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Sound-associated open-field tests as a potential measurement for anxiety behaviors

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Abstract.

Anxiety behaviors are currently being considered as animal and human mental health aims for the development of advanced treatments. These behaviors have been linked to fear-based responses; categorized as social and non-social. This review is considering the use of sounds in an open-field test (OFT) to study anxiety and its associated behaviors in dogs. Non-social fear based responses, related to sound or noise were the focus of this study. The potential use of previous animal models were examined to decide if behavior measurements can be translated across species. An analysis of rodent, canine and human behaviors will help understand how open-field tests and its methodology can be translational. Open-field tests were deemed an accurate test to measure sound-associated anxiety behaviors if various factors are considered. Genetics, historical background, dog breed classification, and risk factors should be considered when developing an accurate test that measures anxiety behaviors. Significant differences were found between research-bred and client-owned dogs. Consequently, subjects in future research studies should consider variance in response to sound-associated fear within these two populations.

Introduction.

Behaviors involving anxiety are being considered as we understand factors that affect mental and emotional health of organisms. Anxiety is defined as “an emotional state that lacks a specific triggering event” (de Rivera, Ley, Milgram, & Landsberg, 2017). On the other hand, fear and anxiety are related to one another, since anxiety can result from ongoing fear (VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). Thus, anxiety, for this review, will be defined as an irrational, ongoing state of fear. Anxiety has been observed in humans as well as in dogs. Dogs are highly sensitive to sounds and research has a found that the introduction of a sound stimulus can cause a fear-based response (Gruen, et al., 2015). The objective of this literature review is to see if a sound-associated open-field test (OFT) can be used as a potential measurement of anxiety behaviors in canines (*Canis familiaris*).

Why we use of animal models?

Many examinations involving anxiety behaviors have been done to understand the origin and difference between fearfulness, anxiety and noise sensitivity in animals (Tiira, Sulkama, & Lohi, 2016). Animal models contributed to the development of such treatments, and these affect research studies in humans. Animal models are somewhat representative of human behaviors; however, it varies upon the area of research being conducted. An article in *Current Biology* explains how personality traits in a human can be used to study these in animals is an example of how animal models can represent behaviors in animals and humans (Briffa & Weiss, 2010). Translational models have been created to compare traits and responses in different animals such as dogs and cats (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013). Thus, comparison across species seems to support the idea that fear-based responses can be similar in different species. Research completed on humans and rodents showed fear-based disorders, such as post-traumatic seasonal disorder (PTSD), had similar physiologic mechanisms (VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). Activation of specific parts of the brain, like the amygdala and hippocampus, are seen in both organisms when examining fear conditioning.

Physiological responses are connected to the developments of anxiety behaviors, and can be explained by the release of hormones, such as cortisol, within the hypothalamic-pituitary-adrenal (HPA) axis (Dreschel & Granger, 2005). In dogs, initial stress responses are initiated by cortisol. Studies confirm that diseases are more likely to develop when an animal is anxious, in part, due to the increase in cortisol levels (Dreschel & Granger, 2005). Therefore, understanding the origin and development of anxiety behaviors could give rise to treatments that would treat diseases caused by these behaviors. Rats that activated the hypothalamic-pituitary-adrenal system died sooner than those who did not (Cavigelli and McClintock, 2003). Anxiety developed from the HPA system be linked to detrimental physiological responses and behaviors in animals because of this.

Pharmacotherapy and anxiety behaviors

Pharmacotherapy has been used to treat and prevent anxiety behaviors in the past. Testing of pharmacological drugs to determine the progression or reduction of certain anxiety behaviors has been done to determine the efficiency of a drug. Researchers that administered diazepam to dogs observed a reduction in anxiety behaviors as the animals were treated (Wormald, Lawrence, Carter, & Fisher, 2016). Observations, such as a decrease in exploratory behavior and noise aversion were considered as underlying anxiety behaviors that supported the precious claim. Also, these observations were deemed a repeatable analysis of the testing procedures.

Improvements in pharmacotherapy drugs gives rise to better treatments and further studies within this area. An example of this would be the development of open-field tests, which help identify behaviors within a controlled, yet free[1], environment. Theoretically, subjects can assimilate a normal environment in which an animal will interact in. Hence, the provision of drugs while completing an open-field test can be assumed to be a detailed representation of how the animal's behavior changes (Gruen, et al., 2015).

Open-field tests

What is an open-field test?

Open field tests are describes as a "commonly used tool for the measurement of animal behavior" (Walsh & Cummins, 1976). The review of such lead to the specificity[2] of tests to consider various aspects of animal behaviors. There are various ways to measure anxiety levels in animals. As discussed before, the measurements of hormone levels can indicate how stressed and anxious an animal is. Various open-field tests can be done, depending on the behaviors they would consider; common methods include grid assignments, the use of ethograms, and heat map analysis. Scholars found that the use of grid assignments can be extremely useful to observe behaviors (Gruen, et al., 2015). Open-field studies allow for an accurate measurement of an animal's response in an artificial, controlled area whilst providing them the freedom to react in

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[1] Freedom to exert normal behaviors.

[2] Evaluates the probability of correctly identifying the absence of some condition or disease state.

an open environment. Researchers have validated the use of open-field tests to measure fear responses and anxiety behaviors in canines (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013; Gruen, et al., 2015; Sherman, et al., 2015). Pharmacological studies have also been validated using open field tests in dogs and cats as well (de Rivera, Ley, Milgram, & Landsberg, 2017; Wormald, Lawrence, Carter, & Fisher, 2016).

Measurements

Observed behaviors in open-field tests include ethograms. An ethogram is a catalog or table of defined behaviors observed within an organism or species used in research (Lestel, 2011). The use of these can provide an insight of behaviors related to anxiety and can be used to quantify them later on. Table 1 lists behaviors commonly quantified in open-field tests.

Movement	
Distance moved	Time spent moving
Freezing	Rearing
Grooming	Escape attempts
Inactivity duration	Latency to leave area
Location	
Time spent in center	Crosses into corner
Distance from stimulus object	Time spent interacting with stimulus
Autonomic nervous system	
Defecation	Urination
Heart rate	Respiratory rate

Table 1. Parameters measured for open-field tests. Modified from Gould, Dao, & Kovacsics, 2009; Walsh & Cummins, 1976.

Quantifying physiological responses, may also be valuable. These involve the release of hormones from the hypothalamic-pituitary-adrenal axis, such as cortisol, epinephrine and norepinephrine; activated by the sympathetic nervous system. An increase in heart rate, respiration and salivation are signs of an activated sympathetic nervous system. Panting and pacing are also associated to an increase in cortisol levels that can leads to the measurement of fear and anxiety behaviors (Sherman, et al., 2015; Butler, Sargisson, & Elliffe, 2011). Physiological measurements can also be taken into consideration to determine the level and progression of the anxiolytic state. Bodily fluids such as an animal’s blood, urine, feces or saliva can be analyzed.

Human interaction tests are often conducted in conjunction with an open-field test. These tests try to identify an animal’s trait anxiety in relation to a specific situation, or event. Studies involving human interaction tests consider how physiological responses vary depending on the time and intensity of each interaction (Stellato, et al., 2019). At the same time, human interaction tests measure the engagement of the animal with humans through various ways. A study conducted by de Rivera, Ley, Milgram, & Landsberg, (2017) point out the amount of distance traveled by cats is related to how fearful an animal remains. Inactivity duration indicated how fearful an animal remains; a fearful animal will remain inactive for shorter periods of time.

Primordial use of animal models

The use of rodents, such as mice and rats, has been common in formerly behavioral research studies done. According to Gould, Dao, & Kovacsics, (2009), investigators have been shifting towards the use of mice instead of rats as research studies have progressed. Thus, establishing that behavioral research does not limit its studies to a single species; it evolves depending on what animal model researchers find beneficial. As a result, guinea pig, cat, dog, or pig animal models are also used to measure behaviors. The use of rodents is generally preferred due to their shorter generation interval period and reproduction capacity, besides other factors. Testing practices in rodents vary; they depend on multiple parameters and can consider many factors. Parameters associated with movement, location and the autonomic nervous system are commonly used to assess anxiety-related phenotypes (Gould, Dao, & Kovacsics, 2009). An example of a testing method using such parameters is an open-field test. The previously mentioned study also points out that open-field tests should not be used to ascertain baseline behaviors; these behaviors should be measured before the principal experiment takes place. However, rodent behaviors measured in open-field tests have been found to properly measure anxiety after they monitor their normal behaviors in a familiar environment.

OFT observed behaviors in rodents can be studied and compared to those of other species to be able to translate results obtained from testing methods. In the past, research models have been directly translated from rodents to humans. However, recent studies have considered more than one animal model before translating testing methods to humans. Fear-based conditioning and associated behaviors are an example of this. A previous study done by VanElzakker, Dahlgren, Davis, Dubois, & Shin, (2014), found that rodents developed certain behaviors associated with fear-based responses and anxiety; freezing, or fear-potentiated startle behaviors were observed. However, the importance of this study lies in the resemblances across species they were able to confirm. Comparison between the rodent and human’s fear-potentiated startle was quantified and they found that behavioral responses tend to overlap. Even though, similarities can be found in some studies, such as the one mentioned above, scientists usually utilize more than one animal model to confirm behaviors and their causes.

Behavioral research with rodents encompasses many areas, including the effects of drugs on behaviors. Evidently, resemblances in rodent pharmacological studies with other species could confirm behavioral responses as well as develop treatments for all in the future. Therapeutic drugs, such as benzodiazepines, have been commonly used as anxiolytic that could somewhat prevent or limit the development of fear and anxiety behaviors. When comparing the use of rodents to cat models, similarities are prevalent. de Rivera, Ley, Milgram, & Landsberg, (2017), conducted a study that used diazepam[3] to measure fear and anxiety in cats. The amount of distance traveled, rearing, pacing, sniffing and urinating were behaviors measured in cats during an open-field anxiety test. Both species, were found to have longer periods of inactivity

[3] Anxiolytic drug.

and a shorter amount fo distance traveled, which were associated with anxiety behaviors (de Rivera, Ley, Milgram, & Landsberg, 2017; Gould, Dao, & Kovacsics, 2009). Subsequently, assumptions regarding the use of pharmacological therapies to observe behaviors across species can be deemed correct.

Anxiety behavior considerations

The importance of distinguishing the difference between state and trait anxiety is essential for the understanding of the behavior itself. As previously stated, the treatment of animal behaviors can be translated to those of humans. For this review, state and trait anxiety definitions can be assumed to be parallel to humans. Trait anxiety relates to the reaction in a consistent way, depending on an individual's predisposition to react in a certain way. Whilst state anxiety is "characterized by physiological arousal and consciously perceived feelings of apprehension, dread, and tension" (Endler & Kocovski, 2001). Therefore, their difference lies in the predisposition of an animal's reaction to a situation or event. It is important to consider these to understand the cause of anxiety behaviors and develop proper tests according to the classification of such behaviors. Ideally, the difference between state and trait anxiety should give way to various types of testing.

Examination of personality characteristics can also play a role in how behaviors are measured. Recognition of individual personalities is imperative for behavioral studies as it provides normal behaviors on an individual basis that can be applied on a larger scale. Briffa & Weiss, (2010) concur on the idea that traits can be measured through long-term observations, rating or repeated measurements of several traits. In addition, an animal's personality traits might influence the response when exposed to a treatment. Previous studies have developed ways in which examiners can measure reactive behaviors in a more appropriate manner as a consequence (Åkerberg, et al., 2012; Sih, et al., 2015). At the same time, the origin and background of potential dog subjects should be acknowledged. Domestic dogs bred for research purposes, versus ones that were previously owned, may respond significantly differently to a stimulus; standard research dog breeds include few breeds. Differences in fear sensitivities were found in various dog breeds Blackwell, et. al. (2013), so it's fair to assume that research dogs might respond differently to fear than those that are not. Hence, it's recommended that researchers perform a primary study observing personality traits, dog breeds, and behaviors before developing a complex behavioral study.

Open-field tests in canines

Anxiety behaviors measured in canines

Anxiety behaviors in canines can be categorized into adaptive and maladaptive behaviors. Adaptive behaviors are considered normal, whilst maladaptive behaviors can impair an animal's ability to respond to its environment. Factors that affect these behaviors include, both, physiological and emotional causes leading to similar responses. Thus, research in this area tends to point out the differences in fear-based responses. Tiira, Sulkama, & Lohi, 2016, categorize fear responses into two areas; social (fear of humans and dogs) and non-social (sounds, situations, or objects) fearfullness. A previous study pointed out that fear responses can be normal, however, it’s important to note when these responses change into a persistent state of fear (Tiira, Sulkama, & Lohi, 2016). As previously mentioned, anxiety is as an irrational state of fear that remains. Consequently, it may persist even when the cause of a fear-based response is removed from its environment. Previous studies done in military dogs have observed maladaptive behaviors that lead to behavioral debilitation (Gruen, et al., 2015). At the same time, they established a connection between maladaptive behaviors and the habituation of dogs leading to a decreased state of anxiety.

Reactive behaviors are another example of anxiety-related behaviors in dogs. Reactive behaviors can include the startle reflex, which seems to be a common area that tends to be examined when contemplating fear and anxiety. The startle reflex is defined as a physiological response caused by a novel stimulus, which can lead to a physical response (Fox, 1963) Observations of a startling response may serve as a measurement for fear-based behaviors as a result. A previous study evaluated the speed of recovery after a startling stimulus was introduced (Foyer, et. al., 2016). Based on this, we can understand how startle responses can be measured in a dog population to determine the level of fear and anxiety within a test.

Sound-association

On the other hand, noise sensitivity can lead to reactive behaviors that develop; a sound is hypothetically associated with a traumatic or unpleasant event in an animal’s life. Noises are often considered in canine studies due to their increased hearing capacity, in comparison to that of humans. Studies have found that the hearing ability across dog breeds is similar in nature (Heffner, 1983). A dog’s sensitivity to sound should be representative of a population of dogs. Also, their increased sensitivity to sounds or noises can have a fear-based physiological response leading to anxiety. It’s safe to assume fear-based responses associated with sounds are an appropriate measure of anxiety behaviors.

Some studies have considered various causes for anxiety related behaviors. Canines are known to be sensitive to sounds associated with fireworks, thunderstorms, and gunshots, which lead to anxiety behaviors within a household (Sherman & Mills, 2008). Figure 1 supports this claim and gives other causes for the development of noise sensitivity, and as a consequence reactive behavior. Researchers have found that “distress responses to sounds take a variety of forms, ranging from more mild reactions, such as panting, hiding, hyperactivity, or escape attempts, to more extreme reactions, such as destructiveness and self-trauma” (Sherman & Mills, 2008). Previous studies have also found that such behaviors can have a negative outcome in working dogs as well (Rooney, Clark, & Casey, 2016). For instance, guide and working dogs that exert fear-associated behaviors do not perform as well as dogs that remain calm.

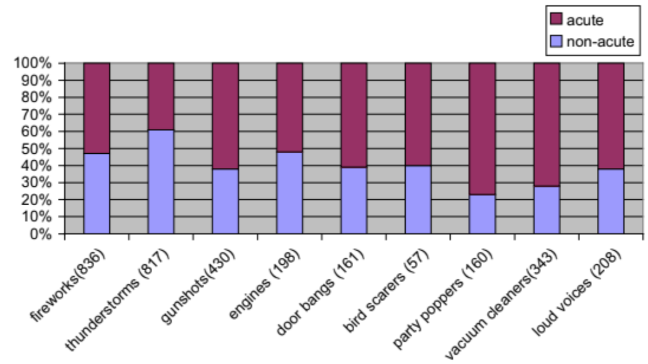

Fig.1 "Onset history (acute versus nonacute or gradual) for the nine most commonly reported noise sensitivities in a population of 3516 dogs. Numbers in brackets refer to the number of dogs affected in each column. The distribution of acute versus nonacute onset across the different stimuli is not even (P<.001)." (Sherman & Mills, 2008)

As stated above, sounds may be used as a novel stimulus causing fear responses. Novel and startling stimulus created is believed to be a state of fear in domestic dogs that primed behaviors associated with this (King, Hemsworth, & Coleman, 2003). Current knowledge supports this idea as studies show the prevalence of fear responses after a noise occurs. Therefore, sound-associated open-field tests can be beneficial for the investigation of animal behaviors. In rodents, photo beams, electric sensors, and computer-based tracking systems allow examiners understand the motor activity and capacity of animals in a controlled environment. However, these methods are not directly translated meaning researchers must develop a way in which they can be translated across species. Comparison of the startle reflex in rodents and humans lead to the belief that this response can be translated between species (VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). The investigators found that behavioral biomarkers can be determined to aid in the treatment of anxiety disorders associated to the startle reflex of a rodent or human. In military dogs, sound-associated open-field tests showed an adaptation to its environment (Gruen, et al., 2015). Sixteen Labrador retrievers aged 2-4 years to an open-field test with a thunderstorm auditory stimulus. Data collected pointed to the conditioning of such sounds in an open-field test after the anxiety responses were accurately measured. Consequently, the use of sound-associated open-field tests are considered advantageous when researching fear and anxiety behaviors.

Considerations of open-field tests

Differences upon breed and origin

Dogs bred for research

Beagles are a common breed utilized in research studies; other common breeds include Labrador retrievers, or German shepherds (Gruen, et al., 2015; Sherman, et al., 2015). Availability of a research animal's genetic composition, historical background and risk factors are more likely to be found in a research setting. Thus, research animals serve as an appealing model for the development of behavioral tests as researchers are aware of factors that affect their reactions. In a sense, the predictability dog breed behaviors can be quantified and then used for experiments in the future. Statistical analyses done on the inactivity frequency and inactivity duration, in beagle dogs, are an example of how anxiety behaviors can be calculated and validated (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013).

Investigators have found thunderstorm recordings can be a useful tool to exert a sound-associated fear response in dogs. A previous study showed that beagles had several responses to thunderstorm recordings and used this to determine the effects of an anxiolytic drug (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013).Such responses included an increase in inactivity frequency and inactivity duration once a thunderstorm recording was played. At the same time, they found a significant decrease in mean distance traveled when they heard the recordings. The response of the dogs is as expected; it confirms the use of an acoustic novel stimulus as a cause for anxiety behaviors. In the case of research dogs, drug administration is highly regulated and considered by a committee. This allows for pharmacotherapy drugs to provide an idea of the effects of canine behaviors.

Araujo et. al., (2013), utilized thunderstorm recordings as a novel sound introduction within a beagle colony study as well. The sound recordings were played in an open-field arena whilst the dogs were recorded. In this case, the study measured the latency and frequency of inactivity duration; the variable factor remained on the thunderstorm recording, it could be present or absent. Figure 2 shows how the introduction of thunderstorm sounds provoked a higher activity pattern in laboratory dogs (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013). As stated above, anxiety behaviors involve a higher activity level in animals. This study exemplifies how sound-associated anxiety behaviors are easily observed in laboratory dog breeds. The distinction of novel sound stimuli present or absent in nature has not been considered by studies, however, it may play a role in the intensity of a response. Research dogs may lack exposure to natural[4] sounds, meaning this novel stimulus is introduced for the first time in a research setting. Therefore, experimental conclusions should consider various factors in a research setting, especially if they wish to be representative of a dog population.

[4] From nature; linked to natural phenomenon or nature-related sounds.

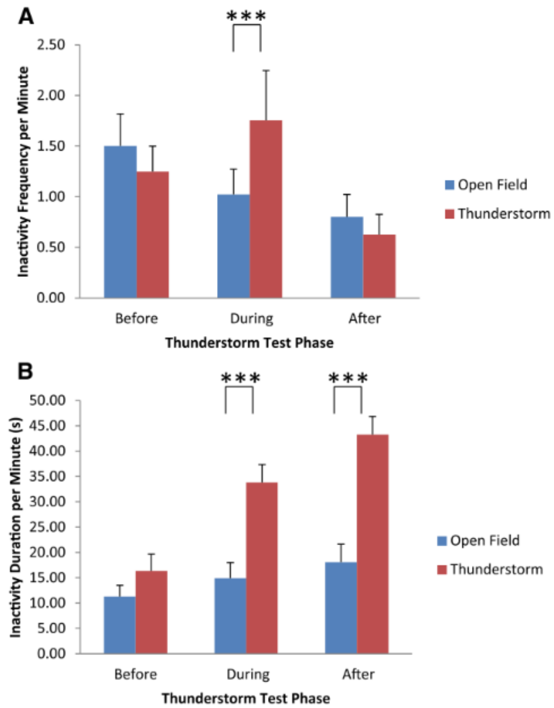

Fig. 2 "A) Mean inactivity frequency and (B) duration per minute over the open-field and thunderstorm tests are depicted in the same subjects (n=30). Inactivity frequency was significantly increased during the thunderstorm test compared with that during the open-field test ($P<0.001$), and this reflected increased inactivity frequency during thunder on the thunderstorm test compared with that on the open-field test. Inactivity duration was significantly increased during and after thunder on the thunderstorm test compared with that on the open-field test ($P< 0.001$). Error bars represent the standard error of mean." (Araujo, de Rivera, Landsberg, Adams, & Milgram, 2013)

On the other hand, startle tests have been conducted in laboratory dog breeds, such as Labrador Retrievers. Foyer, et. al., (2016), measured levels of cortisol in military dogs after a set of acoustic, visual and gradual visual novel stimuli were introduced. Salivary cortisol levels were measured as the open field acoustic startle test took place. Scientists found that examinations that observe startling responses can discern fear and anxiety behaviors in research animals (King, Hemsworth, & Coleman, 2003; Gruen, et al., 2015; Taylor & Mills, 2006). Furthermore, researchers concluded that cortisol levels were higher before they introduced the novel stimulus, which contradicts the expected physiological response to fear. So, this study shows that physiological responses can be measured in laboratory dogs, however, they may not be easy to predict. Breeding programs may have factored in, according to Foyer, et. al., (2016). Limitations for further studies with dogs bred for research must take into account genetic factors as it pertains to resilience and tolerance of stimulus.

Canine military research experiments have also been performed to determine the reaction of Labrador retrievers in a combat environment. The ability of a dog to easily identify an improvised explosive device (IED) requires training that will allow it to habituate to such environment and limit the sound-based fear maladaptive responses (Gruen, et al., 2015). A total of sixteen Labrador retrievers were recruited to participate in a series of subtasks that would measure their reactivity to novel stimulus, which included sounds. Gruen, et. al., (2015) established convergent[5] validity[6] as it related to military behavioral and physiological responses to fear. The confirmation of validation in behavioral studies using research bred dogs can be achieved.

Client-owned dogs

Previously owned or shelter dogs have been used as research models as well. (Flannigan & Dodman, 2001; Stellato, et al., 2019). The comparison of such animals to a domestic dog population can be found representative and results can be applied in a veterinary setting. On the downside, researchers deter from using previously owned dogs due to the lack of background and historical knowledge. At the same time, animals may come in with anxiety behaviors, which would makes the development of an experiment more complex. Even though, more factors need to be considered, the availability and cost of client-owned dogs is more appealing to examiners. It's important to note that statistical analyses have also been done with shelter dog colonies (Butler, Sargisson, & Elliffe, 2011).As a consequence, previously owned dogs can serve as models of anxiety behaviors can also be properly measured and quantified.

Behavioral studies done with client-owned dogs may occur in a laboratory setting, or indirectly[7]. Tests discussed in the research bred dogs are also seen with client-based dogs. Startling tests conducted on domestic dogs have shown to accurately[8] measure physiological responses to novel stimuli (King, Hemsworth, & Coleman, 2003). The control of the environment is key when developing these tests. The startle response is best observed when the novel stimuli is the only variable factor within the test. The control of the animal's environment is usually recommended when working with client-based canines. Startling test conducted as temperament tests provide an accurate insight into the animal's reactive behaviors. Belief that these tests have validity and can be convergent is stated by some studies (Taylor & Mills, 2006). Consequently, temperament tests could serve to ascertain baseline[9] behaviors.

Temperament tests have been commonly used in a veterinary or shelter setting to measure fear-related responses (Stellato, et al., 2019). In these cases, it was beneficial to develop a requirement criteria to recruit dogs that would benefit the study. Responses to loud noise can be considered to develop a valid temperament test. Such research can later be applied in a non-laboratory setting, and be confirmed or validated for the general dog population. Taylor & Mills considered noise stability as a common temperament subtest that has validation to it due to the "underlying behavioral system" present in the individual dog (2006). Although, these tests are used to determine the aggressive behavior of a dog, the basis of the behavior is fear nonetheless. In theory, then, these tests can be linked to properly determining anxiety behaviors in dogs as well.

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[5] Exhibiting convergence in from, function, or development (Merriam-Webster).
[6] Whether the concepts used in research represent the theoretical notions the research is grappling with (Social Research Glossary).
[7] A third party is involved: the owners. Questionnaires and interviews are methods to gather data.
[8] Reflects reality as far as possible devoid of mistakes and taking account of bias. (Social Research Glossary)
[9] Normal. "conforming to a type, standard, or regular pattern" (Merriam-Webster)

A previous study allowed for domestic dogs to participate in a 7 minute physical examination after the novel stimulus was presented (Stellato, et al., 2019). Behaviors such as avoidance, vocalizations and trembling were measured to understand the level of fear in the dogs. This study concluded that the introduction of a background noise can aid in the conditioning of a novel stimuli to prevent the development of anxiety. Working dogs, such as military or service dogs, are constantly exposed to novel sounds that may activate their sympathetic nervous system. Thus, the use of previously owned, working dogs might serve as an animal model to understand sound-based fear responses and anxiety behaviors.

In many cases, studies done with client-based dogs are accomplished through questionnaires and interviews. A previous study interviewed dog owners in order to understand the behaviors associated with a novel sound stimulus (Blackwell, Bradshaw, & Casey, 2013). They found that anxiety behaviors (reference Table 2) were observed as a dog was exposed to a loud noise, such as fireworks or thunder. Behavioral observations were determined by the owner of the dog and data analysis was based solely on their perspective. Storengen & Lingaas, (2015) conducted another study with seventeen dog breeds that supports the idea of noise as a cause for anxiety behaviors in canines. Consequently, anxiety behaviors are assumed to be connected to the presence of a noise or acoustic stimulus in previously owned dogs. Even though research studies validate questionnaires, scientists point out the studies “underestimate the actual prevalence since an unexperienced owner may miss signs of fear in the dog.” (Storengen & Lingaas, 2015). As a result, researchers have shifted towards the collection of data from the animal itself.

Behavioral signs	**Number (%) of dogs reported to show behavior**
Bark	70(38%)
Howl	5(3%)
Try and escape	33(18%%)
Scratch at doors	5(3%)
Tremble/shake	79(43%)
Destroy objects	4(2%)
Seek out people	64(35%)
Urinate/defecate indoors	3(2%)
Hide	58(32%)
Salivate	11(6%)
Other	20(11%)

Table 2. Range of behavioral signs after a sound stimulus is introduced. Data was collected from interviews conducted with dog owners. Modified from Blackwell, Bradshaw, & Casey, 2013.

Alternatively, risk factors should be studied before because the dogs may develop stereotypies, or become habituated to a stimulus, which could affect fear responses. Risk factors that lead to separation anxiety in dogs were looked into (Flannigan & Dodman, 2001). Noise phobias were linked to separation anxiety and other anxiety-related behaviors in dogs. An estimated half of dog participating in their study responded to noise fearfully (Flannigan &

Dodman, 2001). At the same time, another study considered the frequency of anxiety behaviors in animals alone and in combination of one another. Researchers concluded anxiety-associated behaviors can enhance the development of similar behaviors (Overall, Dunham, & Frank, 2001). The previous research mentioned describes the "interaction of multiple pathologic to noises" depends on the physiological, specifically neurochemical, state of the animal in question.

Implications for biomedical research

The understanding of personality traits in canines seems to be an important consideration allowing for more accurate behavioral results (Briffa & Weiss, 2010; Sih, et al., 2015). Even though studies have shown the benefit of establishing baseline[10] behaviors, examiners continue to develop canine behavioral studies without understanding individual personality traits of the animal. The validity of these studies can often be questioned if there is a lack of baseline behaviors. Both, validity and reliability[11], are appealing to researchers as it allows them to determine if the test has accurate measures whilst entirely considering all aspects of the measurement (Taylor & Mills, 2006). Collection of genetic data is recommended to increase validity and reliability in animal behavior studies.

Behaviors might be influenced by breeding programs or other genetic factors that might increase the presence, or absence of adaptive or maladaptive behaviors (Foyer, et al., 2016; Åkerberg, et al., 2012). Further research also might consider how various factors might contribute to the development of anxiety-associated behaviors, such as comorbidity. Fearfulness was linked to a "high comorbidity between noise sensitivity and separation anxiety", which should be considered when considering either one (Tiira, Sulkama, & Lohi, 2016). At the same time, genetics and co-morbidity should be considered as a novel sound stimulus is introduced. Data supports the idea that sound can play a role in increasing fear-based physiological responses (Gruen, et al., 2015; Fox, 1963). Thus, sound-associated tests need to consider risk factors related to the dog's genetic susceptability to acoustic startles.

Nowadays, past research done through interviews and questionnaires seems to be uncommon (Blackwell, Bradshaw, & Casey, 2013; Storengen & Lingaas, 2015). Animal models considering drug administration are developed as a consequence (de Rivera, Ley, Milgram, & Landsberg, 2017). Investigators have found that the results are hypothetically translated to other species, due to the similarity in physiology (VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). Additional research should be considered that implements a substantial link between species fear-based response and behaviors.

Development of experimental study

We conducted a behavioral study to observe fear and anxiety behaviors within a Beagle dog colony. A total of 8 female Beagle dogs participated. The tests were conducted in an open-field test arena, where a grid design was created on the floor; it consisted of a 7 by 7 grid assignment. At least two researchers were always present; one was the designated handler whilst

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[10] "A usually initial set of critical observations or data used for comparison or a control" (Merriam-Webster)
[11] Refers to the consistency of a measure (Research Methods in Psychology).

the other oversaw the room preparation before every test took place. A crate was placed in the room to provide a hide area. Standardization consisted of randomization of tests per day; the order of the tests varied per dog per day. Researchers were blinded to the order in which dogs were tested each day.

These were a total of four tests being conducted and the order of such were randomized per day. Each test lasted 5 minutes and were done one after the other, with a minimum of one-minute intervals in between each test. Tests were categorized as: control, play-reward, olfactory and acoustic startle test. The constant in all of these were the placement of the hide area and cameras positioned in the wall, which would record each session of tests being conducted. An auditory startle sound was introduced twice; at minute one and three. The sound was created by dropping a metal dog food bowl in front of the door by the hallway. Figure 3 shows a simplified image of the test arena when the acoustic startle test was taking place.

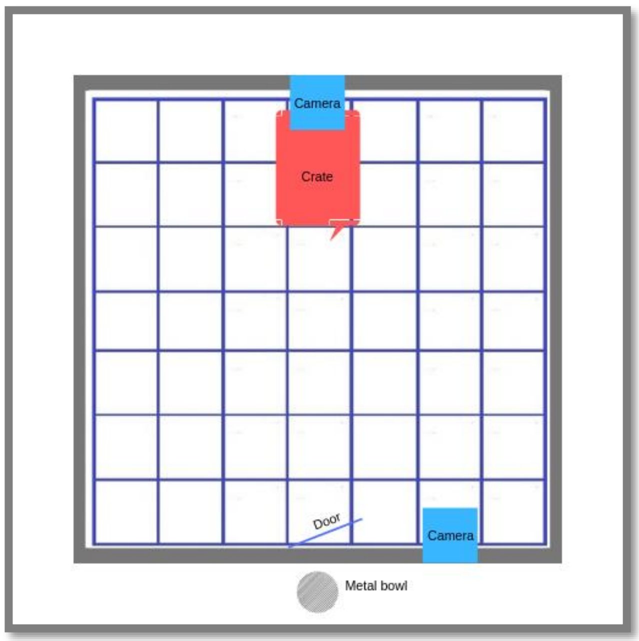

Fig. 3 Open-field test arena when conducting the acoustic startle test.

Conclusion.

Open-field tests can explore the physiological mechanisms that take place in response to fear, and develop observable behaviors expected. Experimental designs, in the future, should consider the role genetics have in the development of reactive behaviors, and how this can be considered in studies. Therefore, future research should consider how they recruit dogs, and their breed pedigree to avoid invalidating their results. The genetic origin and interrelation of such behaviors should be considered when developing a proper open-field study since behaviors may involve comorbidity.

It's important to note that researchers have also shifted their focus to a direct approach, where animal behaviors are measured in a laboratory setting. Laboratory experiments provide a higher level of control and regulation of parameters. Scientists have been able to reach more consistent results related to sound-associated anxiety behaviors by controlling the origin and restriction of sound in their environment. The origin of sounds can be from natural or man-made sound recordings in an open-field test to measure fear responses. In summary, sound-associated open-field testing provides an opportunity for researchers to measure anxiety behaviors in dogs as it measures physiological responses in various ways.

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