

UNDER PRESSURE:
THE EFFECT OF ENVIRONMENTAL STRESS ON COGNITION AND
BEHAVIOR IN SHELTER DOGS

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ABSTRACT

As an obligate social species, dogs experience stress in the animal shelter due to a significant lack of human interaction. Chronic stress can lead to dysregulation of the HPA axis which affects physiological measures of stress, so behavior may be a more accurate indicator of chronic stress among dogs in the shelter. Chronic stress also impacts cognition, so evaluating the performance of shelter dogs with a spatial memory task may give insight to stress level. This study sought to determine if stress behaviors in shelter dogs decrease as a result of participation in a cognitive task, and if poor performance on a cognitive task correlates with more stress behaviors. Behavioral observations were conducted for 36 adoptable dogs at the Humane Society of Southern Arizona and 20 dogs were subjected to a cognitive test on the second day of behavioral observations. Stress behaviors were coded using an ethogram and grouped into two categories using principal component analysis: active stress and vocal stress. The proportion of choices correct were recorded for the cognitive task and analyzed with reference to each dog's principal component stress score. There was a weak negative relationship between cognitive test score and both active and vocal stress score, which suggests that poor cognitive performance may be indicative of dogs experiencing greater stress. However, there was not a significant difference between active and vocal stress scores among the control and experimental groups, which may be due to the nature of the cognitive task being brief with little increase in human interaction compared to the control condition.

INTRODUCTION

I. Domestic Dog

The domestic dog is arguably one of the most socially adept species, attributed to their communication and attunement with humans. Dogs evolved from wolves and the primary theory of wolf domestication lies in the notion that humans were the catalysts in their domestication. This theory, the cross-species adoption hypothesis, states that humans hand-reared wolf pups leading to a cooperative social system (Serpell, 2021). The wolves that chose to stay in close proximity to humans formed close social bonds with humans, which is the basis of what makes dogs so unique. Social interaction between dogs and humans has been documented extensively, beginning with the finding that dogs require very little exposure to humans to form social bonds (Scott and Fuller, 1965). Furthermore, research shows that a dog's relationship with its caregiver is analogous to parent-child attachment behavior given that dogs are more likely to play and explore in the presence of their owner than in the presence of a stranger (Topál et al., 1998). Dogs quickly integrated into human society as social partners for humans and recent studies with dog puppies suggest that the ability of dogs to form social bonds with humans is inherited. Despite similar performance on tests of memory and inhibitory control, dog puppies are more attuned to human communicative cues than wolf puppies raised by humans. This suggests that dogs' communicative abilities with humans is greater than that of wolves' as a result of domestication (Salomons et al., 2021).

The companion dog has become a staple in today's society with nearly 40% of American households owning at least one dog (AVMA, 2022). The companion dog's popularity stems from thousands of years of domestication which has allowed us to select for specific qualities such as temperament and other phenotypic characteristics like size, color, and fur length. Most companion dogs are kept as pets in the home, but many companion dogs are trained as therapy or working dogs because of their cooperative communicative skills with humans. In fact, it has been shown that there is a genetic basis to dogs' ability to communicate with humans and this occurs early in their development at 8 weeks of age (Bray et al., 2021). Since companion dogs are social by nature, they thrive in the presence of humans. This means that a dog's welfare is put at risk when they are deprived of human interaction. Although welfare can be impacted when other species-typical needs like physical activity and mental stimulation are not met, socialization is arguably the most important need for a dog. Research shows that socialization

between dogs and humans is even more important for dogs than socialization between other conspecifics. For example, when assessing conspecific interaction, the removal of a longstanding kennelmate between adult dogs results in minimal cortisol elevation compared to a control group (Tuber et al., 1996). Tuber et al. (1996) further demonstrates that dogs tested in a novel environment with a familiar human caretaker showed significantly decreased glucocorticoid levels than when alone or with a longstanding kennel mate in the same novel environment, thus highlighting the significance of the human-dog social bond. Human-animal interaction, especially that between humans and dogs, is a growing field of research given the evidence that dogs are highly attuned to human communication. The ability for dogs to engage in meaningful social interactions with humans is disrupted by the shelter environment, in which dogs are deprived of many of the necessary interactions needed to maintain welfare.

II. Dogs in the Shelter

Dogs end up in animal shelters for a variety of reasons, whether they were found as a stray or relinquished by previous owners. It is now estimated that over three million dogs enter animal shelters in the United States each year (ASPCA, 2022). While shelters may differ in operation, there is a common goal to get pets adopted as quickly as possible. However, this goal is not always achieved. The amount of time a dog is housed in the shelter, termed length of stay, can vary especially depending on the type of shelter. The two main types of shelter policies are open and closed admissions. Traditional shelters, often operated by municipalities, generally have open admissions policies meaning they are obligated to take all animals brought to their doors, regardless of the condition the animal is in or the reason for its arrival. In contrast, privately funded shelters generally adopt a closed admissions policy meaning that they are not obligated to accept all animals for admission. Reasons for denying an animal admission to a shelter may include if the shelter is already at maximum capacity, if the animal appears to be unhealthy, or if the animal appears to defy the shelter's admission criteria. Dogs in shelters with open admissions policies generally have a shorter length of stay since there is a large turnover of animals. This means that euthanasia may be used as a means to create room for more animals. Conversely, dogs in shelters with closed admissions policies generally have a longer length of stay and these shelters tend to use euthanasia only when necessary. While not all shelters follow these trends, it is important to understand that the type of shelter will likely impact a shelter dog's length of stay. The welfare of dogs becomes a greater issue with increased time spent in the shelter. This is due to the fact that prolonged social isolation as a

result of confinement can lead to chronic stress, which is especially evident for dogs as an obligate social species.

There are efforts in place at many shelters to help mitigate the effects of social isolation. Some shelters may implement group housing to increase social interaction between dogs, in which there are known benefits. In particular, outdoor group housing in beagles resulted in significantly less stress behaviors than in beagles housed individually indoors (Beerda et al., 1998). However, group housing comes with risks for dogs with unknown histories. Most shelters offer both an indoor and outdoor kennel space for dogs which gives dogs the ability to choose where they want to be. This is a critical feature in the shelter environment as choice is something that shelter dogs are often deprived of. Additionally, some shelters may implement playgroups among the dogs in their care to capitalize on both physical and social enrichment. There are different approaches to running playgroups and while the intentions of playgroups are to alleviate stress in shelter dogs, sometimes playgroups can actually be more stressful for dogs. For example, there may be too many dogs in the yard at once which prevents dogs from having their own personal space. Playgroups also require careful planning and supervision to ensure the safety of all dogs, but not all shelter staff are properly trained in understanding dog behavior. If a dog feels threatened by another dog, its ability to flee is compromised so it will have to resort to fighting in order to escape. This not only puts the dogs, but also the shelter staff, at risk for injury.

While shelters have the best intentions for the dogs in their care, the severe physical and social restrictions of the shelter limit dogs from expressing natural behaviors. Even when shelter dogs are given the ability to socialize with other dogs, they are still lacking significant human interaction. This lack of human interaction is arguably the main contributor to stress in shelter dogs, and prolonged lack of human interaction can lead to detrimental behavioral and health effects in shelter dogs.

III. What is stress?

The topic of stress is of particular interest among scientists because there is no universal definition for it. The term “stress” was first explored by Hans Selye in its relation to physical health and emotional states. Stress in both humans and animals has been studied extensively

as it is often perceived in a negative context. Chronic stress can lead to elevated blood pressure and increased risk of heart attacks in addition to accumulation of fat tissue and weight gain (Harvard Health Publishing, 2020). However, stress is not always a bad thing. There are two types of stress: eustress and distress. Eustress is a beneficial form of stress that increases one's ability to interact effectively in the environment. In dogs, eustress has been documented as increased excitement and arousal leading into an agility competition (Pastore et al., 2011). In people, eustress is often defined in the context of anticipation for an event, such as the first day of a new job. On the other hand, distress is the type of stress with negative implications which may make an animal unable to cope in its environment. Distress is often observed among dogs in the shelter environment with repeated exposure to stressors. A dog's inability to cope with stress is commonly displayed with stereotypical behaviors such as pacing or excessive grooming. Selye also emphasized the difference between stress and stressor, being that stressors are the stimuli that elicit a stress response (AIS, 2021). The ability to identify stressors has important implications for welfare, such as environmental modification to mitigate negative stress responses. Stress can further be broken down into four categories: acute, episodic, chronic, and traumatic. Acute stress is a type of stress that occurs suddenly with the stressor being easily identifiable. Episodic stress occurs from acute stressors that occur frequently or routinely. Chronic stress develops from repetitive and prolonged exposure to stressors and traumatic stress occurs from an intense physical or emotional experience.

From a biological standpoint stress is a normal, adaptive, physiological state. Stress has impacts on diverse physiological systems including the autonomic nervous system, neuroendocrine system, and immune system (Moberg and Mench, 2000). Behavior is consequently affected by these physiological systems, but behavioral responses differ on an individual level. An immediate stress response is characterized by the autonomic nervous system as the mobilization of energy to meet a challenge when the body shifts from parasympathetic to sympathetic nervous activity. Parasympathetic nervous activity includes food digestion and energy storage and occurs when the body is at rest. It is also referred to as the "rest and digest" stage as the body works to restore homeostasis. Sympathetic nervous activity is characterized by increased heart rate, blood pressure, gastro-intestinal activity, and the release of adrenaline in the presence of a threat. It is known as the "fight or flight" response and this process utilizes energy that has been built up during the parasympathetic stage (Fritz et al., 2008). Though there is often a negative connotation associated with the term "stress," a stress response is actually beneficial because it allows animals to survive in the wild. For

example, animals will shift to sympathetic activity in the presence of a predator to either fight the predator or escape from it. Stress becomes maladaptive when the response is disproportionate to the situation due to prolonged or repetitive exposure to stressors.

The second part of the stress response involves the neuroendocrine system with activation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis serves to maintain the body's sympathetic nervous system response. The hypothalamus releases corticotropin-releasing hormone and vasopressin which prompts the release of adrenocorticotropic hormone from the pituitary gland. Cortisol is released from the adrenal gland as a result (Harvard Health Publishing, 2020). Cortisol is the primary hormone associated with stress and has been used in numerous studies to assess animal welfare (Tuber et al., 1996, Hennessey et al., 1997, 1998, 2001, Beerda et al., 2000, Coppola et al., 2006, Stephen and Ledger, 2006, Rooney et al., 2007, Dalla Villa et al., 2013, Jones et al., 2014, Epstein et al., 2021). Cortisol release is slower than that of catecholamines like adrenaline and can take several hours after a stressful event to take effect (McEwen and Sapolsky, 1995). It is important to analyze glucocorticoid levels with caution as glucocorticoids have a circadian rhythm in addition to being released following stressful situations. They may also be indicative of activity or arousal levels (Dawkins, 2003). Glucocorticoids are often at higher levels during time of waking (Hennessey, 2013). Furthermore, these rhythms can be dysregulated by prolonged stress (Palme and Möstl, 2002). Elevated glucocorticoids are a short-term adaptation to stress, but chronic elevation of glucocorticoids like cortisol can have negative effects on physical health such as the inability to fight infections, immune suppression, and hyperglycemia (Moberg and Mench, 2000). Chronic stress also affects welfare as it is associated with anxiety and depression (Mumtaz et al., 2018, Moberg and Mench, 2000). A major problem with chronic stress is that it can lead to dysregulation of the HPA axis, meaning baseline levels of glucocorticoids are not restored (Hennessey, 2013). Negative feedback mechanisms to keep glucocorticoid levels in balance can be ineffective when the HPA axis remains activated with chronic stress (Mendoza et al., 2000). Over time, this can manifest into a reduction in glucocorticoid release during stressful situations where an elevated glucocorticoid level would be expected. In shelter dogs it is unclear whether the eventual decrease in glucocorticoid levels is an adjustment to the environment or dysregulation of the HPA axis (Hennessey, 2013). Therefore, cortisol levels may not be indicative of stress level when examined in a dog experiencing chronic stress.

IV. What is stress for a dog?

Stress induces various behavioral responses in dogs that are unique to the individual dog based on factors such as previous experiences and signalment. Chronic stress is the type of stress most often associated with dogs in the shelter. Stress behaviors in the shelter are of particular concern because they are undesirable to potential adopters and may cause the dog to be less adoptable. Furthermore, dogs that spend more time in the shelter are more likely to develop long-term behavioral problems or be euthanized (Wells and Hepper, 1992). The three main behavioral measures of chronic stress are the occurrence of abnormal behavior, absence of normal behavior, and changes in behavior pattern. Evaluating these measures in the shelter environment can be difficult because the previous history and baseline normal behavior of dogs is often unknown. Therefore, there are many studies that evaluate biological indicators of stress through cortisol expression in dogs. Dogs with prolonged exposure to glucocorticoids like cortisol have been shown to display more behaviors indicative of stress (Hennessey et al., 2020). It is important to note that the shelter environment produces a specific type of stressor, known as a psychogenic stressor. These stressors do not directly inflict physical harm on the animal, but rather are a result of novel events (Hennessey et al., 2020). Part of what makes the shelter environment so stressful for dogs is the novel environment itself, as most dogs entering the shelter have not had previous experience in these living conditions. In fact, one study found that cortisol levels of dogs initially entering the shelter were three times higher than that of pet dogs and remained at these high levels for nearly three days before decreasing (Hennessey et al., 1997, 2001). Others report elevated cortisol levels in shelter dogs for several days upon intake before decreasing (Coppola et al., 2006, Stephen and Ledger, 2006). Even dogs with previous habituation to kenneling showed a two-fold increase in cortisol levels when placed in the kennel environment (Rooney et al., 2007). While physiological indicators of stress such as cortisol levels have been successful at determining a dog's stress level, they are not always reliable for assessing long-term stress. In other words, a low cortisol level does not mean that a dog is not stressed. Additionally, increased glucocorticoid levels do not always coincide with increased stress behaviors. For example, a study by Epstein et al. found that dogs exposed to auditory and visual stimulation in the shelter environment showed a decrease in stress behaviors compared to that of a control group, but there was no effect in cortisol level between the two groups (2021). Long-term stress can also lead to dysregulation of the HPA axis, which can be difficult to use as a sole measure of stress. There are other benefits to including behavioral measures of stress as they are non-invasive and easily observable. Due to a lack of

consistency between physiological and behavioral observations in shelter dogs, one cannot be deemed more critical to the evaluation of animal welfare than the other, but they are often used in tandem to evaluate a dog's welfare.

A. Stressors in the shelter environment

As mentioned previously, the shelter environment has proven to be stressful for dogs due to its novelty as a psychogenic stressor. There are several factors including noise levels, unfamiliar environment, lack of control, and unpredictability that induce a stress response in shelter dogs. Noise is considered a chronic stressor in the shelter environment that can increase the frequency of stress behaviors. In particular, noise levels are known to be a factor contributing to cortisol levels. One study found that noise levels were highest in the large dog adoptable kennels, reaching a level of 90dB for over 50% of observations (Coppola et al., 2007). To put this in context, OSHA currently requires a hearing conservation program for workers with noise exposure at or above 85dB (Occupational Noise Exposure, 2021). Considering that dogs can respond to higher frequencies of sound that humans are unable to perceive, this level of sound is likely to have a negative effect on a dog's behavior and corresponding welfare (Barber et al., 2020). Although there is variation between shelters, it is uncommon for a shelter to be completely quiet. There is nearly constant stimulation from shelter workers, volunteers, and members of the public along with dogs moving in and out of their kennels for various reasons. A recent study found that dog-appeasing pheromone does not affect the frequency of barking but decreases the intensity of barking among shelter dogs (Hermiston et al., 2018). Even with such interventions it is very difficult to mitigate noise in the shelter.

Social isolation is arguably the biggest stressor for dogs in the shelter due to their social obligate nature. Paired with increased time in the shelter, it is likely to increase the occurrence of stress-related behaviors. A 2005 study examined the effect of time in the shelter on stress behaviors of single-housed dogs and found that dogs spent more time hiding from the front of the kennel and were less responsive to social stimuli with increasing length of stay (Stephen and Ledger, 2005). Dogs are an obligate-social species, meaning they desire social interaction between both con- and contra specifics. In a study assessing housing conditions in purpose-bred laboratory beagles, it was found that vocalizations increased with social isolation, but not in response to spatial separation (Hetts et al., 1992). There are also numerous studies evaluating

the relationship between humans and dogs and how these relationships affect biological parameters. One study found that plasma glucocorticoid levels in shelter dogs decreased in the presence of human caretaker but did not change when housed with a littermate since the age of eight weeks (Hennessey et al., 2020). Additionally, salivary cortisol levels were lower in dogs that received human interaction upon the second day of entering the shelter than dogs that did not receive human interaction (Coppola et al., 2006). This emphasizes that it is not just the act of confinement that causes stress in dogs, but rather the lack of social interaction with humans as a result of confinement. When dogs are confined to a kennel without sufficient opportunities to interact with people, they subsequently develop more stress.

Common in-kennel stress behaviors include lowered body posture, averted eye gaze, backwards movement away from a stimulus, whining, and excessive grooming and yawning (Deldalle and Gaunet, 2013). Additionally, increased locomotor activity, paw lifting, and urination have been observed in stressed dogs (Beerda et al., 2000). Increased vocalizations in the form of whining, howling, growling, and barking are known to be indicative of stress. In fact, a study that examined the behavior of dogs housed in shelters for 6 weeks from intake found the most observed behavior in nearly 25% of dogs was excessive barking (Stephen and Ledger, 2005). Behaviors that are likely to increase the length of stay, the total time a dog spends in the shelter before being adopted, include rubbing along the kennel wall, standing, growling, repetitive pacing, and orientation toward the back of the kennel (Protopopova et al., 2014). These behaviors parallel stress behaviors listed in other studies, which suggests that stress behaviors have a negative effect on adoptability and may lead to a longer length of stay. Chronic stress can also lead to behavior problems, which not only affect adoptability but also the safety of both the dog and people. It is common for long-stay shelter dogs to develop dog reactivity or aggression and it is almost impossible for shelter staff to predict how these behaviors will change outside of the shelter, making adoption counseling difficult. Sometimes euthanasia is the only option for shelter dogs who have developed severe behavioral problems. Additionally, continued exposure to stressors negatively impacts a dog's welfare and quality of life. Welfare is not only measured by physical health, but also by the ability of an animal to express its natural behaviors. When a dog is confined to a kennel, it is deprived of social interaction which is vital to their obligate social nature and part of what makes them so special. Compromised welfare and exposure to chronic stressors weakens the immune system, making dogs more susceptible to illness (Moberg and Mench 2000). Spread of disease is already a concern among shelters due

to the sheer number of animals entering the shelter carrying potential disease and the layout of the shelter itself with often insufficient ventilation.

V. Cognition

In addition to effects on glucocorticoid levels and subsequent behavior, chronic stress also affects cognition. The loss of neurons in the hippocampus, which has a significant role in memory, has been documented with prolonged exposure to stress, resulting in irreversible hippocampal dysfunction (McEwen and Sapolsky, 1995). This parallels the pattern of neuronal loss seen in aging animals, but is at an accelerated rate in cases of extreme stress.

There are two types of steroid hormone receptors in the brain, both of which are abundant in the hippocampus: mineralocorticoid receptors and glucocorticoid receptors. Mineralocorticoid receptors bind cortisol with higher affinity and are involved in behavioral reactivity in novel situations while glucocorticoid receptors aid in consolidating learned information (De Kloet et al., 1999). When hormones like cortisol bind to receptors in the hippocampus, the receptors coordinate the most adaptive response to the situation. However, repeated exposure to stressors is detrimental and impacts the brain's ability to cope with stress (De Kloet et al., 1999). There are multiple studies that examine aging and the role of the HPA axis, and chronic stress is thought to mimic aging effects on the HPA axis (McEwen and Sapolsky, 1995). Both aged rats and dogs show a reduction in the binding capacity of mineralocorticoid receptors, thereby resulting in a reduced mineralocorticoid: glucocorticoid receptor ratio (Rothuizen et al., 1991). This reduced ratio increases the basal activity of the HPA axis, leading to hyperadrenocorticism (Rothuizen et al., 1991). Chronic stress increases the release of glucocorticoids, which over time result in the inability to effectively turn off stress responses. As seen in aging, this also leads to hyperadrenocorticism and hippocampal neuronal loss which negatively affects memory function (Rothuizen et al., 1991).

There is evidence that social isolation affects cognitive ability in rodents (Liu et al., 2020, Koike et al., 2009). In particular, social isolation stress in early life experiences have been linked to neurological disorders including memory loss (Mumtaz et al., 2018). Adult mice housed individually performed more poorly on cognitive tasks than group-housed mice (Liu et al., 2020). Moreover, social isolation rearing in mice caused learning and memory deficits (Koike et al., 2009). Research is lacking in regard to social isolation and cognitive ability in dogs, but studies

with rodents have implications for human neurological disorders which may parallel that of dogs.

A dog's performance on a cognitive memory task may give insight as to how stressed a dog is in the shelter environment. Dogs experiencing chronic stress often develop a stress response that is disproportionate to the situation in terms of arousal level. This can be explained using the Yerkes-Dodson law with performance graphed on the y-axis and arousal graphed on the x-axis. The relationship between performance and arousal produces an inverted-U shape which illustrates that performance is optimized when an individual is neither under nor over aroused (Mind Tools, n.d.). This concept can be applied to the stress shelter dogs experience since these dogs are almost always at a peak arousal level from constant environmental stimulation. In this case it is not the under arousal, but rather the constant over arousal from the shelter environment that may push a dog into a state of fatigue or exhaustion. This leads to a stressful state in which a dog's performance will steadily decline. As previously discussed, stress can be measured through both physiological and behavioral means, but behavioral measures may be more insightful for long-stay dogs experiencing chronic stress due to potential dysregulation of the HPA axis. Moreover, the task itself may serve as a form of cognitive enrichment to mitigate cognitive decline associated with chronic stress. A two-year study with laboratory beagles found that beagles who experienced behavioral enrichment through cognitive testing had improved discrimination learning compared to the control group, indicating that this intervention improved cognition with age (Milgrim et al., 2005). If cognitive enrichment can decrease the effects of neuronal loss seen with aging, an effect that is mimicked by chronic stress, then cognitive enrichment may also reduce stress by decreasing the presence and/or frequency of stress behaviors.

VI. Conclusion

Given that shelter dogs are likely to experience stress from the shelter environment and consequently from a lack of human interaction, they are likely to develop stress behaviors with increasing length of stay. Since stress behaviors can be perceived negatively by potential adopters, stress has negative implications for both the animal shelter and the dog itself as the shelter will have to house the dog for a longer period of time and the dog will endure more stress. Considering the relationship between physiological stress response and cognitive

function, measuring cognitive ability in shelter dogs may serve as a way to better understand the level of stress a dog is experiencing. This information could be used to advocate for chronically stressed dogs in need of foster placement or to better adapt kennel spaces to minimize stressors. This study aims to evaluate the behavioral responses of dogs housed in a shelter and their subsequent performance on a cognitive memory task in effort to determine if performance correlates with stress behaviors and if stress behaviors decrease as a result of participation in the cognitive task.

METHODS

Subjects:

The subjects of this study were 36 adoptable dogs (20 treatment, 16 control) at the Humane Society of Southern Arizona (HSSA) from August 1, 2021- December 16, 2021. These dogs were vetted for adoption by both behavioral and medical staff before data collection took place. History was obtained for each dog including sex and reproductive status, age, intake source (stray, owner surrender or transfer), date of intake, and date available for adoption.

The inclusion criteria for dogs in the study were as follows:

- Dog must be between 6 months and 10 years old
- Dog must be available for adoption between 4 and 30 days; permissible if the dog was taken off adoption for medical hold, as long as the adoptable window still fell within the 4-30 day period. This means the dog may have physically been in the shelter for longer than 30 days.
- Dog must be medically and behaviorally cleared during the time of data collection

Housing:

The dogs in the study were housed in one of four kennels all located on the adoption floor. The four kennels used in this study were kennels 15, 16, 25, and 26 (figure 1). The dimensions of the interior portion of the kennel were 6 ft x 6 ft and the dimensions of the exterior portion of the kennel were 8 ft x 6 ft. A 5 ft wall divided each row of kennels, so a dog was unable to see other dogs from its kennel. All kennels had a guillotine door leading to the exterior portion. Public viewing of adoptable dogs at the Humane Society was limited to the interior portion only.

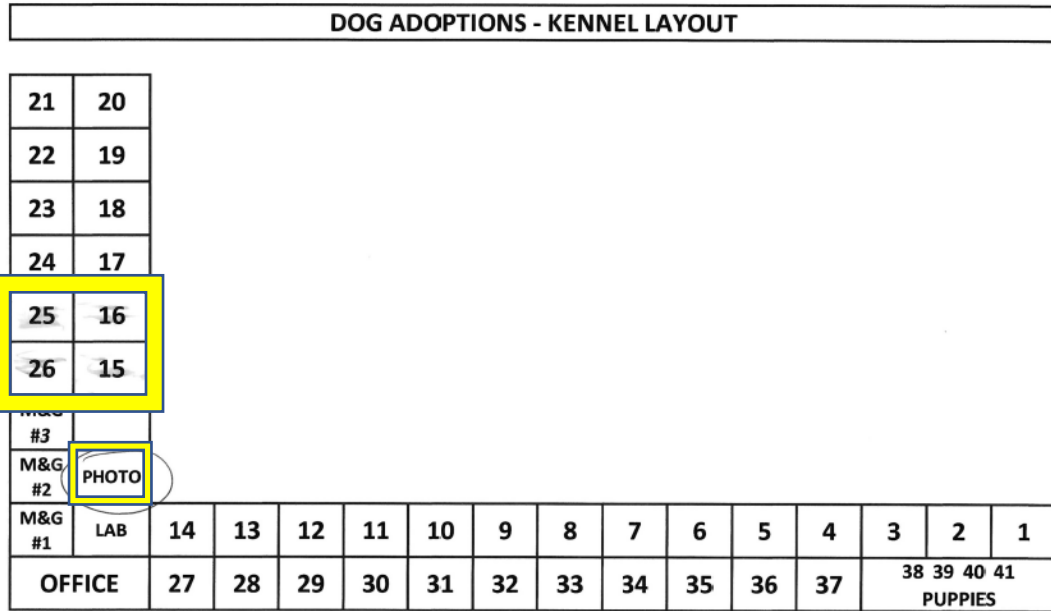


Figure 1. Layout of adoptable dog kennels courtesy of HSSA. Kennels 15, 16, 25, and 26 housed the dogs in this study. The photo room was used for cognitive testing.

Experiment:

Behavioral observations were recorded using a Reolink security camera system between 11am and 12pm on Tuesday, Wednesday, and Thursday from August 1, 2021-December 16, 2021. The cameras were secured to the dividing wall using a flexible tripod. During this time, the guillotine door was closed and dogs were limited to the interior portion of the kennel only to allow for uninterrupted behavioral observation. Dogs selected each week for the study were moved to one of the four kennels no earlier than Monday afternoon and no later than Tuesday at 8am. Data collection began on Tuesday at 11am each week.

Up to two dogs were selected for the experimental condition and the remaining dogs were designated as the control group each week. Dogs in the experimental group were selected randomly using a random number generator. Dogs with specialized dietary needs were excluded from the test group and made part of the control group. Dogs selected for the experimental group were brought to the photo room (11.4 ft x 13.5 ft) on Wednesday morning between 7am and 8am each week. The room was located close to the kennels (figure 1) but served to minimize distractions as there were no windows. Upon entry the dogs were given two minutes to acclimate to the room with the experimenter and assistant present. During this time

the dog was free to explore the room off-leash. The experimenter offered the dog a treat from her hand and subsequently placed a treat in one of the boxes centered at the front edge of the mat. Raw hot dog slices were used as the treat and the experimenter was the only individual to hand the dog treats. It was noted whether the dog ate the treat from the experimenter's hand and/or from the box during the acclimation period. After two minutes, the experimenter brought the dog to the door while the assistant positioned three opaque boxes on the mat using velcro (figure 2).

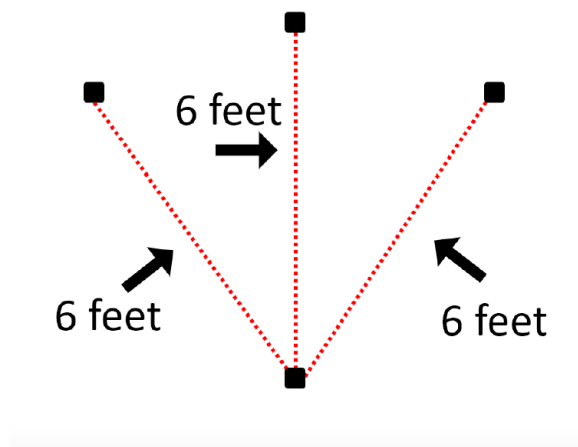


Figure 2. Array setup for the opaque boxes used in the cognitive task courtesy of the Dog Aging Project (DAP). Each box is placed 6 feet from the start line as shown in the figure.

Warmups:

Dogs in the experimental condition participated in a cognitive task measuring spatial memory. The test involved three boxes which were placed in an array 6ft from the start line (figure 2). The experimenter began at the start line and walked the dog in a counterclockwise loop around box 1 and placed one treat inside the box. The dog was prevented from eating the treat during this time as the experimenter kept a short leash. Once back at the start line, the assistant began a 20s timer and the experimenter loosened the dog's leash to allow the dog to search for the treat. The outcome for each warmup trial was recorded as either success (1) or fail (0). Success was defined as the dog putting its head over or into the correct box (choice) within the 20s period. Success was not contingent upon the dog eating the treat. Fail was defined as the dog not making a choice during the 20s period. Two more warm up trials using box 2 and box 3 were completed using the same procedure as warmup 1. If a dog failed a warmup trial, the experimenter guided the dog to the correct location and allowed the dog to eat the treat before

moving onto the next warmup trial. It was required that a dog be successful on 2 of 3 consecutive warm up trials to move onto testing. If a dog did not successfully complete 2/3 of warm up trials, a second set of 3 warm up trials was administered following the same order as the first set (box 1, box 2, box 3). Dogs were required to successfully complete 2 trials within a round of 3 trials to move onto testing.

Test:

Dogs successful in warmups moved onto test trials as described in the “1-2-3 Treat” game of the Dog Aging Project. The Dog Aging Project is a nation-wide initiative aimed at collecting data from pet dogs to better understand how factors such as cognition affect aging (Creedy et al., 2022). The premise of this task is that the dog should remember the location of the baited box in which they were not initially able to eat the treat from. There were 9 trials administered without repeats, with the exception of repeats for experimenter error (EE). The experimenter kept the dog on her right side and walked around the boxes in a counterclockwise loop as in warmups, making sure to keep the dog on her right side away from the boxes. The experimenter carried 3 treats and baited each of the three boxes when walking, allowing the dog to eat from only two of the boxes once the treat was placed inside. For the first round, the experimenter baited box 1 and did not allow the dog to eat from it but allowed the dog to eat from boxes 2 and 3 once baited. This is displayed in figure 3, with the red “X” indicating the baited box. The experimenter returned with the dog to the start line and positioned the dog at the center of the start line facing the boxes. The assistant began the 20s timer once the dog was correctly positioned at the start line. The experimenter then loosened the dog’s leash while maintaining a hold on the leash to allow the dog to search for the baited treat in box 1. Holding the leash prevented the dog from making multiple choices per trial. A dog’s choice was recorded as the box number that the dog searched first. A choice was defined as the dog putting their snout over or inside the box, even if they did not eat the treat. If the dog did not make a choice during the 20s period, the choice was recorded as “no choice” (NC). The remaining 8 trials were repeated following this method, with the baited box changing between trials as indicated in table 1.

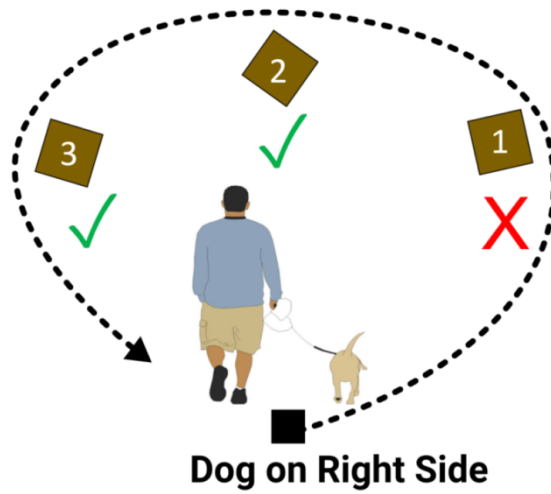


Figure 3. Experimental set up for trial 1 of DAP “1-2-3 Treat” Game courtesy of DAP. Box 1 with the red “X” is baited while boxes 2 and 3 with the green check marks indicate the dog was able to eat the treat immediately from the boxes.

Round	Baited Box
1	1
2	2
3	3
4	2
5	3
6	1
7	3
8	2
9	1

Table 1. Order of baited box location for 9 trials of cognitive test.

Coding:

An ethogram was developed to record each dogs' in-kennel behaviors. The two categories of the ethogram were "states" and "events" displayed in tables 2 and 3, respectively. State behaviors were coded based on duration and event behaviors were coded based on occurrence. This ethogram was adapted from several studies which evaluated behaviors indicative of poor welfare, behaviors influencing length of stay, and behaviors used to develop a behavioral stress assessment score in shelter dogs (Stephen and Ledger, 2005, Protopopova et al., 2014, Jones et al., 2014). Behavioral observations using the ethogram were made for all dogs (experimental and control conditions). Two 5-minute video segments across three consecutive days of data collection were coded for each dog (n=216). This time segment was consistent upon all dogs and all days of observations (11:20-11:25am and 11:35-11:40am). All coding was completed in excel. 16 students at the University of Arizona enrolled in an upper division applied animal behavior course were taught how to code videos using the ethogram by the experimenter. Students were assigned to code either states or events for 4-6 dogs assigned randomly. The students' coding was compared to the experimenter's coding to determine reliability across all measures of the ethogram. Several behaviors were dropped prior to analysis due to low frequency and/or poor inter-rater agreement. The state behaviors, pacing ($r = 0.99$) and panting ($r = 0.972$), were dropped due to low frequency despite high correlation coefficients. Event behaviors including full body shake ($r = 0.703$), full body stretch ($r = 0.812$), self-scratching ($r = 0.702$), and growling ($r = -0.00562$) were dropped.

The remaining behaviors were analyzed through principal component analysis (PCA). A coefficient was calculated for each principal component category. Larger positive values indicated that the dog had performed more of the behaviors in a category while larger negative values indicated that the dog was observed to have performed fewer of the behaviors in a category. Each video received its own PCA score for each behavioral category. These scores were evaluated to compare how the behavior of the dog changed across the three-day observation period and whether the condition (experimental or control) affected the presence or absence of stress behaviors.

Behavior	Operational Definition
STATES (Code duration of behavior)	
MAINTENANCE (Mutually exclusive) <i>*To change behavior, new behavior must occur for a minimum of 3 seconds</i>	
Resting	Limbs are tucked under or extended in front of the dog's body. Limbs may be extended while the dog lays laterally. Head can be elevated or lowered, eyes opened or closed. Body remains stationary, but head can move
Standing	Dog is supported upright with all four legs; feet stationary
Sitting	Dog is supported by two extended front legs and two flexed back legs
Walking	Dog uses all limbs to move from one location in the kennel to another
Pacing	Dog moves around the kennel in a fixed route (circular or linear pattern) at least 2 times
Other	Dog is not doing any of the above maintenance behaviors; is instead doing event to be coded individually or other behavior not listed in ethogram
KENNEL POSITION (Code duration)	
Front of kennel	More than half of the dog's body is positioned in front half of the kennel
Back of kennel	More than half of the dog's body is positioned in back half of the kennel
BARKING (Code duration) <i>*Barking must stop for at least 10 seconds to code separate observations</i>	
Barking	Vocalization of short duration and low frequency
PANTING (Code duration) <i>*Panting must stop for at least 10 seconds to code separate observations</i>	
Panting	Open mouth with tongue extended; audible and/or observable breathing

Table 2. Ethogram for state behaviors in shelter dogs.

Behavior	Operational Definition
EVENTS (Code occurrence of behavior)	
VOCALIZATION	
Whining	Dogs' lips are closed and ears are back while making a long, high-pitched sound varying in intensity; cyclic vocalization
Growling	Low pitched rumbling vocalization; lips pursed or slightly open
Howling	Prolonged high amplitude vocalization of varying pitch. The dog's head is bent back and its neck is straight with the nose being the highest point.
MOVEMENT	
Jumping	Dog is standing on hind limbs and one or both front limbs make contact with the kennel door or wall
Lip licking	Dog uses tongue to lick upper lip and/or nose
Yawning	Opens mouth widely so lips are pulled back as dog inhales
Full body stretch	Extending both front limbs with head and shoulders lowered and hips as highest point; may be followed by shifting weight to front limbs as one or more back limbs are extended
Full body shake	Motions head and body side to side repeatedly and rapidly
Scratching self	Paw makes repeated contact with body or face; head may be angled in direction of moving limb

Table 3. Ethogram for event behaviors in shelter dogs.

RESULTS

Parallel analysis suggested that the behavioral observation data could be summarized using two principal components. The principal components analysis was performed using a varimax rotation, to maximize variance in item loadings across components. Component 1 was primarily loaded positively by standing ($r = 0.79$), walking ($r = 0.62$), barking ($r = 0.79$), lip licking ($r = 0.70$), and yawning ($r = 0.53$) and negatively by resting ($r = -0.85$) and position in the back of the kennel ($r = -0.72$). Thus, we interpreted scores on this component as reflecting active stress behaviors. Component 2 was primarily loaded positively by sitting ($r = 0.75$), barking ($r = 0.22$), whining ($r = 0.47$), and howling ($r = 0.73$) and negatively by resting ($r = -0.33$) and position in the back of the kennel ($r = -0.22$). Thus, we interpreted scores on this component as reflecting vocal stress behaviors.

The average active stress and average vocal stress principal component scores were calculated for each group, experimental ($n=20$) and control ($n=16$) and plotted against time. Average active stress scores decreased across the three-day observation period for both groups (figure 4). Average vocal stress increased across the three-day observation period for both groups, although the control group had a decrease in vocal stress score from day 1 to day 2 (figure 5).

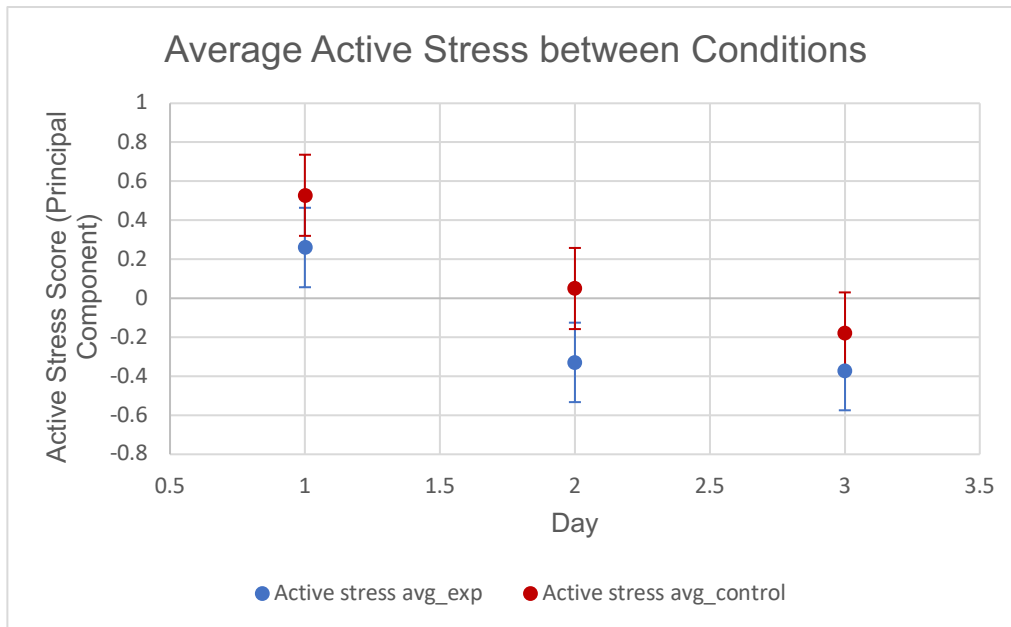


Figure 4. Average active stress scores vs time for both conditions.

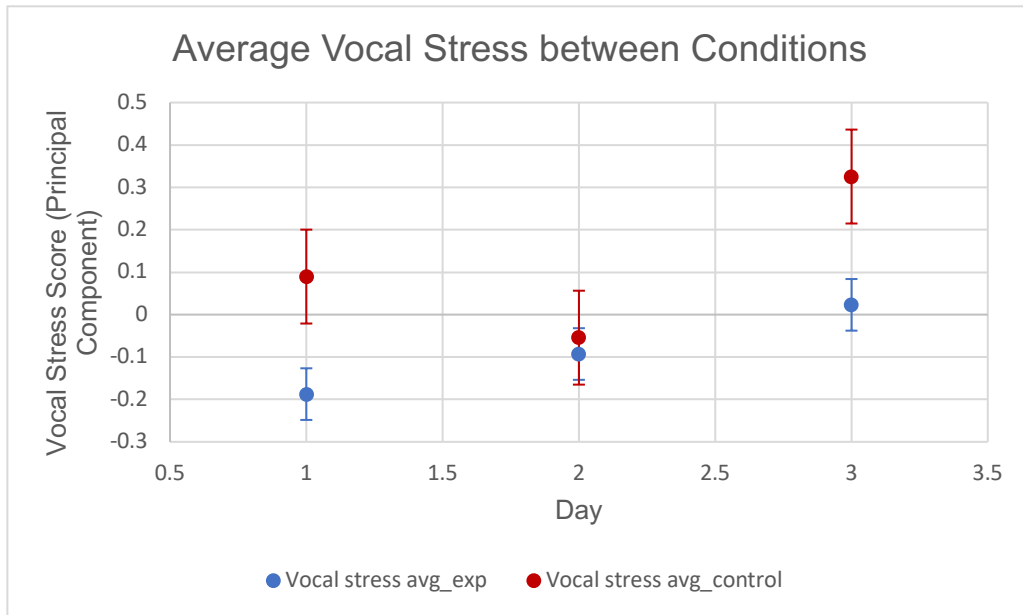


Figure 5. Average vocal stress scores vs time for both conditions.

The cognitive test scores (proportion correct) for experimental dogs were plotted against the dogs' individual active stress and vocal stress principal component scores for days 1-3 (figure 6). Only experimental dogs that completed 5 or more trials were included in the analysis (n=17). The graphs show a slight negative correlation between cognitive score and both active stress and vocal stress scores across all days. This means that dogs with a high cognitive test score were likely to have a lower active stress or vocal stress score while dogs with a low cognitive test score were likely to have a higher active stress or vocal stress score. However, there is not sufficient evidence to conclude that the cognitive test score reliably predicts stress score. This is indicated by the large range of values above and below the trend line.

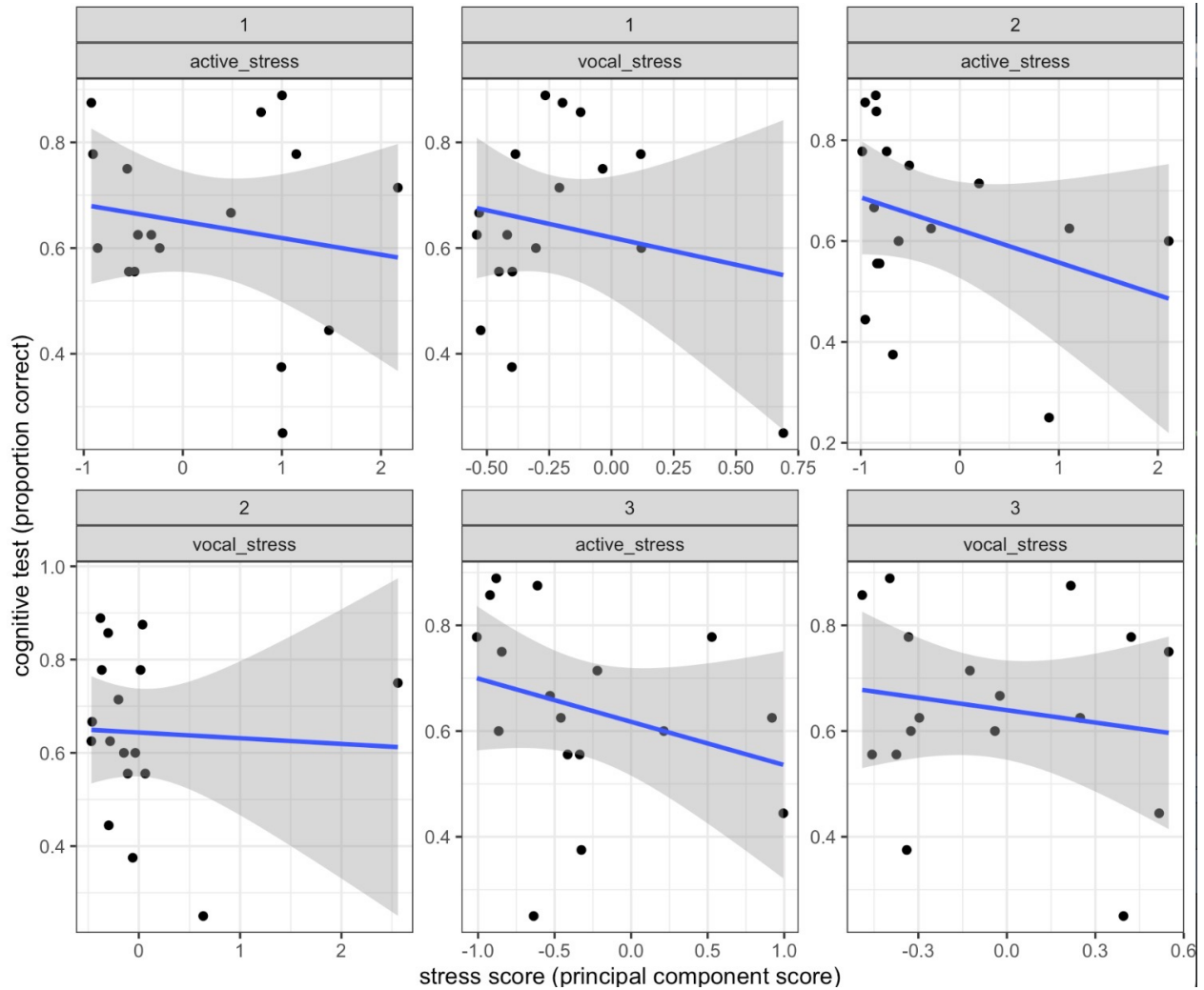


Figure 6. Cognitive test score as a function of active and vocal stress scores by day.

The active stress and vocal stress principal component scores across the three-day observation period were also compared between dogs in the experimental group that completed 4 or less trials ($n=3$) and dogs that completed 5 or more trials ($n=17$). These results are displayed in figure 7. Average active stress scores for dogs that completed 4 or less trials had a greater decrease than the average active stress scores for dogs that completed 5 or more trials. Dogs that completed 4 or less trials had a greater increase in average vocal stress scores than dogs that completed 5 or more trials.

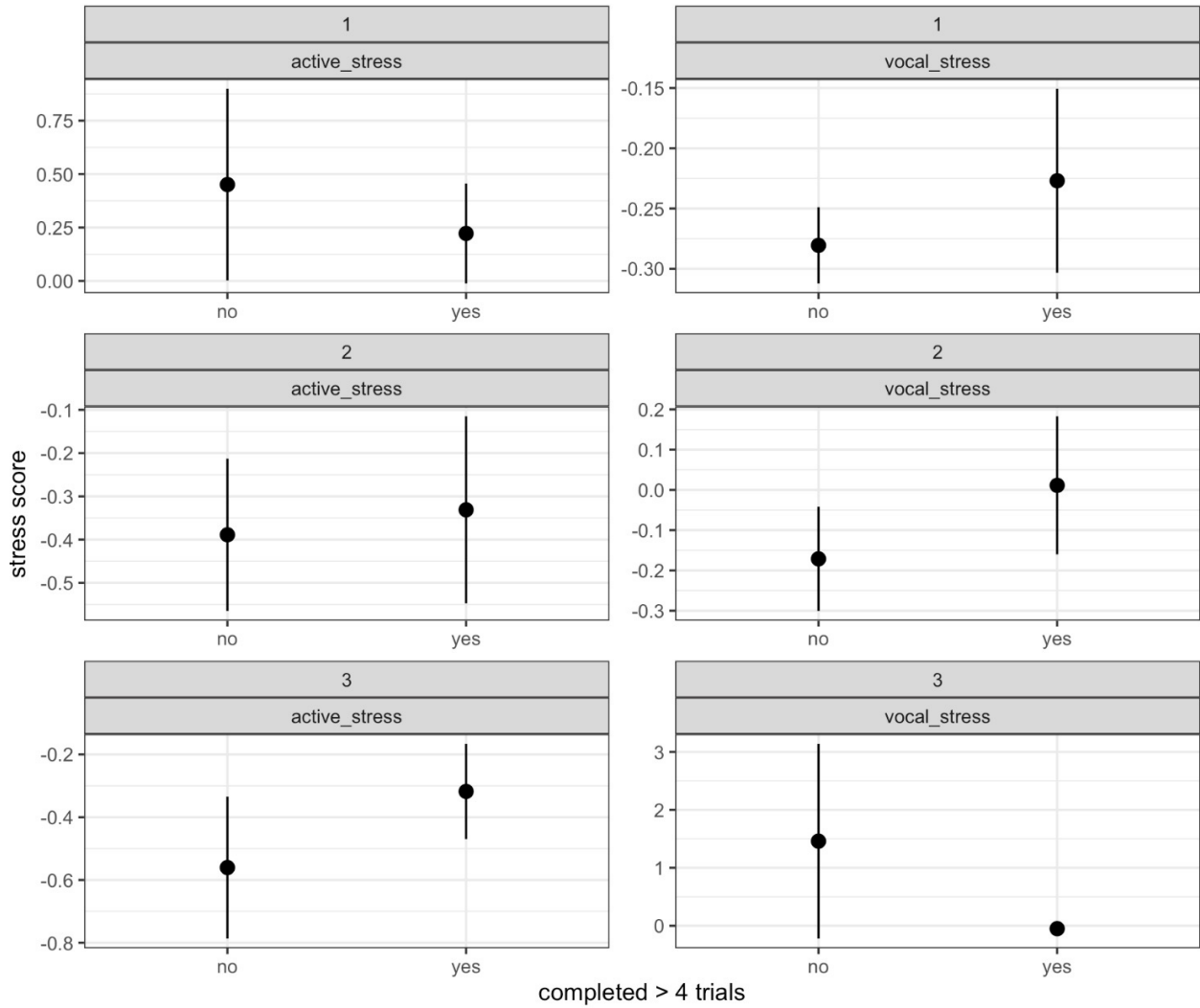


Figure 7. Stress score by day as a function of number of trials completed.

DISCUSSION

STRESS BEHAVIORS BETWEEN CONDITIONS:

It was predicted that dogs that participated in the cognitive task (experimental group) would have fewer stress behaviors overall compared to dogs in the control group because of participation in the task itself. Not only does the cognitive task serve as a potential method to identify stress levels in shelter dogs, but it also serves as a form of enrichment by increasing the amount of human interaction a dog receives aside from twice daily walks by shelter volunteers. Previous literature shows that shelter dogs who experience greater levels of human interaction have lower cortisol levels than dogs that experience less human interaction (Hennessey et al., 1997, Coppola et al., 2006). Although this study does not evaluate cortisol levels, it can be speculated that dogs with increased human interaction will be less stressed than dogs with routine levels of human interaction.

Two different categories of behavior, active stress and vocal stress, were assessed to identify stress level between both groups over time. Average active stress scores determined by principal component analysis for the experimental and control groups across time are displayed in figure 1. While the average active stress scores for both conditions decrease from days 1-3, it was expected that the average active stress score from day 2 to day 3 would have the most profound effect. This is because day 2 is the day in which the cognitive task was administered to the experimental group of dogs. Dogs with this intervention were more likely to benefit from increased human interaction since they are an obligate social species, and presumably display fewer stress behaviors on the day following the cognitive test (day 3). However, this was not the case, as the control group of dogs had a greater decrease in average active stress score from day 2 to day 3 than the experimental group of dogs. While there were individual dogs in the experimental group that showed a large decrease in active stress scores from day 2-3, the average active stress score for experimental dogs from day 2-3 decreased by a standard deviation of less than 0.1, while the average active stress score for control dogs from day 2-3 decreased by a standard deviation greater than 0.1. Therefore, the dogs in the control condition seemed to be slightly less stressed on day 3 than dogs in the experimental condition, on average.

It is also important to consider the discrepancies between locomotion, or active stress behaviors, and resting behaviors in their relation to a dog's stress level. There are multiple studies which evaluate the locomotor activity of dogs in the kennel but have found conflicting results. For instance, it has been shown that dogs housed individually without a kennelmate have both increased locomotor activity (Hetts et al., 1992, Beerda et al., 2000) and decreased locomotor activity (Hubrecht et al., 1992). More recently, it has been suggested that there is a significant correlation between salivary cortisol and maximum activity level in shelter dogs, meaning dogs with high salivary cortisol levels display more activity in the kennel (Jones et al., 2014). Therefore, increased activity level may serve as a good indicator for stress in shelter dogs. Even though an increase in active behaviors such as walking, jumping, and pacing are likely to mean a dog is more stressed, a significant decrease in activity level can also mean that a dog is stressed. In other words, it cannot be assumed that dogs who spend the majority of their time resting in the kennel are not stressed. Rather, both significant increases and decreases in activity level can be indicative of stress and must be considered when evaluating a dog's welfare. Resting behaviors were coded for in this study but not included in the principal component scores due to a lack of correlation with other variables. Therefore, measuring active stress behaviors among the control and experimental dogs seemed to be a more reliable indicator of stress in this experiment because the analysis grouped multiple active stress behaviors with positive correlations.

The second behavior category evaluated was vocal stress. Multiple studies confirm that increased vocalizations are a sign of stress among dogs in the shelter (Hetts et al., 1992, Beerda et al., 1998, Protopopva et al., 2014, Hermiston et al., 2018, Epstein et al., 2021). Vocalizations included in the vocal stress principal component analysis of this experiment were barking, whining, and howling. Figure 2 displays the average vocal stress principal component scores among conditions as a function of time. It was also predicted that dogs in the experimental condition would have an overall decrease in vocal stress score, especially from day 2-3 as a result of participation in the cognitive task. However, unlike the average active stress scores, there was an overall increase in average vocal stress scores in both the experimental and control groups from day 2-3. The increase in average vocal stress score in the control condition was greater than the increase in average vocal stress score for the experimental condition. It was expected that vocal stress scores would be higher for the control condition because these dogs did not participate in the cognitive task and were presumed to be more stressed because they received less human interaction.

To best compare the effect of the cognitive task on a dog's stress level, the stress scores for both active and vocal stress should be very similar for both conditions on day 1, the day prior to the administration of the cognitive task. However, there was a relatively large difference between both average active and average vocal stress scores on day 1. This makes it more difficult to compare the effect of treatment. Despite this, the greatest difference in behavior was expected to occur from day 2 to day 3, the day of the cognitive task and the day following the cognitive test, respectively. The average vocal stress score for both conditions was very similar on day 2, which serves as a good standard to compare the effect of the cognitive task on stress vocalizations on day 3. This was not the case for average active stress score, as the experimental condition had an average active stress score of -0.37 on day 2 and the control condition had an average active stress score of 0.05 on day 2.

A possible explanation for these results lies in the unpredictable nature of the shelter environment itself. It is nearly impossible to control traffic through the adoption floor on any given day as well as the amount of people visiting the shelter. This study had originally been designed so that recordings would take place when the Humane Society was closed to the public, but the shelter's hours changed during week 5 of the study. Therefore, behavioral observations were conducted during a time in which the public was allowed to peruse adoptable dogs in their kennels. The change in hours of operation affected the majority of dogs in the study as these dogs likely experienced heavier foot traffic through the kennels and potentially more attention from the public. Standard recording times were developed to help minimize the effect of outside stimulation, but it could not be avoided in all cases. Additionally, the Humane Society was closed to the public on various holidays like Veteran's Day. Veteran's Day fell on day 3 of data collection which may have affected a dog's stress level with less environmental stimuli. Despite this change, multiple dogs were observed each week and dogs within the same week of observation were subjected to the same environmental stimuli. Therefore, it may be of interest to compare stress scores among dogs by week as opposed to all dogs in the study simultaneously. This may or may not affect the trends observed in active and vocal stress. As previously mentioned, it was predicted that both active and vocal stress would decrease across time for the experimental condition and either increase or have less of a decrease across time for the control condition. However, there was a substantial increase in vocal stress from day 2-3 paired with a decrease in active stress from day 2-3 for both conditions. These results are somewhat conflicting as it would be expected that dogs displaying less active stress behaviors would also display less vocalizations indicative of stress or vice versa. This could potentially be

explained by the type of environmental stimulation presented to dogs from day 2-3. In general, people walking or standing in front of the kennels seemed to stimulate more active behaviors in dogs while dogs who experienced outside sounds without visual access to the source of the sound may have performed more vocalizations.

Another possible explanation for the cognitive task having little to no effect on the presence of stress behaviors in the experimental group is the nature of the task itself. The cognitive task was very brief, lasting approximately 10 minutes for each dog, which may not have been enough time to produce a measurable effect on stress behaviors. Therefore, it is not clear if one administration of the cognitive task provided enough mental stimulation and human interaction to mitigate stress behavior. Future considerations for this study include measuring the effect of the cognitive task as a long-term intervention in shelter dogs to see if stress behaviors decrease with repeated testing over time.

It is also important to recognize that a dog's observable behavior is affected by genetics, signalment, previous experiences, and the current environment. Therefore, individual dogs may react differently to the same stimuli presented. The dogs in this experiment came from multiple sources (found as a stray, relinquished to the shelter by owners, or transferred from another shelter). A dog's previous history and especially any previous experience with kenneling or confinement will likely impact their response to stimuli from the shelter environment. For example, a dog that has never been confined to a kennel before may be more stressed than dogs with previous kennel experience. Another way to analyze active stress and vocal stress scores across time could be to separate the dogs based on their source and compare the effect of source on the presence or absence of stress behaviors.

PERFORMANCE ON COGNITIVE TASK FOR EXPERIMENTAL DOGS:

Performance on the cognitive task was assessed for dogs in the experimental group since dogs in the control group did not complete the cognitive task. It was predicted that dogs that scored higher on the cognitive task, indicated by a greater proportion of choices correct, would display fewer behaviors indicative of stress. Likewise, dogs that scored poorly on the cognitive task would display more behaviors indicative of stress. These predictions were based on the observation that chronic stress, indicated by prolonged elevation of glucocorticoids, leads

to the loss of hippocampal neurons involved in memory development (McEwen and Sapolsky, 1995). Therefore, dogs experiencing chronic stress may be likely to have hippocampal neuronal loss, which impedes memory. This particular cognitive task was chosen because it involves spatial memory, a cognitive process that relies on the hippocampus.

The results are displayed in figure 3 which plots cognitive score as a function of stress score across the three-day behavioral observation period. Active stress and vocal stress scores are displayed individually by day. Broadly, there is a weak negative relationship between cognitive test score and stress score among dogs in the experimental condition. In other words, dogs with a high cognitive test score were likely to have a lower stress score (both active and vocal stress) across all days. However, there are outliers in the data in which dogs with a low stress score performed poorly on the cognitive task and dogs with a high stress score performed well on the cognitive task. There seems to be a slightly greater correlation between cognitive score and active stress than cognitive score and vocal stress, indicated by overall steeper slopes in the active stress graphs. This suggests that cognitive score may be more predictive of active stress behavior than vocal stress behavior. This observation is supported by the initial prediction that poor cognitive performance is representative of greater levels of stress. However, the relationship between the two is not significant, meaning there is not enough evidence to conclude that there is an effect between cognitive test score and stress. One reason for this is the limitation of the study with a small sample size. Dogs included in the analysis were required to remain in the shelter for the full 3-day observation period. So, if a dog was adopted at any point during this time, they were dropped from the data analysis even if they had already participated in the cognitive task. In turn, this drastically decreased the sample size.

Another factor to consider when analyzing cognitive performance is the general cognitive ability of dogs, especially among breeds. Although breed was not recorded in this study, it was unlikely that any two dogs had the same genetics. It is not fair to assume that all breeds have the same baseline cognitive ability, and breed will likely affect a dog's performance on the cognitive task. In fact, studies that pair behavioral data with breed-typical genotypic data have found there are specific genes expressed in brain tissue that account for breed differences in cognitive measures (MacLean et al. 2019, Gnanadesikan et al. 2020). Furthermore, some cognitive traits such as inhibitory control and communication are heritable among certain dog breeds, which can further impact cognitive performance (Gnanadeskin et al. 2020). There are also general stereotypes surrounding dog breeds that may reflect behavior, but these

stereotypes must be interpreted with caution because there can be a large variance of behavior within a breed. For example, German Shepherds and Rottweilers are commonly perceived as protective breeds and may be genetically predisposed to be more vigilant to their surroundings. This could cause these dogs to be more susceptible to stress in the shelter environment if they are able to pick up on more external stimuli and stressors. In turn, breeds that are more susceptible to stress may perform more poorly on the cognitive task compared to breeds that are less susceptible to stress. In this case, a lower baseline cognitive ability may actually be of benefit to dogs in the shelter environment because it would prevent a dog from picking up on many of the stressors presented. Therefore, a low cognitive score may not always be indicative of stress level, but rather more indicative of a dog's perception of its environment. Additionally, the cognitive test was brief, lasting 10 minutes on average, so it does not serve as a complete representation of a dog's memory capacity. In other words, memory function cannot be determined from this task alone, but the task can give insight into a dog's cognitive capabilities.

Lastly, it is important to reflect on the true meaning of "chronic stress." Chronic stress is generally defined as stress that persists for a prolonged period of time, but what actually is this time frame? Chronic stress is of concern in the shelter environment because it can lead to eventual dysregulation of the HPA axis, which makes it difficult to measure stress physiologically (Hennessey, 2013). This dysregulation of the HPA axis has implications for memory function which is pertinent in analyzing cognitive performance. As per the inclusion criteria, dogs in this study must have been available for adoption between 4 and 30 days, but could have physically been in the shelter longer than 30 days. At what point does a dog begin experiencing chronic stress? It may be of interest to compare cognitive performance between dogs according to their length of stay, as previous studies demonstrate that cortisol levels plateau 9-10 days after shelter intake (Coppola et al., 2006). If consistent cortisol levels are indicative of chronic stress, it may be meaningful to compare cognitive performance of dogs in the shelter less than 10 days and greater than 10 days to assess acute vs chronic stress. Dogs in the current experiment with a higher stress score and lower cognitive score may be experiencing chronic stress, while dogs with both a high stress score and high cognitive score may be experiencing acute stress.

COMPLETION OF COGNITIVE TEST TRIALS:

Figure 4 compares the active stress score and vocal stress score across time for dogs in the experimental group that completed 0-4 trials and 5-9 trials. This was in effort to determine if there is a relationship between stress levels and whether or not the dog could complete the majority of the cognitive task. Dogs that completed less trials were presumably too stressed, distracted by external noise in the shelter, or simply disinterested in the task. It was expected that these dogs would have higher active stress and vocal stress scores compared to dogs that completed 5-9 trials. While both groups had a decrease in active stress behaviors from day 2-3, dogs who completed 5-9 trials had less of a decrease in active stress than dogs who completed 0-4 trials. In contrast, dogs who completed 5-9 trials had little to no vocal stress from day 2-3 while dogs who completed 0-4 trials had a large increase in vocal stress from day 2-3. In fact, vocal stress among dogs who completed 0-4 trials on day 3 is about 1.5 standard deviations higher than dogs who completed 5-9 trials. This suggests that dogs who were unable to successfully complete more than half of the test trials experienced a type of stressor eliciting vocal stress responses as opposed to active stress responses. While these results are particularly interesting on day 3, they are not statistically significant and may not be the most reliable representation of stress given the small sample size for dogs who completed less than five trials (n=3).

CONCLUSION:

Overall, there is not significant evidence to suggest that stress behaviors decrease as a result of participation in the cognitive task. This is due to the fact that it is difficult to compare stress between conditions with different stress scores on day 1 as well as the opposing trends in average active stress and average vocal stress. In terms of performance on the cognitive task, there is a weak negative correlation between cognitive performance and stress score among experimental dogs from day 1-3. This suggests that poor cognitive performance may be indicative of dogs experiencing greater stress but is not conclusive as there are many outside variables affecting the data such as a dog's previous experience, breed, and the small sample size of the study. Additionally, the effect of the cognitive task on stress behaviors is likely minimal due to the brief nature of the task itself. If the test was administered more often as a form of enrichment, there may be a more significant effect on the presence and frequency of stress-related behaviors. Therefore, future considerations for this study include measuring the

effect of cognitive enrichment as a long-term intervention to not only increase mental stimulation, but also the amount of human interaction a dog receives. Since a lack of human interaction is thought to be the greatest stressor shelter dogs experience, making human interaction a greater component of the study may give more insight to stress level. This cognitive task is quick and easy to administer and has the potential to give shelter staff important information about a dog's stress level. Knowledge of a dog's stress level, especially in the shelter environment, can be of use when allocating resources for enrichment and advocating particular dogs for foster placement. Therefore, it would be in the interest of shelter staff to develop a method to identify stress levels in shelter dogs to not only maximize efficiency of resources, but also to put the welfare of shelter dogs at the forefront of their mission.

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