

Efficacy of L-Theanine in the treatment of noise phobias in dogs: preliminary results

Aim of the study - The treatment of noise phobias in dogs consists of behavioural therapy and, sometimes, of pharmacologic support. Aim of this study is to determine the efficacy of L-Theanine, a natural substance derived from the tea plant, used in conjunction with behavioural therapy in the treatment of such disorders and to assess any consequent change in blood cortisol levels.

Materials and methods - Twenty subjects with a specific noise phobia were selected through a behavioural examination and thereafter divided into two groups (“ANX” and “NOANX”). For 63 days, the “ANX” group received behavioural therapy in conjunction with L-Theanine, while the “NOANX” group received only behavioural therapy. All subjects underwent blood sampling, before and after treatment, for the evaluation of blood cortisol. Twice a week owners were asked to fill in a questionnaire containing a list of symptoms related to the phobic state. Two telephone interviews were made to assess adherence to therapy. Statistical analysis of the data collected was carried out.

Results and discussion - A statistically significant reduction in the intensity of phobic symptoms was observed in both groups; a decrease in certain specific behaviours (panting, drooling, lip licking, yawning, drawing the attention of the owner, vocalizing, exhibiting compulsive behaviours) was observed only in the “ANX” group. This is suggestive of the fact that L-Theanine may be useful in the treatment of sound phobias in the dog. No difference was detected in the concentration of blood cortisol.



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INTRODUCTION

Noise fear and phobias are among the most common behavioural problems in the dog. However, these disorders are frequently overlooked or poorly managed by the owners, who may not perceive them as problems until the behaviours manifested by the fearful or phobic animal do not become exaggerated and socially intolerable.¹ Fear is an emotional response to a stimulus that the animal perceives as potentially dan-

gerous; this is an adaptive response, as it allows the animal to avoid potentially dangerous situations and thus increases its chances of survival.² Fear can be triggered by different causes and is often the result of various factors.

For example, specific aversive experiences may be associated with the development of intense fears. In other cases, inadequate exposure to specific stimuli during the behavioural development of the subject, such as

Table 1 - Phobic manifestations in the dog

Panting	Open mouth breathing
Pacing	Walking to and fro aimlessly
Trembling	Involuntary movements characterized by rhythmic oscillations of body segments
Yawning	Accessory respiratory act characterized by rapid chest expansion, forced mouth opening and, in most cases, palpebral constriction
Remaining close to the owner	Remain in the proximity of the owner
Seeking attention	Search for interaction with the owner
Hiding	Withdrawing from eyesight, go into hiding
Escaping	Fleeing from a specific situation
Drooling	Excessive salivation
Lip licking	Passing the tongue over the lips
Vocalizing	Barking, whining or howling
Destructive behaviour	To break or damage objects
Inappropriate elimination	Vomiting, defecation, urination
Presence of compulsive behaviours	Repetitive or stereotypic behaviours (such as licking parts of the body, circling)
Aggressiveness	Growling, biting or trying to bite

to places, objects, or persons, may trigger the onset of a fear response in later stages of the animal's life.^{2,4,5,6,7} Genetics may also play a role in the development of certain fears; studies have shown that puppies born to parents with fear of noises are more likely to incur in the same problem compared to puppies born to fearless parents.⁸ However, studies on the genetic predisposition to develop certain phobias often resulted in conflicting results. For some Authors,^{9,10,11} shepherd dogs, Golden Retrievers and Labradors are more prone to various types of phobia, while other studies have not confirmed it.¹² Dogs coming from animal shelters are apparently more prone to such behavioural disorders.^{12,13,14,15,16,17} In a study on noise fear and phobias,¹⁸ the analyzed data indicate a greater manifestation of thunderstorm phobia in mongrels than in purebred dogs; however, this observation may in fact be related to the fact that many mongrels come from animal shelters.

A sudden, extreme and intense fear is classified as a phobia. The manifestations of phobias are always excessively intense, symptoms persist even after the disappearance of the triggering stimulus and, sometimes, the phobic reaction can be triggered in the absence of a specific stimulating factor.^{1,19,20,21,22}

The symptoms exhibited by phobic dogs consist of a sudden, non-gradual, exaggerated response to a stimulus, with a strong impulse to flee or escape from the source of the stimulus by seeking to hide, with anxious

behaviours such as panting, vocalizing, urination or defecation, increased vigilance, trembling and exhibition of destructive behaviours. Sometimes the intensity of these symptoms is such that phobic states may be compared to panic attacks in humans.^{23,24,25} Unlike fear, which may be adaptive, phobia is considered "maladaptive", as it interferes with the normal functioning of the living organism.⁴ It has been hypothesized that once a phobic event has been experienced, every subsequent event associated with the same experience or with its memory is sufficient to trigger the response.^{4,7} One of the most common dog phobias is the one specific to sounds, meaning an intense and extreme fear response to an unexpected, loud and non-gradual noise; this phobia consists of an intense and active attempt of avoidance, with anxious behaviours associated with the activation of the sympathetic component of the autonomous nervous system.⁴ The noises that most frequently cause phobic reactions in the dog are those caused by thunderstorms, fireworks, gunshots and traffic.^{1,22}

The treatment of this behavioural disorder should include a specific behavioural therapy, based on a programme of desensitization and counterconditioning. Systematic desensitization requires a gradual and controlled exposure to noise stimuli in order to eradicate the manifestation of fear-related behaviours. The counterconditioning phase consists in reinforcing a calm and controlled behaviour in response to the trig-

gering noise stimulus, by means of a positive emotional stimulus. In this way the animal is conditioned to experience a positive feeling in direct competition with the previous state of fear.^{1,2}

Behavioural therapy may also be performed in conjunction with a pharmacologic intervention, typically with substances in the pharmacologic class of tricyclic antidepressants, benzodiazepines, monoamine oxidase inhibitors or of selective serotonin reuptake inhibitors.^{4,20,22} To support behavioural therapy, some Authors consider useful the use of non-conventional medications, such as pheromones,^{26,27} while for others, to date, the number of studies available is not sufficient to confirm the beneficial effect of synthetic pheromones on canine phobias.^{28,29}

L-Theanine (N-ethyl-L-glutamine), the left-handed isomer of theanine, is a natural substance obtained in 2001 the approval for use in the treatment of certain behavioural disorders in companion animals. This molecule is a nutraceutical, used to reduce and alleviate stress conditions and certain anxiety-related disorders in small animals.^{30,31} L-Theanine inhibits the reuptake of glutamate and increases GABA levels, generating an anti-stress effect and a sense of well-being, and has neuroprotective effects in the hippocampus by blocking the multiple receptor subtypes of glutamate.^{30,32,33,34} Furthermore, it has been shown that in the rat, L-Theanine is able to increase serotonin levels in the striatum, hippocampus and hypothalamus, and dopamine levels in the striatum. In humans and in the rat, L-Theanine is able to regulate blood pressure, improve memory and learning skills (closely related to the action of dopamine and serotonin).^{30,35,36,37,38} Anxitane[®] tablets (*Virbac Animal*

Fears and phobias of loud noises are very common in the dog, but are often overlooked by the owner.

Health), used in this study, is a nutraceutical containing 99.95% of L-Theanine.³⁸ The tablets are palatable and have no side effects even when administered at five times the recommended dose (http://virbacvet.com/images/resources/other/anxitane_firstintention.pdf).

The aims of this study were to assess the potential efficacy of L-Theanine (Anxitane[®]) in conjunction with behavioural therapy in the treatment of noise phobias in the dog, to evaluate any difference in cortisol levels in the blood of dogs with noise phobia compared to non-phobic subjects, and to highlight the possible impact of L-Theanine on these hormone levels.

MATERIALS AND METHODS

Recruitment of subjects

Dogs with noise phobias were recruited following a behavioural examination carried out at the *Clinic for behavioural problems in dogs and cats* of the University of Milan (Università degli Studi di Milano).

In total, 20 subjects were taken into consideration, heterogeneous in terms of breed, age and gender, and suffering from specific phobias against thunderstorms or fireworks; dogs with concomitant physical disorders, over 10 years of age or with the presence of other behavioural disorders were excluded from the study.

PROTOCOL GUIDELINES

Prior to the study, all the dog owners received information on the research methodology used and were trained to recognize phobia symptoms in the dog²³ (Table 1); in the early stages of the protocol they were also assisted by an expert. The 20 owners received the same behavioural instructions (ignore demands of attention and inappropriate phobic behaviours, reward relaxed behaviours). Using a randomized block design,³⁹ two age- and gender-balanced groups were created, “ANX” and “NOANX”, of 10 dogs each. The “ANX” group consisted of six spayed females and four intact males, mean age of 5.3 ± 1.88 years; the “NOANX” group consisted of six spayed females and four intact males, mean age of $5.7 \pm 2:00$ years. Both groups underwent a desensitization and counterconditioning protocol, using an audio simulation reproducing the sounds responsible for the phobic response in dogs (“*Sound CD for behaviour therapy*” - *The Company of Animals*):^{4,19} owners were asked to play the audio simulation to their dogs at the minimum volume capable of triggering a phobic response in the animal; while listening to the sound CD, owners had to entertain the dog with a pleasant activity, such as playing together or allowing the dog to eat, depending on the character of each subject.¹⁹ In our study, within the “ANX” group 3 owners associated a recreational activity to the listening of the CD, while the remaining owners used food; in the “NOANX” group, while listening to the audio file 4 dogs were involved in recreational activities and 6 received food. Owners repeated such protocol twice a week for a total of 18 times (63 days). In addition to the standard behavioural therapy (desensitization using the audio CD and counterconditioning), subjects belonging to the “ANX” group received an oral therapy with L-Theanine (Anxitane[®]), twice daily at the dose recommended by the manufacturer (dogs weighing less than 10 kg: Anxitane[®] S, ½ tablet twice daily; dogs weighing between 10 and 25 kg: Anxitane[®] M/L, ½ tablet twice daily; dogs weighing more than 25 kg: Anxitane[®] M/L, 1 tablet twice daily) for the entire duration of the study.

Subjects belonging to the “NOANX” group received only the standard behavioural therapy (desensitization using the audio CD and counterconditioning). At the end of each session, all the owners (both those of group “ANX” and those of group “NOANX”) completed a questionnaire designed to provide information on the specific reactions of the dogs to the audio simulation, based on a score system ranging between 1 and 5 and related the frequency and intensity of symptoms, with 1 representing the absence of symptoms and 5 the maximum possible severity of phobic manifestations. The owners’ compliance was assessed by means of two telephone interviews, the first halfway through the study and the second at the end of it.

During these interviews an investigation was made on the owners’ opinion on the initial explanation given for the treatment, on the difficulties encountered in implementing it, on the presence of side effects and on problems related to the administration of Anxitane® tablets.

The relaxing effects of L-Theanine may help to reduce stress and anxiety in the dog.

Two blood samples were collected in all the dogs (at the *Clinic for behavioural problems in dogs and cats* and in the time slot between 14:00 and 16:00 hrs), the first at the time of the first behavioural examination, before initiation of therapy, and the second at the end of treatment (day 63), with the aim of assessing the clinical status of the animals and to assay blood cortisol levels with RIA methodology (Radio Immune Assay).^{24,40} As for the assessment of cortisol levels, a control group was included in the study in addition to groups “ANX” and “NOANX”, consisting of 10 dogs (6 spayed females and 4 intact males, mean age of $5.6 \pm 2:00$ years) with no behavioural disorders. Blood samples were collected in the same location and within the same time slot as for the dogs belonging to groups “ANX” and “NOANX”.

The study period was divided into three time intervals: time 1 (T1), corresponding to the time interval between day 1 and day 21; time 2 (T2), corresponding to the time interval between day 22 and day 42; time 3 (T3), corresponding to the time interval between day 43 and day 63.

The dogs in the study were divided into two groups: the first received behavioural therapy in conjunction with the administration of L-Theanine, the second only behavioural therapy.

STATISTICAL ANALYSIS

All data were entered into an Excel file and statistically analyzed with SPSS 16.0 software (SPSS, 2007). Descriptive measures were used to calculate the mean and the standard deviation of the scores assigned to individual phobic behaviours and to cortisol levels. Mann-Whitney and Friedman tests were performed to compare the behaviour scores of groups “ANX” and “NOANX”. The differences in the concentration of cortisol before and after treatment were analyzed using the Wilcoxon test (SPSS, 2007).

RESULTS

The dogs’ phobic-response variation over time in both groups was evaluated by comparing the mean scores resulting from the questions proposed in the questionnaires administered to the dog owners at times 1, 2 and 3. This comparison revealed a statistically significant decrease in phobic manifestations at both T2 and T3 (Fig. 1).

The change over time of specific phobic signs (in periods T1, T2, and T3) was then assessed: trembling (Fig. 2), seeking contact with the owner, hiding, pacing (Fig. 3) and escape behaviour (Fig. 4) were significantly

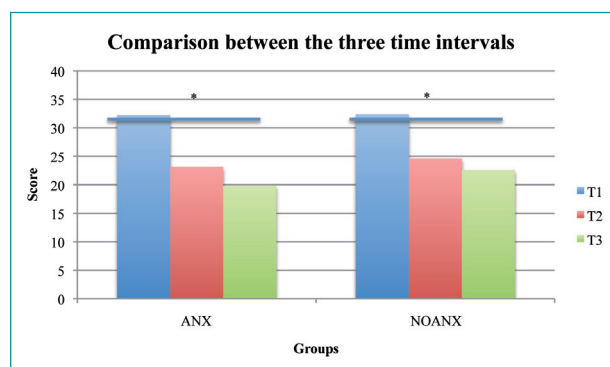


Figure 1 - Mean total scores of phobic symptoms in the three time intervals.

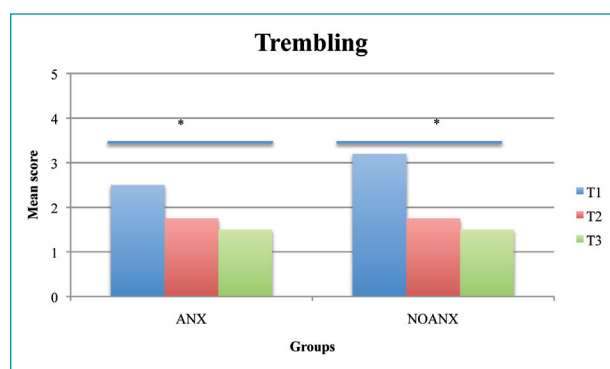


Figure 2 - Trembling behaviour in the two dog groups in the three time intervals.

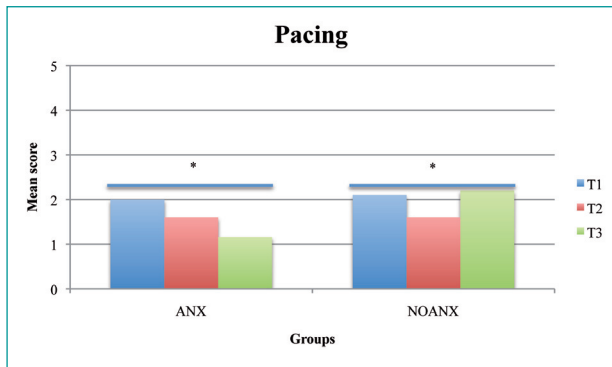


Figure 3 - Pacing behaviour in the two dog groups in the three time intervals.

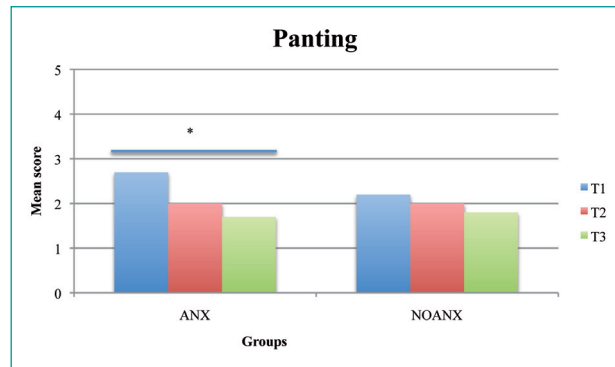


Figure 5 - Panting behaviour in the two dog groups in the three time intervals.

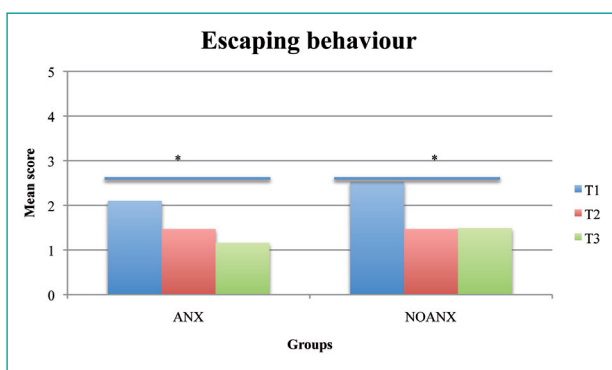


Figure 4 - Escaping behaviour in the two dog groups in the three time intervals.

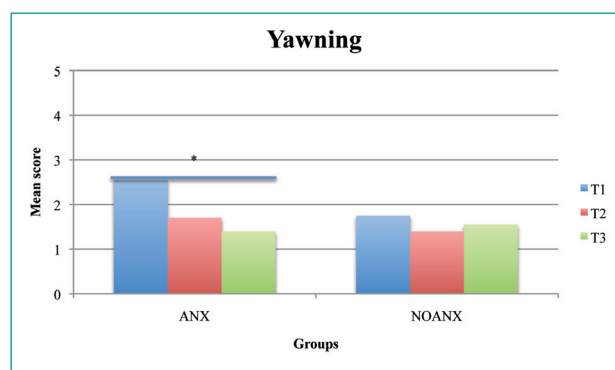


Figure 6 - Yawning behaviour in the two dog groups in the three time intervals.

decreased in both groups (“ANX” and “NOANX”); destructive behaviours, inappropriate elimination and aggressive behaviours did not instead shown any significant decrease in either the ANX or the NOANX groups. The comparison between the variation of individual phobic behaviours over time revealed that only dogs in the ANX group showed a significant reduction in the score of the following behaviours: panting (Fig. 5), yawning (Fig. 6), drooling, drawing the owner’s attention, vocalizing, exhibition of compulsive behaviours.

Analysis of the answers given by the owners during the two telephone interviews provided information regarding the owners’ adherence to the protocol: all 20 owners followed the study protocol accurately, considered it of easy execution and appreciated the clarity of the instructions given on the use of the CD. No owner reported the presence of side effects or problems in administering Anxitane® tablets. Blood tests did not identify any clinical problems in the subjects of the study. Cortisol levels were within physiologic limits in both groups and comparable to those of the dogs in the control group (10-60 ng/ml). No significant variations on such levels were detected after treatment.

DISCUSSION

The study detected an improvement in phobic symptoms in the dogs of both the “ANX” and the “NOANX” treated groups. As shown in the literature, the results obtained confirm that behavioural therapy (desensitization and counterconditioning), in accordance with the methodology used by the Authors of the article, is to be considered essential for the treatment of noise phobias. In order to achieve optimal results, it is important for the veterinarian to teach the owner how to recognize the signs of stress, fear and phobia exhibited by the dog. In fact, during the standard desensitization and counterconditioning protocol the dog should be exposed to the sound stimuli in a gradual and controlled way, in order to allow desensitization to the stimulus, conditioning to elicit a positive response and extinguishment of fear-related behaviours.^{1,22} The use of a score assigned to each phobic behaviour allows to make the owner’s interpretation as objective as possible.²² The owner’s compliance is also important, in order to ensure that the treatment protocol is followed regularly and for the entire period necessary for the healing of the animal. The treatment protocol must therefore be explained clearly, it must be of easy

execution and the owner must be aware of the time interval necessary for clinical improvements.²²

The ANX group presented a significant improvement in some phobic signs (panting, drooling, lip licking, yawning, drawing attention, vocalizing and compulsive behaviours), a result not found in the NOANX group. This improvement may be related to the reduction or alleviation of conditions of anxiety and stress induced by L-Theanine. Our study confirms the usefulness of Anxitane® in the treatment of dogs with anxiety- and fear-related behaviours, as reported in the literature.^{30,32,41,42} Anxitane® can therefore be considered a valid support in conjunction with behavioural therapy

in dogs with phobia of loud noises. Furthermore, the palatability of the tablets makes the product easy to administer and the absence of contraindications and side effects in all the subjects of the study allows for its safe administration.

Our study did not show any difference in blood cortisol values between dogs with noise phobias and subjects in the control group, neither differences between the values found in the first and second sampling. Although some studies have shown that the rise in cortisol levels is correlated with the body's response to stress, and that it can hence be considered as a valid parameter for the assessment of conditions of stress,^{40,43} other studies report that in the dog such variations nevertheless fall within the range of normal values.² In addition, the level of plasma cortisol may be affected by multiple factors, such as the circadian rhythm, gender and age; a considerable individual variation in its production is also present.²

In addition to a general improvement in both groups, subjects who took L-Theanine presented a significant decrease of some phobic signs.

KEY POINTS

- Phobias of loud noises are a common behavioural problem in the dog; the treatment of choice consists of a specific behavioural therapy.
- With regard to this disorder, the pilot study compared a desensitization and counterconditioning protocol used in conjunction with the administration of L-Theanine versus behavioural therapy alone.
- The results showed that L-Theanine may be considered a valid support for behavioural therapy in the presence of noise phobias.

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