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Modified open-field test with odor search stimulus: Anticipated canine motivation and behavioral outcomes between anxious and non-anxious dogs

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Abstract

Fear is an emotion that is necessary to survive, but when it's prolonged and frequent, it can cause suffering in both animals and humans. Fear and anxiety are interrelated; therefore, fear can cause anxiety and anxiety can cause fear. Treatments for anxiety behaviors are currently an ongoing process, in order to improve the mental health of the animal. This review is focused on trying to understand anxiety behaviors through the use of a modified open-field test. More specifically, an odor search stimulus is used in conjunction with dogs on L-DOPA treatments. Previous animal models, particularly the mouse and rat, have been used for many behavioral tests, including ones to treat anxiety. Such open-field tests were examined and briefly analyzed to decide whether the behavioral measurements used could be translated across species, more particularly with the canine. Modified open-field tests were deemed applicable to measure anxiety behaviors in the canine. However, the odor stimulus in the open-field test modification is novel. An increased capacity for odor detection in the canine deems a plausible factor to consider when noting the canine's motivation and behavioral outcomes to the odor-search stimulus. Significant results were found in both military and cancer-detector dogs for the motivation to search for a novel odor stimulus. There were also studies done in which olfactory stimuli are used to stimulate exploratory motivation, such as in zoo animals. Consequently, all studies examined concluded that future research studies within these topic areas should continue to be evaluated for better research design and training programs. This paper will address the anticipated canine motivation to pursue the odor search stimulus and the behavioral outcomes that can be measured to differentiate between anxious and non-anxious dogs. Modifying the standard open-field test with olfactory stimuli will strengthen its relevance for use in dogs. In this review I will provide an overview of the open-field test, appraise the open-field test assumptions and logic in relation to rodents, show the applicability of the open-field test in relation to canine subjects, and provide the experimental design for a pilot study that dealt with dogs on L-DOPA treatments.

Introduction

This paper will address the anticipated canine motivation to pursue the odor search stimulus and the behavioral outcomes that can be measured to differentiate between anxious and non-anxious dogs. In order to accurately measure canine anxiety, a pilot study with dogs was conduced using a modified open-field test. The dogs tested were currently on L-DOPA treatments. L-DOPA is a precursor to the neurotransmitters dopamine, norepinephrine, and epinephrine and treats the symptoms of Parkinson's disease (PD) by crossing the blood brain barrier and increasing dopamine concentrations. Parkinson's disease is characterized by a loss of dopamine neurons in the nigrostriatal pathway and is likely involved in the onset of anxiety and depression. The symptoms involved with PD patients include tremor, rigidity, and bradykinesia, which is evident in canines as well as other species. Since the loss of dopamine neurons can lead to anxiety-related behaviors, behaviors associated with anxiety in dogs can include pacing, whining, drooling (outside the context of investigating odor), or shaking, to name a few. These behaviors can be quantified using an ethogram. In terms of the odor search stimulus used in the modified open-field test, the ethogram would need to focus on the canine's behavioral and physical behaviors exhibited during the study. The less fearful dogs will show interest in exploring the odor stimulus, as this is a novel modification. These behaviors exhibited by non-anxious dogs can include resting, grooming, and exploring, while it is expected that anxious dogs will not show interest in exploring the room and odor stimulus.

What is anxiety?

Anxiety is a reaction to a prospective or imagined danger or uncertainty (Sherman et al., 2008). Fear is a normal response to uncertain situations, while anxiety is an excessive form of fear that can turn into a phobia. Fear and anxiety are among the most fundamental emotions required to survive or cope in potentially dangerous or harmful situations (Bateson, 2011; Hohoff, 2009). However, a fundamental emotion such as anxiety may turn into a pathology when prolonged and generalized (Tiira et al., 2016). Anxiety in dogs can be categorized according to general fearfulness, separation anxiety, or aging related anxiety. Fear related anxiety might arise from the presence of a new or strange environment, unwanted loud noises, or unusual or strange people. Separation anxiety often manifests itself in undesirable behaviors, such as urinating or defecating in unwanted places, destroying property, or excessive barking. Age related anxiety affects senior-aged dogs and leads to a decline in cognitive function, learning, perception, and awareness. The way in which an animal behaviorally and physiologically responds to a stressful situation can be termed a “coping style” (Koolhaas et al., 1999).

Signs of anxiety in dogs

The signs of anxiety in dogs include physiologic signs (eg, increased respiratory and heart rate, vasomotor changes, trembling or paralysis, increased salivation or sweating, gastrointestinal disturbances) and behavior signs. The behavioral signs may include changes in activity (eg, immobility, pacing, circling, restlessness); changes in nearest neighbor distances (eg, remaining close to a person or conspecific); or changes in appetite, including anorexia (Sherman et al., 2008). If owners do not recognize anxiety in their dogs, they will be unable to prevent unwanted, related stimuli. Canine anxiety can predispose to aggressive behavior and can be measured both in response to preconditioned stimuli, such as thunderstorm simulations (Araujo et al., 2013) and also in response to relatively unconditioned stimuli (Wormald et al., 2016).

Current treatments of anxiety

Anxiety is an evolved response to help animals survive. However, anxiety can become a problem when the duration, frequency, or intensity affects the anima’s wellbeing. Anxiety is considered to be a highly pathological condition because it modifies the animal’s relationship with the environment, humans, and other animals, eventually harming its welfare (Overall, 2000). Some authors, such as Overall (1996) and Landsberg (2001), report that anxiolytic pharmacological therapy associated with a behavioral modification program is the best therapy for anxiety disorders in dogs. The medication promotes the animal’s recovery rate by allowing implementation of a behavioral modification program at the same time as anxiety is reduced

(Pineda et al., 2018). Currently, benzodiazepines are one of the most commonly used medications for treating anxiety in dogs. Benzodiazepines act on GABA-A receptors, specifically ligand-gated chloride channels. They are also deemed positive allosteric modulators, since they bind to a regulatory site and increase the effects of ligand binding. This in effect slows the actions of the central nervous system, inducing a state of relaxation. Some antidepressants, such as selective serotonin reuptake inhibitors, are useful in veterinary medicine because the high selectivity of the serotonin system works well for treating anxiety behavior, with few adverse effects (Fitzgerald and Bronstein, 2013). Diazepam is an anxiolytic drug commonly used for pharmacological validation of paradigms used to measure anxiety in a range of mammalian species (Ohl, 2003). In previous studies, diazepam has reduced some of the behavioral signs of anxiety-related behavior problems (Herron et al., 2008).

A pilot study was conducted to provide information about the mean responses and variability between and within dogs. The purpose of this was to provide behavioral and physical descriptions of dogs during periods of L-DOPA treatments and control periods when L-DOPA was not present. L-DOPA is expected to increase dopamine concentrations in the brain, leading to a decrease in anxious behaviors. In order to quantify the results, an ethogram would need to be created. An ethogram is a catalogue or inventory of behaviors or actions exhibited by an animal used in ethology. The behaviors in an ethogram are usually defined to be mutually exclusive and objective. In terms of the odor search stimulus, the ethogram should focus on the physical and behavioral traits the dog is expressing. For example, the amount of times the dog paces, whines, ears or tail are tucked, any abnormal urination or defecation amounts, the amount of times and time spent in the crate, excessive drooling or yawning are a few examples for anxious behaviors that could be measured. Non-anxious behaviors that could be measured include grooming, resting, or the distance traveled by freely exploring the room and odor stimulus using the taped-out grid lines. The primary objective of the behavior test was to determine sensitivity and specificity of a modified open-field test as a measure of anxiety in dogs. This paper will address the anticipated canine motivation to pursue the odor search stimulus and the behavioral outcomes that can be measured to differentiate between anxious and non-anxious dogs.

What is an open-field test?

An open-field test (OFT) is a common measure of exploratory behavior and general activity in both mice and rats, where both the quality and quantity of the activity can be measured (Gould et al., 2009). The open-field (OF) is an enclosure, generally circular, square, or rectangular in shape with surrounding walls to prevent the animal from escaping. The OFT is a commonly used mechanism to measure a number of facets of behavior beyond simple locomotion, such as a test for anxiety or exploration (Gould, 2009). Many behavioral tests of anxiety are based on the animal's locomotion and body activity. It has been suggested that two factors influence anxiety-like behavior in the open-field; the first is social isolation resulting from the physical separation from cage mates when performing the tests, and the second is the stress created by the brightly lit, unprotected, novel test environment (Prut and Belzung 2003). Thus, the test has a number of uses and is included in almost every thorough analysis of rodent behavior.

Assumptions and logic of open-field test with rodents

Behavioral paradigms designed to measured both trait and state anxiety has been developed in rodents (Ohl, 2003). These paradigms include the open field test, elevated plus maze, and the free exploratory paradigm (Teixeira-Silva et al., 2009). The assessment of anxiety-related behavior in animal models is based on the assumption that anxiety in animals is comparable to anxiety in humans (Ohl, 2003). However, it cannot be proven than experiences that an animal has related to anxiety are the same for a human. Distinct behavioral patterns in rodents indicate anxiety, such as behavioral and peripheral changes presumed to accompany high sympathetic nervous activity (Hall, 1936), meaning that an analogy between human and rodent anxiety may be assumed. Additionally, anxiety in both humans and laboratory animals can be described as a non-unitary phenomenon because it includes both innate (trait) anxiety and situation-evoked (state) anxiety. Modeling anxiety in animals is critically dependent on the test system used, such as, a test for anxiety in rodents has to allow the animal to display natural, anxiety-related behavior. It is important to note that test-retest repeatability of behavioral measures is believed to indicate a correlation with a behavioral trait (Teixeira-Silva et al., 2009). Test-retest repeatability is the reliability of a test measured over time, and measures test consistency. Trait anxiety does not vary from moment to moment; therefore, any model used to evaluate it must be stable over time (Andreatini, 2000). Although evidence exists that the open field may be useful in detecting genetic or pharmacological effects on anxiety (Treit and Fundytus, 1989), some studies also report a lack of sensitivity for anxiety-modulations of this test (Saudou, et al., 1994). Given the difficulties of interpreting the OFT and the lack of independent validation, some authors have called for caution when using the OFT to measure personality traits (Carter et al., 2016).

How does/does not the open-field test apply to other species, like the dog?

Many previous studies measuring the fear or anxiety of a dog occurs largely in the presence of a potentially conditioned stimulus, such as an unfamiliar dog (Svartberg, 2005), or human (Planta and De Meester, 2007), or thunderstorm (Araujo et al., 2013). For this reason any past experiences (both positive and negative) and socialization of the dog, may strongly affect the behavior exhibited. Just as behavioral paradigms are designed to measure both trait and state anxiety in rodents, some studies have measured aspects of canine anxiety using paradigms. The simplicity of the settings of the OFT, with rapid and easy measurements of behaviors has made the OFT popular for measuring activity and exploration in a variety of animals beyond rodents (Perals et al., 2017). To make the OFT applicable to other species, a modified open-field test has been used. This involves adding novel objects to the arena and quantifying the time it takes the animal to visit the objects.

How will the modified open-field test for odor search stimulus differ between anxious and non-anxious dogs?

What is olfaction?

The most important characteristic of the canine is its sense of smell (Jenkins et al., 2018). The major components of the olfactory system are the nasal cavity, olfactory epithelium and receptors, the vomernasal organ, and the olfactory bulb. The nasal cavity is comprised of two chambers and three turbinates that are highly vascularized and contribute to increased mucosal surface area. The olfactory epithelium is comprised of the neurepithelium lining the cribriform plate, dorsal septum, dorsal and middle turbinates, and pseudostratified columnar epithelium, with millions of olfactory receptor cells (Jenkins et al., 2018). There are also supporting sustentacular cells of the olfactory epithelium that regulates the composition of mucous, insulates in between olfactory receptor cells, and protects the epithelium from damage. Olfactory receptor cells project directly to the olfactory bulb and contain cilia that have surface odor receptors. The vomernasal organ lies along the ventrorostal aspect of the nasal septum, and provides additional odor detection for chemical signals that stimulate physiological and/or behavioral changes in the environment. The vomernasal organ also functions in the detection of pheromones and plays a role in reproduction and social behavior. The olfactory bulb is a paired structure that functions primarily as a relay station, and to filter sensory input (Evans HE, 2013). The olfactory bulb has both a sensory role and a modulatory role in the hypothalamus, limbic system, and forebrain. The olfactory cortex is located within the medial temporal lobes and functions to receive sensory input from the olfactory bulb, permit conscious awareness of odor, identification of odor, odor memory, and odor localization in lower animals (Jenkins et al., 2018).

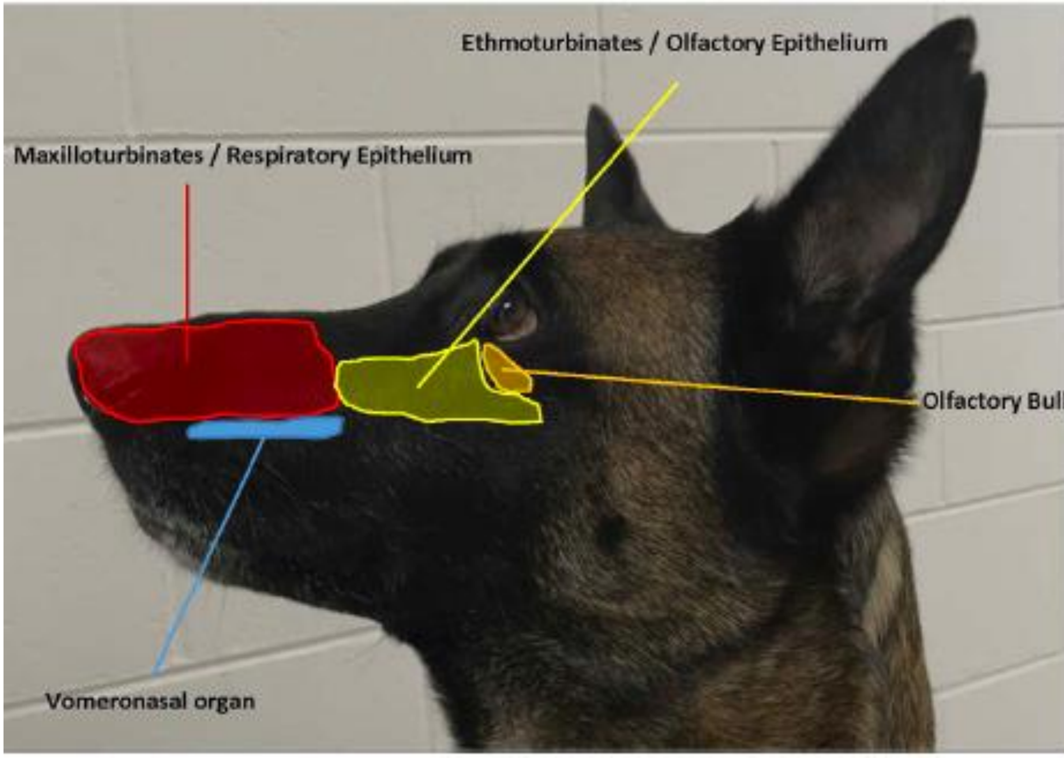

Figure 2: Left exterior view demonstrating placement of interior structures associated with olfaction (Jenkins et al., 2018).

Dogs have a significantly large surface area of olfactory epithelium, with approximately 30% more olfactory receptors, than a human, that can recognize a wide variety of odorants. The canine's capacity for odor detection is as much as 10,000-100,000 times that of the average human, and the canine detectability for volatile organic compounds is one part per trillion. Dogs can have the capability for excellent odor localization, even in the presence of significant background odor, likely due to the larger nasal cavity size as compared to other species (Barrios et al., 2014). During inspiration, 12-13% of air flow travels to the olfactory portion of the nose, and the remaining airflow is directed toward the nasopharynx where it exits the nasal cavity (Craven et al., 2010). Through active sniffing, or the production of short, sharp breaths at 4-7 Hz, the dog has improved airflow sampling and odorant collection. When a canine is sniffing, air within approximately 1 cm of the nostril is drawn toward the naris, and the high velocity of airflow is transported to the dorsal nasal cavity where it turns 180 degrees and flows back over the ethmoturbinates (Craven et al., 2010). Each nostril samples air separately, yielding bilateral odor samples that assist in odor source localization (Craven et al., 2010). Sniffing is advantageous compared to normal inhalation because it provides unidirectional laminar flow to the dorsal meatus and sensory epithelium of the ethmoturbinates, increases the sensitivity to odors, drives activity in the olfactory cortex, and affects odorant intensity and identification (Gazit et al., 2003). Olfactory cues provide information about predators, food, mates, and pathogens, to name a few. Due to the ability to find the source of a scent even in the presence of competing scents, the canine has been long used by humans for odor identification and discrimination (Bregeras et al., 2016). This sensitivity, unique capability to detect a target odor among a myriad of odors in an operational environment, and the ability of the dog to learn by operant conditioning has made the working canine an intrinsic component of law enforcement, military, search and rescue, and medical/ assistance operations worldwide (Jenkins et al., 2018).

Studies in which olfactory stimuli is used to stimulate exploratory motivation

Olfactory enrichment has been recognized as an effective way to promote exploration and increase activity levels in primates, wild cats, and deer found in zoos (Clark and King, 2008). Studies suggest that increased quantity and variety of stimulation in zoo environments also impact the behavior of species exhibited in zoos (Fay and Miller, 2015). The goal of environmental enrichment is to allow zoo animals to engage in as many opportunities to display species-appropriate behavior. In a recent study done by Fay and Miller (2015), the addition of different scents resulted in an increase in time spent in the location closest to scent dispersal and decreased the amount of time animals were standing and resting. This suggests that olfactory enrichment in the form of scents might be an appropriate animal management technique to decrease predictability and introduce novelty into a zoo environment (Fay and Miller, 2015). For example, Wells and Egli (2004) found that nutmeg, catnip, and body odor of prey were all found to increase activity levels and exploration in black-footed cats. Olfactory stimulations can be very beneficial to many different species. Because many species are driven by their sense of smell, they use olfactory signals to communicate, locate prey, attract mates, and find food (Wells, 2009). Another olfactory enrichment study done by Resende and Pedretti et al (2011) looked at the benefits of cinnamon and catnip at reducing the amount of pacing that Oncilla Cats showed in captivity and the results were that it greatly reduced the amount of pacing that was done. Future research is still needed to better understand the effects of olfactory enrichment on zoo animals. But using olfactory cues can be generalized to a single statement: animals that are kept in captivity need enrichment activities to live better lives. A study was done looking at calming odors (lavender and chamomile) and stimulating odors (rosemary and peppermint) effect on the behavior of kenneled dogs (Myatt, 2014). It was found with the calming odors that lavender was effective in encouraging behavior that was indicative of relaxation in dogs, such as less barking and more resting, and the stimulation odors of peppermint made the dogs more active, so there was more movement (Wells, 2004). This is an interesting approach, as a calming odor could be used in setting to decrease anxious behaviors in dogs, while a stimulating odor could be used to increase movement and exploration in non-anxious dogs.

Modified open-field test: Pilot Study

The modified open-field test used in the pilot study focused on adding a novel odor stimulus to the testing environment. Odor was added to the open-field test as a way to measure anxiety in dogs, by proving a motivation to search. Using an odor stimulus helped the researchers discriminate between anxious and non-anxious dogs by measuring the movement and amount of times the dogs interacted with the odor, by way of the grids. The L-DOPA treatments fit into this study because the dogs were currently on L-DOPA treatments when the pilot study was conducted, as a way to decrease any anxious behaviors in the dogs. Performing the modified open-field test was a way to measure the outcomes while on L-DOPA. The modified open-field test for odor was conducted in the behavior testing room. Cameras were mounted to record behavior and a grid of white tape on the floor was used to measure the dog's movement in the room. The dogs were given five minutes to explore the room, and each dog was tested individually once per day for a maximum of four days per week until the commencement of the L-DOPA treatments. The odor search stimulus test was conducted using two Kong toys with 1-2 drops of salmon oil placed inside the toys, and placed in the grid within the room. The end result was to measure the amount of times the dogs went to the Kong toys and freely explored the room. This is an accurate measure of anxiety in canines- where they able to freely explore the stimulus, or where they anxiously waiting until the test was over with? Just as an olfactory stimulus was used in zoo settings to stimulate exploratory motivation, salmon oil was used as the novel odor stimulus in the L-DOPA study because it is accessible, cheap, and the researchers believed the dogs would find the fish smell appetizing, since fish oil is finding its way into more dog food labels as an ingredient. A crate was also placed inside the testing room along one wall. The crate was available to provide protection and a sanctuary for the anxious dogs that did not feel comfortable enough to freely explore the room, or interact with the odor filled Kong toys. The researchers used the modified open-field test to identify concerning levels of canine stress. Behaviors associated with anxiety can include pacing, whining, drooling (outside the context of investigating odor), or shaking, to name a few. The less fearful dogs will show interest in exploring the salmon oil, as this is a novel odor.

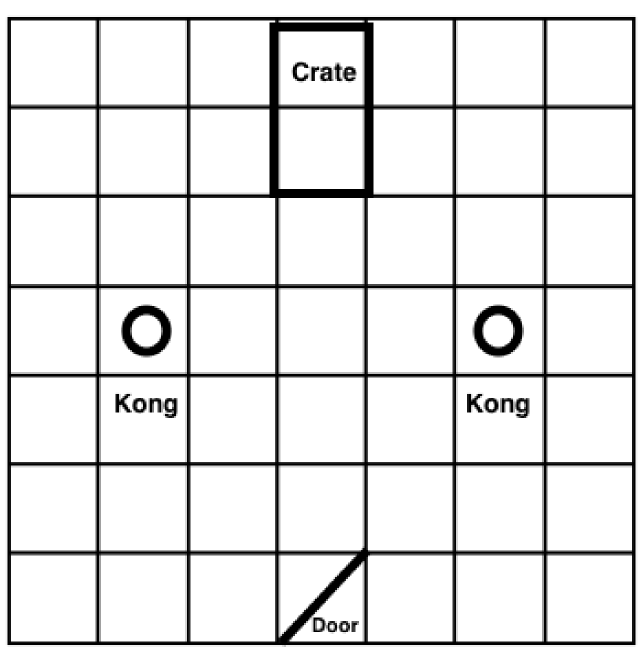

Figure 1: Modified open-field test with odor search stimulus setup

Canine motivation anticipated pursuing the odor search stimulus

Military dogs

Detector dog-handler teams are currently employed by a multitude of national and local law enforcement agencies, private organizations, and militaries throughout the world (Williams and Johnston, 2002). The outstanding sensitivity of the canine olfactory system has been acknowledged by using sniffer dogs in military and civilian service for detection of a variety of odors (Lesniak et al., 2008). These dogs are used to detect a broad variety of substances including narcotics and human remains in the military field. Another well established role for dogs is scent detection of land mines, improvised explosive devices, undetonated munitions, and other explosive materials that pose a risk to civilian and military populations (Lazarowski and Dorman, 2013). Although dogs seem to be remarkable effective at detecting a variety of targets, little is known about how they accomplish detection tasks, the effectiveness with which they do so, or how to optimize their performance (Williams and Johnston, 2002). For many years, the only reported work examining the sensitivity of dogs to explosives was a study conducted by Becker et al. in 1962, and more recently Johnston et al (1995) and Waggoner (1997) generated psychometric functions describing the detection of smokeless power by mixed breed dogs, and finally Williams et al. (1997,1998) determined the odor detection signature for a number of explosives. Dogs are clearly able to detect the odors required by different agencies, the effects on detection performance of training multiple odors for detection have yet to be examined. A study done in 2002 showed that training dogs to detect as many as 10 odors in a fixed search scenario did not approach the point at which detection performance began to deteriorate (Williams and Johnston, 2002). It was also shown that dogs were able to learn additional odors with increasing ease. These results suggest that there are no detrimental effects on refresher training requirements. Furthermore, training dogs to detect explosives presents several challenges: the types of explosives can vary widely from region to region, the use of homemade explosives has recently become more common than commercial and military explosives, and most target odors encountered by dogs under field conditions are comprised of a combination of many different substances (Lazarowski and Dorman, 2013). These results prove that there is a motivation to seek an odor stimulus with proper training. In relation to anxiety-related behaviors, military dogs can also develop PTSD, a form of canine anxiety. The symptoms can vary widely, but can include increased or decreased responsiveness to the environment, changes in the relationship with the handler, failure to perform work-related tasks, escape or avoidance behavior, depression, and general signs of fear, stress, and anxiety.

Cancer detection dogs

Using odor detection dogs could potentially be a valuable cancer screening method. Cancers have worldwide high mortality rates, primarily due to late diagnoses. Regular screening for early symptoms of cancer can reduce the mortality rate; however, contemporary screening methods are not ideal (Walczak et al., 2012). The X-ray or sputum cytology in lung cancer, and mammography used for early detection of breast cancer, has an unsatisfactory sensitivity; mammographies ranging from 39% to 66% (Shen and Zelen, 2001). In contrast, more advanced methods, such as computed tomography, runs into a problem of overdiagnosis, resulting in unnecessary fear and more invasive procedures. There is, therefore, a place for new, noninvasive, even unconventional cancer screening methods that would be cheap and affordable for society, which could be applicable in the veterinary oncology clinic (Walczak et al., 2012). Early diagnoses of cancer using effective screening methods are crucial for successful treatment (Pirrone and Albertini, 2017). It can be expected that dogs will be widely used for cancer detectors. However, before canine cancer screening can be adopted in clinic practices, additional studies need to be done to determine the procedures with the best-reduced error rate and highest degree of accuracy. Studies on special training of dogs to detect different cancers using various odor samples (breath, urine, cancer tissue) have provided promising results, suggesting that dogs may play a critical role in cancer research and diagnosis (Pirrone and Albertini, 2017). Previous reports from Williams and Pembroke (1989) and Church and Williams (2001), showed how dogs, after appropriate training, might be able to discriminate breath, urine, or feces of tumor-tissue samples of patients with cancer (e.g. lung, breast, prostate, skin, and ovarian cancers) from respective samples taken from healthy volunteers. Melanoma was the first type of cancer that canine olfactory detection of human malignancy was initiated. A woman was encouraged to get a skin lesion on her leg examined after her dog constantly sniffed at it, where chemical markers for melanoma were found in body fluids (Pirrone and Albertini, 2017). This suggests that volatile compounds may be released from melanoma cells on the skin surface in amounts sufficient for lesion localization by the canine olfactory system. Additionally, a man had a lesion from a patch of eczema on the outer side of his left thigh excised after his pet dog began to persistently show interest in it. The histological assessment revealed a basal cell carcinoma. There are various studies and accounts documented where dogs were able to use olfactory cues to help diagnose certain cancers. However, the practical use of dogs is still limited by a lack of validated cancer-derived metabolites and by a lack of sensing technologies optimized to their detection (Lavra et al., 2015). There is mounting evidence that dogs may be trained, rapidly and cost-effectively, to recognize the characteristic odor signature of various forms of cancer in body samples from cancer patients (Pirrone and Albertini, 2017). These results are proof of why adding an olfactory exploratory stimulus can strengthen the open-field test with dogs because the odor stimulus used is a novel modification. Just as the cancer-detector dogs are interested in their owner's unique body marks and lesions, dogs used in a modified open-field test will be equally as interested in searching for a novel odor.

Discussion

The purpose of the pilot study was to determine sensitivity and specificity of a modified open-field test, specifically one that focused on the odor search stimulus, as a measure of anxiety in dogs. The modified open-field test with odor search stimulus is a novel modification, but the drive to perform the study was to provide information about mean response and variability between and within anxious and non-anxious dogs. Since this was only a pilot study, there wouldn't be enough statistical power to identify any differences between anxious and non-anxious dogs, but any results from the study would be used to inform future grant applications for more appropriate modified open-field tests to measure anxiety in dogs. This pilot study focused on using Beagles as the animal in the behavioral tests. The reason Beagles are used in such a big quantity is because of their size. They are easy to handle, equally trusting, loyal, of good temperament, and easy to manipulate. Future studies could be done to differentiate anxiety in client animals versus laboratory animals, and if there is a difference in behavioral outcomes.

The canine motivation anticipated pursing the odor search stimulus is due to the importance of olfaction to animals and the many jobs a dog has due to the significance of olfactory cues. The anticipated motivation to be interested in the salmon oil is because of the exceptional capacity for odor detection in dogs. Because of a dog's keen sense of smell, they have been used in the military, law enforcement, as guide dogs, and as cancer detectors, to name a few. This presents the multitude of olfactory cues used in a dog's life and the motivation to use their sense of smell to go to work and do what is expected and good of them. The canine motivation in relation to anxiety is that the dogs used for the study should have the motivation to freely move around the testing room to search and investigate the Kong toys filled with the odor stimulus. The behavioral outcomes that can be measured in relation to anxiety is the amount of times the dogs go to the Kong toys filled with the odor stimulus, the amount of times the dog is exhibiting signs of anxiety, or the amount of times the dog will find sanctuary in the crate. If the dog consistently went to the crate provided in the room during the pilot study, this would indicate the dog was too anxious and didn't feel comfortable enough to explore the testing room.

Conclusion

Olfaction is a powerful tool in the hands of an animal, especially the dog. Dogs have been used in police, guard, herding, search and rescue, military, service, and cancer detectors as a few of the many jobs that motivate them to contribute to their pack and protect their caretakers. The purpose of this review was to help the readers understand different aspects of the modified open-field test, particularly in relation to the odor search stimulus. This topic is significant because odor can be used to stimulate exploratory motivation and decrease pacing and anxiolytic behaviors in dogs. Anxiety is a rising behavioral concern in dogs and there has been much research and discussion conducted on this topic in the animal welfare, veterinary medicine, and ecological aspects of the industry. Most of the research found was on the many ways olfaction motivated dogs to use their keen sense of smell in order to benefit civilians, or the many ways they benefited themselves to live better lives through enrichment, especially when in shelters, laboratories, or captivity. The main take-away for performing the modified open-field test with an odor search stimulus was that dogs on L-DOPA treatments exhibited decreased anxious behaviors and were able to perform the modified open-field test with no trouble.

References

Andreatini, R., & Bacellar, L. F. S. (2000a). Animal Models : Trait or State Measure? The Test-Retest. *Science*, 24(4), 549-560.

Andreatini, R., & Bacellar, L. F. S. (2000b). Animal personality: What are behavioural ecologists measuring? Biological Reviews, 88(2), 465-475.

Araujo, J. A., de Rivera, C., Landsberg, G. M., Adams, P. E., & Milgram, N. W. (2013). Development and validation of a novel laboratory model of sound-induced fear and anxiety in Beagle dogs. *Journal of Veterinary Behavior: Clinical Applications and Research*, *8*(4), 204–212.

Barrios AW, Sánchez-Quinteiro P, Salazar I. (2014). Dog and mouse: toward a balanced view of the mammalian olfactory system. *Front Neuroanat,* 8:106.

Bateson, M., (2011). Anxiety: an evolutionary approach. Canadian Journal of Psychology. 56, 707-715.

Becker, R.F., King, J.E., Markee, J.E., (1962). Studies on olfactory discrimination in dogs. II. Discriminatory behavior in a free environment. J. Comp. Physiol. Psych. 55, 773–780.

Bregeras, M.S., Puaux, D., Gervals, R., Ferry, B. (2016). Rigorous training of dogs leads to high accuracy in human scent matching to sample performance. PLoS One, 11(2).

Church, J., Williams, H., (2001). Another sniffer dog for the clinic? Lancet 358, 930.

Clark, F., and King, A., (2008). *Chemical Signals in Vertebrates 11*. New York, NY: Springer New York.

Craven, BA., Paterson, EG., Settles, GS., (2010). The fluid dynamics of canine olfaction: unique nasal a irflow patterns as an explanation of macrosmia. Journal of the Royal Society Interface. 2(47), 933-43.

Evans, HE, and de LaHunta, A. (2013). Miller's Anatomy of the Dog. 4th ed. St. Louis, MO: Elsevier Saunders. Print..

Fay, C., & Miller, L. (2015). Utilizing Scents as Environmental Enrichment: Preference Assessment and Application with Rothschild Giraffe. *Animal Behavior and Cognition*, *2*(3), 285–291.

Fitzgerald, K. T., & Bronstein, A. C. (2013). Selective Serotonin Reuptake Inhibitor Exposure. *Topics in Companion Animal Medicine*, 28(1), 13-17.

Gazit, I., Terkel, J., (2003). Explosives detection by sniffer dogs following strenuous physical activity. Appl. Anim. Behav. Sci., 81, 149-61.

Gould, T. D., Dao, D. T., & Kovacsics, C. E. (2009). The open field test. *Neuromethods*, 42(1), 1-20.

Hall CS. Emotional behavior in the rat. III. (1936). The relationship between emotionality and ambulatory activity. J Comp Physiol Psychol; 22:345–52.

Herron, M. E., Shofer, F. S., & Reisner, I. R. (2008). Retrospective evaluation of the effects of diazepam in dogs with anxiety-related. *JAVMA*, *233*(9), 1420–1424.

Hohoff, C., (2009). Anxiety in mice and men: a comparison. J. Neural. Transm. 116, 679-687.

Hurst, J. L., Beynon, R. J., Roberts, S. C., & Wyatt, T. D. (Eds.). (2008). *Chemical Signals in Vertebrates 11.*

Jenkins, E. K., DeChant, M. T., & Perry, E. B. (2018). When the Nose Doesn't Know: Canine Olfactory Function Associated With Health, Management, and Potential Links to Microbiota. *Frontiers in Veterinary Science*, *5*.

Johnston, J.M., Wagoner, P., Williams, M., Jackson, J., Jones, M., Myers, L.J., Hallowell, S.F., Petrousky, J.A., (1995). Canine olfactory capability for detecting NG smokeless powder. In Midkif, C.R. (Ed.), Proceedings of the 5th International Symposium on Analysis and Detection of Explosives. Bureau of Alcohol Tobacco and Firearms, Washington, DC.

Koolhaas J. M., Korte S. M., De Boer S. F., Van Der Vegt B. J., Van Reenen C. G., Hopster H., De Jong IC., Ruis MA., and Blokhuis HJ. 1999). Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.

Landsberg, GM (2001) Clomipramine--beyond separation anxiety. Journal of the American Animal Hospital Association: July/August 2001, Vol. 37, No. 4, pp. 313-318.

Lavra, L., Catini, A., Ulivieri, A., Capuano, R., Beghernajad Salehi, L., Sciacchitano, S., Bartolazzi, A., Nardis, S., Paolesse, R., Martinelli, E., Di Natale, C., (2015). Investigation of VOCs associated with different characteristics of breast cancer cells. Sci. Rep. 5, 13246.

Lazarowski, L., & Dorman, D. C. (2013). Explosives detection by military working dogs: Olfactory generalization from components to mixtures. *Applied Animal Behaviour Science*, *151*, 84–93.

Lesniak, A., Walczak, M., Jezierski, T., Sacharczuk, M., Gawkowski, M., & Jaszczak, K. (2008). Canine olfactory receptor gene polymorphism and its relation to odor detection performance by sniffer dogs. *Journal of Heredity*, *99*(5), 518–527.

Myatt, A. (2014). An Olfactory Enrichment Study At The Ashland Cat Shelter: A capstone project submitted in partial fulfillment of graduating from the Academic Honors. N. Page. Print.

Ohl, F. (2003). Testing for anxiety. *Clinical Neuroscience Research*. Elsevier BV, 3(4-5), 233-238.

Overall, KL.(2000). Natural animal models of human psychiatric conditions: assessment of mechanisms and validity. Prog Neuropsychopharmacol Biol Psychiatry, 24:727–776.

Perals, D., Griffin, A. S., Bartomeus, I., & Sol, D. (2017). Revisiting the open-field test: what does it really tell us about animal personality? *Animal Behaviour*, *123*, 69–79.

Pineda, S., Anzola, B., Ruso, V., Ibáñez, M., & Olivares, Á. (2018). Pharmacological therapy with a combination of alprazolam and fluoxetine and use of the trace element lithium gluconate for treating anxiety disorders and aggression in dogs. *Journal of Veterinary Behavior*, *28*, 30–34.

Pirrone, F., & Albertini, M. (2017). Olfactory detection of cancer by trained sniffer dogs: A systematic review of the literature. *Journal of Veterinary Behavior: Clinical Applications and Research*. Elsevier USA.

Planta, J., De Meester, R.,(2007). Validity of the Socially Accetable Behavior (SAB) test as a measure of aggression in dogs towards non-familiar humans. Vlaams Diergeneeskundig Tijdschrift 76, 359-268.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*. Elsevier.

Resende, Leticia de S., Karla C. Pedretti, et al. (2011). Influence of Cinnamon and Catnip on the Stereotypical Pacing of Oncilla Cats (*Leopardus tigrinus*) in Captivity. *Journal of Applied Animal Welfare Science*, 14 247-254.

Saudou F, Ait Amara D, Dierich A, et al., (1994). Enhanced aggressive behavior in mice lacking 5-HT1b receptor. Science, 265: 1875–8.

Shen, Y., Zelen, M., (2001). Screening sensitivity and sojourn time for breast cancer early detection clinical trials: mammograms and physical examinations. J. Clin. Oncol. 19, 3490-3499.

Sherman, B. L., & Mills, D. S. (2008). Canine Anxieties and Phobias: An Update on Separation Anxiety and Noise Aversions. *Veterinary Clinics of North America - Small Animal Practice*, 38(5),1081-1106

Svartberg, K., (2005). A comparison of behaviour in test and everyday life evidence of three consistent boldness-related personality traits in dogs. Appl. Anim. Behav. Sci. 91, 103-128.

Teixeira-Silva, Goes, T.C., Antunes, F.D., (2009). Trait and state anxiety in animal models: i there correlation? Neuroscience. Lett. 450, 266-269.

Tiira, K., Sulkama, S., & Lohi, H. (2016). Prevalence, comorbidity, and behavioral variation in canine anxiety. *Journal of Veterinary Behavior: Clinical Applications and Research*, *16*, 36–44.

Treit D, Fundytus M., (1989). Thigmotaxis as a test of anxiolytic activity in rats. Pharmacology Biochemistry Behavior, 31:959–62.

Waggoner, L.P., (1997). Olfactory Sensitivity of the dog (Canis lupus familiaris) to the vapor from cocaine hydrochloride and methyl-benzoate. Unpublished doctoral dissertation, Auburn University, AL.

Walczak, M., Jezierski, T., Górecka-Bruzda, A., Sobczyńska, M., & Ensminger, J. (2012). Impact of individual training parameters and manner of taking breath odor samples on the reliability of canines as cancer screeners. *Journal of Veterinary Behavior: Clinical Applications and Research*, *7*(5), 283–294.

Wells, Deborah L, (2004) A review of environmental enrichment for kenneled dogs, *Canis familiaris*. *Applied Animal Behaviour Science,* 85 307-317.

Wells, D.L. and Egli, J.M. (2004) The influence of olfactory enrichment on the behaviour of blackfooted cats, Felis nigripes. *Applied Animal Behavior Science.* 85, 107–119.

Williams, H., Pembroke, A., (1989). Sniffer dogs in the melanoma clinic. Lancet 333, 734.

Williams, M., & Johnston, J. M. (2002). Training and maintaining the performance of dogs (Canis familiaris) on an increasing number of odor discriminations in a controlled setting. *Applied Animal Behaviour Science*, *78*(1), 55–65.

Williams, M., Johnston, J.M., Cicoria, M., Paletz, E., Waggoner, L.P., Edge, C.C., (1998). Canine Detection Odor Signatures for Explosives. In: DePersia, A.T., Pennella, L.J. (Eds.), Proceedings of the SPIE Series on Enforcement and Security Technologies, Vol. 3575, p. 291–301.

Williams, M., Johnston, J.M., Waggoner, L.P., Jackson, J., Jones, M., Boussom, T., Halalowell, S.F., Petrousky, J.A., (1997). Determination of the canine odor detection signature for a selected nitroglycerin based smokeless powder. In: Proceedings of the 13th Annual Security Technology Symposium and Exhibition, Virginia Beach, VA.

Wormald, D., Lawrence, A. J., Carter, G., & Fisher, A. D. (2016). Validation of modified open field behaviour as a measure of trait anxiety in the dog. *Applied Animal Behaviour Science*, *179*, 95–102.