a novel environment: Nikaido and Nakashima, 2011) to strongly stressful ones (e.g. the forced swimming model of depression in rats: Nakatomi et al., 2008). Besides studies on laboratory animals, green odour has been shown to have stress-

alleviating effects, such as a decrease in vocalizations and concentration of plasma cortisol, and an increase in the duration of rumination in steers undergoing isolation stress in a novel environment (Sutoh et al., 2013). Moreover, in human adults green odor reduces nociception and fatigue-like response, while not affecting normal sensory, motor and emotional function under non-stress conditions (Oka et al., 2008).

Although there is evidence for a green odour stress-alleviation effect in various animal species, no data are available for domestic dogs.

The exceptional olfactory ability of dogs is well known. The canine nasal cavity contains hundreds of millions of sensory neurons, and a slower and more laminar flow in the olfactory recess (Craven et al., 2010) allowing more time for odorants to bind to receptors found on the sensory neurons in the olfactory epithelium (Green et al., 2012). Exposure to certain odours may influence dogs' behaviour. For instance, aromatherapy with lavender is effective in reducing excitement in domestic dogs during car transportation (Wells, 2006). Diffused lavender and chamomile have been found to increase relaxation, evidenced by increased resting and decreased barking in sheltered dogs, while peppermint and rosemary increased their time of alertness (Graham et al., 2005).

The use of air fresheners with different fragrances (including green odour) in homes is very common. Considering that dogs live in home

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environments, the odour perceived may influence their behaviour and welfare.

The aim of the study was to investigate the possible effects of the exposure to a green odour fragrance in domestic dogs in a mildly stressful situation, i.e. under social isolation in a novel environment. The hypothesis is that, as found in other species, green odour may have a calming effect on dogs, thus evoking more relaxed behaviours and a lower increase in cortisol levels compared to the same mildly stressful situation in the absence of green odour.

Social isolation in a novel environment is a well-known cause of stress in dogs (Mariti et al., 2013a,b). Similarly to that performed in steers (Sutoh et al., 2013), it may represent a suitable condition for a study on the effectiveness of green odour in domestic dogs. The stress response of dogs to separation from their owner during the Ainsworth's Strange Situation Test (ASST; for a review see e.g. Payne et al., 2016) and its modifications (Parthasarathy and Crowell-Davis, 2006; Mariti et al., 2013a,b; Rehn et al., 2013) has long been documented. A shorter and simpler version of this test was used to create a brief social isolation, and dogs' behaviour before, during and after separation (reunion) was assessed.

2. Materials and methods

2.1. Subjects

The inclusion criteria for tested dogs were: being more than 20 months old; living in a home environment; having been lived with the owner for at least 1 year; being clinically healthy and not under pharmacological treatment with psychoactive drugs. Furthermore, none of the female dogs could be in estrus, nor pregnant at or around the time of testing. After the tests, it was checked with the owner that air fresheners with green odour were not used at home or in other environments frequently visited by their dog.

Sixteen companion dogs and their owners (11 women and 5 men) were involved in the study. The characteristics of individual dogs were variable: 13 females, of which 9 neutered, and 3 males, of which 2 neutered; most dogs were mixed-breeds (69.2%), and the rest belonged to various breeds (1 Border Collie, 1 Italian Pointing Dog, 1 Australian Kelpie, and 1 Flat Coated Retriever); the age ranged 20–138 months (mean \pm standard deviation, 84.7 ± 40.6 months); as for the size, 3 dogs were small, 7 were medium, and 3 were big; the weight ranged 12–35 kg (37.5 ± 12.2 kg); they had been living with the owner for 3–11 years (7.2 ± 3.1 years).

2.2. Experimental setting

All the tests were conducted in a relatively bare room, unfamiliar to the dogs, at the Dipartimento di Scienze Veterinarie - University of Pisa (Italy), over a period of 8 months. The room (4.50 x 4.30 m) had two doors (although the owner could enter/exit only from one) and it was equipped with: two chairs (one for the owner and the other one left empty, as a control for exploration to owner's chair); three toys (a ball, a rope and a dog toy); a table; an automatic aerosol dispenser on the wall (out of the dog's reach); and a videocamera (GO-Pro Hero 3+) (see Fig. 1).

Two dispensers were available to the researchers, and one of them was used for each test. In the test without green odour, an empty dispenser was used. In the test with green odour, the dispenser located in the experimental room contained and spread the LawnScape fragrance (Adar Fragrances®, Agan Aroma & Fine Chemicals, Ashdod, Israel), that creates a green note effect. According to the producer, the fragrance is comprised of a significant proportion of cis-3-hexenol and trans-2-hexenal along with other green note aroma ingredients such as trans-3-hexenol, oakmoss and cis-3-hexenol-acetate. Moreover, because the core elements of the fragrance are quite volatile and diffuse rapidly, other aroma ingredients are added which stabilize the compound and

prevent rapid diffusion. This enables a long-lasting effect in the room or nearby outdoors. To the human nose, LawnScape evokes the scent of fresh cut grass and green leaves, and for this reason it is used as an air freshener.

2.3. Behavioural test

Before starting the procedure, the participating owners were introduced to the study, their role, and what to expect by participating. They were then asked to complete a consent form. It is well recognized that dogs' behaviour is strongly affected by owners' behaviour (Rehn et al., 2014; Siniscalchi et al., 2018). In order to avoid that owners would modify their behaviour according to the presence of green odour in the room, before the tests owners were told that the purpose of the study was to analyse dog explorative behaviour; the complete aim was disclosed after the participation at the two tests.

Each dog was tested twice, once in the presence and once in the absence of green odour. The behavioural test was created for this study, and it consisted in a modified, reduced version of the Ainsworth Strange Situation Test (original protocol in humans: Ainsworth and Bell, 1970; for its use in dogs see the review: Payne et al., 2016).

The behavioural test was composed of three 3-minute episodes: sitting before.

- Episode 1 (pre-separation): the owner led the dog into the experimental room, unfamiliar to all dogs, closed the door, unleashed the dog, left the leash on a table and sat on one of the two chairs available in the room.
- Episode 2 (separation): the owner left the room without saying anything to the dog.
- Episode 3 (reunion): the owner came back in the room and he/she could greet the dog if the dog was seeking attention. Then the owner sat in the same chair where he/she was sitting before.

In all episodes, the dog was free to move in the room. Throughout the behavioural test owners were instructed to ignore the dog, but they could not move the dog away if the dog approached them. An operator, who remained out of the experimental room, led the owner throughout the test.

Due to dogs acting as their own control, all the dogs underwent the test twice, once with the green odour (ODOUR, experimental condition) and once without it (NON-ODOUR, control condition) in the experimental room.

In order to avoid a possible order effect, the order of execution was inverted for half of the sample: 6 female and 2 male dogs started the test with the green odour; 7 female and 1 male dogs started the test without the green odour. For each dog, at least 20 days passed between one trial and the other.

An automatic aerosol dispenser on the wall was present in all tests. In the NON-ODOUR condition the dispenser was empty and had never been used; in the ODOUR condition the dispenser with the odour was put in the room 24 h before the test and the odour was sprayed out 30 s before starting each test.

As the green odour persisted in the environment for a few days, a washout period of 20 days was respected between tests with the green odour and test without it.

At the end of each test, the floor was mopped and washed only with water, since any kind of disinfectants may have interfered with the fragrance of the green odour.

2.4. Physiological and behavioural assessments

In order to measure cortisol levels, all dogs underwent two saliva collections in each test: one before the beginning of the behavioural test (basal cortisol) and the other one 17 min after its end (i.e. 20 min after

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