Venipuncture-related Stress in Dogs and Evaluation of the Fear Free™ FAS Scale as a Non-Invasive Stress Measurement Tool for Dogs in the Veterinary Hospital

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Abstract

Recognizing and measuring stress quickly and non-invasively in the veterinary context is desirable for guiding interventions to improve canine health and welfare during the provision of veterinary care. The Fear Free™ Fear, Anxiety and Stress (FAS) Scale serves this purpose but is not validated. We collected behavioral and physiological measures (urine cortisol:creatinine ratios, heart rate, and heart rate variability) of stress in 40 privately-owned dogs visiting a Fear Free Certified™ veterinary hospital for venipuncture with a credentialed technician. Visits were video-recorded to code 11 observable behaviors and score stress using the FAS Scale and Clinic Dog Stress Scale 1 (CDSS). There was moderate agreement between FAS and CDSS scores (73.78%, Cohen's kappa = 0.55). While the number of intervals during which a dog experienced the venipuncture condition did not correlate with post-visit UCCR, veterinary visit duration was positively correlated with percent change in UCCR (rho = 0.34, p = 0.04) and post-visit UCCR was significantly higher than baseline UCCR (mean difference = 43.80, p = 0.0000051, 95% CI: 27.09 - 60.51). UCCR did not correlate significantly with FAS; however, max FAS scores were moderate to high for most dogs during venipuncture. Given the weak evidence for validity of the FAS Scale and moderate interobserver agreement (mean IOA = 76.04%), modifications to optimize the scale for clinical application should be considered, such as reduction of scoring to basic categorical levels (low, moderate, high), incorporation of arousal and emotional valence, focus on a single species with inclusion of visual references for common behavioral presentations of FAS, and more robust education and training in its use and application.

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Introduction

The topic of veterinary visit stress has intrigued scientists and practitioners since the mid- to late 20th century (Stanford, 1981), and in more recent years has been the subject of research aiming to assess and mitigate sources of stress in dogs visiting the veterinary hospital (Squair et al., 2024; Squair et al., 2023). Indicators of stress such as observable behaviors (Csoltova et al., 2017; Döring et al., 2009; Edwards et al., 2022), cortisol (Beerda et al., 1996; Hekman et al., 2012; Van Vonderen et al., 1998), heart rate (Bragg et al., 2015; Csoltova et al., 2017; Edwards et al., 2022), heart rate variability (Grigg et al., 2022), and other biomarkers have been used to attempt to quantify or otherwise describe the level of stress experienced by dogs in the veterinary hospital. This information is useful for veterinary professionals aiming to reduce patient stress through various interventions.

Physiological measures of stress take time and effort to obtain, and the collection of these measures may be a source of stress (Beerda et al., 1996). For this reason, observable behaviors provide the least invasive, most easily attainable information about a dog's level of stress. Several dog stress behavior scales have been developed for research and clinical use (Hekman et al., 2012; Hernander, 2008; Mercier et al., 2023; Overall, 2013; Srithunyarat et al., 2018); however, only the Fear Free™ Fear, Anxiety, and Stress (FAS) Scale (Martin & Martin, 2022) is widely used in veterinary medicine, and it has yet to be validated. A single, easy-to-use, validated dog stress scale for veterinary professionals would be incredibly valuable to help practitioners quickly identify when it is appropriate to implement stress-reducing interventions during the veterinary visit. Reduction of stress in canine patients has implications for canine welfare (Mellor et al., 2020) as well as the health, safety, and welfare of the people who care for them (Everett et al., 2025; Garcia-Sanchez et al., 2024; Volk et al., 2011).

Stress-Mediating Systems

The stress response is mediated by two systems: the sympatho-adrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis. The SAM axis is associated with the fight-or-flight response, which is characterized by an instant hormonal response to a stressful stimulus that primes the animal for action. Epinephrine/adrenaline and norepinephrine/noradrenaline are released to adapt the body's processes, resulting in several changes which include higher arousal, breakdown of glycogen to glucose for energy, and peripheral vasoconstriction. The HPA axis sends the glucocorticoids, or steroid hormones, cortisol and corticosterone into action, but this stress response occurs over several minutes and reaches peak effect 20 minutes or more after identification of a threat. Glucocorticoids produce several effects within the body as well, notably changing behavior, enhancing the formation and consolidation of memories, increasing glucose in the blood, and inhibiting immune system function (Romero & Butler, 2007).

Veterinary-Related Stress

Research shows the hospital environment alone can cause significant elevations in vital signs such as heart rate, respiratory rate, blood pressure, and rectal temperature (Bragg et al., 2015) and elicit observable fear and stress-related behaviors in many, if not a majority of, canine patients (Döring et al., 2009; Hernander, 2008; Mariti et al., 2015; Stanford, 1981). These changes not only impact the veterinarian's ability to accurately assess the physical health of the animal, but also present concern for overall canine health and wellbeing (Mellor et al., 2020).

While aspects of the veterinary visit such as physical examination (Csoltova et al., 2017; Döring et al., 2009; Edwards et al., 2022; Jokela et al., 2023; Lind et al., 2017) or handling and restraint (Grigg et al., 2022; Scalia et al., 2017; Squair et al., 2024; Squair et al., 2023) might be stressful, venipuncture may be one of the most stressful procedures commonly performed during a visit. Venipuncture is performed in both well and sick veterinary patients and involves the insertion of a needle into a vein for the collection of blood samples or injection of therapeutic agents. Because it often involves physical restraint and some level of pain or discomfort, venipuncture has the potential to elicit an acute stress response (Knol et al., 1992).

The stress of more invasive veterinary procedures such as venipuncture remains largely unstudied, especially in pet dogs. In humans, research in women showed both psychological and physiological stress responses when blood was collected via finger prick and venipuncture from the median cubital vein, suggesting possible significance of both anatomical sampling location and method of collection (Lorenz, 2021). Furthermore, research suggests that familiarity with venipuncture may be a key factor influencing the degree of physiological stress response. Research on laboratory beagles has shown nonsignificant changes in parameters such as cortisol in dogs already acclimated to routine venipuncture (Knol et al., 1992) and stabilization of hormones such as epinephrine in dogs after a 4-week acclimation period to the procedure (Slaughter et al., 2002). Similarly, laboratory beagles and privately-owned blood donor dogs did not differ significantly in physiological variables or in stress scores assigned during blood sampling procedures (Srithunyarat et al., 2017). Most pet dogs, however, do not experience acclimation to venipuncture, and thus are more likely to experience an acute stress response when subjected to the procedure (Srithunyarat et al., 2018). These studies provide evidence to support the claim that venipuncture is likely stressful for many pet dogs.

Measuring Stress in Dogs

Since veterinary visits have been established to be inherently stressful for many dogs, reliable measures of stress are desirable. There are two primary methods for assessing stress in dogs: physiological measures and behavioral measures. Acquiring physiological measures of stress is invasive by nature, so the use of less invasive methods is favorable to avoid further exacerbating stress during sample collection or measurement. Behavioral measures, on the other hand, are completely non-invasive, requiring no physical contact to obtain. Often, both physiological and behavioral measures are collected for comparison, as is done in the present study. Some of the less-invasive physiological measures of stress include cortisol, heart rate, and heart rate variability.

Cortisol

Cortisol is a favored biomarker for evaluating stress in dogs and other animals. The advantages of measuring urinary cortisol levels include low daily variation (Kolevská et al., 2003); flexible window of sample acquisition for peak cortisol and metabolite concentrations, which occur in dogs within 2-4 hours of HPA axis stimulation (Schatz & Palme, 2001); and minimally invasive sampling technique when collecting naturally voided urine. Urinary cortisol is typically measured alongside urinary creatinine and reported as a ratio of cortisol:creatinine (UCCR) to account for the effect of urine concentration on urinary cortisol levels (Llera et al., 2024). The validity of UCCR as a physiological measure of stress has been repeatedly established (Beerda et al., 1996; Beerda et al., 2000; Part et al., 2014; Rooney et al., 2007) and shows the HPA axis activity over the preceding 4-8 hours (Casey, 2006).

Despite its advantages, cortisol does present several challenges for interpretation. Cortisol is often inconsistent from one individual to the next (Palme et al., 2005) and within an individual when measured at different points in time (Clark et al., 1997). Disease processes affecting the HPA axis such as hyperadrenocorticism, or Cushing’s disease, and hypoadrenocorticism, or Addison’s disease, directly impact cortisol levels (Vertloo, 2024, July-a, 2024, July-b). To further complicate interpretation of cortisol levels, it should be noted that increased cortisol concentrations cannot be solely attributed to negative stress, or distress. For example, exercise may increase or decrease cortisol levels. Sled dogs subjected to highly strenuous exercise had significantly different urinary cortisol and UCCR values when compared with a control group that did not exercise (Durocher et al., 2007). In contrast, other studies found no significant difference in cortisol levels of rested versus lightly exercised dogs (Clark et al., 1997; Eiermann et al., 2025). In fact, exercise with and without social play has been associated with decreasing cortisol levels in dogs (Clark et al., 1997; King et al., 2010). This contrast in findings suggests that significant increases in cortisol levels related to exercise may not be seen in the average pet dog but cannot be ruled out. Because of the possible confounding variables affecting cortisol interpretation at the individual and group level, other parameters continue to be explored, including heart rate and heart rate variability.

Heart Rate and Heart Rate Variability

Heart rate (HR) is measured as the number of heart beats per minute (bpm), while heart rate variability (HRV) is a term that encompasses a variety of analyses applied to the measurement of the time intervals between heart beats (Shaffer & Ginsberg, 2017). Heart rate variability measures provide information about the sympathetic and parasympathetic response expressed by the autonomic nervous system, as well as other physiological changes related to blood pressure and respiration (Fernandes & Seara, 2021; Shaffer & Ginsberg, 2017). High HRV can be indicative of physiological disease, but in otherwise healthy individuals, a higher HRV serves as a metric suggestive of resilience and coping ability (Shaffer & Ginsberg, 2017). Because HR may be associated with positive or negative affective states and indicates a general state of arousal (Beerda et al., 1998), assessment of HRV may be a better indicator of psychological stress as it reflects more specific autonomic nervous system activity and neurocardiac function (Boissy et al., 2007; Shaffer & Ginsberg, 2017). A small number of studies have attempted to correlate HRV and affective state, but their study designs vary and their results have contradicted one another (Katayama et al., 2016; Maros et al., 2008; Zupan et al., 2016). Most recently, Flint and colleagues (2024) noted a significantly higher HRV in dogs exposed to a scenario designed to inflict negative emotion in a low arousal state, but not in a high arousal state, so arousal level may impact interpretation of this metric and be responsible for contradictions in earlier studies.

An exhaustive discussion of all possible calculations of HRV is beyond the scope of this paper; we provide a concise review of relevant time-domain calculations and refer the reader to Shaffer and Ginsberg (2017) for a comprehensive review. Within the time-domain method of analysis, standard deviation of consecutive R-R intervals for which artifacts have been removed (SDNN) reflects sympathetic and parasympathetic activity within the measured time period, and the root mean square of successive R-R interval differences (RMSSD) represents a standard deviation between heart beats which correlates with parasympathetic activity (Fernandes & Seara, 2021; Shaffer & Ginsberg, 2017). Time-domain data should only be compared with data from similar time scales and within individuals as no reference interval for normal values currently exists for dogs, and longer time intervals result in higher HRV values (Fernandes & Seara, 2021; Shaffer & Ginsberg, 2017). The standard minimum measurement time for short-term HRV analysis is 5 minutes (Shaffer & Ginsberg, 2017).

Measurement of HR and HRV can be performed relatively non-invasively using chest-strap style human heart rate monitors. Polar® human heart rate monitors have some evidence of validity for use in dogs that are stationary (Jonckheer-Sheehy et al., 2012) or in motion (Essner et al., 2013). Because no small dogs were included in the study and the maximum heart rate achieved was 180 bpm, predictions of reliability in small dogs and dogs with heart rates higher than 180 bpm are limited (Essner et al., 2013). Polar® heart rate monitors are available for purchase through many public sources and software to capture the data is freely available, thus these devices are accessible and useful for capturing HR and HRV data in dogs in both the veterinary and home setting.

Behavioral Measures of Stress in Dogs

Along with physiological measures, behavioral observations are often used to assess stress levels in dogs. This is particularly important because while physiological measures may normalize, behavioral indicators may remain. For example, the behavioral observations of general restlessness and one dog's "freeze" response improved but did not resolve over the course of one study, though the dog's physiological measures improved (Slaughter et al., 2002). Additionally, behavioral measures are non-invasive and thus quite favorable in terms of overall stress reduction during sampling.

An understanding of canine behavior is fundamental for those working with dogs. Identification of individual dog behaviors and behavioral displays are documented at least as early as 1872 by Charles Darwin (Simpson, 1997) and continued research in this area has helped identify specific behaviors and postures that suggest a dog is experiencing fear, anxiety, or stress. Table 1 lists the behaviors of interest for the present study alongside supporting literature.

Table 1

Fear, Anxiety, and Stress Behaviors in the Literature

Behavior	FAS Scale Score and Description	Source(s)
Yawn	Not mentioned	Beerda et al. (1998) Rooney et al. (2007)
Lip lick	1	Beerda et al. (1997) Beerda et al. (1998) Schilder and van der Borg (2004) Rooney et al. (2007) Hekman et al. (2012) Rooney et al. (2009)
Paw lift	1	Beerda et al. (1997) Schilder and van der Borg (2004) Rooney et al. (2007) Rooney et al. (2009)
Eat	1 2 3 (roughly, may refuse) 4 (may or may not)	Luño et al. (2018), emotional eating in dogs
Pant	1 (relaxed) 2 (tighter mouth) 3 (increased frequency) 4 (excessive)	Beerda et al. (1997) Rooney et al. (2007) Hekman et al. (2012)
Ears back	2 (slightly back or to side) 3 (increased frequency) 4 (back)	Schilder and van der Borg (2004), lowered
Tail low	2 (down) 3 4 (tucked)	Beerda et al. (1997), lowered Schilder and van der Borg (2004), lowered Jokela et al. (2023), tucked
Avoidance	4 (escape, resist restraint, move away)	Solomon and Wynne (1953), escape Schilder and van der Borg (2004) Dreschel and Granger (2005), hiding Rooney et al. (2009), hiding
Tremble	4	Dreschel and Granger (2005) Rooney et al. (2009)

Behavior	FAS Scale Score and Description	Source(s)
		Jokela et al. (2023)
Vocalize	5 (bark, growl)	Solomon and Wynne (1953), yelp Beerda et al. (1997) Schilder and van der Borg (2004), yelp, squeal, bark Dreschel and Granger (2005), whine Rooney et al. (2007), howl, whine, bark Rooney et al. (2009)
Aggression	5 (lunge, snarl, snap)	Schilder and van der Borg (2004) Rooney et al. (2009)

Of course, the context in which behavior is performed is relevant for evaluation (Beerda et al., 1998). The implications of context are two-fold: external environmental factors may influence behavior, and they may be relevant for the interpreter to consider when decoding the behavioral message. Some external environmental factors influencing dog behavior include presence of the owner (Gácsi et al., 2013; Palestrini et al., 2005) or other familiar person (Norling, 2012), a stranger or threat (Beerda et al., 1998; Gácsi et al., 2013), and the physical location itself. Most, if not all, of these factors exist within the veterinary hospital, complicating interpretation of behavior. Considering the unique combination of effects of the veterinary environment and unfamiliar people and procedures on canine behavior, as well as the potential impact on canine health and welfare, there is a need for a veterinary-specific behavioral stress scale for measuring stress in this context.

Development and Validation of Dog Stress Scales

Physiological measures of stress require specialized equipment and, in some cases, laboratory testing, making utilization of these measures during the visit to the veterinary hospital impractical. Given the time delay in determination and interpretation of results of physiological measures of stress, observable behaviors are generally accepted as a more immediate measure of stress in dogs (Polgár et al., 2019). For this reason, several stress assessment scales and scoring systems have been developed for use in research and clinical settings. We will focus our review on those designed for use in the veterinary context.

In 2009, Dr. Marty Becker began developing Fear Free™ to advocate for the emotional welfare of veterinary patients through practitioner recognition and attentiveness to behavioral signs of fear, anxiety, and stress (Dale, 2019). The Fear, Anxiety and Stress (FAS) Scale (Figure 1) was developed by Debbie Martin, LVT, VTS (Behavior) and Kenneth Martin, DVM, DACVB as a key component of the Fear Free™ Veterinary Professional Certification Program, which was launched in the United States in 2016 (Martin & Martin, 2022). It is a tool for assessment and scoring of canine and feline patient stress based on the number, frequency, duration, and magnitude of some commonly recognized stress-related behaviors. The FAS Scale allows veterinary professionals to relatively objectively assess the level of patient stress in a manner similar to that of a widely used acute pain scale (Hellyer et al., 2006).

Figure 1

Fear Free™ FAS Scale

Several other previously developed scales have been used in research and clinical settings. Overall (2013) adapted behavioral stress assessment tools from other sources (Döring et al., 2009; Hellyer et al., 2007; Hernander, 2008) to create six Clinic Dog Stress Scales (CDSS), which are intended for use by veterinary teams and dog owners across different aspects of the veterinary visit and veterinary procedures. Clinic Dog Stress Scale 1 scores general demeanor on a scale of 0-5 similar to the FAS Scale (Overall, 2013). Another stress scale is a version of the visual analog scale (VAS) originally created by Norling (2012) which was modified and used by

Srithunyarat et al. (2018). The purpose of the VAS is to score stress behaviors as mild, moderate, or severe during blood and saliva collection from dogs, with an emphasis on avoidance behaviors (Srithunyarat et al., 2018). Examples include mild stress behaviors such as moving away and lifting the paw, moderate behaviors intensifying to growling and raising hackles, and severe behaviors further escalating to overt aggression and inability to handle (Srithunyarat et al., 2018). Lastly, the stress research tool (SRT) is a scale that was developed based on the results of an experiment which identified three behaviors most correlated with salivary cortisol: head resting, panting, and lip licking (Hekman et al., 2012).

The existing behavior-based dog stress scales vary in their ease-of-use and application in the veterinary context. Overall's (2013) multiple scales require tallying of scores across the visit to determine an overall score at the end of the visit, allowing for observation of behavior change over time. The VAS and SRT focus on a few specific behaviors and so do not encompass the individual variations in presentation of stress behaviors (Hekman et al., 2012; Srithunyarat et al., 2018). A single validated and practical dog stress scale for use in the veterinary hospital, as the Fear Free™ FAS Scale is intended to be, is needed.

Though the Fear Free™ FAS Scale is the first assessment tool of its kind to be widely used in veterinary medicine, it has yet to be validated. Validity is the assessment of a measure's representation of a variable (Jhangiani et al., 2019). There are several types of validity; however, for our purposes we will focus on criterion validity, which uses the relationship between an established measure of a variable, or criterion, and a proposed measure of the variable, or construct, to determine validity of the proposed measure (Jhangiani et al., 2019; McLeod, 2023). Three types of criterion validity are of interest in the present study: concurrent validity, where construct and criterion are measured simultaneously; predictive validity, where construct is measured first and criterion is measured later; and convergent validity, where an existing validated measure serves as the criterion to correlate with the construct (Jhangiani et al., 2019).

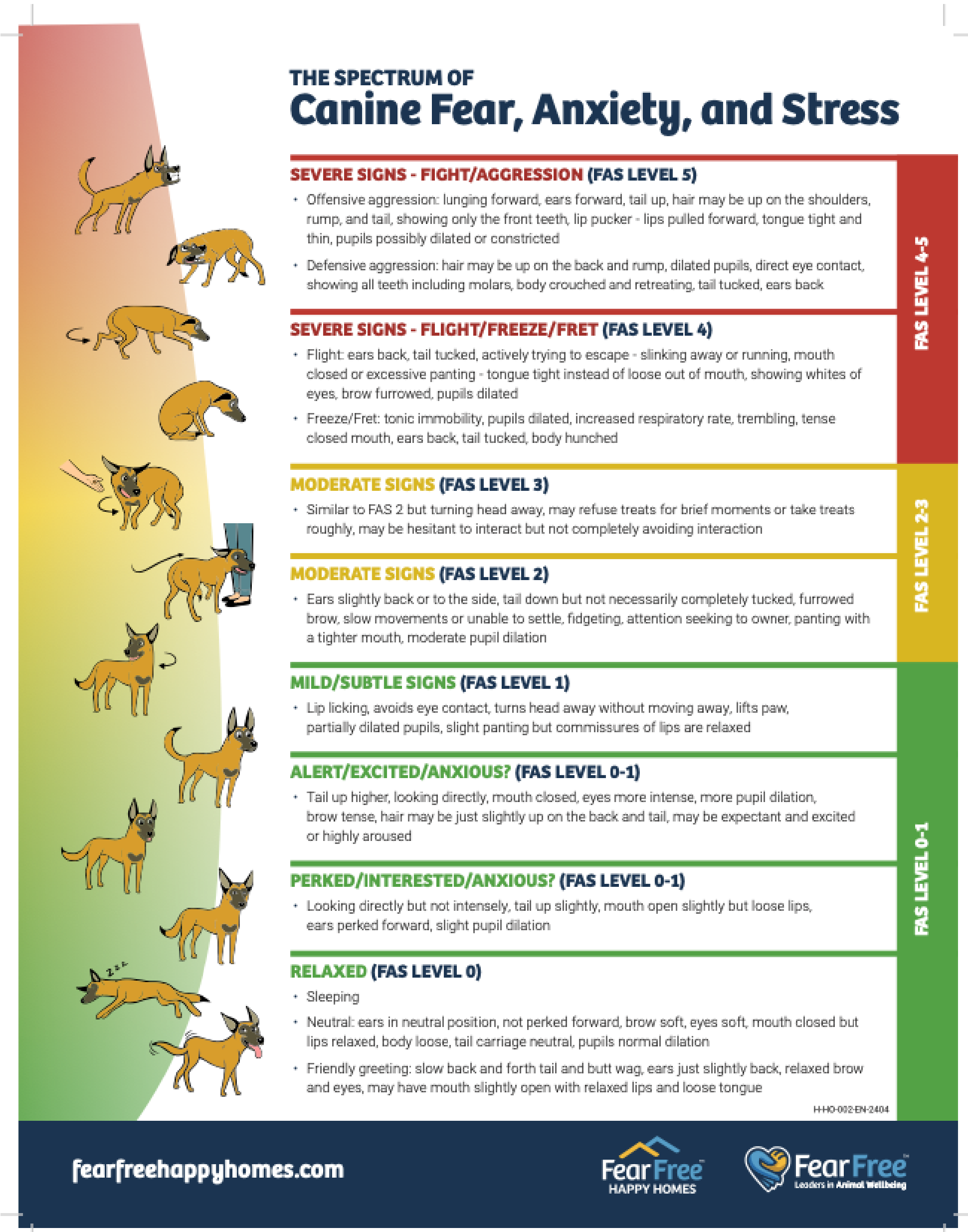
Of the scales mentioned, only the VAS has evidence of criterion validity. Overall's (2013) CDSS 1 has been compared against urinary cortisol to assess validity; however, the study has three significant limitations. The study sample size was limited to six dogs, urinary cortisol without creatinine was used as the physiological measure of stress, and no statistical analyses were reported (Țurcanu & Papuc, 2016). Another study using two subjective Likert scales adapted from Overall's (2013) CDSS did not collect other measures of stress, so criterion validity cannot be confirmed and overall validity of the scale is limited to face validity (Mercier et al., 2023). For the SRT, researchers noted positive but nonsignificant correlation between salivary cortisol and both panting and lip licking (Hekman et al., 2012). The VAS has evidence of validity using salivary catestatin as criterion to measure physiological stress response against behavioral stress response during saliva and blood sampling in a group of dogs unfamiliar with the veterinary hospital and venipuncture procedure (Srithunyarat et al., 2018). Salivary catestatin and VAS score for saliva sampling were significantly positively correlated (Srithunyarat et al., 2018).

At this time, no published studies have utilized or evaluated the Fear Free™ FAS Scale in its original version for reliability or validity; it has only been used as a reference in the creation of new stress scales for use in clinical research (Mandese et al., 2021; Nixon et al., 2025). Another Fear Free™ FAS resource, the Spectrum of Canine Fear, Anxiety, and Stress (Figure 2), was adapted from the FAS Scale and quite recently achieved some validity compared against the previously-validated Lincoln Canine Anxiety Scale (LCAS) (Gatehouse et al., 2025).

Validation of the Fear Free™ FAS Scale is needed to support continued use of the scale as a measure of acute canine stress in the veterinary hospital.

Figure 2

Fear Free Handout: The Spectrum of Canine Fear, Anxiety, and Stress



Purpose

The purpose of the present study was to investigate the physiological effects of a potentially stressful veterinary visit on dogs and to assess the validity of the Fear Free™ FAS Scale as a tool for efficient, non-invasive stress measurement in dogs in the veterinary hospital setting. Three physiological measures of stress were selected as variables to assess the physiological response and serve as criteria for concurrent and predictive validity of the FAS Scale: heart rate (HR) and heart rate variability (HRV), both of which are variables representative of the stress response by the SAM axis, and urine cortisol:creatinine ratio (UCCR), which is representative of the HPA axis response. Forty dogs and their owners were recruited to follow a three-day protocol of urine sample collection plus a veterinary visit on Day 2 for a blood draw. Veterinary visits were video-recorded to code observable behaviors and score stress using the FAS Scale and Clinic Dog Stress Scale 1. We hypothesized that 1) UCCR would increase following a veterinary visit where venipuncture was performed, 2) FAS scores would agree with CDSS scores, 3) FAS scores would positively correlate with HR and change in UCCR, and 4) FAS scores would negatively correlate with HRV.

Method

Participants

Dog-owning clients of Clarendon Animal Care in Arlington, Virginia were given the opportunity to volunteer for this study if their dog(s) met the following criteria: healthy with no significant acute or chronic illness, owned for more than 3 months, aged between 2-10 years, not on any medication (other than heartworm, flea, and tick prevention, NSAIDs, or Apoquel), and sedation not required for venipuncture. We recruited participants through hospital media including monthly newsletters, flyers posted within client-facing spaces in the hospital, social media, and direct emails to owners with dogs seemingly meeting these criteria based on a targeted search of the hospital's patient database. A financial incentive of $20 off the cost of routine heartworm testing was offered to encourage enrollment.

Setting

Sampling occurred both in the subject's home environment and within one of four similar examination rooms at the Clarendon Animal Care South Arlington location. Clarendon Animal Care is a Fear Free Certified™ Veterinary Practice, so venipuncture procedures followed Fear Free™ protocols to prevent and mitigate fear, anxiety, and stress as much as possible. Dogs remained in exam rooms with their owners for the duration of the visit. We encouraged owner involvement in the procedure by aiding in gentle restraint and/or comforting and distracting by petting, speaking to, and feeding their dog. Venipuncture was always performed by the researcher (AC) on a non-slip surface on the floor or exam table, depending on patient size and comfort. We used canine-appeasing pheromone (Zenidog® Gel Diffuser, Virbac; ThunderEase® Calming Diffuser for Dogs, ThunderWorks®, Ceva Animal Health LLC) and calming music to help create a Fear Free™ environment.

Data Collection

Coordination and Preparation

Data collection occurred between October 2023 – December 2024. Communication with owners occurred primarily through email and phone. We scheduled participants for a technician appointment to complete venipuncture with the researcher on a Wednesday of their choosing between 0745h-0945h. These appointments were strictly limited to the collection of blood for routine lab testing; no other services were rendered during these appointments. Coordination of urine sampling supply pickup or delivery was completed at the time of appointment scheduling.

Owners were given the option to pick up supplies from the hospital or have them delivered to their home. The instructions provided to owners included directions for collection, labeling, and storage of urine, as well as an outline of the technician appointment procedure, and a handout explaining how to provide Fear Free™ travel to the hospital. Coordination of retrieval of samples from owners varied; some owners chose to return samples to the hospital, others opted to have the researcher pick up the samples from their home.

Urine Sampling

We collected urine samples to non-invasively measure HPA axis activity prior to and following venipuncture. Urine sampling occurred for each dog across three consecutive days: Tuesday (Day 1), Wednesday (Day 2), and Thursday (Day 3). On Day 1, owners collected their dog's first morning urine sample. On Day 2, owners collected their dog's first morning urine sample, completed the veterinary technician appointment, and collected a post-visit urine sample approximately 6-8 hours after the appointment. On Day 3, owners collected their dog's first morning urine sample to complete the urine sampling process. Urine samples were transferred to test tubes, labeled, and stored frozen until submission to the lab for testing.

Heart Rate/Heart Rate Variability Sampling

Heart rate and heart rate variability data were captured to measure SAM axis activity during the veterinary visit using a Polar® H10 heart rate monitor connected to the researcher's Apple iPhone 13 Pro via Bluetooth using the HRV Logger app (Developer A.S.M.A. B.V., © Marco Altini 2013, versions 5.1.1 and 5.1.2). The features computation window was set to 30 seconds to match the intervals used for video scoring. We followed the app developer's recommendation to discard RR-intervals which differed from the previous interval by more than 25 percent.

Veterinary Visit Procedure

The general technician appointment procedure intentionally closely mimicked the typical flow of a veterinary appointment. Owners sent a text message to the researcher upon arrival to the hospital and waited to enter the building until signaled by the researcher to do so. The researcher escorted owners and dogs to the scale in the lobby to obtain the dog's weight, led them into an exam room, briefly outlined the plan for the visit, and exited the exam room. Video recording began as dogs entered the exam room. Dogs underwent a 5-minute acclimation period in the room beginning after the researcher exited the exam room to wait nearby in the treatment area. During acclimation, owners were encouraged to drop their dog's leash and allow them to freely explore the room while the owner did whatever they would normally do while waiting for the veterinary team to begin the appointment.

After the 5-minute acclimation period elapsed, the researcher re-entered the room. Next, the researcher fit the dog with a Polar® H10 human heart rate monitor (HRM) to capture heart rate and heart rate variability. To encourage the dog to accept application of the HRM and promote a sense of calm, we pre-treated it with dog-appeasing pheromone (ThunderEase® Dog Calming Spray, powered by Adaptil®). Contact between the HRM and the skin was achieved using ultrasound gel and water; no fur was shaved. For some dogs whose chests were smaller than the tightest setting allowable, we secured any slack in the strap using a clothespin-style clip over the back. In the smallest dogs, the HRM was double wrapped around the chest.

Once the HRM was secured, the researcher used a cotton ball moistened with diluted 2% chlorhexidine solution to apply topical lidocaine 2.5%/prilocaine 2.5% cream to possible venipuncture sites. These varied between dogs, dependent on the dog's comfort with handling of different areas of the body as well as vessel prominence. Possible venipuncture sites included jugular, cephalic, and lateral saphenous veins. The researcher asked general questions about the dog's recent stress level and health, verified the owner was able to collect the first two urine samples, and explained the plan for accomplishing venipuncture.

We used the least amount of restraint possible, ranging from simple food distraction with owner petting or gently holding the dog to additional assistance provided by experienced Fear Free Certified™ veterinary assistants, licensed veterinary technicians, and veterinarians employed by Clarendon Animal Care. We used a fresh needle for every venipuncture attempt. If at any time the dog's FAS increased to a score of four or higher and remained elevated despite interventions, the visit was discontinued. Some dogs required multiple attempts to successfully complete blood collection due to difficulty accessing the vein, equipment failure, or FAS score, and in two cases was unsuccessful altogether due to sustained high FAS.

Finally, owners completed payment in the exam room, and we removed the HRM. Owners departed and were instructed to attempt to delay urination for 6-8 hours, reduce exposure to any potential aversive stimuli, and keep their dog calm and rested to limit variables affecting cortisol secretion. All instruments and surfaces in the exam rooms were cleaned with Rescue® activated hydrogen peroxide cleaner between patients, and the HRM was re-sprayed with dog-appeasing pheromone (ThunderEase® Dog Calming Spray, powered by Adaptil®).

Behavior Coding

The researcher and three observers reviewed audio/video recordings of the veterinary visits, beginning when the dog and owner enter the exam room and ending when the dog and owner exit the exam room. Volunteer observers were 1) an Elite Fear Free Certified™ licensed veterinary technician and Online Master of Agricultural and Life Sciences student with similar experience, education, and training (AB); 2) a Level 2 Fear Free Certified ™ licensed veterinary technician with a special interest in behavior (SH); and 3) an experienced veterinary assistant who has been Elite Fear Free Certified™ for over 3 years (LB). Starting at time point 00:30 and continuing at 30 second intervals for the duration of the video, each observer independently recorded the maximum FAS and CDSS scores reached during the previous 30 seconds. They also completed partial interval recording for the occurrence of behaviors listed and defined in Table 2. If a dog could not be observed for at least 10 seconds of the interval, a “U” was recorded to indicate inability to score.

The researcher noted when an interval met the criteria for one of five conditions. If an interval met criteria for more than one condition, the condition that covered the greater duration of the interval was recorded. Conditions include acclimation (ACC), owner and dog alone in exam room prior to beginning the appointment; heart rate monitor and lidocaine/prilocaine cream application (HL), researcher fitting the HRM on the dog and applying lidocaine/prilocaine cream to possible venipuncture sites; pre-venipuncture (PRE-V), researcher, client, and dog in room prior to venipuncture but not interacting except for petting; venipuncture (VENI), performing venipuncture from beginning to touch the limb where blood would be collected to application of a light bandage after venipuncture; and post-venipuncture (POST-V), researcher, client, and dog in room after venipuncture completed but not interacting except for petting. Intervals that did not meet criteria for any of the conditions were recorded as no condition (NC), such as the time between multiple venipuncture attempts, or time when researcher left the room to seek assistance with restraint.

Table 2

Ethogram

Behavior	Description
Lip lick	Licking the outside of the dog's own mouth when not eating or drinking
Yawn	Opening the mouth to its greatest extent for a single breath
Paw lift	Dog lifts own paw off the surface the dog is standing or sitting upon, may be tightly flexed or loosely hanging, purpose of behavior is not locomotion or movement
Ears back	Ears move behind midline to any degree for any amount of time
Eat	Consuming a food item
Tail low	Tail is held down below the individual's baseline or tucked under the body
Avoidance	Leans or moves any portion of or the entire body away from the veterinary professional when approached or vet professional moves toward dog; struggles against or resists restraint
Pant	Breathing with the mouth open
Tremble	Body is visibly shivering, not to include intentional "shake off" behavior
Vocalize	Growl, bark, whine, whimper, yelp
Aggress	Lunge, bare teeth or lift lips to show the teeth, snarl, snap or bite air, bite to human

Data Preparation

Participant Exclusion

The FAS was deemed too high by the researcher for dog 30 to attempt application of the HRM and lidocaine/prilocaine cream so the appointment was stopped. This determination was made based on the dog's consistent evasion of approaching veterinary staff, panting with a tight mouth, refusal of food or treats, and attempts to hide (FAS score = 4). Additionally, the veterinary visit procedure was preceded by a >20-minute wait in the lobby due to a late appointment arrival, straying significantly from the research protocol. All physiological and behavioral data from dog 30 is excluded from analysis, but stress scores are included only for evaluation of convergent validity between the FAS and CDSS scales. Data from the remaining 39 dogs is included in study analyses.

Stress Scale Scores

Stress scale scores were converted to their equivalent color score (0-1 = low, 2-3 = moderate, 4-5 = high). We calculated the maximum and mode FAS and CDSS scores for each dog to assess whether the degree or frequency of stress level experienced during the veterinary visit might have a stronger correlation with physiological measures of stress. All recorded visits were scored by the researcher. For interobserver agreement, 11 videos were randomly selected for scoring by one of three observers.

Behavioral Data

We used a partial interval recording method to code 11 observable behaviors. We calculated the frequency of occurrence of behaviors for each dog by counting the number of intervals during which the dog performed the behavior at least once and divided that number by the total number of intervals observed for that dog. Behavioral results are presented as a proportion of intervals in which the behavior was performed.

Heart Rate and Heart Rate Variability Measures

Heart rate and heart rate variability data were analyzed with Kubios HRV Scientific Lite version 4.1.2. We removed the first and final 2 minutes of each recording to account for any potential invalid data recorded as the HRM was being applied or removed. Recordings less than 5 minutes in duration were excluded. Recordings with a minimum 5-minute duration were further pre-processed. We applied automatic beat correction at the software's "Threshold (medium)" setting. Dogs with data determined to be low quality (>5% of beats corrected) by the software were excluded from analysis. Data from 7 dogs met criteria for analysis.

Urine Cortisol:Creatinine Ratios

We submitted urine samples to the Smithsonian Conservation Biology Institute Wildlife Endocrinology Laboratory for analysis. Urine samples were kept frozen at -20°C until time of sample preparation, then thawed and diluted with EIA assay buffer to 1:50 for creatinine determination using the Jaffe method modified for a 96 well microplate. Some highly concentrated samples required dilution to 1:20 or 1:30. For cortisol, urine samples were diluted 1:200 to be run on the Cortisol EIA (R4866). Urine cortisol:creatinine ratios (UCCR) were calculated and reported for each sample, expressed as cortisol ng/mg creatinine.

We calculated a baseline UCCR for each dog by taking the mean of urine samples 1 and 2. Sample 1 was collected as the first morning urine on Day 1 between 0527h-1100h. Sample 2 was collected as the first morning urine on Day 2 between 0514h-0900h. Sample 1 was discarded for dogs 4 and 6 due to an inexplicably and unusually high value (>900), and for dog 12 due to an error in sampling time (owner collected second urine of the day in the afternoon). For these three dogs, the UCCR for Sample 2 served as the baseline value. Sample collection time for sample 2 from dog 36 could not be confirmed, so the UCCR for sample 1 was used for baseline.

Sample 3 (post-visit) served as a measure of stress experienced during the veterinary visit, collected on Day 2 as the first urine following the veterinary visit. Samples were collected between 1240h-2000h, ranging 3.42h-10.75h after the visit. Sample 4 (recovery) served as a potential measure of residual HPA axis activity, collected on Day 3 at first morning urination between 0258h-1020h.

Statistical Handling of Data

Statistical analyses were performed using Microsoft Excel for Mac (Version 16.100.3) and R Studio (Version 2025.09.0+387). We ran Shapiro-Wilk tests and visually inspected histograms and scatter plots to assess the normality of the dataset. Because most data were not normally distributed and for continuity of comparison with similar studies, we opted for nonparametric testing for much of the data. All correlational relationships are described as weak (0-0.3), moderate (0.31-0.7), or strong (0.71-1) (Gatehouse et al., 2025). A p-value of <0.05 is considered statistically significant, though preference is given to confidence intervals where possible. Interval-by-interval interobserver agreement (IOA) and Cohen's kappa are reported for stress scores and behavior coding for transparency; however, percent agreement is likely the more practical value to interpret for our data. Cohen's kappa is interpreted as excellent (0.81-1.00), substantial (0.61-0.80), moderate (0.41-0.60), fair (0.21-0.40), slight (0.00-0.20), or poor (<0.00) (Landis & Koch, 1977). Cohen's kappa is weighted for stress scores and unweighted for behavioral data.

Ethical Note

All procedures involving animals were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (VT IACUC protocol #23-165). Participant recruitment was facilitated by a small financial incentive afforded in part by the donation of SNAP 4Dx+ tests by IDEXX Laboratories. This research was funded in part by a grant from Fear Free LLC. The primary researcher provided contracted services between December 2021 – January 2025 and is currently employed full-time by Fear Free LLC. All research procedures and data analyses were performed independently of Fear Free LLC; neither the company nor any of its representatives contributed to the study design, data collection, analysis, preparation or interpretation of results.

Results

Participant and Veterinary Visit Summary Data

A total of 40 dogs of varying breeds and sizes participated in this study. Median dog age was 4.40 years (Range 2.25 – 9.67 years). Median dog weight was 24.66 kilograms (Range 2.91 – 64.91 kg). Almost all dogs (95%) were reproductively altered with 37.5% (n=15) of the total sample represented by spayed females, 57.5% (n=23) neutered males, and 5% (n=2) intact males. The signalment of each dog is presented in Table 3.

Table 3

Participant Dog Signalment

ID	Sex	Age	Breed	Weight (kg)
01	FS	2y7m	Lab Mix	26.64
02	MN	5y10m	Pit Bull Mix	23.27
03	MN	4y	Spinone Italiano	37.36
04	FS	5y7m	Toy Poodle	2.91
05	MN	2y3m	Shih Tzu Mix	4.68
06	MN	2y4m	Pembroke Welsh Corgi	18.09
07	MN	3y2m	Cavalier King Charles Spaniel	9.77
08	FS	5y5m	Pit Bull Mix	21.91
09	MN	3y4m	German Shepherd	40.09
10	MN	3y4m	Shepherd Mix	32.73
11	FS	8y	Boxer Mix	25.09
12	MN	3y2m	Labrador Retriever	31.82
13	MN	2y9m	Shetland Sheepdog	10.45
14	MN	6y11m	American Foxhound	33.91

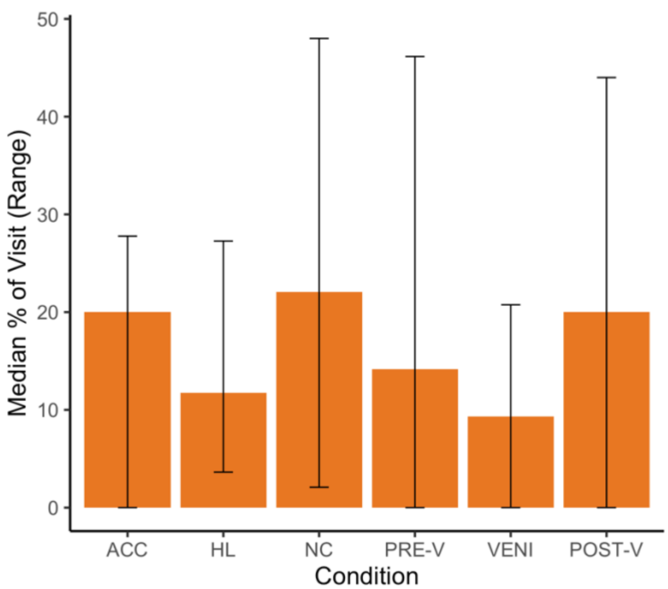
ID	Sex	Age	Breed	Weight (kg)
15	FS	5y5m	Shih Tzu Mix	5.00
16	FS	5y5m	Hound Mix	29.59
17	MN	2y9m	Shepherd Mix	23.27
18	FS	3y3m	Mixed	24.23
19	MN	7y6m	Lab Mix	30.27
20	FS	4y2m	Goldendoodle	20.77
21	MN	5y10m	Lab Mix	27.50
22	FS	4y6m	Bloodhound	47.36
23	MN	6y1m	Rhodesian Ridgeback	45.73
24	MN	5y9m	Great Pyrenees Mix	32.55
25	MN	4y2m	Tree Walker Coonhound	33.41
26	FS	3y1m	Maltipoo	3.36
27	MN	4y8m	Chihuahua Mix	5.50
28	MN	3y2m	Goldendoodle	23.00
29	FS	2y4m	Cavapoo	5.64
30	FS	3y2m	Labradoodle	12.91
31	MN	4y2m	Coonhound	26.45
32	FS	6y1m	Labrador Retriever	33.27
33	MN	2y4m	Boxer Mix	23.73
34	MN	3y	Shepherd Mix	25.36
35	MN	2y4m	Mixed	18.45
36	FS	9y8m	Mixed	22.73
37	MN	3y5m	Yorkshire Terrier	6.00
38	FS	6y11m	Golden Retriever	25.45
39	MI	4y	Leonberger	54.55
40	MI	4y1m	Leonberger	64.91

Note. FS = female spayed, MN = male neutered, MI = male intact. Age is notated as number of years (y) and months (m) at the time of the veterinary visit.

Median veterinary visit duration from exam room entry through exam room exit was 25.44 minutes (Range 18.10 - 42.88 minutes). The median percent of the veterinary visit dogs spent in each condition is displayed in Figure 3. Venipuncture was attempted in 39 dogs and blood was collected successfully in 37 dogs. Median number of VENI intervals was 4.00 (Range 2.00 – 14.00). Due to technical issues with the camera, one dog's acclimation period was not captured on video, and videos ended abruptly during the post-venipuncture condition for two dogs. Overall, dogs spent less of the visit in the VENI and HL conditions than other conditions.

Figure 3

Median Percentage of Veterinary Visit Spent in Each Condition



Note. This figure shows the median percentage of the veterinary visit dogs spent in each condition. Error bars represent the range. Conditions are ACC = acclimation, HL = HRM and lidocaine/prilocaine cream application, NC = no condition, PRE-V = pre-venipuncture, VENI = venipuncture, POST-V = post-venipuncture.

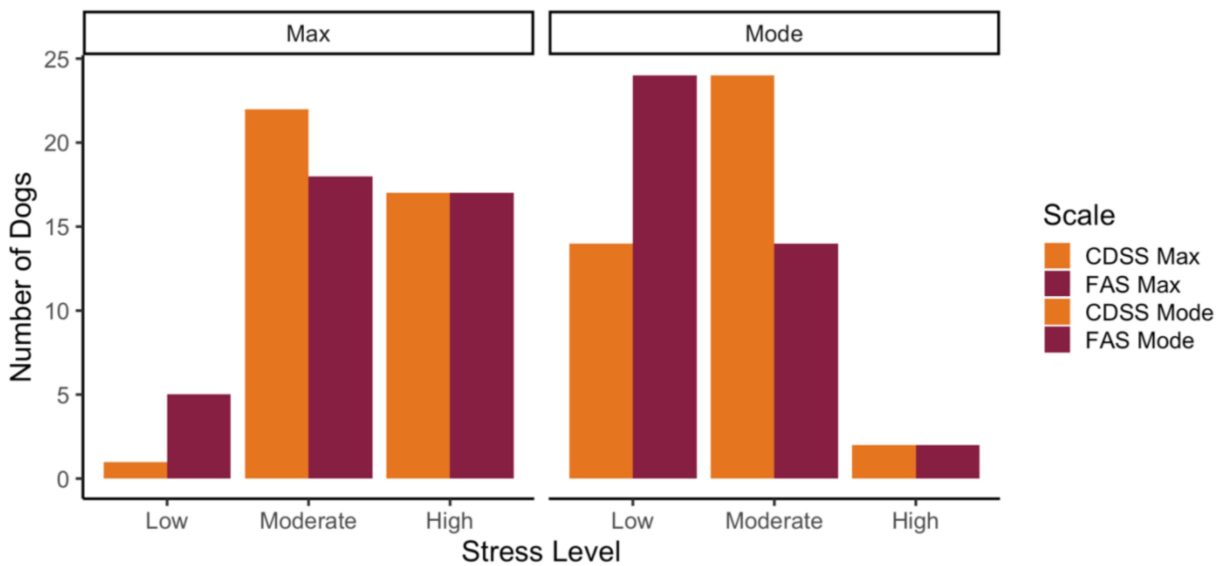
Behavioral Measures of Stress

Stress Scale Scores

The maximum and mode categorical stress scores for all dogs are presented in Figure 4. Most dogs experienced moderate to high stress at some point during the veterinary visit (FAS, n = 35; CDSS, n = 39) but were scored low to moderate stress in most scored intervals (FAS and CDSS, n = 38).

Figure 4

Maximum and Mode Stress Scores

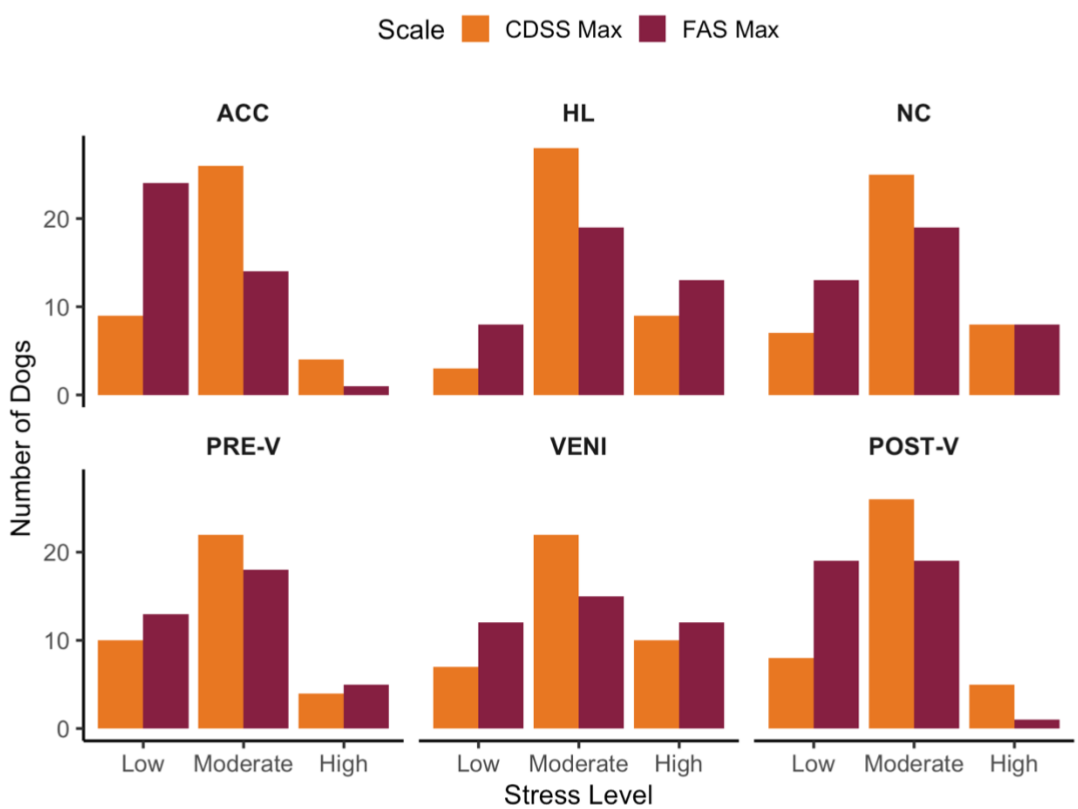


Note. The left side of this figure shows the number of dogs whose maximum stress score during the veterinary visit was low, moderate, or high. On the right, this figure shows the number of dogs whose most frequently recorded stress score during the visit was low, moderate, or high.

Maximum stress scores for dogs in each condition are displayed in Figure 5. Across all conditions, CDSS was moderate for most dogs. Most dogs had moderate to high FAS in the HL and VENI conditions, but more dogs had low FAS in the VENI condition than in the HL condition.

Figure 5

Maximum Stress Scores by Condition

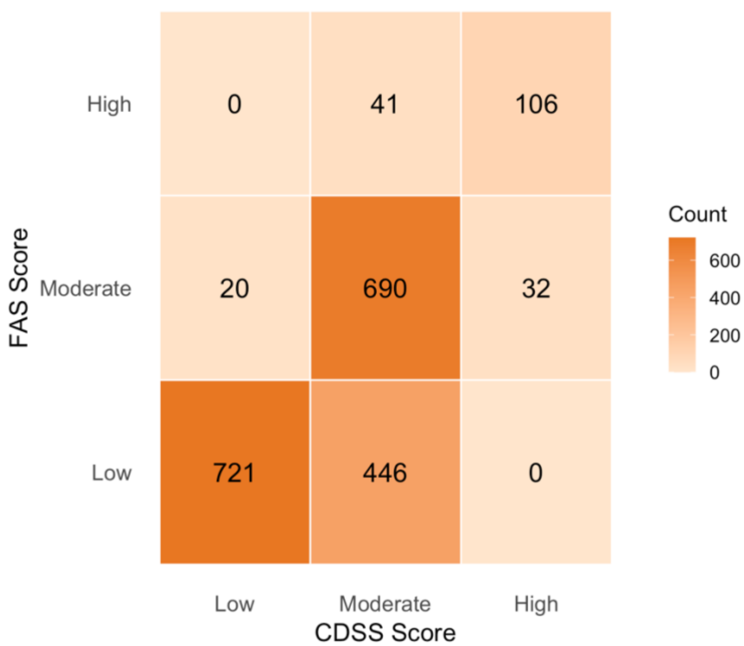


Note. This figure shows the number of dogs whose maximum recorded stress score during each condition of the veterinary visit was low, moderate, or high. Conditions are ACC = acclimation, HL = HRM and lidocaine/prilocaine cream application, NC = no condition, PRE-V = pre-venipuncture, VENI = venipuncture, POST-V = post-venipuncture.

We hypothesized that FAS and CDSS scores would agree, affording some validity to the Fear Free™ FAS Scale. We calculated the percentage of all scored intervals with complete agreement between FAS and CDSS scores. With missing data omitted, 2056 scored intervals for all 40 participant dogs were included. There was moderate agreement between scores from the two scales (73.78%, Cohen's kappa = 0.55). Distribution of scores and agreement is visualized in Figure 6. Disagreement occurred most frequently where CDSS was scored moderate and FAS was scored low.

Figure 6

FAS and CDSS Score Agreement Heatmap



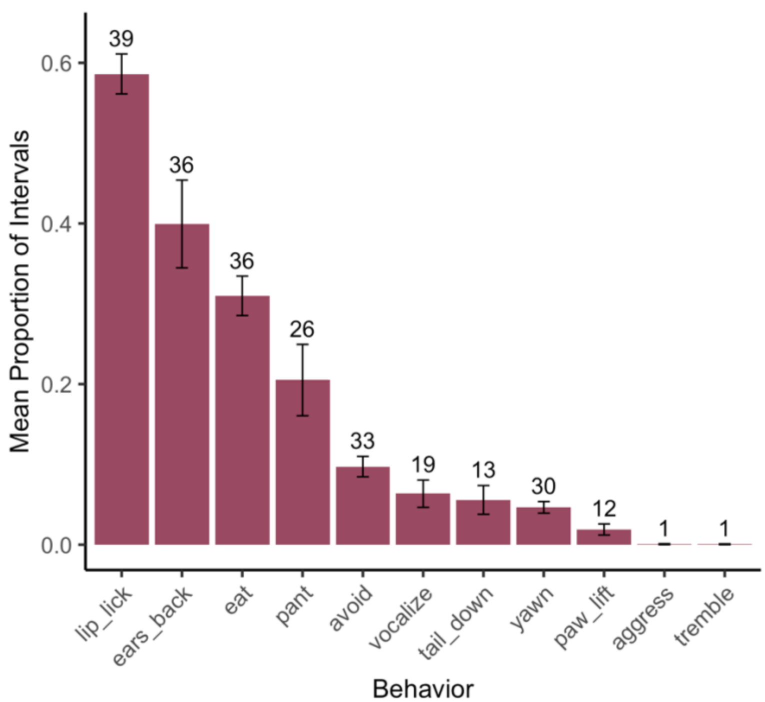
Note. This figure shows the number of intervals each possible pair of scores was recorded across all observed intervals for all dogs during the veterinary visit.

Behavioral Data

We completed partial interval recording for 11 behaviors. The mean proportion of intervals each behavior was performed by dogs during the veterinary visit is displayed in Figure 7. Lip licking was the most frequently observed behavior, performed by all dogs included in the analysis (n=39). More than half of all dogs displayed ears back, eating, panting, and avoidance behavior. Aggression and trembling were performed by one dog each.

Figure 7

Mean Proportion of Intervals Behaviors Performed

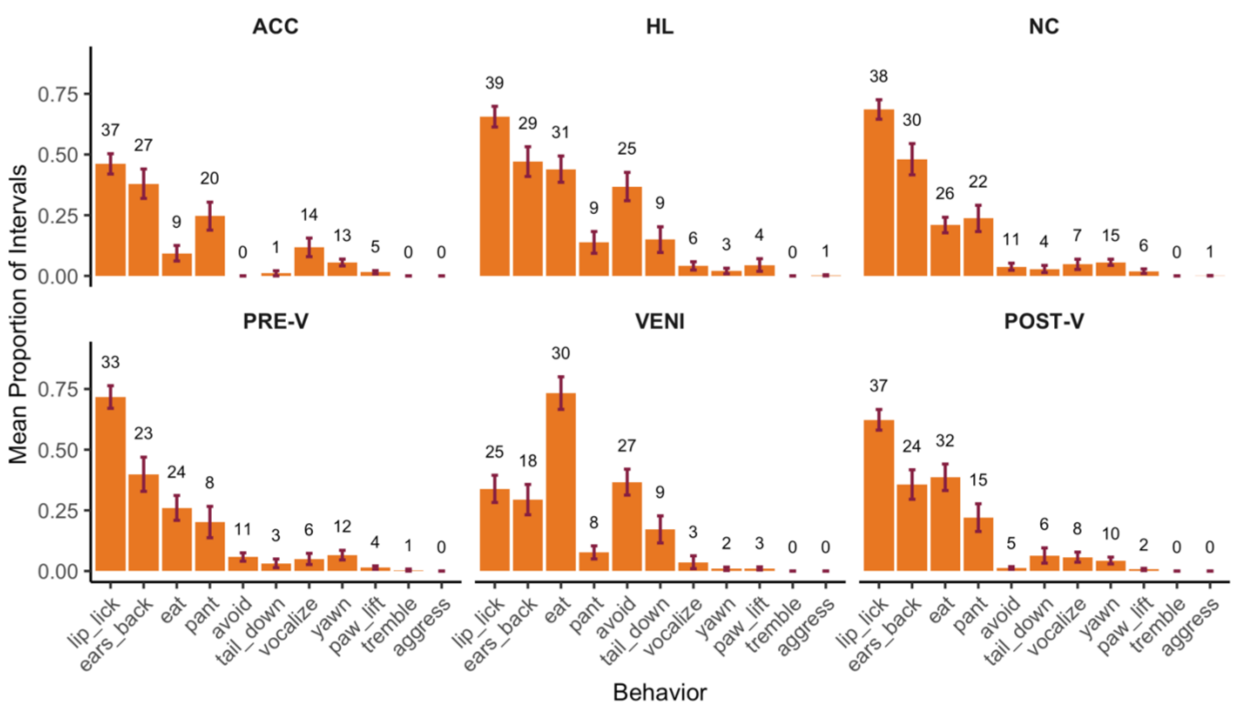


Note. This figure shows the mean proportion of intervals observed for each dog where a behavior was performed at least once. Error bars represent the standard error of the mean. Numbers above the error bars indicate the total number of dogs that displayed the behavior at least one time.

The mean proportion of intervals each behavior was performed by dogs during each condition of the veterinary visit is displayed in Figure 8. Missing data were omitted. Lip licking behavior was the most frequently performed behavior in all conditions except VENI, where eating was the most frequent. Of the 36 dogs who ate during the veterinary visit, 30 dogs ate during venipuncture. Avoidance was most frequently observed during HL and VENI. Vocalization was most commonly performed during ACC.

Figure 8

Mean Proportion of Intervals Behaviors Performed by Condition



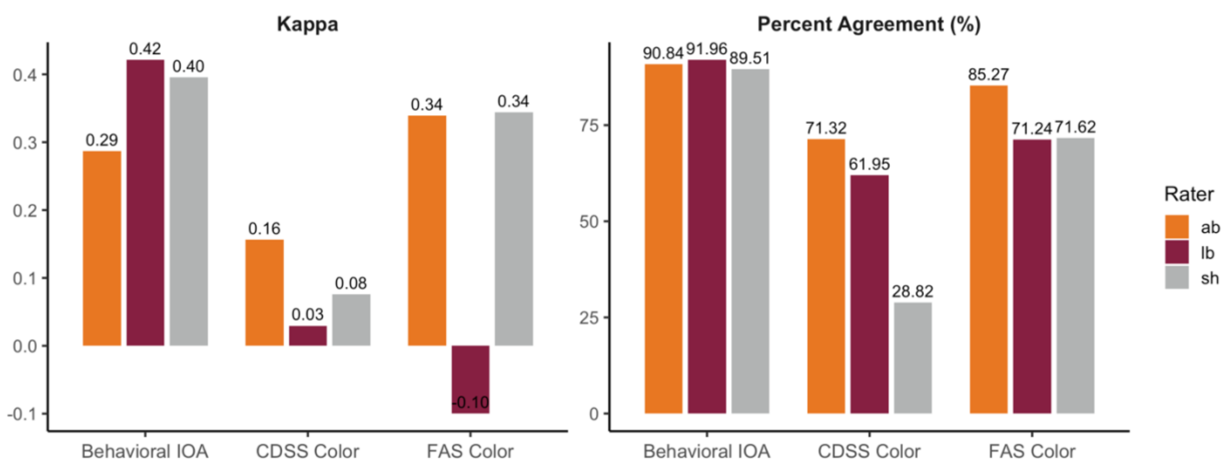
Note. This figure shows the mean proportion of intervals observed for each dog in each condition during the veterinary visit where a behavior was performed at least once. Conditions are ACC = acclimation, HL = HRM and lidocaine/prilocaine cream application, NC = no condition, PRE-V = pre-venipuncture, VENI = venipuncture, POST-V = post-venipuncture. Error bars represent the standard error of the mean. Numbers above the error bars indicate the total number of dogs that displayed the behavior at least one time.

Interobserver Agreement for Stress Scores and Behavioral Data

Interobserver agreement (IOA) by rater for stress scores and behaviors is presented in Figure 9. Mean IOA for FAS scores was 76.04%, and for CDSS scores mean IOA was 54.03%. Mean IOA for all behaviors was 90.77%.

Figure 9

Interval-by-Interval Interobserver Agreement for Stress Scores and Behaviors



Note. This figure displays Cohen's kappa, unweighted for behavioral IOA and weighted for CDSS and FAS, and percent interval-by-interval agreement between researcher (AC) and observers (AB, LB, SH).

Physiological Measures of Stress

Heart Rate and Heart Rate Variability

Due to the small subset of dogs whose HR and HRV data was viable, a summary of the measures for all dogs is presented in Table 4 alongside FAS and UCCR data. All HR and HRV data encompassed the VENI condition but was inconsistent in capturing data during other conditions from dog to dog. Limited interpretation of this data is included due to the variation in sample length and portions of the veterinary visit occurring during data capture.

Table 4

Summary of Data for Dogs with HR/HRV Data

Dog ID	HR/HRV Sample Length (min)	FAS Mode (Max)	UCCR (% change)	HR (bpm) Mean ± SD (Range)	RMSSD (ms)	SDNN (ms)
01	10	G (G)	12%	97 ± 12 (54-133)	53.28	67.6
04	6	Y (R)	11%	160 ± 19 (118-235)	28.69	41.37
10	8	Y (R)	6%	107 ± 10 (79-146)	41.93	51.41
11	11	Y (R)	131%	139 ± 14 (107-215)	17.78	39.97
14	19	G (Y)	51%	120 ± 13 (93-179)	47.63	54.29
23	7	G (Y)	24%	110 ± 11 (84-163)	35.27	48.9
33	8	G (G)	37%	116 ± 11 (81-174)	36.52	48.78

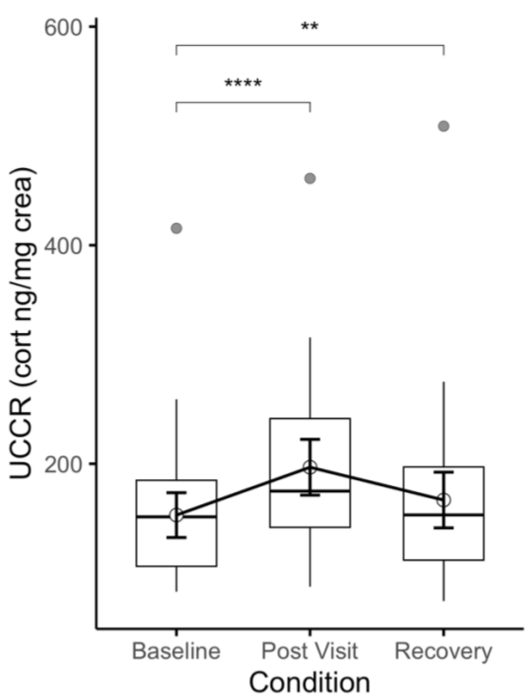
Note. The FAS mode and max scores are indicated by the initial of their corresponding color level, where G = green/low, Y = yellow/moderate, and R = red/high stress.

Urine Cortisol:Creatinine Ratios

We hypothesized that a veterinary visit involving venipuncture constitutes a stressful experience and would result in an increased UCCR. Values for 39 dogs included in the analysis ranged from 82.89 – 415.45 in the baseline condition, 87.55 – 461.11 in the post-visit condition, and 74.33 – 508.9 in the recovery condition. A comparison of average UCCR values across all conditions is presented in Figure 10. Data were normally distributed according to a Shapiro-Wilk test (W = 0.93, p = 0.019) so we performed a paired t-test to compare baseline and post-visit values. Post-visit UCCR differed significantly from baseline UCCR (mean difference = 43.80, p = 0.0000051, 95% CI: 27.09 - 60.51). Although a Shapiro-Wilk test did not support assumption of normal distribution (W = 0.96, p = 0.23), we followed the same testing protocol and performed a paired t-test to compare baseline and recovery values. Recovery UCCR differed significantly from baseline UCCR (mean difference = 13.79, p = 0.0059, 95% CI: 4.22 - 23.37), however clinical or practical significance of this difference is unlikely.

Figure 10

Comparison of UCCR Values by Condition



Note. This figure shows median and IQR for the raw UCCR values for 39 dogs across baseline, post-visit, and recovery conditions. The outlier points represent the data from a single dog (Dog 4). Mean points for each condition are represented by open circles and connected by a line. Error bars represent 95% confidence intervals. Statistically significant differences between groups denoted by ** = $p \leq 0.01$, **** = $p \leq 0.0001$.

To account for the wide range of values and individual variation, descriptive statistics were computed using the percent change in UCCR from baseline to post-visit. Median change in UCCR was 24% (Range -56% to +131%). We also fit a linear mixed model to UCCR data with condition, dog age (years), weight (kg), and sex/reproductive status as fixed effects, and individual dog ID as a random effect. Dog ID was responsible for 72.12% of the total variance, and no significant effect was found for age (+10.41 cortisol ng/mg creatinine per 1 year of age, SE = 5.89, $p = 0.09$) or sex/reproductive status (MN: -25.02 cortisol ng/mg creatinine, SE = 21.55, $p = 0.25$; MI: +74.04 cortisol ng/mg creatinine, SE = 57.46, $p = 0.21$). The effect of weight was statistically significant (-2.94 cortisol ng/mg creatinine per kg, SE = 0.87, $p = 0.002$), however clinically irrelevant.

Comparing Measures of Stress

We hypothesized that FAS scores would correlate positively with UCCR and HR and negatively with HRV, which would validate the Fear Free™ FAS Scale. For these analyses, we used the percent change in UCCR from baseline to post-visit. We also determined the max FAS and mode FAS for each dog to assess the magnitude and frequency, respectively, of FAS response. We evaluated relationships in the data using Spearman's rank correlation.

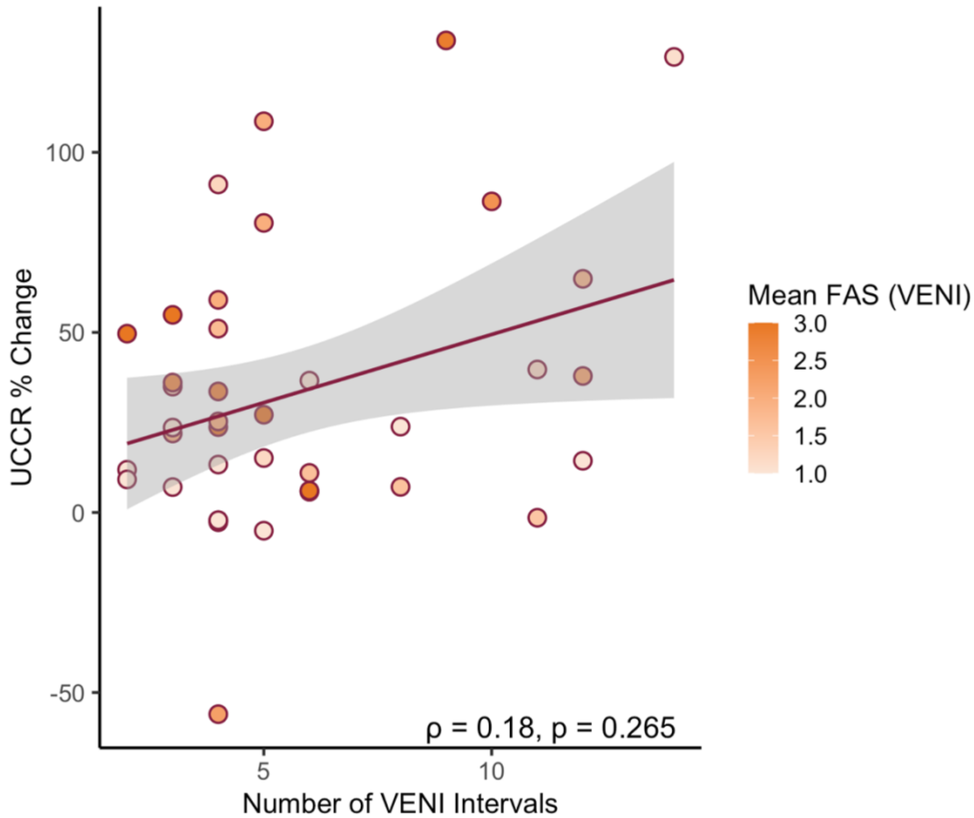
Correlation was weak and nonsignificant between FAS mode and percent change in UCCR (rho = 0.13, p = 0.45) and FAS max and percent change in UCCR (rho = 0.22, p = 0.19). There was moderate positive correlation between FAS mode and HR (rho = 0.43, p = 0.33) and FAS max and HR (rho = 0.51, p = 0.24), however these results were not statistically significant. There was moderate negative correlation between FAS mode and RMSSD (rho = -0.58, p = 0.17), FAS max and RMSSD (rho = -0.60, p = 0.15), FAS mode and SDNN (rho = -0.58, p = 0.17), and FAS max and SDNN (rho = -0.53, p = 0.22) but again, the results were not statistically significant.

Veterinary Visit Duration, Venipuncture, and UCCR

To investigate the effect of venipuncture duration on physiological stress response, we compared percent change in UCCR with the number of intervals of venipuncture for each dog using Spearman's rank correlation. This relationship is visualized in Figure 11. There was a nonsignificant weak positive correlation (rho = 0.18, p = 0.27). We then looked at the effect of the veterinary visit duration on percent change in UCCR and found a significant moderate positive correlation (rho = 0.34, p = 0.04). This relationship is visualized in Figure 12.

Figure 11

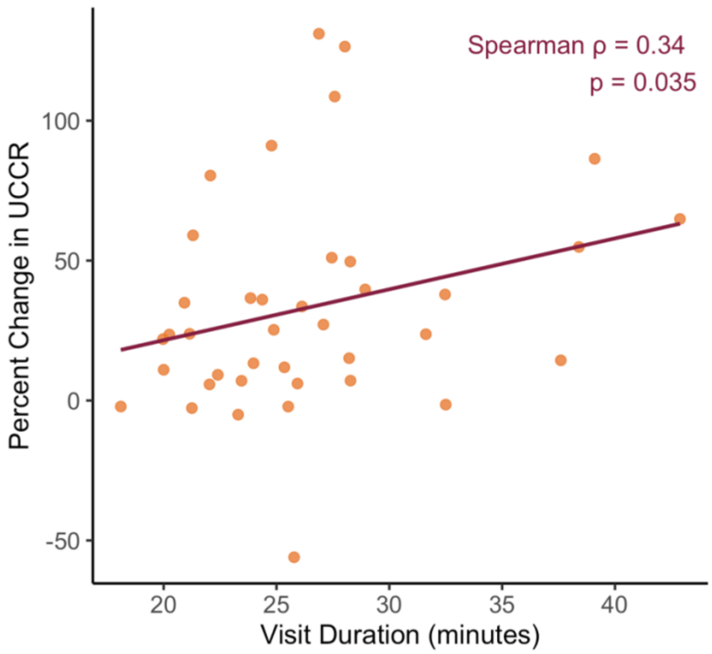
Comparison of Percent Change in UCCR, Venipuncture Intervals, and Mean FAS During Venipuncture



Note. This figure displays the percent change in UCCR from baseline to post-visit for dogs (n = 39) compared with the number of venipuncture intervals the dog experienced. A gradient fill for each dog's dot indicates the mean FAS the dog experienced during the venipuncture condition.

Figure 12

Correlation of Veterinary Visit Duration and Percent Change in UCCR



Note. This figure shows the correlation between veterinary visit duration and percent change in UCCR from baseline to post-visit condition for each dog.

Exploratory Analysis of Data

We performed an exploratory analysis of correlational relationships between individual behavior frequencies, stress scores, and physiological measures of stress using a Spearman's rank correlation matrix with Holm adjustment and alpha = 0.05. From this matrix, we identified 12 moderate to highly correlated pairs with statistical significance ($p < 0.05$) to further evaluate using a 95% confidence interval. Pairs are displayed in Table 5. Of these 12 pairs, two remained significant: SDNN and post-visit UCCR value, and mean HR and post-visit UCCR value. Given this finding, we calculated Spearman's rho for FAS and post-visit UCCR values. These results showed weak and nonsignificant correlation between both FAS mode and post-visit UCCR (rho = 0.05, p = 0.75) and FAS max and post-visit UCCR (rho = 0.17, p = 0.30).

Table 5

Results of Exploratory Analysis

	Spearman's rho	p-value	95% CI lower	95% CI upper
eating/uccr_baseline	-0.38	0.02	-0.62	-0.08
tail_down/uccr_baseline	-0.32	0.05	-0.58	-0.01
hrv_rmssd/uccr_post_visit	-0.82	0.02	-0.97	-0.18
hrv_sdnn/uccr_post_visit	**-0.86**	**0.01**	**-0.98**	**-0.29**
hr_mean/uccr_post_visit	**0.86**	**0.01**	**0.29**	**0.98**
paw_lift/uccr_post_visit	-0.35	0.03	-0.60	-0.04
hrv_sdnn/uccr_recovery	-0.79	0.04	-0.97	-0.08
tail_down/uccr_recovery	-0.4	0.01	-0.63	-0.09
tail_down/lip_lick	0.34	0.04	0.03	0.59
ears_back/paw_lift	0.38	0.02	0.08	0.62
vocalize/eating	0.32	0.05	0.00	0.57
pant/tail_down	-0.33	0.04	-0.59	-0.02

Discussion

To evaluate the validity of the Fear Free™ FAS Scale as a non-invasive stress measurement tool for use in dogs visiting the veterinary hospital, we collected and evaluated several physiological and behavioral measures of stress in dogs before, during, and after a visit to the veterinary hospital for venipuncture, which we presumed would constitute a stressful experience. We hypothesized that UCCR would increase following such a visit, FAS and CDSS scores would agree, UCCR and HR would correlate positively with FAS scores, and HRV would correlate negatively with FAS scores. Based on UCCR and stress scores, veterinary visits involving venipuncture appear to be stress-inducing for most dogs, but overall veterinary visit duration may be a significant factor to consider. Additionally, while our data does not provide strong support, it does grant some evidence of validity to the FAS Scale.

Veterinary Visit Stress

Stress Scale Scores

To attempt to evoke a stress response in the dogs in our study, we subjected them to a veterinary visit involving venipuncture. When video recordings of the visits were scored using Clinic Dog Stress Scale 1 and the Fear Free™ FAS Scale, we found that most dogs in our study experienced moderate to high stress levels at some point during the veterinary visit but maintained low to moderate stress levels in most intervals across the visit. Dogs were most commonly scored moderate to high stress not only during venipuncture, as expected, but also during the application of the HRM and lidocaine/prilocaine cream. This is likely due to the handling by the veterinary team associated with these procedures; all other conditions did not involve contact between dog and veterinary staff except when attention was solicited by the dog. Interestingly, more dogs were scored as low FAS while having blood drawn than when the HRM and lidocaine/prilocaine cream were applied. This could have been related to increased provision of food during the blood draw or discomfort associated with application of cold ultrasound gel and lidocaine/prilocaine cream. Dogs known to experience high FAS for veterinary visits involving venipuncture were excluded from our study, so our sample population heterogeneity is limited with respect to anticipated stress level. Even so, our stress score results suggest that venipuncture is stressful for most pet dogs, which is in agreement with a previous study which found pet dogs to exhibit more behavioral signs of stress and an increased physiological stress response when compared with blood donor dogs and laboratory dogs (Srithunyarat et al., 2018).

Urine Cortisol:Creatinine Ratios

We used UCCR to measure participant dogs' physiological stress at baseline prior to the veterinary visit, post-veterinary visit, and at recovery on the day after the veterinary visit. Our results support our hypothesis that UCCR would increase following the veterinary visit. We observed a significant increase in post-visit UCCR over baseline UCCR values. We also observed a statistically significant difference between baseline and recovery values, but the difference between these mean values was clinically irrelevant. This finding agrees with an earlier study which suggested a possible impact of duration of exposure to a veterinary-related stressor on UCCR levels in dogs (Van Vonderen et al., 1998). Van Vonderen and colleagues (1998) found veterinary visits for routine vaccination to cause a relatively mild increase in UCCR in some dogs, and overnight hospitalization to cause a significant increase in UCCR values which was sustained up to 12 hours after discharge in some dogs; however, physiological recovery occurred in both groups within 24 hours of the stressful veterinary visit, similar to what we observed in the present study.

When we looked at the relationship between venipuncture and physiological stress response, we did not find a significant relationship between the number of venipuncture intervals and percent change in UCCR. Instead, we found a significant moderate positive correlation between veterinary visit duration and percent change in UCCR. In our study, we cannot determine whether increased veterinary visit duration may have occurred secondary to the dog's stress level, or vice versa. Dogs exhibiting moderate to high levels of stress often required additional veterinary team members to assist with restraint, which added time to the visit when the researcher left the room to seek help. Some dogs needed more than one attempt at venipuncture to successfully have blood collected, which also added time to the visit. Venipuncture lasted no longer than seven minutes with a median duration of roughly one to two minutes. Although we can estimate venipuncture based on the number of intervals during which venipuncture occurred, we did not directly measure duration of the venipuncture condition or record the number of venipuncture attempts. The effects of these variables on dog stress merit further investigation. Given the significant increase in post-visit UCCR and moderate to high stress scores recorded for a majority of dogs during the venipuncture condition of the veterinary visit, our results indicate that veterinary visits involving venipuncture are likely to be stressful for most dogs. Additionally, physiological recovery from a stressful veterinary visit may be achieved within 24 hours, especially when the duration of the exposure to a stressor is relatively short.

FAS Scale Validity

Stress Scale Scores

We assessed validity of the FAS Scale from multiple angles. For convergent validity, we used CDSS 1 and the FAS Scale to score the video recordings of participant dogs' veterinary visits every 30 seconds. Our results showed moderate agreement between the CDSS and FAS Scale when scoring was converted from a 0-5 scale to a condensed categorical scale of low (0-1), moderate (2-3), or high (4-5) stress, similar to what was done by Mercier et al. (2023) to improve inter-rater reliability of the CDSS. There are several reasons why strong agreement might not have been achieved. The CDSS 1 is a succinct, simple scale utilizing very general criteria to aid front desk staff in scoring stress upon entry to the veterinary hospital. The FAS Scale, on the other hand, is more detailed and intended for use by all veterinary staff throughout the veterinary visit. Perhaps most notably, there are discrepancies between the language used in the descriptions of behavior observed at score levels in the scales. Our results indicate a moderate CDSS score was commonly assigned in the same interval as a low FAS score, which can be explained by the verbiage used within the scales. A CDSS score of 2 (moderate) is defined simply as "alert, but calm and cooperative" and a score of 3 (moderate) might include "panting slowly" (Overall, 2013). Similar language exists in the FAS Scale where a score of 0 (low) is indicated by calm behavior and a score of 1 (low) might include relaxed panting (Martin & Martin, 2022). There were 26 dogs that panted during the veterinary visit, which likely caused a significant discrepancy between FAS and CDSS scores, as well as interobserver agreement. Another key difference between the scales is that CDSS 1 does not include aggressive behavior. An FAS score of 5 indicates aggression, so a CDSS score of 5 is not equivalent. For these reasons, strong agreement would not reasonably be expected between the scales, and moderate agreement may be a good indicator of convergent validity.

Physiological Measures of Stress

When we compared UCCR with FAS and CDSS scores, no correlation was noted; however, stress scores for most dogs peaked at moderate to high levels and dogs were scored at low to moderate levels most frequently during their veterinary visit. Only five dogs (Dogs 1, 6, 19, 21, and 33) were scored as low stress for the duration of their visit, and of these dogs the UCCR change from baseline to post-visit for all except Dog 33 was minimal, ranging between a 3% decrease and a 14% increase. Only two dogs in our study were scored high stress in most intervals, one of whom did not experience any condition except acclimation and was excluded from UCCR analyses. This may have kept the mean UCCR for our sample population relatively low compared to what we might expect to see with a greater number of dogs frequently scored as high stress. Even so, a stress scale solely encompassing behaviors equivalent to FAS scores of 4-5, or high stress, achieved only moderate correlation with physiological measures of stress (Srithunyarat et al., 2018). Our exclusion criteria were similar to other studies which disqualified dogs known to experience higher FAS and/or display aggressive behavior due to ethical and safety concerns (Gatehouse et al., 2025; Mercier et al., 2023; Nixon et al., 2025). More research is needed in a larger population of dogs displaying high stress to determine concrete predictive validity of the FAS Scale, but ethical concerns may prevent further investigation.

Due to technical difficulties with the Polar® H10 human HRM and HRV Logger App, our HR and HRV data was limited to seven dogs. For this reason, evaluation of HR and HRV was limited to inclusion in an exploratory analysis of correlational relationships between physiological and behavioral measures of stress. Our results indicate moderate positive correlation between FAS score and mean HR, as well as moderate negative correlation between FAS score and HRV measures, which would support our hypotheses; however, the results were nonsignificant. As a result, we cannot confidently confirm concurrent validity of the FAS Scale using these measures.

Our exploratory analysis of correlational relationships amongst all variables uncovered strong significant correlations between mean HR and post-visit UCCR (positive correlation), and HRV (SDNN) and post-visit UCCR (negative correlation). This was unexpected given the small proportion of our sample population that had usable HR/HRV data. While we did not directly assess arousal or emotional valence in our study, we can draw some parallels to recent research in this area. Flint and colleagues (2024) observed greater increases in HR in high arousal scenarios, with the greatest increase occurring in those that had a positive emotional valence, and no effect of emotional valence in low arousal scenarios. This is consistent with previous research which found HR to be a general indicator of arousal, and not specific to stress or severity of stress (Beerda et al., 1998). Because we observed a strong positive correlation between mean HR and post-visit UCCR, we can conclude that this relationship is likely reflective of arousal, but does not give information regarding the emotional valence of the dogs. Flint et al. (2024) also found that HRV (SDRR, a measure similar to SDNN, and RMSSD) was not significantly influenced except in low arousal scenarios with negative emotional valence, and in these cases the HRV was increased. This is the opposite effect we expect to see as a higher HRV is typically attributed to lower stress. This may have been due to difficulty with measuring HRV at higher HR and associated artifacts (Bidoli et al., 2022), but could suggest that HRV is not a reliable indicator of stress in conditions where arousal is high. Although our results show a strong negative correlation between post-visit UCCR and HRV as might be expected, the challenges of interpretation preclude any significant conclusions.

Several limitations exist in the interpretation of our physiological data. First, both physiological and psychological stress affect cortisol secretion by the HPA axis (Beerda et al., 1996). Though we accounted for these by excluding dogs from our study who had diagnosed neuropsychological or endocrine disease, or for whom medication known to affect cortisol levels had been prescribed, it is possible that a disease process may have been occurring but undetected or undiagnosed, or administration of medication was not reported by dog owners. Because UCCR is reflective of cortisol secretion over the previous 4-8 hours (Casey, 2006), we also cannot distinguish whether events prior to or following the veterinary visit activated or attenuated a significant response from the HPA axis. This could include transport to and from the veterinary hospital, a known stressor for some dogs (Herbel et al., 2020). Although we asked owners not to allow dogs to participate in significant activity, we cannot confirm whether dogs engaged in exercise or social play following the veterinary visit, which could have decreased (Clark et al., 1997; King et al., 2010) or increased cortisol levels (Durocher et al., 2007). Similarly, memory from a recent veterinary visit could have affected the stress response as many of the dogs in our study had been seen within the previous week to month for their annual exam and vaccinations (Hernander, 2008). We assessed physiological, but not psychological, recovery from a stressful veterinary visit; this is an area of research that deserves attention.

Finally, challenges exist with obtaining and evaluating HRV measures. Technical difficulty with the HRV Logger App prevented data capture for some dogs. Contact with the skin and properly fitting human equipment to dogs proved challenging in the present study because fur was not shaved, and excessive motion often caused the HRM to slip. These and other factors, including dog weight and age, may increase the rate of artifacts in HRV data recordings (Bidoli et al., 2022). Provision of food may also confound physiological indicators of stress, as increased heart rate has been observed in dogs provided with food in scenarios designed to invoke low levels of arousal and positive emotions (Flint et al., 2024). Importantly, Flint et al. (2024) did not include food provision in negative emotional scenarios of high or low arousal, so we cannot rule out potential changes to heart rate in such scenarios. All dogs were offered food during our study and most ate during venipuncture, so this effect could have contributed to our data loss. Because respiration rates can influence time-domain HRV measures (Shaffer & Ginsberg, 2017), the effect of panting should be considered when evaluating HRV data. Panting was observed in more than half of our participant dogs and thus could have affected our HRV data. Other variables that impact HRV measures in humans include age, sex, and general health (Shaffer & Ginsberg, 2017); however, the available data for dogs does not support parallelism between species, as no differences between sex, reproductive status, age, or weight were found in a study aiming to establish reference intervals for HRV in small and medium dogs (Baisan et al., 2020). Moreover, group- or population-level evaluation of HR and HRV data is complicated by the experience of the individual, including the presence, absence, combination or order of exposure to factors such as the owner, a stranger, or a threat (Gacsi 2013). Finally, interpretation of any HRV data in dogs must be done with marked scrutiny. There is no standard method for pre-processing HRV data, and this can significantly impact the resulting calculated measures (Von Borell et al., 2007). Additional research is needed to determine canine-specific factors that may impact the interpretation of HRV data, and determining a standardized method of pre-processing data should be prioritized.

Behavioral Measures

Observable behavior is an accepted indicator of fear, anxiety, or stress in dogs, but quantifying the degree of stress using behavioral stress scales has rarely been successful (Mills et al., 2020). Measuring stress in dogs using behavior alone presents several challenges, which is why behavioral measures of stress are often evaluated alongside physiological measures of stress. We calculated the proportion of intervals during which dogs performed 11 stress-related behaviors for any duration and found that most dogs performed six of the 11 behaviors at least once. All but one of the behaviors (yawning) are included on the FAS Scale, so behavior data and stress scores were not compared due to bias. When we compared stress-related behaviors with physiological measures from our study subjects, our results did not indicate any relationship between these various measures of stress. This finding agrees with prior research in which UCCR was not correlated with any behavioral measure of stress (Part et al., 2014; Rooney et al., 2007), nor was its variation measurable according to the type of behavior observed (Van Vonderen et al., 1998). Sample population sizes were relatively small in this and prior studies, and, given the diverse presentation of behaviors in these smaller groups, a very large sample population is probably necessary to illuminate any potential relationship between UCCR or other physiological measures of stress and specific stress behaviors. Additionally, our findings agree with prior research which found significant variation in cortisol within and between individual dogs (Alberghina et al., 2019; Clark et al., 1997; Palme et al., 2005; Stephen & Ledger, 2006) but no significant effect of age or sex (Part et al., 2014; Stephen & Ledger, 2006; Van Vonderen et al., 1998), so the individual variation in behavioral presentation and physiological response complicates the identification of trends.

As mentioned, using behavior to measure stress is an imperfect science. We video-recorded veterinary visits to code behavior, which resulted in some limited visibility of subject dogs if they were blocked by objects, people, or blind spots in the exam room. Some behaviors were difficult to detect (e.g., trembling), and the phenotype of some dogs caused difficulty as well (e.g., black or darkly colored dogs, docked tails). The method used to measure behavior quantitatively may impact interpretation. We used partial interval recording to estimate the frequency of behavior, which can overestimate the occurrence of behavior. Moreover, the rate, frequency, or magnitude of a particular behavior may vary depending upon the level of stress a dog is experiencing, so the relationship between level of stress and behavioral measure may not be linear and might not have been captured in our data collection. For example, lip licking behavior may be multifunctional and relatively specific to the level of stress, serving as an appeasement signal used commonly in greetings toward humans, submissive communication in less stressful situations, and observed at less frequent rates in higher stress situations (Firnkes et al., 2017). We observed lip licking in 39 dogs and it was the most frequently performed behavior in all conditions, except during venipuncture, when eating was the most frequently performed behavior. This was likely due to the use of food as a positive distractor during venipuncture, whereas it was typically withheld or offered infrequently during the rest of the veterinary visit. Thus, the comparison of lip licking across conditions is impossible given that food provisioning also varied. Nevertheless, the commonality of the lip licking behavior amongst the dogs in our sample population indicates that it may be a significant behavior to attend to when assessing stress in any dog.

Similar to lip licking, eating might be variable and provide insight into the level of stress for the individual under observation. Emotional eating might be particularly pertinent; emotional eating is described as the motivation to eat in response to negative emotion or stress (Luño et al., 2018). The literature on emotional eating behavior in dogs is scarce; however, patterns of emotional eating have been noted in humans and other mammals (McMillan, 2013). Both negative and positive emotions can impact eating behavior and food selection in humans (Blechert et al., 2014), and a survey of dog owners found nearly 82% observed emotional eating in relation to negative emotions experienced by their dogs (Luño et al., 2018). In our study, a majority of dogs ate during the venipuncture condition, and only a few who ate during other conditions did not eat during venipuncture. A strength of the FAS Scale is that it takes these variable behavioral presentations into consideration. For example, it describes reduced willingness to eat or take treats as a potential indicator of stress but also includes increased roughness of treat-taking as a signal that the dog's stress level is increasing. Other differences in stress behavior presentation across the FAS Scale are outlined in Table 1. Our findings do not support any strong conclusions surrounding this or any specific stress behavior; however, given the limited existing literature on emotional eating behavior in dogs and the potential implications of misunderstanding this behavior, canine eating behavior should be explored further.

In addition to variation of presentation of stress behaviors across levels of stress, individual expression of these behaviors may vary widely. This diversity in individual expression of stress-related behavior is perhaps most profoundly illustrated by Rooney et al. (2007) who, despite a homogeneous sample population of dogs with similar genetics and early life experiences, report wide individual variation in behavior patterns observed. In their study of 31 one-year-old male labrador retrievers which had been raised naïve or habituated to a kennel from 8 weeks of age, Rooney and colleagues (2007) did not identify a consistent behavior pattern indicative of stress across the sample population. Additionally, behavior is shaped in part by learning, thus early similarities in behavioral response to a stimulus may change over time and exhibit increasingly wide individual variation (Godbout et al., 2007; Rooney et al., 2007). Another study revealed differences in behavioral response to an acute stressor dependent upon the level of chronic stress dogs experienced (Beerda et al., 2000), adding to the complexity of assessing stress using observable behaviors. Considering the existing evidence and the results of the present study, we suggest that the FAS Scale is a valid clinical tool for assessment of the presence of fear, anxiety, or stress in dogs, but we did not find evidence to support a claim of validity for measuring or quantifying specific levels of stress in dogs in the veterinary hospital.

FAS Scale versus FAS Spectrum

While no other studies have evaluated the validity of the FAS Scale, two recent studies have evaluated the reliability and validity of the FAS Spectrum, a tool similar to the FAS Scale but with a few key differences. The FAS Spectrum encompasses seven scores and includes illustrations of some behavioral presentations characteristic of each score. The “green” or low level of FAS is expanded to four possible scores, whereas the “yellow” (moderate) and “red” (high) levels retain the same two possible scores as described in the FAS Scale. Observers in each study ranged from naïve to expert in the use of the FAS Spectrum, and study results indicated moderate interobserver agreement (Zhao et al., 2024) and excellent interrater reliability (Gatehouse et al., 2025). In contrast, one board-certified veterinary behaviorist and three veterinary behavior residents with extensive experience in interpretation of dog behavior and prior training in the use of the CDSS only achieved fair to moderate interrater reliability when the scale was simplified as previously described (Mercier et al., 2023). Similarly, our IOA for FAS scores was moderate in terms of percent agreement, but somewhat poor for CDSS scores. This is likely due in large part to the lack of experience raters had with the CDSS but may also be related to the limited criteria provided for each score.

Some evidence of validity is afforded to the FAS Spectrum when evaluated against the Lincoln Canine Anxiety Scale (LCAS) (Gatehouse et al., 2025). It should be noted, though, that the LCAS has previously been validated for use in dogs with noise phobia during a noise stimulus event in their home environment; it was not designed for, nor has it been applied in, the veterinary hospital context (Mills et al., 2020). Additionally, although interrater reliability was strong, participants scored FAS incorrectly more than half the time, and few felt the scale was easy to use (Gatehouse et al., 2025). Neither Zhao and colleagues (2024) nor Gatehouse and colleagues (2025) evaluated reliability or agreement with scores collapsed to their respective categorical scale as was done in the present study, and although Gatehouse and colleagues (2025) calculated the absolute error of incorrect scores, these results were not reported in full. Physiological measures of stress were not collected to allow for additional evaluation of validity or comparison of such results with the findings of the present study. Therefore, we cannot conclude whether the FAS Scale or the FAS Spectrum might be the more valid or reliable tool; however, both seem to be more reliable and accessible stress assessment tools than the CDSS.

Implications and Future Directions

Our results indicate that veterinary visits involving venipuncture are stressful for most dogs even when Fear Free™ interventions are in place, but the elevation in stress is likely brief. This is consistent with existing research that has found positive effects for interventions such as non-slip surfaces and food or treats for scale mounting (Score, 2024; Squair et al., 2024; Squair et al., 2023), use of topical anesthetic and minimal restraint plus provision of food for venipuncture (Squair et al., 2024; Squair et al., 2023), and presence during or inclusion of dog owners in veterinary examination procedures (Csoltova et al., 2017; Girault et al., 2022; Helsly et al., 2022; Stellato et al., 2020). Fear Free™ interventions should be implemented whenever possible to positively impact canine health and welfare. Because related studies often exclude dogs known to experience higher stress or exhibit aggressive behavior, more research is needed to identify non-pharmaceutical interventions that may benefit dogs with more intensive intervention needs.

A disadvantage of the FAS Scale is its linear design based on a continuum of known stress behaviors; it does not account for the influence of core affective state on behavior and physiological parameters (Flint et al., 2024). Russell (2003) describes core affect in humans to be a combination of feelings of pleasure or displeasure and arousal or energy, each occurring along a continuum. A similar framework for application in animal welfare assessment presents positive and negative emotional valence along a horizontal continuum, and high and low arousal along a vertical continuum, creating four quadrants of core affective states (Mendl et al., 2010). Based on this framework, recent research suggests that cortisol is most significantly increased when dogs experience negative emotional valence paired with high arousal (Flint et al., 2024). It should be noted, though, that both positive and negative valence increased cortisol, with negative valence prompting a greater increase in cortisol levels regardless of arousal, and no significant effect of arousal on cortisol was observed in positive emotional valence states (Flint et al., 2024). Some of the increased post-visit UCCR values observed in our study may be reflective of core affective state, but interpretation of affective state based on the FAS Scale is difficult due to its focus on behaviors indicative of fear, anxiety, and stress. For example, Dog 33 displayed behaviors consistent with low FAS for the duration of the visit but experienced a 37% increase in UCCR post-visit. This might be interpreted as psychological arousal with a positive emotional valence. Dog 1 was also scored as low FAS for the visit duration and had a 12% increase in UCCR post-visit. This might be interpreted as low psychological arousal and positive emotional valence. Attentiveness to behaviors indicative of low stress, such as head resting (Hekman et al., 2012), could improve the FAS Scale. Currently, low-stress behavioral indicators on the FAS Scale are limited to willingness to eat or interact with the veterinary team. This may be problematic given the discussion on emotional eating, and presence of a stranger which is a known potential stressor (Beerda et al., 1998; Gácsi et al., 2013). Incorporation of emotional valence, psychological arousal, and behaviors associated with low stress and coping ability in the FAS Scale could improve the veterinary team's assessment of canine stress in the hospital, thus leading to more effective and targeted interventions and improving overall outcomes with respect to patient care during present and future visits.

Conclusion

The present study highlights the limitations individual variation in behavior and physiology place on the design, utilization, and validation of canine stress measurement tools. Researchers previously suggested that validation of a sole method of assessing an animal's stress level is unlikely, in part due to the variability of stressors, and also due to variability in the individual response (Mills et al., 2014). Additional difficulty in interpretation arises from the impact of arousal on physiological measures of stress, the effect of which cannot be parsed out (Beerda et al., 1998; Mills et al., 2014). A validated, reliable, non-invasive stress measurement tool for clinical use may be achievable with targeted improvements to the existing Fear Free™ FAS Scale and more substantial training in its use. Results from this and prior studies suggest key areas for improvement of the FAS Scale include improved training in the use of the scale through more robust educational programs, inclusion of visuals to aid in identifying behaviors associated with score levels, and modifying scoring to be based on wider categorical scores rather than a more specific numerical scale.

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