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Assessment of Physiological Stress to Environmental Challenges by Measurement of Salivary Cortisol in Dogs

개에서 환경 변화에 따른 타액 코티솔 측정을 통한 생리학적 스트레스의 평가

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Abstract

Stress is an important indicator of animal welfare. Considering the welfare, as well as the physical health of domesticated animals, is an important perspective in animal health, and determining the appropriateness

of particular situations or environments for animals is becoming an important topic of discussion.

The level of stress experienced by animals can be comprehensively evaluated by considering their behavioral responses, physiological changes, and a diverse range of immune responses. In particular, physiological changes can be expressed as relatively accurate numerical values, which makes this an objective indicator of stress. Among the physical changes associated with stress, cortisol concentrations are regarded as a trustworthy indicator of immediate stress responses. Especially, the use of salivary cortisol concentrations is on the rise, given the relatively noninvasive nature of such measurements.

A diverse range of behavioral problems are being reported in companion dogs. Among such problems, separation anxiety (SA) is very common. Separation anxiety constitutes a representative behavioral issue that often leads to non-adoption, abandonment, and abuse, and requires a combination of chemical therapy and behavioral modification therapy. It is important to prevent unmanaged stress from increasing exponentially. For this reason, this study evaluated whether separation anxiety-induced cortisol concentrations could be lowered through exposure to the smell and voice of the owners. Twenty-eight dogs with SA were divided into three groups and their salivary cortisol concentration along the period (PRE: pre-separation period; SP1–4: separation period; POST: post-separation period) were assessed: group 1 (control), group 2 (with owner's clothes during the separation period; SP) and group 3 (a recording of the owner's voice was played during SP).

In group 1, the cortisol at SP1 was 1.68 ± 0.27 times greater than that at PRE and 2.99 ± 0.50 times greater than at POST. In group 2, The cortisol level at SP1 was 1.17 ± 0.11 times greater than that at PRE and 2.06 ± 0.41 times greater than at that at POST. In group 3, The cortisol level at SP1 was 1.10 ± 0.18 times greater than that at PRE and 1.62 ± 0.14 times greater than that at POST. The ratio of the concentration at SP1 to that at PRE (SP1/PRE) was significantly different among groups (p<0.05). Likewise, the ratio (SP1/POST) differed significantly among groups (p<0.01). In addition, when comparing the differences in concentrations between PRE and SP1 among groups (SP1 – PRE), these levels were significantly different (p<0.05). In the same manner, the differences in concentrations between POST and SP1 among groups (SP1 – POST), there were significant differences (p<0.05).

The results indicated that exposing dogs to the smell and voice of their owners could significantly reduce the increase in dogs' physiological stress levels in response to separation. As such, we recommend the collection of dog owners' smell and voices as a method of managing stress, along with other known techniques.

Moreover, this study also evaluated whether low sociality could lead to increased stress caused by exposure to unfamiliar people and environments, ultimately leading to ethological issues.

The differences in the sociability and physiological stress response of dogs were evaluated. A total of 37 healthy dogs (21 companion dogs = C group; 16 shelter dogs = S group) were included in the study. The dogs were divided into two groups according to the results of the behavior test (H group = dogs with high sociability; L group = dogs with low sociability). In the H group, the salivary cortisol concentration at P1 was 0.3848 ± 0.0969 μg/dl and at P2 was 0.3577 ± 0.0981 μg/dl. In the L group, the concentration at P1 was 0.5593 ± 0.0755 μg/dl, and the concentration at P2 was 0.6527 ± 0.0781 μg/dl. The ratio of the concentration at P2 to that at P1 (P2/P1) were significantly different between the groups (p=0.008). Likewise, the differences in the concentration between P1 and P2 (P2 − P1) were −0.0272 ± 0.03 μg/dl in the H group and 0.0933 ± 0.0371 μg/dl in the L group. These levels were significantly different (p=0.003).

Dogs with low sociality per their behavioral assessment had higher cortisol concentrations when interacting with unfamiliar people, compared to their counterparts with higher sociality. This indicates that dogs with low sociality may engage in inappropriate behavior by being overly stressed by unfamiliar people and environments. Sociality constitutes a lifelong characteristic that is formed during puppyhood, which is the period of dog socialization, and is also influenced by the dog's innate character. As such, it is important for owners to encourage dogs to positively accept a diverse range of people and environments during socialization periods. Dogs with positive socialization are better able to manage stress caused by diverse environments throughout the course of their lives, and it appears likely that behavioral problems caused by low sociality can be prevented.

Lastly, this study evaluated the stress experienced by dogs in pet drying rooms (PDR; PDR-20000S, Izu Korea, Seoul, Korea), in order to evaluate whether this convenient method for drying dog hair would lead to lower stress compared to common pet dryers (CD; APST2031, A-plus ENC, Incheon, Korea). Drying dogs' hair is an essential part of caring a dog; however, the excessive noise and heat may lead to stress and resulting difficulties in caring dogs.

The cortisol level after the drying was 2.29 ± 0.30 times greater than that before the drying with CD and 1.21 ± 0.09 times greater with PDR. This ratio of the concentration was significantly different between the groups ($p<0.005$). Likewise, the concentration after drying increased 0.30 ± 0.07 μg/dl more than that before drying with CD and 0.07 ± 0.03 μg/dl with PDR. There was significant difference between them ($p<0.05$).

For the reason, PDR found to induce a low level of stress compared to CD, and could be useful in large-scale dog facilities due to improved efficiency and lower costs.

It is expected that the results from this study will aid in evaluating the welfare of dogs by measuring the stress levels of companion dogs in various environments, and will provide a fundamental basis for stress management in dogs.

Keywords: dog, salivary cortisol, physiological stress, animal behavior, ethology, animal welfare

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CONTENTS

CHAPTER I.

Literature review

CHAPTER II.

Evaluation of effects of olfactory and auditory stimulation on separation anxiety by salivary cortisol measurement in dogs

CHAPTER III.

Relationship between sociability toward humans and physiological stress in dogs

CHAPTER IV.

Comparison of stress levels induced by two types of pet dryers (standing type and box type) using salivary cortisol measurement in dogs

Abbreviations

ANOVA	Analysis of variance
DSCC	Desensitization and Counter-Conditioning
ELISA	Enzyme-linked immunosorbent assay
H–P–A	Hypothalamus–pituitary gland–adrenal gland
hr	Hour
min	Minute
rpm	Revolution per minute
SE	Standard error

List of figures

List of tables

GENERAL INTRODUCTION

Physiological changes in animals can be measured to indirectly predict the state of wellbeing of the animals (Bodnariu, 2008). Among the various physiological measurements, estimation of salivary cortisol, instead of measuring serum cortisol, is recognized as a convenient tool to quickly assess stress level (Dreschel and Granger, 2009). Therefore, salivary cortisol is currently widely used to assess stress in animals under various conditions (Beerda et al., 1996; 1998; Hellhammer et al., 2009). This research used salivary cortisol for the evaluation of the stress caused by separation anxiety and sociality issues, both of which are currently considered important with respect to pet dogs. Further, whether the pet dry room, which is currently increasing in demand, is a better choice for drying dog hair, for both its convenience to humans, as well as its benefits on the animal, was evaluated using salivary cortisol.

Separation anxiety in pet dogs refers to the canine behavioral problems that occur in the absence of the owner (Appleby and Pluijmakers, 2004). Such behaviors include excessive barking, object destruction, self-harming, and disregard of housetraining, through which the dogs try to relieve the stress caused by the owner’s absence (Landsberg et al., 2013; Palestrini et al., 2010). Many methods have been suggested to treat these behaviors, but most of them are time consuming and require the use of drugs, creating the need to find a simpler solution (Lansberg et al., 2013; King et al., 2004; Schwartz, 2003). In this research, salivary cortisol was used to evaluate whether the body odor or the voice of the owner could reduce the stress from separation anxiety, in pet dogs.

Sociality refers to the characteristics of dogs to react amicably towards the same or different breeds of dogs (Jakovcevic et al., 2012). Sociality is established during a short socialization period and maintained throughout the life (Battaglia, 2009). Higher sociality enables the dog to adapt flexibly to strange people, situations, and environment. Therefore, sociality is emphasized as an important characteristic in working dogs, abandoned dogs meant for adoption, and pet dogs living with people (Jones and Gosling, 2005). The Ainsworth strange situation test for humans is modified for dogs to evaluate their sociality (Barrera et al., 2010; Coppola et al., 2006; Jakovcevic et al., 2012). In this research, the behavioral test was used to evaluate sociality and the resulting changes in the salivary cortisol level was compared to identify whether highly social dogs are physiologically less stressed.

The pet dry room is a equipment developed to allow more convenient and efficient drying of dog hair. To evaluate whether this equipment, which is developed for human convenience, also results in less stress in the animals, and is, thus, a suitable choice for both humans and animals, pet dry rooms and conventional pet dryers were used to dry dog hair to evaluate the levels of stress using salivary cortisol.

CHAPTER I.

Literature review

1. Measurement of stress in mammals

There is a recent trend of increasing public interest in the welfare of animals that are being bred, and as people begin to recognize that animal welfare is linked to human welfare, its importance has been receiving more attention (Beerda et al., 1998). While a general definition of welfare has not yet been established, Broom's definition is the most widely used in assessing welfare: welfare of an individual is its state as regards its attempts to cope with its environment at a physiological, behavioral and medical level (Broom, 1986). Although a clear correlation between stress and welfare has not been identified, stress indicators are considered key tools for estimating welfare status (Beerda et al., 1999b). Accordingly, by measuring stress levels in animals via various behavioral and physiological indicators, the welfare status of those animals may be assessed indirectly (Bergamasco et al., 2010; Bodnariu, 2008; Part et al., 2014).

It is already well known that people tend to show a variety of stress responses when they face low levels of welfare, and it is believed that this may be true in other animals as well (Hilton, 1989; Jørgensen et al., 1990;

Stafleu et al., 1992). Evaluating the physiological stress of animals is the one of the most critical ways to assess their welfare (Beerda et al., 2009). Stress that animals experience under negative situations is expressed in various ways and can be assessed largely through their behavioral and physiological responses.

Dogs are among the animals that show stress responses when placed in negative environments (Beerda et al., 1998; Haverbeke et al., 2008; Sales et al., 1997). Recent studies have focused on assessing stress levels of dogs when they are placed in specific situations. Accordingly, a variety of stress measurement methods are being used to the assess the welfare of dogs. Recent studies have assessed behavioral and physiological stress in experimental, abandoned, working, and pet dogs under various environmental challenges (Bergamasco et al., 2010; Döring et al., 2014; Haverbeke et al., 2008; Hennessy et al., 2001; Hiby et al., 2006; Palma et al., 2005; Rehn et al., 2014).

Behavioral responses of dogs under stressful conditions are as follows (Beerda et al., 1997; 1999a; Diederich and Giffroy, 2006; Haverbeke et al., 2008): First, their locomotor activity may increase. Relevant activities include nosing, body shaking, and yawning, the frequencies of all of which increase under stress conditions. Second, stressed dogs typically show low body posture. Last, auto-grooming, vocalizing, urinating, and defecating are sometimes observed, along with an increase in repetitive behavior without specific function or purpose, which is known as stereotypic behavior. Typical examples of such behavior include circling and pacing. In particular, such stereotypic behaviors are recognized as key behavioral indicators for measuring welfare levels under long-term stress conditions. In some cases, however, dogs may show inactivity instead of these behaviors.

Recent studies related to physiological stress can be divided largely into those that examined acute stress versus those that examined chronic stress (Beerda et al., 1997). There have been studies on identifying stress by measuring immediate hormonal responses to acute stress under specific situations (Beerda et al., 1996; 2000; Bergamasco et al., 2010). Assessment of chronic stress has involved predicting welfare levels related to being placed under long-term stress conditions (Beerda et al., 1999a; 1999b).

Physiological parameters associated with acute stress include heart rate, arterial blood pressure, movement (which can be measured with an accelerometer), and hormonal indicators such as cortisol and catecholamine (Beerda et al., 1996; 1998; Bodnariu, 2008; Kobelt et al., 2003; Möstl and Palme, 2002; Jones et al., 2014; Vincent and Michell, 1993). Since cortisol, which responds immediately to stress conditions through the hypothalamic–pituitary–adrenal (H–P–A) axis, does not show statistically significant differences based on age, sex, or breed, it can serve as a stress indicator with a certain degree of objectivity (Coppola et al., 2006). In particular, salivary cortisol assessment has received attention as a relatively reliable stress indicator based on the fact that, unlike some other indicators, its measurement is non-invasive and therefore does not cause physiological changes (Hellhammer et al., 2009). It is already known that salivary cortisol shows a concentration difference of about 1/10 of serum cortisol, and it reflects response in blood within 5 min to allow immediate identification of serum cortisol change (Ross et al., 2010; Vincent and Michell, 1992). Especially, some studies revealed that using multiple breeds of dogs in various ages and sexes to assess stress effects on various stimulations was not found to have a significant effect on salivary cortisol level (Bennett and Hayssen, 2010; Bergamasco et al., 2010; Coppola et al., 2006). Therefore, it is widely accepted as an alternative to serum cortisol in measuring the stress response (Bennett and Hayssen, 2010; Ross et al., 2010; Dreschel and Granger, 2009; Hekman et al., 2012).

There have also been studies on developing more accurate sampling methods for obtaining enough saliva from dogs to obtain reliable salivary cortisol concentrations (Dreschel and Granger, 2009). The method of using a stimulant, meaning food, to get dogs to salivate is not recommended because the saliva may be contaminated by the food, so values may not be accurate. Increasing the amount of saliva produced by using citric acid or by having the dogs smell beef also has an impact on cortisol values. On the other hand, hydrocellulose, a collecting material, has been confirmed to absorb saliva well without affecting salivary cortisol concentration, and thus, it may be a useful sampling tool.

2. Separation anxiety and stress in dogs

The state in which dogs exhibit behavioral problems caused by severe anxiety due to their caregivers being absent or unapproachable is referred to as separation anxiety (Landsberg et al., 2013). This can cause severe stress in both the animal and the caregiver, and it is believed to be one of the main behavioral problems that lead to abandonment, cancellation of adoption, or abuse of the animal (Segurson et al., 2005). Studies have found that sex, breed, and age do not have significant impacts on separation anxiety, but it is known to occur more commonly when animals are placed in shelters (Flannigan and Dodman, 2001). Having just one human household member has also been identified as a risk factor (Flannigan and Dodman, 2001). Separation anxiety is also known to occur when the caregiver's availability does not satisfy the specific needs of the dog (Konok et al., 2015). According to a recent retrospective study, about 14.4% of canine admissions to behavior clinics are due to separation anxiety (Bamberger and Houpt, 2006). Therefore, separation anxiety is considered one of the most common behavioral problems in pet dogs.

Problem behaviors caused by separation anxiety include vocalizing (barking, whining, or howling) destructive behaviors (toward some objects, or self-traumatic behavior), and house soiling, as dogs tend to relieve the anxiety caused by the absence of the caregiver through such behaviors (Appleby and Pluijmakers, 2004; Landsberg et al., 2013; Palestrini et al., 2010; Rehn and Keeling, 2011). In contrast, some dogs may instead show depressive symptoms and decreased activity levels, as well as digestive problems such as loss of appetite, drooling, vomiting, and diarrhea (Flannigan and Dodman, 2001). Separation anxiety may be triggered along with other fear and anxiety problems when there is a change in the caregiver's routine, the dog's family is in the process of moving, the dog visits a new environment, the dog is locked in a kennel, a new pet is introduced into the family, there is a change in social relationships due to a new family member, or cognitive impairment or other medical issues (Landsberg et al., 2013). General characteristics of separation anxiety include hyper-attachment to an individual family member, where the behavioral problems caused by anxiety are exhibited only when that family member is not present or approachable, even if that time is very short. Excessive greeting behavior may be exhibited as well (Flannigan and Dodman, 2001; Landsberg et al., 2013).

Various methods have been explored to address the problems associated with separation anxiety (Landsberg et al., 2013; Takeuchi et al., 2000). According to a recent study, caregivers preferred solutions that involved methods that required as little time as possible, such as giving a chew toy to the dog when being separated, not punishing the dog, and increasing the length of walks. However, compliance with behavioral modification methods such as desensitization and counter-conditioning (DSCC) or uncoupling departure cues, which require a considerable time commitment, was not high (Takeuchi et al., 2000). Administering drug therapy (clomipramine) together with behavioral therapy has also been recommended (King et al., 2004).

3. Sociability and stress in dogs

Sociability is defined as the attention or attitude of an animal toward another animal, whether than animal is a conspecific or a heterospecific (Jakovcevic et al., 2010). Among various temperaments of dogs, sociability is a key trait that allow dogs to experience less stress and respond more flexibly when faced with changing environments such as working or being adopted into a new family (Klausz et al., 2014; Valsecchi et al., 2011). Establishing positive social relationships with people is essential for dogs to successfully interact with future owners (Landsberg et al., 2012). Unsociable dogs may harm humans and society. Furthermore, dogs can become extremely stressed when introduced to unfamiliar surroundings and strangers, and this situation may severely threaten the welfare of dogs.

The period when dogs become sociable is closely associated with the period of myelination and maturation of the spinal cord, which is about 3–12 weeks after birth (Landsberg et al., 2013). Puppies can quickly learn social behaviors during this period, and thus, dogs that have positive experiences with a variety of people, animals, and environments during this period have a higher probability of adapting more easily to new ones later (Battaglia, 2009; Uzunova et al., 2010). The successful socialization of puppies through proper education is the primary factor that determines the formation of their behavioral patterns (Uzunova et al., 2010). Additionally, these experiences contribute not only to shaping the characteristics of dogs that are not completely genetically determined, such as aggressiveness, playfulness, fearfulness or possessiveness, but also to the ability of dogs to solve problems (Strandberg et al., 2005). Moreover, personality traits that are formed during this period tend to persist when the dogs become adults (Svartberg et al., 2005). In particular, sociability may be determined early in life over a short time period and remain stable over the entire life of a dog (Svartberg et al., 2005). The possibility that sociability may increase over time in dogs that were relatively unsociable early in life, is highly unlikely (Svartberg, 2007).

In particular, sociality is an important characteristic that can determine whether a working dog or an abandoned and re-adopted dog can successfully adapt to new environments (Barrera et al., 2010; Parlma et al., 2005; Serpell and Hsu, 2000; Valsecchi et al., 2011). By assessing the behavioral and physiological responses of abandoned dogs towards people and other stressors, behavioral problems that may occur in the new environment may be predicted (Cafazzo et al., 2014; Hennessy et al., 2001). Therefore, a wide diversity of tests used to assess sociality have been designed (Diederich and Giffony, 2006).

Dogs, one of the first domesticated animals, have evolved as members of human society, and as a result have been selected to maintain a strong social bond with humans (Ed, 1995). Sociality towards humans can be assessed by numerous behavioral and physiological indicators (Barrera et al., 2010; Palestrini et al., 2005; Rehn et al., 2014; Uvnäs-Moberg, 1997). Ainsworth's strange situation test, which was already well-known in human studies, is a laboratory procedure designed to investigate the level of attachment between a mother and infant (Ainsworth and Bell, 1970), and modified versions of this behavioral test can be used to assess sociality in dogs (Palestrini et al., 2005; Prato-Previde et al., 2003). One study assessed sociality by observing gazing behavior, one of the key non-verbal behaviors for communicating with humans, together with a modified version of Ainsworth's strange situation test (Jakovcevic et al., 2012).

To assess sociality through stress arising from unfamiliar people and/or environments, modified behavioral tests based on Ainsworth's strange situation test are used in combination with physiological indicators. In other words, to assess sociality, stress-related behaviors and physiological indicators are analyzed by repeating the process of placing the dog either together with or separated from a stranger or a caregiver with whom the dog has formed an attachment (Jakovcevic et al., 2012; Palestrini et al., 2005;

Svartberga and Forkman, 2002). Studies have shown that dogs with higher sociability have lower heart rates and other stress indicators when separated from caregivers and communicating with strangers than dogs with low sociability (Barrera et al., 2010; Bergamaso et al., 2010; Palestrinin et al., 2005; Plato-Previde et al., 2003; Uvnäs-Moberg, 1997). By conducting experiments with dogs classified as having high and low sociality, based on their behaviors towards strangers (fear-appeasement behaviors, sociability-related behaviors, and other behaviors), the effects of sociality on the personalities and associated stress levels of dogs have been identified (Barrera et al., 2010; Jakovcevic et al., 2012). Physiological stress levels due to contact or the formation of relationships with strangers may indicate whether the animal perceives the relationship with humans positively. Thus, physiological stress levels can be used to predict future behaviors in a variety of situations (Bergamasco et al., 2010; Uvnäs-Moberg, 1997)

4. Stress assessment of other environmental challenges

Besides interactions with unfamiliar people, stress from various environmental challenges that are faced in living with humans is also important in determining the welfare of dogs (Hennessy et al., 2001; Hiby et al., 2006; Palma et al., 2005; Serpell and Hsu, 2001; Valsecchi et al., 2011). By assessing behavioral and physiological stress from various environmental challenges that dogs may experience in living with humans, stress responses to working or re-adoption can be predicted as well (Hennessy et al., 2001; Hiby et al., 2006). Personality tests that assess behaviors of working or pet dogs in response to unfamiliar spaces, restraint, unfamiliar objects such as umbrellas, wearing a collar, and unfamiliar sounds have already become universally accepted (Diederich and Giffroy, 2006; Palma et al., 2005; Serpell and Hsu, 2001; Taylor and Mills, 2006). By also assessing associated stress, the adaptability of dogs to strange situations may be predicted.

CHAPTER II.

Evaluation of Effects of Olfactory and Auditory Stimulation on Separation Anxiety by Salivary Cortisol Measurement in Dogs

Abstract

Separation anxiety (SA) is a serious behavioral problem in dogs. In this study, salivary cortisol was studied to determine if the owner's odor or voice could reduce SA in dogs. Twenty-eight dogs with SA were divided into three groups: group 1 (control), group 2 (with owner's clothes during the separation period; SP) and group 3 (a recording of the owner's voice was played during SP). The dog's saliva was collected after the owner and their dog were in the experimental room for 5 min (PRE). The dog was then separated from the owner for 20 min and saliva collected four times at intervals of 5 min (SP1–4). Finally, the owner was allowed back into the room to calm the dog for 5 min, after which saliva was collected (POST). Evaluation of salivary cortisol concentrations by ELISA revealed that the ratios of SP1 concentration to PRE or POST concentrations were significantly higher in group 1 than in group 2 or 3. Additionally, the concentrations of SP1 – PRE and SP1 – POST among

groups differed significantly. These findings indicate that the owner’s odor or voice may be helpful to managing stress in dogs with SA.

1. Introduction

Separation anxiety (SA) is defined as any problematic behavior or group of behaviors that occurs exclusively in companion dogs in the owner's physical or virtual absence (Palestini et al., 2010). Problematic behaviors that occur during the owner's absence are common and make up a significant proportion of the caseloads of behavioral specialists (Appleby and Pluijmakers, 2004). SA disorder is distressing for the owner as well as the dogs (Landsberg et al., 2013; Schwantz, 2003). Dogs with SA exhibit their stress via undesirable behaviors, such as excessive vocalization, urination or defecation in inappropriate places, self-harming behavior and/or general destruction (Palsetini et al., 2013).

SA accounts for approximately 10 to 20% of the cases referred to dog behaviorists (Lansberg et al., 2013), but in older dogs, this proportion may rise to 50% (Mills et al., 2005). From 1991 to 2001, 1644 dogs were taken to the behavior clinic at Cornell University, 14.4% of which were diagnosed with SA (Bamberger and Houpt, 2006). In addition, SA is the third most common problem at referral practices in three countries (Canada, USA, and Australia) (Mills et al., 2005). These problematic behaviors have the potential to lead owners to abandon or abuse their dogs. To prevent such tragic situations, appropriate diagnostic methods and practical solutions must be developed.

Many studies have been conducted in an attempt to better diagnose and treat this problem (Appleby and Pluijmakers, 2004; Flannigan and Dodman 2001; Parthasarathy and Crowell-Davis, 2006; Simpson et al., 2007; Takeuchi et al., 2000). The first step is to evaluate the extent to which the dogs are stressed due to the absence of their owners and confirm whether various practical solutions can reduce stress.

Recently, there have been many discussions regarding the methods used to evaluate acute or chronic stress in dogs, and non-invasive methods of measuring stress have been received increased attention because they improve animal welfare while providing more reliable results (Beerda et al., 1990; 1996). In particular, use of salivary cortisol to evaluate stress has attracted much attention and is considered very useful (Bergamasco et al., 2010; Freschel et al., 2009; Haverbeke et al., 2008; Hekman et al., 2012) because it is relatively easy to take saliva samples and use them to measure stress levels while inducing a minimum amount of physiological changes in the subjects (Dreschel and Granger, 2009; Kobelt et al., 2003). Salivary cortisol is a better measure of adrenal cortical function and a better physiological indicator of stress than plasma cortisol because it is a direct reflection of the biologically active portion of the total cortisol level (Coppola et al., 2006). Additionally, the concentrations found in saliva were between 5% and 10% of those in found the plasma (Vincent and Michell, 1992). Therefore, although it is a very easy and non-invasive method, salivary cortisol can be a good indicator of acute stress response because it responds very quickly, even when no physiological changes occur in the blood (Beerda et al., 2000; Bodnariu, 2008; Hekman et al., 2012).

Although there are many successful management strategies for SA, some dogs require long-term treatment (King et al., 2004), and in most cases, drug therapy will be needed to address the dogs' intense anxiety (Landsberg et al., 2013). As a result, some owners may abandon their dogs rather than seeking treatment.

Therefore, this experiment was conducted to reduce stress in dogs in the absence of their owners by using the owners' odors or voices. To accomplish this, stress levels were assessed by measuring salivary cortisol levels before, during, and after the dogs were separated from their owners in an unfamiliar environment. The results of this study may determine whether olfactory or auditory stimulation originating from a dog's owner can relieve the stress induced by SA in the dogs.

2. Materials and methods

2.1. Subjects

A total of 28 healthy dogs (two intact males, eight neutered males, 15 intact females, and three neutered females) were included in this study. All dogs were housed indoors and privately owned. The mean weight was 4.74 ± 0.41 kg, and the mean age was 4.47 ± 0.56 years, ranging from 1 to 12 years of age. The mean length of time since adoption was 3.63 ± 0.51 years. Five Maltese, five Toy Poodles, nine Pomeranians, three Japanese Spitzs, three Shih Tzus, one American Cocker Spaniel, one Coton de Tulear, and one mixed breed were used. Nine dogs were adopted from other private owners at under 6 months old, 15 dogs were adopted from a pet shop within 6 months of age, three dogs were readopted in adulthood after being abandoned or from shelters, and one dog was bought from a certified breeding kennel at under 6 months of age. All dogs were selected on the basis of the results of a simple SA questionnaire (Palestrini et al., 2010) How does your dog behave when you are absent? If they exhibited at least one behavior such as excessive vocalization, destructive behavior toward themselves or the environment, and/or house soiling, the dogs were assumed to have SA. The experiment was immediately stopped and the dog was excluded from experimental analysis if the subject showed immoderate excitement or aggressiveness. This study was approved by the Institutional Animal Care and Use Committees (IACUC) in Seoul National University.

2.2. Testing facility

The experiment was conducted in an empty room (3.5 m × 3.7 m) at Seoul National University. In the room, one chair for the owner was situated at 0.9 m from the door, and a blanket (0.45 m × 1.45 m) was placed by the chair. A camera was installed at a position by the door (110 IS; Canon, Japan) and all experiments were recorded. The floor of the room was a non-slip texture.

2.3. Preparing for test

The subjects were divided into three groups according to weight, age and breed. The characteristics of each group are presented in Table 1. Group 1 (n=10) was a control group to assess salivary cortisol levels of dogs separated from the owners without any treatment. Group 2 (n=9) was an ‘olfactory group’ to determine the effects of the owner’s odor on SA. For this test, T-shirts were sent to the owners at least one week before the appointed test day and the owners were asked to wear the T-shirts without cleaning or washing them until the day of the test, then bring the shirt on the test day. During the experiment, the T-shirt was placed on the blanket during the separation period. Group 3 (n=9) was the ‘auditory group,’ which was evaluated to confirm the effects of the owner’s voice on a dog’s SA. Prior to test day, the owners were asked to read a story approximately 10 min long to their dogs before going to bed every night for at least one week. In addition, they were instructed to record their voice while reading the story at least once and send it to the experimenter. Voice recorded files were edited to be of uniform length and volume. At the time of testing, the recorded voice was played twice during the separation period.

2.4. Experimental procedures and data collection

The day before test day, the experimental room was cleaned to remove any possible stray odors. Before the test, the owner was asked to walk with the dog slowly for 10 min to allow the dog to urinate or defecate and relax. To prevent dilution of the saliva, the dog was restricted food and water from 2 hr before the test until the test ended. The total time of the experiment was 30 min. The experimenter was in the testing room throughout the experimental period to monitor the subject’s condition and state. To rule out the response of the dog to the experimenter, the experimenter did not engage in eye contact or physical movement during the experimental period. First, the owner and the dog were introduced into the testing room and given time to adapt to their new surroundings. The owner sat in the chair, while the dog was allowed to move freely around the room. Five minutes later, the owner left the room after the first saliva sample was collected (pre-separation period; PRE). During the 20 min separation period (SP), saliva samples were collected four times at intervals of 5 min (SP1–4). During SP, the dog moved freely around the room and was not allowed contact with the experimenter, except during collection of saliva. After 20 min SP, the owner reentered the room and was given 5 min to calm the dog. The last saliva sample was collected after this 5 min relaxation period (post-separation period; POST).

Saliva was collected by keeping a Salivabio infant swab (Salimetrics, USA) in the dog's mouth for 1 min, after which the swab was stored in a swab storage tube (Salimetrics) and refrigerated. Within one hour, the refrigerated saliva tubes were centrifuged at 4,000 rpm for 15 min, then stored at –70 ℃. Frozen saliva samples were thawed at room temperature for 10 min, then centrifuged at 4,000 rpm for 15 min. The absorbance of each sample was measured using an expanded range high sensitivity salivary cortisol ELISA kit (Salimetrics), and the salivary cortisol concentrations were calculated as μg/dl.

2.5. Statistical analysis

The calculated salivary cortisol levels in the samples were analyzed using the SPSS software (ver. 21.0; SPSS, USA). The alpha value was set at 0.05 in all cases, and all analyses involved two-tailed tests. Differences between two groups at same period were analyzed by the Mann-Whitney U test, and three groups were analyzed by the Kruskal-Wallis test. The concentrations from different periods within the group were analyzed by the Wilcoxon signed-ranked test, and concentrations from all periods were analyzed by the Friedman test.

3. Results

3.1. Subjects analysis

There were no significant differences among groups in weight, age, or time since adoption (Table 1).

3.2. Cortisol concentration and statistical results

In group 1, the salivary cortisol concentration at PRE was 0.59 ± 0.13 μg/dl, while that at POST was 0.35 ± 0.09 μg/dl, which was significantly different ($p<0.01$). The SP1 cortisol concentration was 0.84 ± 0.16 μg/dl, which was significantly higher than that of PRE ($p<0.01$) or POST ($p<0.01$). During SP, concentrations decreased by 0.64 ± 0.17 μg/dl, 0.52 ± 0.12 μg/dl and 0.42 ± 0.09 μg/dl, respectively. Changes along periods were significantly different ($p<0.001$). The cortisol at SP1 was 1.68 ± 0.27 times greater than that at PRE and 2.99 ± 0.50 times greater than at POST. Furthermore, the cortisol level at SP1 increased to 0.25 ± 0.06 μg/dl, which was higher than that at PRE and 0.49 ± 0.11 μg/dl more than that at POST.

In group 2, the PRE cortisol concentration was 0.47 ± 0.09 μg/dl, while that at POST was significantly lower, at 0.29 ± 0.06 μg/dl ($p<0.05$). The SP1 concentration was 0.52 ± 0.09 μg/dl, which represented a non-significant increase from the PRE concentration (p>0.05), but was significantly higher than the concentration at POST (p<0.05). During SP, concentrations were 0.52 ± 0.11 μg/dl, 0.47 ± 0.10 μg/dl and 0.41 ± 0.11 μg/dl, respectively. Changes along the periods were significantly different (p<0.005). The cortisol level at SP1 was 1.17 ± 0.11 times greater than that at PRE and 2.06 ± 0.41 times greater than at that at POST. Furthermore, the cortisol level at SP1 increased by 0.05 ± 0.03 μg/dl relative to that at PRE and 0.23 ± 0.04 μg/dl relative to that at POST.

In group 3, the PRE cortisol concentration was 0.56 ± 0.11 μg/dl, which was significantly higher than the value of 0.37 ± 0.09 μg/dl (p<0.05) observed at POST. The concentration at SP1 was 0.57 ± 0.12 μg/dl, which was significantly higher than that at POST (p<0.01), but not significantly different than that at PRE (p>0.05). During SP, concentrations were 0.52 ± 0.41 μg/dl, 0.50 ± 0.13 μg/dl, and 0.50 ± 0.14 μg/dl. Hormonal changes along periods were significantly different (p<0.005). The cortisol level at SP1 was 1.10 ± 0.18 times greater than that at PRE and 1.62 ± 0.14 times greater than that at POST. Furthermore, the cortisol level at SP1 was 0.01 ± 0.09 μg/dl higher than that at PRE and 0.19 ± 0.06 μg/dl higher than that at POST.

Overall variations in each group with time are shown in Figure 1. There were no significant differences among groups at corresponding sampling times (p>0.05). However, the ratio of the concentration at SP1 to that at PRE (SP1/PRE) was significantly different among groups (p<0.05) (Figure 2A).

Comparison of group 1 and 2 revealed significant differences (p<0.05). In addition, there were significant differences between group 1 and 3 (p<0.05). Likewise, the ratio of the concentration of SP1 to that of POST (SP1/POST) differed significantly among groups (p<0.01) (Figure 2B). Specifically, there were significant differences between group 1 and 2 (p<0.05) and between group 1 and 3 (p<0.05).

In addition, when comparing the differences in concentrations between PRE and SP1 among groups (SP1 – PRE), these levels were significantly different (p<0.05) (Figure 3A). In the same manner, the differences in concentrations between POST and SP1 among groups (SP1 – POST), there were significant differences (p<0.05) (Figure 3B).

4. Discussion

This study was conducted to evaluate the effects of olfactory or auditory stimulation by owners on SA in dogs based on salivary cortisol concentrations. Measurement of the salivary cortisol concentration is an effective method of assessing stress levels in dogs that is also non-invasive and induces minimum physiological changes in the animals (Beerda et al., 1998; 1999b; Haverbeke et al., 2008).

It should be noted that the sample size was very small and included a variety of individual histories, as well as different breeds, sexes, and ages. The dogs also displayed various degrees of SA. Therefore, the results of the examination may not be representative of all dogs with SA. In addition, deviation from the average cortisol level was very large in each dog, indicating that some of the results may not be meaningful or representative of a larger group. Nevertheless, the results revealed clear differences in salivary cortisol concentrations among groups.

The concentrations of PRE were similar in all groups; however, stress levels increased more rapidly in group 1 than in the other groups. During SP, the concentrations in group 2 and 3 were lower than in group 1, although these differences were not statistically significant. Therefore, the ratio of SP1 to PRE (SP1/PRE) or POST (SP1/POST), and the differences in concentration between SP1 and PRE (SP1 – PRE) or POST (SP1 – POST) were estimated to identify relative increases in stress levels because of the owner's absence.

The increase in the ratio of SP1 to PRE in groups 2 and 3 was significantly lower than that in group 1. Stress levels increased immediately after the owners left, while during SP these levels decreased to various degrees in all groups. POST levels were the lowest in all the groups, although some of the dogs were extremely excited by their owner's return.

These results suggest that the PRE cortisol concentration can be used to indicate increased stress due to the unfamiliar surroundings, and that POST cortisol concentration may be assumed as a baseline cortisol level because the owners calmed the dogs gently and almost all dogs rapidly relaxed with 5 min. The increase in concentration at SP1 over that at POST (assumed baseline) was statistically different among groups, and the ratio in groups 2 and 3 was lower than that in group 1. In addition, the differences in concentration between PRE and SP1 or POST and SP1 were significantly different among groups.

These results indicated that stress induced by the owner's departure could be reduced physiologically by allowing the dog to sniff the owner's odor or hear the owner's recorded voice. Accordingly, this method may be useful to owners when applied along with practical training and drug therapy as a way of treating dogs with SA. This method is easy to implement and allows for more efficient management of SA when combined with other techniques. Furthermore, it is worthwhile to improve the dogs' welfare; they may otherwise live the majority of their time at home alone with their anxiety.

In conclusion, companion dogs with SA become stressed in the absence of the owners and exhibited various problematic behaviors including excessive barking, destruction, and improper urination and defecation. This study demonstrated that the owner's odor or voice could reduce separation related stress significantly at the physiological level. Overall, the methods employed herein could be a useful and practical management solution for dogs and owners struggling with SA, especially if applied in combination with other training techniques or drug therapies.

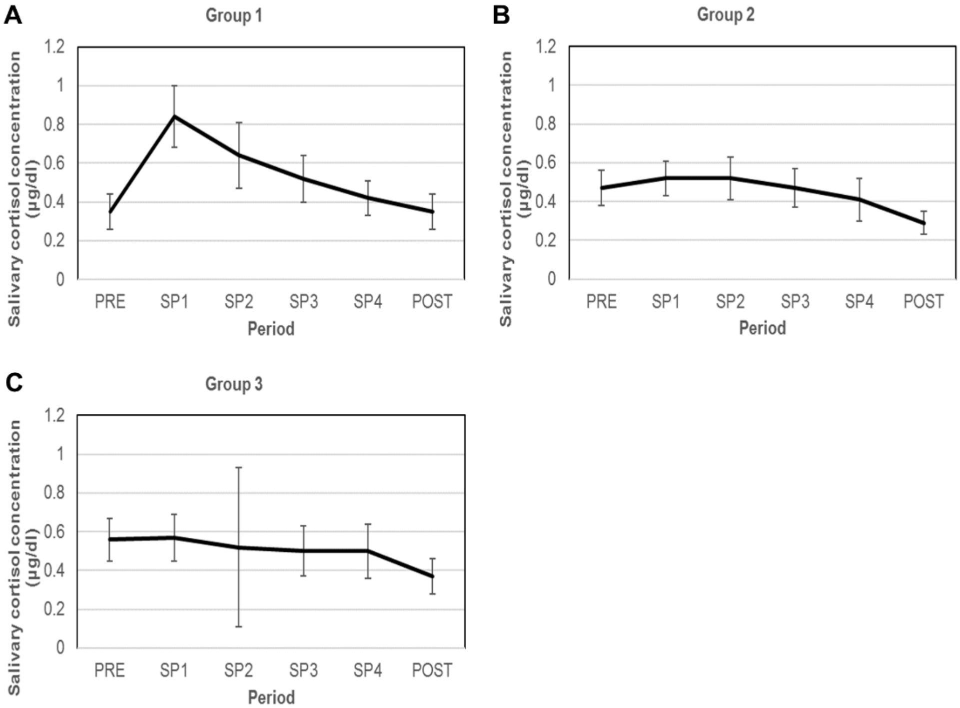


Figure 1. Variation in salivary cortisol level of the three groups.

(A) Variation in salivary cortisol level in group 1. Changes along periods were significantly different (*p*<0.001). (B) Variation in salivary cortisol level in group 2. Changes along the periods were significantly different (*p*<0.005). (C) Variation in salivary cortisol level in group 3. Hormonal changes along periods were significantly different (*p*<0.005). There were no significant differences among groups at corresponding sampling times (*p*>0.05). * *p*<0.05, ** *p*<0.01 in comparison of pre-separation period (PRE) and post-separation period (POST). SP = separation period. (mean ± SE)

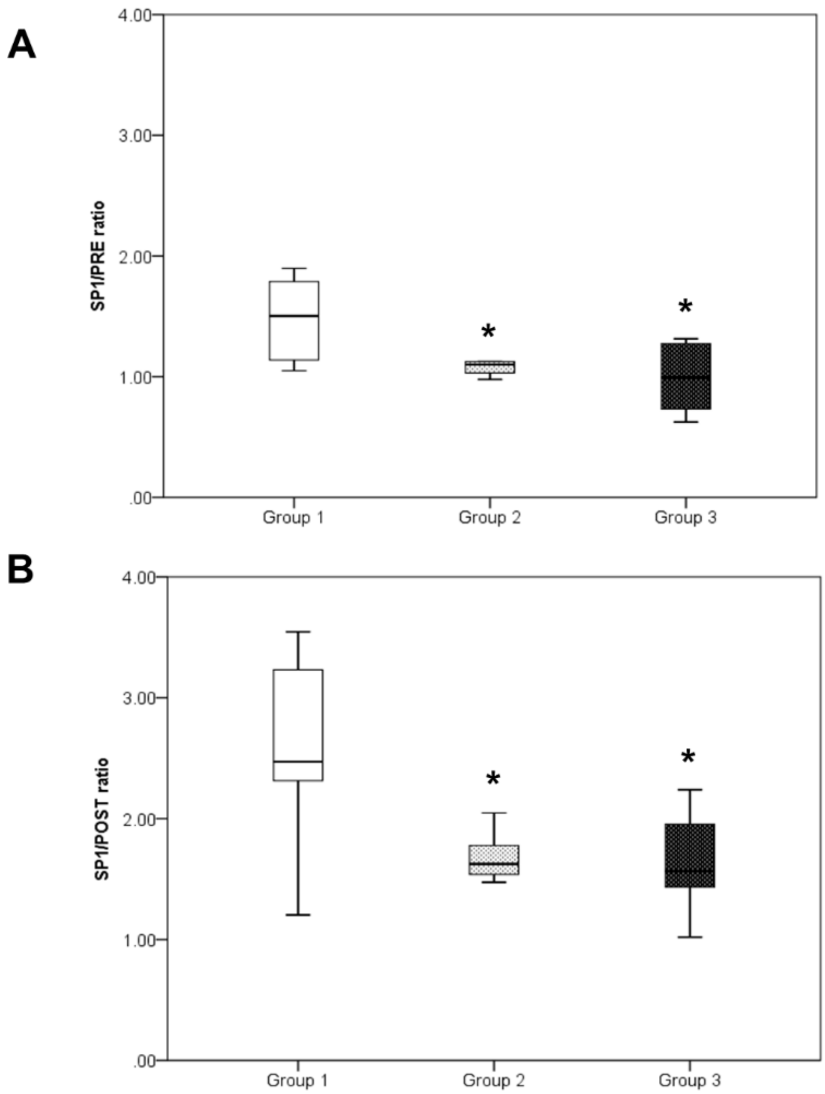


Figure 2. Cortisol concentration ratio of SP1 at different time points.

(A) At SP1 to that at PRE. (B) At SP1 to that at POST. * $p<0.05$ in comparison with group 1. (mean ± SE)

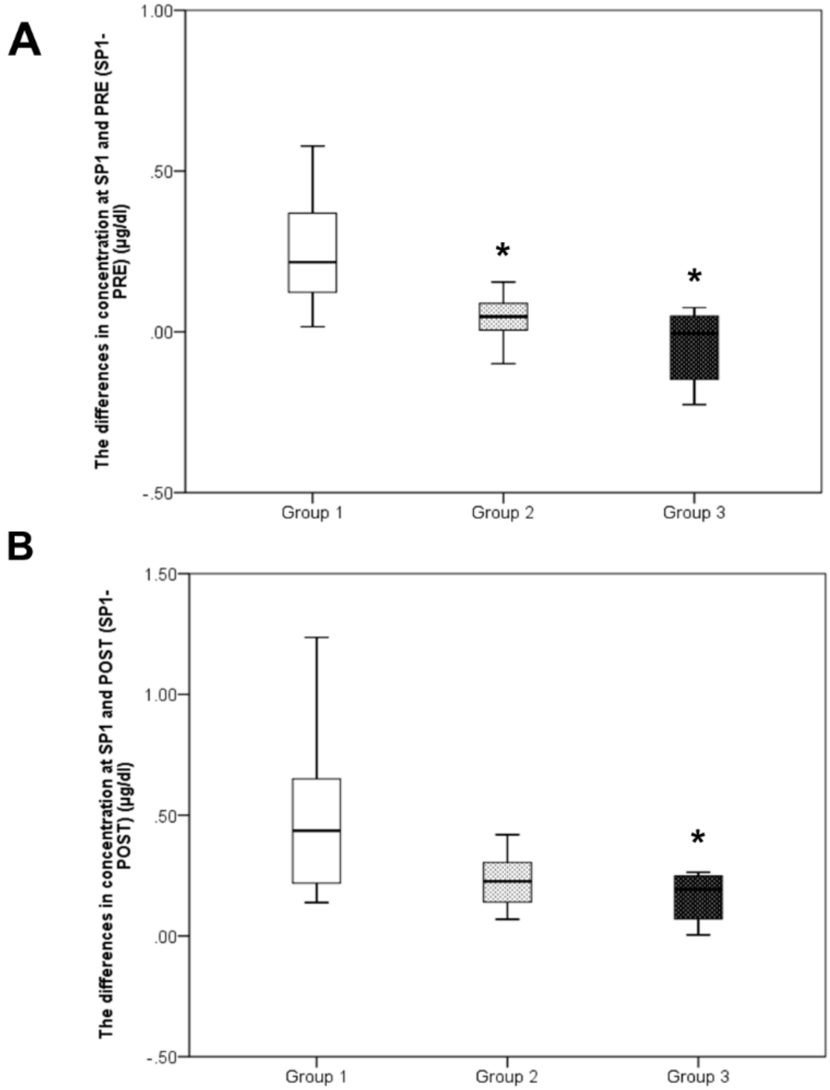


Figure 3. The differences in cortisol concentration between time points.

(A) Between PRE and SP1. (B) Between SP1 and POST. * $p<0.05$ in comparison with group 1. (mean ± SE)

Table 1. Information regarding dogs in the three groups (Kruskal-Wallis test)

Information ╲ Groups	1	2	3
n	10	9	9
Weight (kg)	4.46 ± 0.85[a)]	4.69 ± 0.53[a)]	5.09 ± 0.77[a)]
Age (yrs)	3.85 ± 0.97[b)]	5.67 ± 0.87[b)]	3.96 ± 1.06[b)]
Duration of ownership (yrs)	2.50 ± 0.39[c)]	5.00 ± 0.96[c)]	3.56 ± 1.12[c)]
Breeds (n)	Maltese (2) Toy Poodle (2) Pomeranian (3) Japanese Spitz (2) Shih Tzu (1)	Maltese (2) Toy Poodle (1) Pomeranian (2) Japanese Spitz (1) Shih Tzu (1) Coton de Tulear (1) Mixed breed (1)	Maltese (1) Toy Poodle (2) Pomeranian (4) Shih Tzu (1) American Cocker Spaniel (1)
Sex (n)	MC (5)/ F (5)	M (1)/ MC (2)/F (5)/ FS (1)	M (1)/ MC (1)/ F (5)/ FS (2)

There were no significant differences among groups. Figures in columns marked with the same letters are not significantly different (p>0.05). n = number; yrs = years; M = intact male; MC = male castrated; F = intact female; FS = female spayed.

CHAPTER III.

Relationship between Sociability toward Humans and Physiological Stress in Dogs

Abstract

Sociability is an essential trait for dogs to successfully interact with humans. In this study, the relationship between sociability and physiological stress was examined. Additionally, whether differences exist between companion dogs (C group) and shelter dogs (S group) was examined. Overall, healthy 37 dogs (C group =21 and S group =16) were examined. After 5 min of walking, the dog and the owner (or the chief manager) rested freely in the experimental location for 5 min. The behavioral test with 6 categories was conducted to evaluate sociability over 4 min. The establishment of two groups (H group = dogs with high sociability; L group = dogs with low sociability) was supported by the statistical results of the behavioral tests. Saliva was collected before (P1) and after the test period (P2), and salivary cortisol levels were determined and statistically analyzed. The cortisol concentrations at P2 and the differences in concentrations between P1 and P2 (P2 – P1) in the groups with high sociability were significantly lower than those in the groups with low sociability. These results may demonstrate that sociable dogs adapt

more comfortably to strangers and unfamiliar situations. Meanwhile, there were significant differences in hormonal results between the C and S groups. For this reason, their sociability should be evaluated using behavioral and physiological assessments before re-adoption to ensure their successful adaptation.

1. Introduction

The tendency to be friendly toward strangers has been proposed to be a personality trait in dogs, and several studies have described this trait as sociability (Svartberg, 2007). Although this trait is related to social fearfulness and aggressiveness, it may also contribute to positive interactions with unfamiliar individuals (Svartberg, 2007). More sociable animals, such as those who pay more attention to a person, may be more persistent in their attempts to communicate and may be more easily trained (Jakovcevic et al., 2012). A lack of adequate socialization can result in problematic behaviors, such as aggression, excessive excitability, fearfulness, sexual inadequacy or indifference toward owners (Battaglia et al., 1996).

Some dogs may show sensitive responses to strangers, unfamiliar dogs or other animals or places, resulting in excessive stress, because their chances of experiencing various social situations and human interactions may be limited during the critical early period of their life (Battaglia, 2009; Landsberg et al., 2013; Uzunova et al., 2010). This characteristic defect could also result in the failure of dogs to be adopted from a shelter due to their inability to adapt to a new family and house (Salman et al., 2000). Overall, these situations may severely harm the welfare of dogs.

Many behavioral assessment tests have been used to evaluate temperament and personality in dogs (Diederich and Giffroy, 2006; Jones and

Gosling, 2005; Svartberg et al., 2002; Taylor and Mills, 2006), and multiple studies have identified behaviors and physiological responses in dogs when meeting strangers (Barrera et al., 2010; Bergamasco et al., 2010; Rehn et al., 2014). Some studies have evaluated behavioral traits, especially the sociability of shelter dogs, using various methods to verify the successful re-adoption and adaptation of dogs to new families and surroundings (Palma et al., 2005; Valsecchi et al., 2011). Overall, research has shown that sociability is one of the most important traits to have a successful adaptation.

Although various measures have been used to evaluate physiological stress in dogs and assess animal welfare (van Hooff et al., 2000; Haverbeke et al., 2008; Möstl and Palme, 2002), measuring salivary cortisol levels has been the preferred method, because this simple and non-invasive procedure minimizes additional physiological changes as a result of the measurement itself (Beerda et al., 1996; Hekman et al., 2012; Helhammer et al., 2009; Kobelt et al., 2003). Additionally, measuring salivary cortisol levels reflects physiological changes relatively faster than other methods, allowing immediate stress responses to be monitored (Dreschel and Granger, 2009; Vincent and Michell, 1992). For these reasons, the physiological responses of dogs facing strangers were evaluated based on changes in salivary cortisol levels.

In this study, the dogs analyzed were divided into two groups based on results from a behavioral sociability assessment that took place at an unfamiliar location without their owners. Differences in the degree of physiological stress (as inferred from salivary cortisol levels) resulting from interactions with strangers between dogs with high sociability and low sociability were evaluated.

2. Materials and methods

2.1. Subjects

The differences in the sociability and physiological stress response of companion dogs and shelter dogs were also evaluated. A total of 37 healthy dogs (21 companion dogs = C group; 16 shelter dogs = S group) were included in the study. All of the dogs in the C group were privately owned and were housed indoors. The applications were submitted by owners on voluntary base via SNU VMTH (Veterinary Medical Teaching Hospital in Seoul National University) advertisement and other website. The mean age was 4.57 ± 0.62 years, and the mean weight was 5.67 ± 0.95 kg. Four neutered males, six intact females and 11 neutered females were included. Three Malteses, one Shih Tzu, two Yorkshire Terriers, two Pomeranians, one American Cocker Spaniel, one Jack Russel Terrier, two Chihuahuas, one Japanese Chin, one Shetland Sheepdog, one Border Collie and six mixed breeds were used.

Shelter dogs from Seoul animal rehoming center in Seoul Grand Park were included in the study as the S group. All dogs were neutered (nine males and seven females). The mean estimated age was 2.81 ± 0.44 years, and the mean weight was 3.67 ± 0.17 kg. Six Malteses, three Toy Poodles, two Shih Tzus, one Miniature Pincher, one Yorkshire Terrier, one Pomeranian, one

Japanese Spitz and one mixed breed were included. These individuals had been kept in the center for an average of 2.37 ± 0.38 months.

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University.

2.2. Experimental location

The experiment for the C group was conducted in the rectangular enclosure (1.5 m × 3.5 m) surrounded by a wall (height = 1 m) in an empty room at Seoul National University. In the experimental area, one chair (0.5 m × 0.5 m), for the owner or experimenter (E), was situated 3 m from the wall with the door and 0.5 m from the other three walls. Small signs placed at 1 m in front of the chair were used to designate the experimental area. The experimental procedure for S group was similar and took place in an empty room at Seoul animal rehoming center. The arrangement of the chair and the marks were identical. The rooms used were unfamiliar to all of the dogs and were cleaned before the experiment. All experiments were conducted at similar time around the noon.

2.3. Experimental procedures

Sociability was identified using behavioral assessment tests (Barrera et al., 2010; Jakovcevic et al., 2012).

2.3.1. Period 1 : with owners

The dog was walked for a maximum of 5 min. After walking, the dog and the owner (or the chief manager) were introduced to the testing area and given time to adapt to their new surroundings for 5 min. The owner or the chief manager was allowed to sit in the chair or on the floor for 5 min, hugging the dog or exploring the place only if he or she did not excite the dog. The dogs were restricted food and water for at least 1 hr before the test to prevent the saliva from being diluted.

2.3.2. Period 2 : separation period

Passive phase: E came into the testing area and collected saliva samples from the dog immediately after the owner or the chief manager left the room (phase 1, P1). Saliva was collected by keeping a SalivaBio infant swab (Salimetrics, Carlsbad, CA, U.S.A.) in the dog’s mouth for 1 min. Restraint during sampling without making friendly visual contact, physical contact or name calling was kept to a minimum. After the first sampling, E sat in the chair for 2 min without moving and made visual contact with the dog. When the dog initiated physical contact with E in any way, E petted the dog's head or chin once.

Active phase: After the 2-min passive phase, E stood while making eye contact with the dog and calling him or her by name in a friendly manner. If the dog initiated physical contact with E in any way, E petted the dog's head or chin once. If the dog did not come close to E, the E repeated the dog's name 3 times at intervals of 10 sec. For the S group, the names of dogs that were called out during the experiment were established during their first visit to the center so that they would be familiar to the dogs. After the 2-min active phase (phase 2, P2), saliva was collected in the same manner described previously.

2.3.3. Grouping based on the results of the behavioral tests

A total of 6 categories, three from each phase, were used to evaluate the sociability of the dogs (Barrera et al., 2010; Jakovcevic et al., 2012). The overall measures are shown in Table 1 (PCL = contact latency at passive phase; PTC = time close to E at passive phase; PPC = physical contact at passive phase; ACL = contact latency at active phase; ATC = time close to E at active phase; and APC = physical contact at active phase).

2.3.4. Analysis of salivary cortisol concentration

The swab storage tubes (Salimetrics) were refrigerated and centrifuged at 4,000 rpm for 15 min. The separated saliva samples were stored within 1 hr at –70°C until they were analyzed. Stored, frozen saliva samples were completely thawed at room temperature (10 min) and centrifuged at 4,000 rpm for 15 min. The absorbance of the supernatants from each sample was measured using an expanded-range high-sensitivity salivary cortisol ELISA kit (Salimetrics), and salivary cortisol concentrations were calculated as μg/dl. The optical density of the plates was read at 450 nm using a SUNRISE™ version 3.31 microplate reader (TECAN, Männedorf, Switzerland).

2.4. Statistical analysis

The results of the behavioral scoring in the sociability assessments and the calculated salivary cortisol levels were analyzed using SPSS software version 21 (SPSS, Chicago, IL, U.S.A.). The establishment of two groups (H group = dogs with high sociability; L group = dogs with low sociability) was supported by a K-means cluster analysis of the data from the 6 categories of the behavioral tests. Behavioral results of the groups were analyzed by ANOVA. Two-way repeated measured ANOVA was used to assess the hormonal variation across the period. The differences in the concentration between P1 and P2 (P2 – P1) of the group and the ratio of the concentration at P2 to that at P1 (P2/P1) were analyzed by ANOVA. Because we compared between H and L groups, and between C and S groups, significance level of 0.025 (2-tailed) was adopted throughout the analysis with Bonferroni correction.

3. Results

3.1. Subjects analysis

All dogs included in the study were classified according to their status and results of sociability behavioral test (Table 2). There were no significant differences in body weight or age among the groups (p>0.025).

3.2. Sociability test results

The results from the 6 categories of behavioral tests from the groups are shown in Table 3. There were significant differences between the H group and the L group for each category (PCL: p<0.001, PTC: p<0.001, PPC: p<0.001, ACL: p<0.001, ATC: p<0.001 and APC: p<0.001). However, the C group and the S group were not significantly different in any category (p>0.025).

3.3. Salivary cortisol analysis

Age, weight, sex and breed type of the subjects included in this study were not found to have a significant effect on hormonal results (p>0.05).

The overall hormonal results are shown in Figure 4. In the H group, the salivary cortisol concentration at P1 was 0.3848 ± 0.0969 μg/dl and at P2 was

0.3577 ± 0.0981 μg/dl. In the L group, the concentration at P1 was 0.5593 ± 0.0755 μg/dl, and the concentration at P2 was 0.6527 ± 0.0781 μg/dl. There were no significant differences between the groups across the period (p=0.058). The ratio of the concentration at P2 to that at P1 (P2/P1) were significantly different between the groups (F=8.136, p=0.008) (Figure 5A). Likewise, the differences in the concentration between P1 and P2 (P2 − P1) were −0.0272 ± 0.03 μg/dl in the H group and 0.0933 ± 0.0371 μg/dl in the L group. These levels were significantly different (F=10.667, p=0.003) (Figure 6A).

In the C group, the concentration at P1 was 0.5035 ± 0.0842 μg/dl, and the concentration at P2 was 0.5037 ± 0.0823 μg/dl. In the S group, the cortisol concentration at P1 was 0.4546 ± 0.0907 μg/dl, and the concentration at P2 was 0.5439 ± 0.1082 μg/dl. There were no significant differences between the groups across the period (p=0.303). The ratio of the concentration at P2 to that at P1 (P2/P1) were significantly different between the groups (F=4.167, p<0.05) (Figure 5B). Likewise, the differences in the concentration between P1 and P2 (P2 − P1) were 0.0002 ± 0.0279 μg/dl in the C group and 0.0893 ± 0.0458 μg/dl in the S group. These values were significantly different (F=6.224, p=0.018) (Figure 6B).

4. Discussion

Sociability measurement are important for evaluating the ability of companion dogs to live successfully with humans (Taylor and Mills, 2006).

The first objective of this study was to confirm the relationship between sociability and physiological stress in dogs. The L group showed significantly high levels of stress variation compared with H group. In other words, dogs with low levels of sociability became more physiologically stressed in response to exposure to strangers and unfamiliar situations. Consequently, less sociable dogs may become extremely stressed in unfamiliar situations, such as abandonment, re-adoption, moving to another location or visiting a veterinarian. If the dog fails to adapt to these various situations and exhibits undesirable behaviors, the possibility of abandonment or abuse increases (Salman et al., 2000).

The second objective of this study was to compare companion dogs and shelter dogs in terms of their levels of sociability and physiological stress. Some studies have indicated that social isolation and inadequate surroundings may induce various behavioral problems, such as aggressiveness toward strangers or severe timidity, in shelter dogs (Barrera et al., 2010; Diederich and Giffroy, 2006). In contrast, evaluations of their hormonal changes suggest that dogs in shelters adapt successfully to the shelter environment (Hiby et al., 2006).

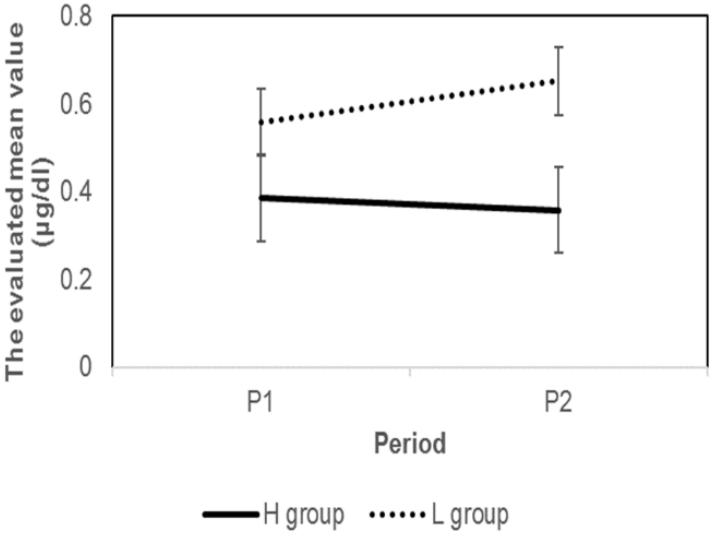
The results of the hormonal tests on shelter dogs included in this study were significantly different from those of companion dogs, though the behavioral tests were statistically similar. The results may indicate that when shelter dogs are adopted by new family, the possibility of failure to adapt to the unfamiliar environment may increase; these dogs are increasingly likely to exhibit unacceptable behaviors in some situations due to the high level of physiological stress they experience. For this reason, the sociability of shelter dogs may need to be confirmed using both behavioral and physiological assessments to predict their ability to successfully adapt to a new environment and to reduce the likelihood of a failed re-adoption.

The majority of the dogs showed higher initial cortisol level than the known basal value, which was assessed in their own homes with normal routine and diet (Bennett and Hayssen, 2010). The dogs included in this study were assumed to be experiencing temporary stress, because they had been moved from their familiar surroundings to the unfamiliar experimental area. For this reason, the differences in the concentrations before and after the move (P2 – P1) among the groups were also statistically analyzed to account for the increased initial stress level. In addition, there was no significant difference in the concentration at P1 according to age, weight, sex and breed type, consistent with previous studies (Bennett and Hayssen, 2010; Bergamasco et al., 2010; Coppola et al., 2006).

Meanwhile, the sociability of the dogs may have to be evaluated according to the behavioral and hormonal results of the study, because of the small sample size and the variety of individual differences. It should be noted that the sample size of the study was small and that dogs with a variety of individual histories were included. Therefore, the results of the study may not be representative.

The results of this experiment show that more sociable dogs experience less physiological stress and consequently have a greater ability than less sociable dogs to adapt to various human-based environments. The methods applied in the study may be used to predict dogs' successful adaptation to unfamiliar environments.

A



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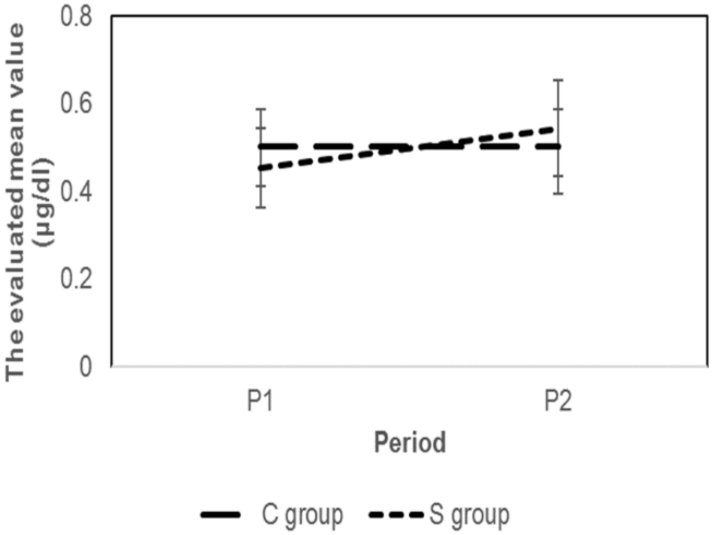

Figure 4. Variation in salivary cortisol level of the groups.

(A) There were no significant differences across the testing period among the groups (p=0.058). H group = dogs with high sociability; L group = dogs with low sociability. (B) There were no significant differences across the testing period among the groups (p=0.303). C group = companion dogs; S group = shelter dogs. (mean ± SE)

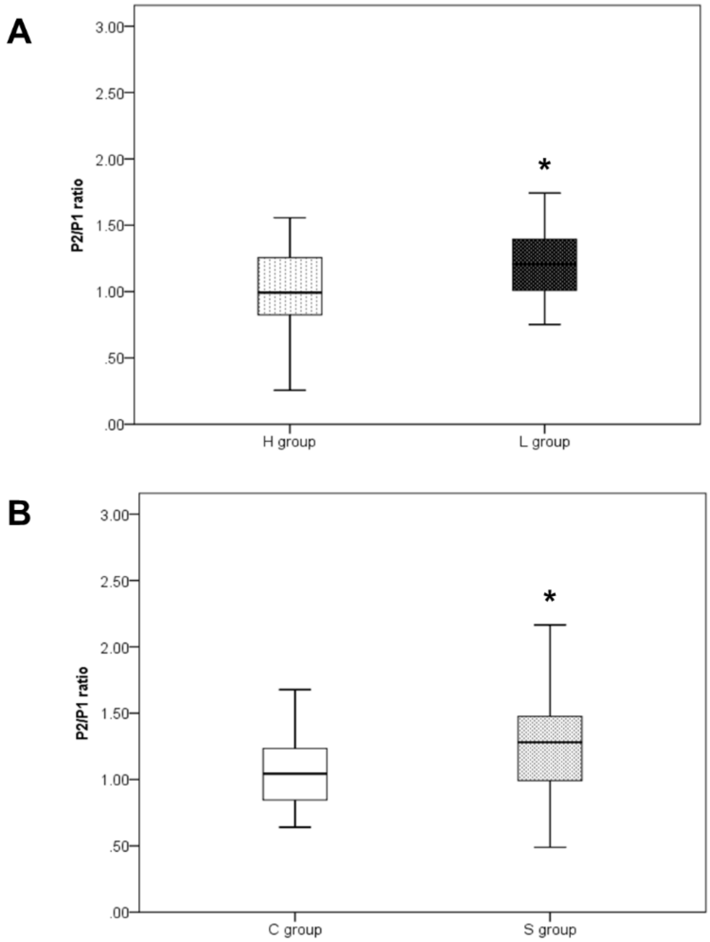

Figure 5. The ratio of the concentration of P2 to that of P1, P2/P1.

(A) There were significant differences between H and L groups. H group = dogs with high sociability; L group = dogs with low sociability. (B) There were significant differences between C and S groups. C group = companion dogs; S group = shelter dogs. * = significantly different with Bonferroni correction. (mean ± SE)

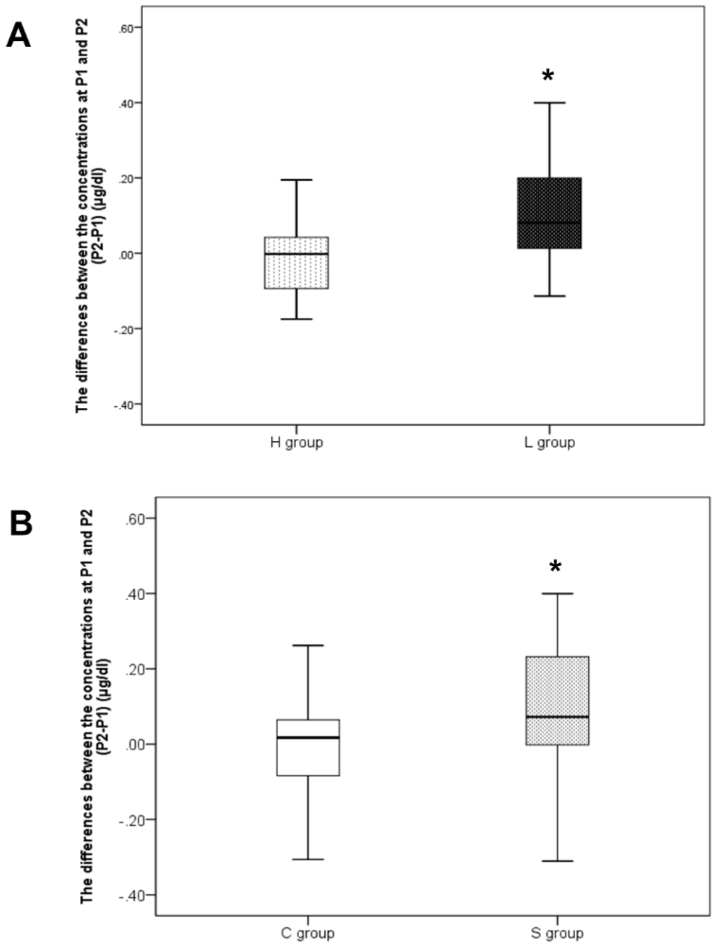

Figure 6. The differences in concentration between P1 and P2 (P2 − P1).

(A) There were significant differences between H and L groups. H group = dogs with high sociability; L group = dogs with low sociability. (B) There were significant differences between C and S groups. C group = companion dogs; S group = shelter dogs. * = significantly different with Bonferroni correction. (mean ± SE)

Table 2. Sociability assessment categories

Measurements ╲ Phase	Passive (P)	Active (A)
Contact latency (CL)	Measured from the time the animal enters the room until it makes physical contact with the E for the first time (sec)	
Time close to E (TC)	< 1m distance (sec)	
Physical contact (PC)	Duration of the physical contact between the E and the subject (sec)	

Table 3. Grouping using data from sociability measures

Measurements \ Groups	C		S	
	H	L	H	L
Information	Companion dogs with high sociability (n=8)	Companion dogs with low sociability (n=13)	Shelter dogs with high sociability (n=9)	Shelter dogs with low sociability (n=7)
Age (yrs)	$5.12 \pm 1.26^{a)}$	$4.23 \pm 0.67^{a)}$	$2.78 \pm 0.66^{a)}$	$2.86 \pm 0.59^{a)}$
Weight (kg)	$6.95 \pm 1.96^{b)}$	$4.88 \pm 0.96^{b)}$	$3.91 \pm 0.23^{b)}$	$3.36 \pm 0.21^{b)}$
Sex (n)	MC (2)/ F (2)/ FS (4)	MC (3)/ F (4)/ FS (6)	MC (4)/ FS (5)	MC (5)/ FS (2)
Breeds (n)	Maltese (1) Shih Tzu (1) Yorkshire Terrier (1) Chihuahua (2) Shetland Sheepdog (1) American Cocker Spaniel (1) Border Collie (1)	Maltese (2) Yorkshire Terrier (1) Chihuahua (1) Pomeranian (2) Jack Russel Terrier (1) Japanese Chin (1) Mixed breed (5)	Maltese (3) Shih Tzu (1) Yorkshire Terrier (1) Toy Poodle (3) Mixed breed (1)	Maltese (3) Shih Tzu (1) Pomeranian (1) Miniature Pinscher (1) Japanese Spitz (1)

n = number; yrs = years; C = companion dogs; S = shelter dogs; H = dogs with high sociability; L = dogs with low sociability; MC = male castrated; F = intact female; FS = female spayed. Figures in columns marked with the same letters are not significantly different.

Table 4. Results from sociability measures in all groups

Groups \ Phase	Passive phase (120 sec)			Active phase (120 sec)		
	CL	TC	PC	CL	TC	PC
H	13.18±4.80[a)]	91.24±5.57[b)]	19.18±4.43[c)]	13.71±6.73[d)]	97.82±6.73[e)]	21.29±6.07[f)]
L	107.90±7.64[a)]	20.15±7.16[b)]	0.35±0.22[c)]	110.00±6.47[d)]	17.80±8.36[e)]	0.70±0.60[f)]
C	67.90±11.98	55.76±10.25	9.81±3.97	79.43±10.74	52.71±11.68	9.71±4.90
S	59.75±14.19	48.94±11.13	7.94±2.87	47.81±14.45	57.00±12.96	10.75±4.07

CL = Contact latency; TC = Time close to the experimenter; PC = Physical contact; H group = dogs with high sociability; L group = dogs with low sociability; C group = companion dogs; S group = shelter dogs. [a)-f)] $p<0.001$. (mean ± SE sec)

CHAPTER IV.

Comparison of Stress Levels Induced by Two Types of Pet Dryers (standing type and box type) Using Salivary Cortisol Measurement in Dogs

Abstract

The common pet dryer (CD) is typically used to dry dogs after bathing, but the excessive heat and noise can induce stress. Dog owners and facilities housing many animals, such as research facilities, have begun to widely adopt the pet dry room (PDR) as a more convenient drying alternative. In the present study, the stress induced by CD or PDR was assessed by measuring the salivary cortisol. Ten healthy Beagles at a research laboratory were included. The mean cortisol level before drying (S1) with CD was 0.25 μg/dl and significantly increased to 0.38 μg/dl 10 minutes after drying (S2) and 0.56 μg/dl 20 minutes after drying (S3). The cortisol level at S1 with PDR was 0.33 μg/dl, and increased to 0.38 μg/dl at S2 and 0.40 μg/dl at S3, but this difference was not statistically significant. Comparing the values from S1, S2 and S3, use of CD or PDR were not significantly different each other. However, the difference between concentrations S1 and S3 and the S3-to-S1 ratio differed significantly between the groups. These results indicate that

PDR may induce less stress in dogs compared with CD. In conclusion, this facility may be convenient for owners or managers, especially those of large-scale facilities such as for laboratory dogs or centers for abandoned dogs, and could be more comfortable for dogs in that they induce less stress. The equipment also has the potential to be widely used in other animal welfare programs.

1. Introduction

Dog owners are commonly instructed to bathe their dogs every 1 to 2 weeks to cleanse the coat and prevent skin disease caused by improper humidity (Miller et al., 2013). It is important to completely dry the dog's coat to prevent pruritus and consequent injury, pain, or infection (Miller et al., 2013; Yoshida et al., 2002). However, the process of drying the coat can be very stressful because dogs are very sensitive to heat and noise (Bruchim et al., 2006; Drobatz and Macintire, 1996; Sales et al., 1997). For this reason, it may be necessary to devise strategies to reduce the stress from common daily activities such as drying the coat. In particular, experimental facilities housing laboratory research dogs may be quite very stressful for the dogs due to isolation (physical or social), severe noise, various experimental procedures, and other events (Balcombe et al., 2004; Cobb et al., 2014; Overal and Dyer, 2005; Part et al., 2014). Numerous attempts and studies have been performed to reduce and minimize the stress experienced by laboratory research dogs using a variety of methods such as socialization and environmental enrichment (Coppinger and Zuccotti, 1999; Hubrecht et al., 1993; Kiddie and Collins, 2014; Overall et al., 2005).

Common pet dryers (CD) are typically used to dry the wet coat after bathing, but the pet dry room (PDR) was recently designed and introduced commercially as a more convenient drying alternative. The box-shaped device is available in several sizes according to the dog breed. The wet dog is placed into the box and dried using wind at an appropriate temperature. Although these devices have been widely used by owners as a more convenient drying alternative, the effects of PDR on dogs have not been studied to date.

Stress is measured in animals in numerous ways. Recently, noninvasive methods have gained favor for evaluating animal welfare (Bodnariu. 2008). Salivary cortisol measurement is a noninvasive method with minimal physiological effects and is very useful for investigating acute stress responses because the test can detect blood cortisol changes within several minutes of a stressful event (Beerda et al., 1996; Dreschel and Granger, 2009).

The purpose of this study was to examine the differences in stress levels using salivary cortisol between bathed dogs that were dried by CD or PDR.

2. Materials and methods

Ten healthy adult Beagle dogs housed in a laboratory animal facility in the College of Veterinary Medicine at Seoul National University were included in the experiment. The mean weight of the dogs was 11.10 ± 1.20 kg, and all were intact males under 2 years old. All were introduced in the lab facility about one year ago and had not thus far been included in any experimental procedures, only receiving regular management treatments like bathing and drying with CD twice at monthly intervals by college students. The experiments were started at 1:00 pm to 4:00 pm every day for two weeks.

The dogs were bathed with warm water for one minute, immediately introduced into the room (within 30 s) and dried using CD (APST2031, A-plus ENC, Incheon, Korea) or PDR (PDR-20000S, Izukorea, Seoul, Korea) for 20 min. CD and PDR equipment had different decibel levels (CD, about 70 decibel; PDR, 60 decibel). Peak temperatures using CD and PDR were 100°C (usually dried at 70°C – 80°C) and 40°C (dried at 25°C), respectively. The size of the PDR was 64 cm × 90 cm × 81 cm for medium-sized breeds such as Beagles.

In the first week of the experiment, the odd-numbered dogs were dried with CD and the others (even-numbered) were dried with PDR. Conversely, in the second week of the experiment, the even-numbered dogs were dried with CD, and the others (odd-numbered) were dried using PDR. All the experiments were done at the same time on weekdays for two weeks.

For CD, the dogs were restrained with leash while the researcher dried their wet coat; however, no restraint was used for PDR, so the dogs were able to move freely within the PDR. During PDR treatment, dogs were able to see outside through the front window.

The initial saliva sample was collected immediately after bathing the dog (S1: before drying), and then, the coat was dried using CD or PDR. The second saliva sample was collected 10 min after the drying process started (S2) and third saliva sample was collected 20 min after the drying process started when drying process was finished (S3).

Saliva was collected by keeping the Salivabio infant swab (Salimetrics, Carlsbad, CA, USA) in the dog's mouth for 1 min and the swab was stored in a swab storage tube (Salimetrics, Carlsbad, CA, USA) and refrigerated. Within one hour, the refrigerated tube was centrifuged at 4000 rpm for 15 min and stored at –70°C. Collected and frozen samples were thawed for 10 min at room temperature and centrifuged at 4000 rpm for 15 min. Using an expanded range high sensitivity salivary cortisol ELISA kit (Salimetrics, Carlsbad, CA, USA) and a SUNRISE™ version 3.31 microplate reader (TECAN, Männedorf, Switzerland), the absorbance of each sample was measured, and the cortisol concentration was calculated as μg/dl.

The calculated salivary cortisol levels in the samples were statistically analyzed using SPSS version 21.0 (SPSS, Chicago, IL, USA). The concentrations from different periods within the group were analyzed by the Friedman test. Differences between two groups at same period were analyzed by Mann-Whitney U test. The alpha value was set at 0.05 in all cases.

The study was approved by the International Animal Care and Use Committee at Seoul National University (SNU-140609-1-1).

3. Results

In the CD group, the cortisol concentration from S1, S2, and S3 was respectively 0.25 ± 0.03 μg/dl; 0.38 ± 0.05 μg/dl; 0.56 ± 0.08 μg/ dl. The concentration significantly increased over time ($x^2 = 11.4$, $p<0.005$).

In the PDR group, the cortisol concentration from S1, S2, and S3 was showing the same increase like in the CD group, respectively, 0.33 ± 0.03 μg/dl; 0.38 ± 0.05 μg/dl; 0.40 ± 0.05 μg/dl. However, there were no significant differences between S1, S2 and S3 ($p>0.05$).

When the values between the CD and PDR groups were analyzed statistically, there were no differences between levels at S1, S2 and S3 ($p>0.05$). Overall variations in each group across the test time are shown in Figure 7.

However, the cortisol level at S3 was 2.29 ± 0.30 times greater than that at S1 in the CD group and 1.21 ± 0.09 times greater in the PDR group. This ratio of the concentration at S3 to that at S1 (S3/S1) was significantly different between the groups ($Z=-2.797$, $p<0.005$) as depicted in Figure 8A. Furthermore, the concentration at S3 increased 0.30 ± 0.07 μg/dl more than that at S1 in the CD group and 0.07 ± 0.03 μg/dl in the PDR group. There was significant difference between them ($Z=-2.419$, $p<0.05$) as depicted in Figure 8B.

4. Discussion

The purpose of this study was to determine the differences in the stress level induced in dogs by CD and PDR when drying the coat, as well as determine which drying method was better for the dogs' welfare. Salivary cortisol is a particularly useful method of quantifying the stress response objectively and of identifying acute stress responses in animals noninvasively (Drobatz and Macintire, 1996; Beerda et al., 1996).

The PDR group was predicted to experience greater stress than the CD group because the subjects were unfamiliar with the PDR device. Most of the subjects were accustomed to the CD drying process, as they are routinely dried by CD twice monthly, whereas PDR was unfamiliar to them. However, the cortisol concentrations at S2 and S3 did not differ significantly between the two groups. However, the cortisol concentrations at S1 and S3 differed significantly between the groups, and the ratio between the cortisol concentrations at S3 and S1 (S3/S1) differed significantly between the groups. These results indicate that the physiological stress induced by PDR was less than that induced by CD. The drying wind used in PDR was at room temperature and was milder and calmer than the air produced by CD, which may underlie the significant difference in cortisol concentrations between the groups.

PDR could be especially useful in facilities housing a large animal population or for dogs exhibiting severe anxiety in response to the current drying methods. In addition, PDR is very convenient to use for owners and facility managers, and costs less than CD for large facilities. The present study results recommend PDR as a drying method due to its potential to reduce stress responses, making it very safe for both owners and dogs. The majority of laboratory research dogs live in extremely poor conditions (Balcombe et al., 2004). They are vulnerable to minimal stimulation and to regular care regimens such as bathing, drying, and other procedures. While these treatments and regimens are very simple and common in pet dogs, laboratory research dogs can respond severely to these procedures. Furthermore, some research dogs are unaccustomed to handling and restraint, and PDR could be a good alternative.

In summary, we surmise that PDR may induce a lower stress response than CD because it produces less heat and noise, and does not require restraint. Wide adoption of PDR in facilities housing large populations, especially laboratory research facilities and animal shelters, is expected to improve the welfare of dogs.

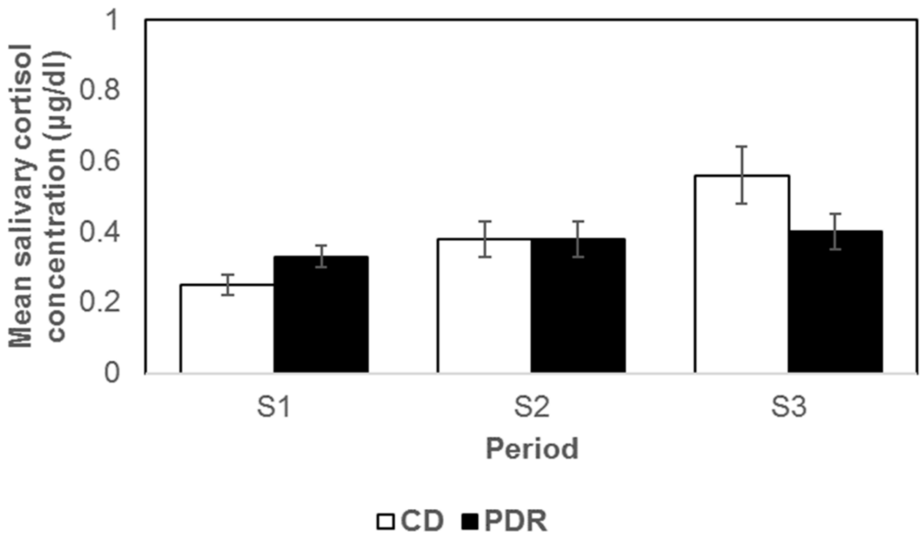

Figure 7. Variation in cortisol concentration between the two groups, CD and PDR.

Changes along periods in CD group were significantly different (p<0.005). When the values between the CD and PDR groups were analyzed statistically, there were no differences between levels at S1, S2 and S3 (p>0.05). (mean ± SE)

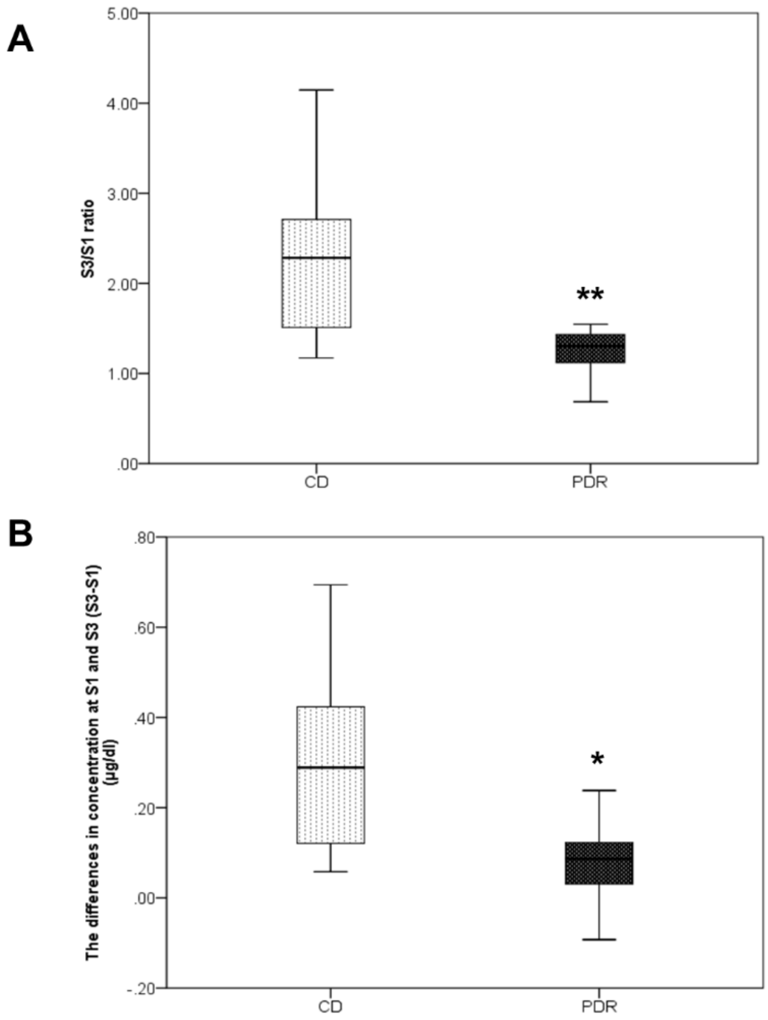

Figure 8. The ratio of the concentration of S3 to that of S1, S3/S1 and the differences in concentrations between S1 and S3 (S3 − S1) of the groups. (A) There were significant differences between groups. (B) There were significant differences between groups. * p<0.05, ** p<0.005 in comparison with CD group. (mean ± SE)

GENERAL CONCLUSION

The relationship between salivary and serum cortisol, used to measure physiological stress, has already been identified. Salivary cortisol is currently recognized as a more reliable tool than serum cortisol because its non-invasive sampling process has less physiological impact on the dogs being tested than serum cortisol testing.

Separation anxiety (SA) in dogs, especially in companion dogs, is a set of behavioral problems that is a current focus of research, and since it can cause severe stress in both the caregiver and dog, it can also lead to social issues such as abandonment and cancellation of adoption. Dogs with SA get stressed in the absence of the owners and exhibit various problematic behaviors including excessive barking, destruction, and improper urination and defecation. To deal with this problem, the stress due to SA needs to be managed by a more fundamental approach. From the fact that stress caused by separation anxiety can be lowered by the bodily scent and voice of the owner, it is expected to be an alternative that can be suggested to owners, in combination with other behavioral modification and drug therapies.

Sociality in dogs refers to their ability to adapt to various environments, and it is closely linked to success of working dogs or abandoned dogs that are re-adopted. It is also recognized as a personality trait that is developed during a specific period called the socialization period and does not change much over the dog's lifetime. It has been confirmed that dogs that have been classified as having good sociality through behavior assessment experience less physiological stress when faced with unfamiliar people or situations. This indicates that having good sociality is advantageous in forming relationships and living in human society while feeling less stress. Assessment of sociality and behavioral and physiological stresses that dogs experience under various situations can allow prediction of welfare levels of dogs and their adaptability to subsequent life.

Assessment of dry stress from pet dry room that recently became commercially available showed that pet dry room, a new tool, can be used to care for dog hair since it did not show a significant difference in stress as compared to regular dryers. Therefore, it is expected that pet dry rooms may be used safely by not only individuals, but also in various large facilities that care for dogs.

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국문초록

개에서 환경 변화에 따른 타액 코티솔 측정을 통한 생리학적 스트레스의 평가

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동물에서의 스트레스 평가는 동물의 복지 수준을 판단할 수 있는 중요한 지표 중 하나이다. 동물이 어느 정도의 스트레스를 받고 있는가는 크게 행동 반응과 생리학적인 변화, 그 외 다양한 면역 반응들을 통해 종합적으로 평가한다. 그 중 생리학적인 변화는 수치적으로 비교적 정확한 값으로 나타내어 질 수 있으므로 보다 객관적인 지표를 제공할 수 있는데, 특히 코티솔

수치를 평가하는 것은 즉각적인 스트레스 반응에 대해 신뢰할만한 지표로 널리 이용되고 있다. 그 중 샘플링으로 인한 생리학적인 변화를 적게 유발하는 비침습적인 방법이라는 점에서 타액에서의 코티솔 측정이 동물에서 스트레스를 평가할 수 있는 유용한 도구로써 연구자들 사이에서 새롭게 주목받고 있다.

반려견에서 다양한 행동 문제들이 보고되고 있다. 그 중 분리불안은 아주 흔하고 파양과 유기, 학대 등을 유발하는 대표적인 행동 문제로 약물 치료와 행동 수정 요법이 복합적으로 이루어져야 한다. 첫 번째 연구에서는 보호자의 체취와 음성을 통해 분리불안으로 인해 폭발적으로 상승하는 스트레스가 관리될 수 있는지 타액 코티솔 수치를 측정함으로써 평가하였다. 스물여덟 마리의 분리불안이 있는 것으로 판단된 반려견들을 세 개의 군(1 군: 대조군, 2 군: 보호자의 채취가 묻은 옷을 보호자와 분리된 후 놓아둔 후각 군; 3 군: 보호자의 녹음된 음성을 보호자와 분리된 후 들려준 청각 군)으로 나누어 각각 보호자와 분리되기 전(PRE, Pre-separation period), 분리된 후 4 회(SP1-4, Separation period, 5 분 간격), 보호자와 재회했을 때(POST, post-separation period)의 타액 코티솔을 측정하고 이를 통계적으로 분석하였다.

1 군에서 SP1 의 코티솔 농도는 PRE 에 비해 1.68 ± 0.27 배 증가하였고 POST 에 비해 2.99 ± 0.50 배 증가하였다. 2 군에서

SP1 에서의 코티솔 농도는 PRE 에 비해 1.17 ± 0.11 배 증가하였고 POST 에 비해 2.06 ± 0.41 배 증가하였다. 3 군에서 SP1 의 코티솔 농도는 PRE 에 비해 1.10 ± 0.18 배 증가하였고 POST 에 비해 1.62 ± 0.14 배 증가하였다. 이 두 비율은 군 별로 통계적으로 유의적인 차이를 보였다(SP1/PRE 비율에서의 $p<0.05$; SP1/POST 비율에서의 $p<0.01$).

또한 PRE 와 SP1 에서의 코티솔 농도의 차이(SP1 – PRE)와 POST 와 SP1 에서의 코티솔 농도의 차이(SP1 – POST) 역시 군 별로 유의적인 차이가 확인되었다(SP1 과 PRE 코티솔 차이에서의 $p<0.05$; SP1 과 POST 코티솔 차이에서의 $p<0.05$).

이 실험의 결과, 보호자의 체취와 음성이 분리에 의한 스트레스의 폭발적 증가를 유의적으로 줄일 수 있음이 확인되었다. 따라서, 다른 알려져 있는 치료 방법들과 더불어 보호자의 체취와 음성 역시 스트레스를 관리할 수 있는 해결책의 한 가지 방법으로 추천할 수 있을 것으로 생각되었다.

두번째 연구에서는, 개에서 이미 형성된 낮은 사회성으로 인해 낯선 사람과 환경에 대해 보다 더 큰 스트레스를 받을 수 있는지 평가하였다. 37 마리의 건강한 개(21 마리의 반려견 = C 군; 16 마리의 유기견 = S 군)가 실험에 포함되었다. 참여한 개들을 행동 평가를 통해 사회성이 높은 군(H 군)과 낮은 군(L 군)으로

나누었다. H 군에서 보호자와 함께 있을 때(P1)에서의 타액 코티솔 농도는 0.3848 ± 0.0969 μg/dl, 낯선 실험자와 상호작용 했을 때(P2) 의 타액 코티솔 농도는 0.3577 ± 0.0981 μg/dl 였다. L 군에서 P1 에서의 타액 코티솔 농도는 0.5593 ± 0.0755 μg/dl, P2 에서의 타액 코티솔 농도는 0.6527 ± 0.0781 μg/dl 로 확인되었다. P1 에 대비한 P2 에서의 타액 코티솔 증가 비율은 군 별로 유의적인 차이를 보였다(p=0.008). 마찬가지로 P1 과 P2 에서의 타액 코티솔 농도 차이는 H 군에서 −0.0272 ± 0.03 μg/dl, L 군에서 0.0933 ± 0.0371 μg/dl 를 나타냈으며 군 간 통계적으로 유의적인 차이가 확인되었다(p=0.003).

행동 평가 실험을 통해 사회성이 낮은 것으로 평가된 개들은 낯선 사람과 낯선 환경에서 상호작용할 때 사회성이 높은 개들보다 높은 타액 코티솔 수치를 보였다. 이는 낮은 사회성으로 인해 낯선 사람이나 환경에 과도한 스트레스를 받아 부적절한 행동을 하게 할 가능성이 보다 높다는 것을 암시한다. 사회성은 개 그 자체의 태생적 성격과 더불어 사회화 시기에 주요하게 형성되어 평생 잘 바뀌지 않는 특성이다. 따라서 사회화가 주로 이루어지는 어린 강아지 시기에 보호자가 관심을 가지고 다양한 사람, 환경을 긍정적으로 접하도록 관리해주는 것이 중요하다. 긍정적으로 사회화가 이루어진 개는 평생 어떤 환경에 처하더라도

그에 따른 스트레스를 비교적 수월하게 관리할 수 있으며, 그로 인한 다양한 행동 문제 역시 예방할 수 있을 가능성이 높다.

마지막으로, 사람에서의 편리성이 동물에서도 마찬가지로 스트레스를 적게 유발하여 사람과 동물 모두의 이익을 가져올 수 있는지를 평가하기 위해 최근 새롭게 출시된 펫 드라이룸(PDR, pet dry room; PDR-2000S, Izu Korea)을 사용하여 젖은 털을 말렸을 때 개들이 받는 스트레스를 평가하였다. 털을 말리는 것은 개를 관리함에 있어 필수적인 과정이나 드라이기로 털을 말리는 과정에서 발생하는 소음과 열로 인한 스트레스로 인해 관리에 어려움을 겪고 있는 경우가 많다.

10 마리의 비글견을 대상으로 각각 일반 펫 드라이기(CD, common pet dryer; APST2031, A-plus ENC)와 PDR 을 이용해 털을 말리고 타액 코티솔을 분석하는 실험을 진행하였다. CD 로 털을 말렸을 경우 코티솔 수치가 초기값에 비해 2.29 ± 0.30 배 증가하였고, PDR 을 이용해 털을 말렸을 경우 코티솔 수치가 1.21 ± 0.09 배 증가하였다. 두 군 간 이 수치는 통계적으로 유의적인 차이가 확인되었다(p<0.005). 마찬가지로 털을 말리기 전후 코티솔 농도 차이를 군 별로 비교했을 때, PDR 을 사용했을 때가 CD 를 사용하였을 때에 비해 유의적으로 적은 값을 보였다(p<0.05)

실험 결과, PDR 은 CD 와 비교했을 때 보다 낮은 수준의 스트레스를 유발하는 것으로 평가되었으며, 그 효율성과 비용적 측면을 고려했을 때 특히 대규모 사육 시설 등에서 유용하게 쓰일 수 있을 것으로 생각되었다.

위의 연구들을 통해 다양한 상황이나 환경에서 반려견이 받는 생리학적인 스트레스를 타액 코티솔을 통해 측정함으로써, 개의 복지 수준을 평가할 수 있음을 확인하였다. 이 연구를 토대로 개에서 다양한 상황에서의 보다 근본적인 스트레스 관리 방향을 제시할 수 있을 것으로 기대된다.

주요어: 개, 타액 코티솔, 생리학적 스트레스, 동물 행동, 동물 복지

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