Response to Acute Stress in Domestic Cats Using Synthetic Analogues of Natural Appeasing Pheromones with Nepeta cataria Extract Rich in Nepetalactone: A Double-blinded, Randomized, Positive Controlled Cross-over Study

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ABSTRACT

Background

Olfactory communication in cats is of major importance. It is rather complex and includes pheromone-driven and classical odour-based communication. Cats use various marking behaviours to transmit pheromone signals: appeasing markings that have a calming effect and alarm markings that can exacerbate stress. On the other hand, classical odours could possibly complete, modify, synergise, or add to pheromone-driven communication in felines, including in the

domestic cat. One of the best known natural odours able to produce an identified behavioural sequence in Felidae is nepetalactone, from Nepeta plant species. A relationship is suspected to exist between nepetalactone and pheromone production in cats. We hypothesised that nepetalactone may have a synergistic or additive action with appeasing pheromones in domestic cats.

Methods

The present study compared the impact of synthetic analogues of feline appeasing pheromones combined with a Nepeta cataria extract rich in nepetalactone (ZENIFEL® spray) to a positive control consisting of synthetic analogues of feline appeasing pheromones alone (FELIWAY® spray) on acute stress in domestic cats.

Results

Differences were observed between the positive control and Zenifel® at various time points. Zenifel® provided a statistically significant reduction in the stress index compared to the positive control ($p=0.029$) during the central stressful event (blood pressure measurement).

Conclusion

The association of synthetic analogues of the F3 fraction of feline facial pheromones and nepetalactone allows better management of moderate acute stress in domestic cats than analogues of feline facial pheromones alone. This may be a useful new tool for the targeted management of moderate behavioural disorders.

BACKGROUND

Communication in domestic carnivores involves multiple modalities, and olfactory communication is among one of the most important.[1,2] This form of communication is rather complex and takes place at various levels. An important difference has been determined in the past 20 years between simple smells and pheromone-driven communication.[1] Pheromones are chemical substances,[1,3] and they are secreted in very small quantities by some species. They have particular olfactory characteristics and do not act as only an olfactory stimulus.[1] In the earliest publications pheromones were classed as natural social odours.[4] Pheromones are also described as olfactory cues and trigger highly species-specific non-learned (innate) social responses.[3] They are detected in several mammals, including cats, by a specific organ, the vomeronasal organ (VNO), which is defined as an accessory olfactory tract. The odour of the pheromones can be a chemical stimulus that induces the opening of the VNO.[1, 5]

The order of carnivora has the greatest variety of glands secreting these chemical signals.[1] Sources of pheromones in Felidae include the perianal complex, facial glands, interdigital glands, genital complex, urine, and faeces.[1,4] In the cat, five different facial pheromone fractions named Fl to F5 have been isolated[1,6] from the sebaceous secretions of the cheeks. The function in territorial marking behaviour of the F2, F3, and F4 fractions of facial pheromones is well described in cats.[1] Synthetic analogues of F3 and F4 are successfully used to manage mild behavioural disorders such as inappropriate urinary marking[3,7,8] and scratching of furniture,[3] facilitate cohabitation,[1] socialisation,[1] avoid fear,[3,9] and possibly reduce the overall sense of anxiety in cats.[9]

On the other hand, olfactory communication in cats cannot be reduced to only the pheromone side. The influence of classical odours is also important in feline behaviour.[2,10] Over recent years, with attention being focused on pheromone-driven communication, this type of olfactory stimulus has been to some extent "forgotten" by veterinary science. Nevertheless, some odours are known to induce a specific repeatable behavioural response with unique behavioural patterns. Multiple chemical substances from diverse biological sources can elicit unique patterns of behaviour in most species of the family Felidae.

One publication[4] cited 14 chemical compounds having a behavioural impact or/and crossed reaction with natural social odours (pheromones). From the chemical point

of view they form three different groups (methylcyclopentanoid monoterpenes, monoterpenoid alkaloids, and 4-methyl-benzofuranones), all able induce what we call the "catnip response" consisting of four components:

1. sniffing
2. licking and chewing with head shaking
3. chin and cheek rubbing, and
4. rolling over and body rubbing.[11]

Although none of these behaviours are specific or unique to this response, the sequence remains almost invariable in domestic and wild felines.[4,12,13] The name "catnip response" comes from the best-known natural odour able to induce this response,[4,14] the cyclic lactone nepetalactone, found in Nepeta plant species (for exemple Nepeta cataria), or its isomeres that are found in Actinidia species (dihydronepetalactone, isodihydronepetalactone, and neonepetalactone).[4] Nepetalactone has also been reported to be found in some other plant species.[4,14]

One of the most interesting questions is the physiological nature of the nepetalactone response in felines. Some results indicate a peripheral nicotinic and central muscarinic cholinoceptive and serotonergic facilitation of the catnip response.[15] Oral administration of encapsulated nepetalactone (20 to 80mg) does not induce the response,16 nor does taste or parenteral administration.[4] The nepetalactone response is restricted to simple olfactory stimulation in cats, without involvement of the vomeronasal organ.[13]

Separately, the behavioural components of this response are also seen in feeding or sexual behaviour. However the response in itself is independent of the presence of the gonads[17] and clearly associated with pleasure in the cat.[15,18,19] The response has not been found in other animals, and is considered to be specific to Felidae.[4] The question arises whether this natural compound could cross-react with pheromone communication in Felidae, and if so, in what way.

The hypothesis has been advanced that the nepetalactone reaction is a response to an additional stimuli in pheromone-driven communication.[4,12] It is still unknown whether it stimulates natural pheromone production, modifies perception, or even simply reinforces the message delivered by the specific olfactory pheromone-driven stimulation. The nepetalactone odour is highly attractive for various felines (domestic and wild)[19] in a dose dependant manner and even at weak dosages for the domestic cat (0.1-0.01 mg), while interest decreases toward the dosage of 0.001 mg.[19] Exposure to nepetalactone containing substances increases overall well-being in cats, facilitates play-like behaviour and social interaction,[2] and induces calming effects.[20]

Based on the currently available literature, we hypothesised that nepetalactone may have a synergistic or additive action with appeasing pheromones, by reinforcing the message delivered by pheromones or stimulating the production of natural appeasing pheromones. In order to verify if there could be an additive or synergistic effect, we conducted a study with domestic cats. The positive control cats received the currently commercially available synthetic analogues of feline appeasing pheromones (FELIWAY® spray) and other cats received a new combination of synthetic analogues of feline appeasing pheromones combined with a Nepeta cataria extract as a source of nepetalactone (ZENIFEL® spray).

METHODS

General Design

This was a double-blinded, crossover, randomised, single centre, positive controlled efficacy study. The study was carried out in accordance with the relevant legislation on animal experimentation and Virbac's chart of Ethics.

Animals

The study was done with 24 healthy adult cats (at least 12 months old), divided into four small groups, all living in an enriched (special feline toys and scratching support material) experimental environment. The

Table 1: *Schedule of events during the test and administration periods*

Test and administration period			
Day	**Blood pressure measurement and human manipulation**	**Product administration**	**Behavioural assessment**
D1	Yes (initial)	No	Yes (4 time points)
D2	No	Yes (3 times)	No
D3	No	Yes (3 times)	No
D4	No	Yes (3 times)	No
D5	Yes (final)	Yes (3 times)	Yes (4 time points)

groups of cats were formed 2 weeks prior to the start of the study. Each group included three females and three males, with only one intact male in each group. The 24 cats included in the study were ranked within sexes in descending order of their human manipulation acceptance score (the lowest possible score was 1, and the greatest 10).

Cats included in the study were considered to be stable from a behavioural point of view with an individual human manipulation score of between 6 and 9, and no difference between groups in their mean score.

Before the study all animals were treated against the most common internal and external parasites, and were tested for common infectious diseases including FIV and FeLV. All cats were naive regarding blood pressure measurement.

Products

The positive control was Feliway® spray 20 ml (Ceva, France), which contains synthetic analogues of Fraction F3 of the feline facial pheromone (10%).

The test product was Zenifel® spray 20 ml (Virbac, France), which contains synthetic analogues of Fraction F3 of the feline facial pheromone (10%) and extract of Nepeta cataria 0.5%.

The two products were administered using identical spray (20ml) applicators labelled in a blinded manner. An identical proportion and amount of F3 fraction contained in the two mixtures used.

Study Schedule

The study included test and administration periods of 5 days (described in Table 1), and a washout period of at least 9 days. Two groups received the test product first, and two groups received the positive control first. All cats then changed to the other product such that each product was tested by all 24 cats. The central stressful event was human manipulation and blood pressure measurement (oscillometric Cardell BP Monitor), which had never been performed before on these cats.

The study was conducted in four identical separate study rooms, each with a high flow ventilation system in which there was no sharing of air between rooms. The cats rotated between the rooms to remove any risk of cross contamination, and thus, an extended carry-over effect. At the beginning of each wash-out period, each room and the material in the room were cleaned in order to eliminate as much as possible the residues of the product. The scratching materials were changed.

Blood pressures were taken before the first administration of the product at the day 1 of the administration period, and at the day 5 of the administration period. The products were sprayed in five applications (product volume 0.15 ml per application) in each corner of the study room on three occasions per administration day (at approximately 8 AM, 12 noon and 4 PM). The products were

Table 2: *Behavioural assessment record: observation sheet during blood pressure measurement with immediate responses*

Acceptance of blood pressure measurement				
Behavioural & somatic signs Behavioural sequence/attitude	Never	Once or for a short period	Few (1< >5) or for a long time	Many (>5) or mostly/all of the time
1. Dilated pupils	4	3	2	1
2. Startled, aroused, hyper vigilant	4	3	2	1
3. Salivation	4	3	2	1
4. Feet sweating	4	3	2	1
5. Trembling	4	3	2	1
6. Polypnea, panting	4	3	2	1
7. Tail flicking-thumping	4	3	2	1
8. Frenetic self-licking	4	3	2	1
9. Emotional elimination (micturition or diarrhoea)	4	3	2	1
10. Attempting to flee	4	3	2	1
11. Facial marking on objects	1	2	3	4
12. Appeased self-grooming	1	2	3	4
13. Spontaneous contact with manipulator (allomarking, play or just contact)	1	2	3	4
14. Sitting on all four legs, tense body posture and backward-rotated ears	4	3	2	1
15. Threatening vocalizations (hissing, spitting, growling) or rolling over with flattened ears (ready to launch a paw strike)	4	3	2	1
16. Paw strike/ Attempting to bite	4	3	2	1

applied to the wall from the bottom corner up to a height of 2 meters.

Behavioural and Stress Assessments

The staff that performed these assessments had received specific behavioural training prior to the onset of the study. Acceptance of blood pressure measurement and overall human interaction/handling was assessed during blood pressure measurement using an appropriate score chart (Table 2).

Another chart was used to access behavioural observations 30 – 40 minutes before manipulation, and then 30 – 40 minutes and 4 – 5 hours after manipulation (Table 3).

Both score charts (Tables 2 and 3) for the behavioural evaluation were prepared by a specialist in behaviour. The mean score per cat obtained from the observations in the charts above provided the wellbeing score of each individual cat. The stress index was obtained by subtracting the actual well being score from the maximum possible score.[4]

Table 3: *Behavioural assessment record: Observation sheet before / after blood pressure measurement (duration 5 minutes)*

Behavioural & somatic signs Behavioural sequence/attitude	Never	Once or for a short period	Few (1< >5) Or for a long time	Many (>5) or mostly/all of the time
1. Dilated pupils	4	3	2	1
2. Startled, aroused, hyper vigilant	4	3	2	1
3. Salivation	4	3	2	1
4. Feet sweating	4	3	2	1
5. Trembling	4	3	2	1
6. Polypnea, panting	4	3	2	1
7. Rolling Skin Syndrome	4	3	2	1
8. Tail flicking-thumping	4	3	2	1
9. Frenetic self-licking	4	3	2	1
10. Cat staring at the observer	4	3	2	1
11. Emotional elimination	4	3	2	1
12. Spraying, Scratching	1	2	3	4
13. Facial marking on objects	1	2	3	4
14. Eating	1	2	3	4
15. Relaxed rest	1	2	3	4
16. Appeased self-grooming	1	2	3	4
17. Spontaneous contact with observer	1	2	3	4
18. Induced contact with observer after the	4	3	2	1
19. Sitting on all four legs, tense body posture	4	3	2	1
20. Threatening vocalizations	4	3	2	1
21. Rolling over with flat-tened ears	4	3	2	1
22. Paw strike/ Attempting to bite	4	3	2	1
23. Inhibition/Hiding, staying at the opposite side	4	3	2	1

In order to assess the impact of the new formulation (Zenifel®), we calculated the mean stress scores per product (Zenifel® and the Feliway® positive control) at different time points (30 – 40 minutes before manipulation, during manipulation, and then 30 – 40 minutes and 4 – 5 hours after manipulation) on day 1 and again on day 5. We then calculated the arithmetical change (day 5 - day 1) and the relative change ((day 5-day 1)/day 1) in stress scores for each group, at each time point, and compared the mean

changes (arithmetical and relative) between groups at each time point.

Statistical Methodology

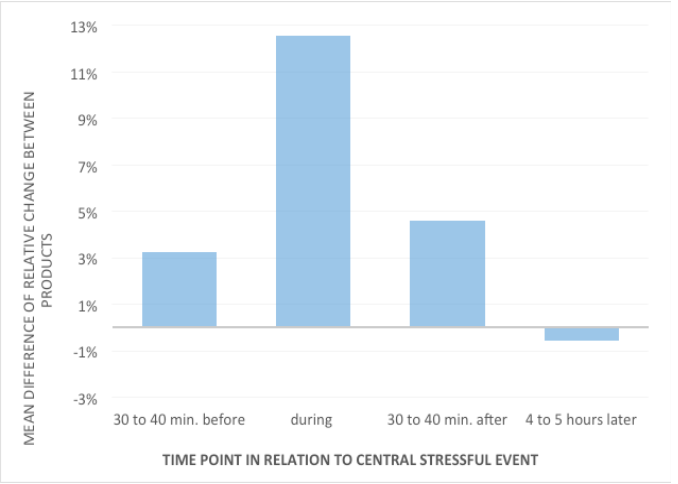
Arithmetical changes were analysed using a Generalized Linear Model, including factors group, sequence, period, and animal within sequence.

RESULTS AND DISCUSSION

The results of the study showed a difference between the positive control and Zenifel® at different time points. At the first and third time points, numerical differences were noted between arithmetical changes for the two products. However, the benefit provided by Zenifel® for the reduction in the stress index, in comparison to the positive control, was not statistically significant at these points (p values 0.83 and 0.75 respectively). During the central stressful event (blood pressure measurement) Zenifel® provided a statistically significant reduction in the stress index compared to the positive control (p=0.029). 4 to 5 hours after the blood pressure measurement a very small numerical difference was noted without statistical significance (p=0.74).

The differences of mean relative change between the two products at each time point are presented in Figure 1. The results of this study, demonstrating a statistically significant reduction of the stress index with Zenifel® when compared with the positive control (Feliway®) in the situation of acute stress, appear to support the hypothesis of a synergistic or additive action between nepetalactone and analogues of feline appeasing pheromones. However, it is not possible to define whether this is due to an interaction between two different substances, or whether nepetalactone stimulates production of natural appeasing pheromones by supranormal stimuli as has been previously speculated.[4]

Figure 1: *Zenifel®-induced proportional reduction in relative stress index normalised on positive control (n=24 cats per product)*



The activity of nepetalactone seems to be confined to olfactory stimulation in cats.[13] Its effect in cats is not induced with systemic or direct gastric administration, while the anatomic positioning of the classical olfactory system and pheromonal system in cats are very close.[1] Another important point supporting stimulation of pheromone delivery is the fact that until now, catnip responses have never been seen in other species.[4,14] The specific nepetalactone response is limited to Felidae sharing the same pheromonal system.

Nepetalactone is a well-known interomone acting as an attractant on cats or as a repellent on various insect pests (mosquitoes, ticks, flies)[21] On the other hand, it is interesting to note that in this study the significant difference was noted during an acute stress situation where the cats would not have had the opportunity to deposit any increase in pheromone production onto inanimate objects in the environment to appease themselves. Until now, it has been believed that fraction F4 of the facial pheromones is required to improve interactions with other living beings.[1] It is, therefore, possible that the combination of the F3 fraction and Nepeta extract rich in nepetalactone could have positive effects both in appeasement as

derived from territorial pheromone marking and also in improving interactions with human beings.

Despite very interesting results in the situation of acute stress, no difference was shown between the two products out-with the period of manipulation.

Two reasons could possibly explain this: a presumed relatively short duration of action of nepetalactone[15] or the fact that the stressful event in this study, being relatively short and of moderate intensity, did not produce a significant effect on cats 30-40 minutes later, nor 4 hours later. In the authors' opinion, the latter is the more likely explanation. It should be noted additionally that in order to obtain suitable behavioural evaluation and acceptable levels of human manipulation, all animals included in the study were stable regarding behaviour and had high initial wellbeing scores. This is not always the case with cats in the field, and especially in in-clinic conditions. This could possibly explain an absence of significant differences out-with the acute stress situation and represents one of the limitations of the study. It is possible that less appeased cats could have shown more variation in the wellbeing/stress index. However it would then have been almost impossible to form homogeneous groups for the purposes of this study.

The classical behavioural response to nepetalactone has been reported to be relatively short.[15] However, less perceptible effects such as calming, play-like behaviour, and interaction stimulation seem to last beyond the behavioural sequence called the "catnip response".[2,20]

A further potential limitation of this study is the absence of a pure nepetalactone group and the choice of Feliway® as the positive control. Feliway® has already been shown to be efficacious in the treatment of moderate behavioural disorders[3,8] and was thus judged to be acceptable as a positive control. The use of plant-based products containing cyclic lactones and able to produce the "nepetalactone response" is worldwide, and there is no need to prove the existence of this response or describe it. Nevertheless, this study design does not permit us to conclude on whether the benefit of nepetalactone in association with facial pheromones is additive or synergistic. Beyond the number of groups or products tested in the present study, the key limitation was the fact that the behavioural evaluation was done by human beings. In order to reduce this limitation, all staff involved in the present trial were trained by a behavioural specialist prior to animal manipulations. The behavioural evaluations were performed using a chart prepared by a specialist in veterinary behaviour, and although these questionnaires are not a standardized tool, in behavioural medicine, such questionnaires are widely accepted.[22,24] The number of separate questions (measures) was high in order to increase the robustness and objectivity of the evaluation.[25]

The measurement of blood pressure may have been an interesting parameter in itself related to stress,[26] and could potentially have provided a less subjective indicator. However, unless multiple measurements are taken, the variability in this parameter is too high to interpret the results, and there is a large effect of habituation, which can be seen when cats have multiple measurements taken over time. In this study, the idea was to use a non-invasive act which is becoming routine in veterinary practice, but which is known to be stressful to cats. The cats were naïve to this procedure, and the limited number of times it was performed avoided any significant effect of habituation. If an alternative stress source was used, and the cats were habituated to blood pressure measurement, this may be an interesting way to include a less subjective parameter for a future study.

The action of nepetalactone is associated with a pleasurable emotional state in domestic cats and other Felidae,[4,15,19] but without a clear behavioural consistency between calming or euphorisant effects.[15,19,20] Moreover, if the theory of reinforcement of

pheromone signalling is correct, it could be suggested that nepetalactone may possibly reinforce multiple feline pheromones, not simply fraction F3.

During this study, no euphorisant effect was reported. Rather, the results were much more related to an overall better tolerance of stress and tolerance of human interaction. This is more desirable in practice and allows targeted calming use of the nepetalactone when it is given in association with the F3 fraction of the facial pheromones, which provide an initial appeasing stimulus. This confirms the interest of this association for the management of moderate stress.

CONCLUSION

The association of the synthetic analogue of the F3 fraction of the feline facial pheromones and a Nepeta spp extract rich in nepetalactone allows better management of moderate acute stress in domestic cats than analogues of feline facial pheromones alone. Such an association could be a new tool for the targeted management of moderate behavioural disorders especially in the case of acute stress. It could also possibly be useful to facilitate adoption/socialisation of kittens and to increase play-like behaviour and social interaction.

Competing Interests and Funding Disclosure

Authors NB, NC, PM, HG and DM are employees of Virbac. Virbac paid for this research which relates to a potential future product in development.

Authors' Contributions

NB, DM conceived the study. CB was involved in the study protocol conception. PM, HG were involved in the conception of tested product. NB, NC, DM were responsible for analysis and interpretation of the data. NB, DM drafted the paper. All authors contributed to critical revision of the paper and approved the final version.

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