



## Research

# The use of an open-field model to assess sound-induced fear and anxiety-associated behaviors in Labrador retrievers



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## ABSTRACT

Previous studies have shown that the playing of thunderstorm recordings during an open-field task elicits fearful or anxious responses in adult beagles. The goal of our study was to apply this open-field test to assess sound-induced behaviors in Labrador retrievers drawn from a pool of candidate-improvised explosive devices (IEDs)-detection dogs. Being robust to fear-inducing sounds and recovering quickly is a critical requirement of these military working dogs. This study presented male and female dogs, with 3 minutes of either ambient noise (days 1, 3, and 5), recorded thunderstorm (day 2), or gunfire (Day 4) sounds in an open-field arena. Behavioral and physiological responses were assessed and compared with control (ambient noise) periods. An observer blinded to sound treatment analyzed video records of the 9-minute daily test sessions. Additional assessments included measurement of distance traveled (activity), heart rate, body temperature, and salivary cortisol concentrations. Overall, there was a decline in distance traveled and heart rate within each day and over the 5-day test period, suggesting that dogs habituated to the open-field arena. Behavioral postures and expressions were assessed using a standardized rubric to score behaviors linked to canine fear and anxiety. These fear/anxiety scores were used to evaluate changes in behaviors after exposure to a sound stressor. Compared with control periods, there was an overall increase in fear/anxiety scores during thunderstorm and gunfire sound stimuli treatment periods. Fear/anxiety scores were correlated with distance traveled and heart rate. Fear/anxiety scores in response to thunderstorm and gunfire were correlated. Dogs showed higher fear/anxiety scores during periods after the sound stimuli compared with control periods. In general, candidate IED-detection Labrador retrievers responded to sound stimuli and recovered quickly, although dogs stratified in their response to sound stimuli. Some dogs were robust to fear/anxiety responses. The results suggest that the open-field sound test may be a useful method to evaluate the suitability of dogs for IED-detection training.

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## Introduction

Fear and anxiety are debilitating conditions that can negatively affect the functionality and well-being of working dogs. Fear is the

awareness of immediate danger, whereas anxiety is the anticipation of future danger usually from prior experiences or unknown or imagined origin (Overall, 2013). Fear and anxiety may be difficult to differentiate behaviorally in animals; the terms are often used interchangeably to describe a constellation of behavioral and physiological responses to external stimuli. Although in some cases, fear and anxiety may be adaptive and enhance survivorship, in other cases, fear and anxiety may impair an animal's function and inhibit learning (Passalacqua et al., 2013). In severe cases or in stressful environments, an exaggerated maladaptive response may occur, leading to behavioral debilitation.

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Fearful or anxious dogs may be hypervigilant even in the absence of specific stimuli and may startle easily; assume low posture (Haverbeke et al., 2008); or show more subtle signs such as yawning, tongue flicking, or lip licking (Scaglia et al., 2013). With specific stimuli, fear-induced physiologic responses resulting from enhanced activation of the hypothalamic-pituitary-adrenal axis, such as release of cortisol, epinephrine, and norepinephrine, may occur (Part et al., 2014). Acute physiological responses may include tachycardia, tachypnea, and increased body temperature (Beerda et al., 1997).

Inappropriate fear or anxiety responses could impair the function of military working dogs (MWD) in a combat situation. Behavioral problems, many resulting from stress effects, were the most common cause of early discharge in MWDs aged younger than 5 years in 1 study (Evans et al., 2007). Identifying dogs susceptible to elevated fear or anxiety response and rejecting them for further training is critically important for MWDs. A standardized behavioral assay to evaluate such dogs before training and deployment would improve MWD effectiveness and welfare.

Recently, an open-field test (OFT) that used recorded thunderstorm sounds was shown to be a robust model of noise-induced fear and anxiety in laboratory beagle dogs (Araujo et al., 2013). The present study modified this OFT model to evaluate physiological and fear-/anxiety-related behavioral responses to loud sounds by Labrador retrievers selected for training as improvised explosive device detection dogs (IDDs). The IDDs are specifically trained to detect improvised explosive devices in combat zones. As such, they need to be resilient to loud sounds, including rapid gunfire, explosives, and other military noise. Our objective was to expose candidate IDDs to the sounds of thunderstorms and gunfire in an OFT, and to use physiologic measures, activity data, and assessment of sound-induced behaviors to evaluate the strengths and limitations of this model for screening candidate IDDs.

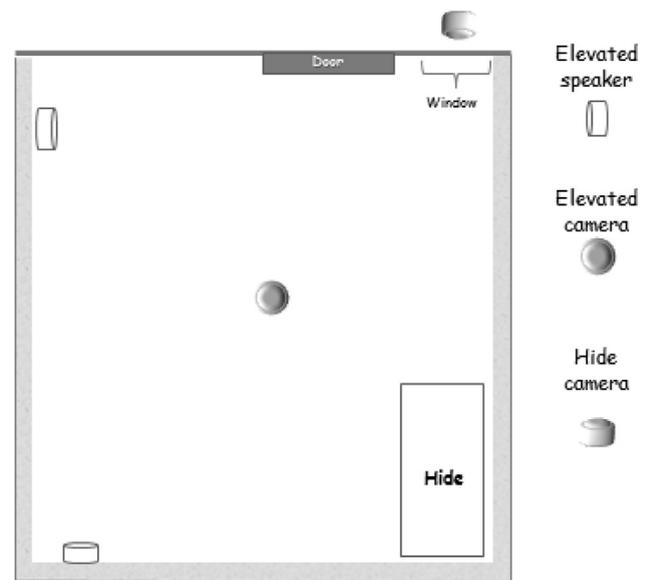
## Materials and methods

### Subjects

The experimental subjects were 16 Labrador retrievers aged between 2 and 4 years. There were 8 intact males, 5 intact females, and 3 spayed females. The dogs had been selected from field trial stock as candidates for IDD training by a private MWD training firm (K2 Solutions, Southern Pines, NC). Additional details regarding their selection, housing, and welfare oversight have been described (Lazarowski et al., 2014). At the time of OFT, all dogs had been in residence for approximately 3.5 months in a dedicated indoor canine facility under veterinary supervision at the North Carolina State University College of Veterinary Medicine Laboratory Animal Resources Unit. They were individually housed in kennels separate from the test areas, and were maintained on a stable regime of feeding, exercise, and rest. The Laboratory Animal Resources Unit is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee and the United States Army Medical Research and Materiel Command Animal Care and Use Review Office.

### OFT arena

The OFT arena (Figure 1) consisted of a room approximately  $3 \times 3$  m, located in a dedicated free-standing building, maintained at an ambient temperature of approximately 20–25°C. The OFT arena had an epoxy-painted cement floor, and was constructed of 3 cement block walls and a fourth modular wall with a door and



**Figure 1.** The open-field test arena (3 m × 3 m) with “hide.” The schematic representation, not to scale, shows the approximate location of the door, 2 elevated speakers, and 2 cameras. One camera was positioned overhead in the center of the arena, and 1 camera was laterally positioned, 0.6 m above the floor level, at an opaque window with a port just large enough to accommodate the camera lens.

narrow viewing panel. The OFT arena was equipped with a hide (W61 × H76 × L91 cm), constructed of high-density polyethylene boards (King StarBoard; King Plastic Corporation, North Port, FL), into which the dogs could retreat. Two video cameras (ICD-49 B/W camera; Ikegami Tsushinki Company, Ltd., Japan) were mounted so that dogs could be visualized at all times while in the OFT arena, including the hide. One camera was mounted overhead in the center of the ceiling, whereas a second horizontal camera was mounted outside the arena, 0.6 m above the OFT floor. The horizontal camera was fitted with an infrared filter and illuminator (IR-ROOM Ultra-Covert 940 nm; Nightvisionexperts.com, Buffalo, NY) and was directed through a camera port in an opaque window to record each dog’s behavior while in the hide and adjacent areas. During recordings, the cameras recorded digital video to a nearby computer equipped with EthoVision XT 7.1 (Noldus Information Technology, Leesburg, VA) dedicated behavioral analysis software. A sanitizing agent Virkon-S (Dupont, Fayetteville, NC) diluted to 0.25% strength was applied to the floor of the OFT arena and allowed to air dry following each dog’s session.

### Sound stimuli and OFT procedures

Digital audio recordings of the sounds of a thunderstorm (Can-Cog Technologies, Toronto, Ontario, Canada) or simulated gun battle (K2 Solutions, Southern Pines, NC) were played through 2 overhead speakers in the OFT arena at standardized sound pressure levels (SPL), measured in decibels (dB). Background sound level (without a dog in the arena) was approximately 46–50 dB SPL. The mean thunderstorm sound level was 88.8 dB SPL; the peak level was 104–105 dB; the A-weighted sound exposure level was 110.9 dBA. The mean gun battle sound level was 95.2 dB; the A-weighted sound exposure level was 117.2 dBA.

The OFT was completed during a 2-week period (8 dogs/week). Testing was performed between 13:00 and 16:00 hours. None of the subjects had been exposed to the OFT arena before testing. Within each group of 8 subjects, males were evaluated before females. Otherwise, the order of the dogs was randomized for each group

(Week 1 or Week 2), and then dogs were tested in the same order each day.

The OFT protocol was identical for all dogs, with the order of sound presentation standardized. Each dog was placed in the open-field arena for a 9-minute session on 5 consecutive test days. Each 9-minute session was divided into three, 3-minute periods (A, B, and C), as illustrated in Table 1. On all days, no sound stimuli were presented during periods A and C. On day 1, no stimulus was presented as this day was designed to acclimate dogs to the OFT arena. On day 2 during period B, the sound of a thunderstorm was played for 3 minutes. On day 4 during period B, the sound of gunfire was played for 3 minutes. Animals were not exposed to sound stimuli on days 3 and 5. The experimental design allowed us to determine each dog's behavioral response over 3 time periods (A, B, and C) on each day, and over the 5 days of the OFT.

### Physiologic measures

Physiologic measures were collected from each dog on each day (Table 1). To detect the effect of exposure to the open-field conditions, heart rate and body temperature data were collected by a trained investigator immediately before and after each 9-minute session. Heart rate was collected by auscultation with a stethoscope over the heart base or by femoral pulse detection. This was followed by measurement of body temperature via digital rectal thermometer. Then, immediately, saliva for cortisol analysis was also collected. These salivary cortisol samples were compared with baseline salivary cortisol levels obtained between the hours of 13:00 and 16:00 on a day approximately 2–3 weeks before commencement of the testing protocol. Saliva was collected with a 15-cm piece of test-specific cotton rope (Salimetrics, State College, PA) by inserting 1 end of the rope into the dog's mouth for approximately 2 minutes. Previously, dogs had been conditioned to accept the rope in their mouths in anticipation of a food reward after saliva collection as described (Sherman et al., 2014). Briefly, dogs were individually trained to sit on cue for a food reward with minimal restraint. Then, once or twice per day for 5 days, dogs were conditioned to allow 1 end of a cotton rope to be placed in the lateral commissure of their mouth while the experimenter held a small piece of odoriferous food treat (Pup-peroni; DelMonte Foods, San Francisco, CA, USA) in a closed hand in front of the dog. The dog was encouraged to sniff the treat to stimulate salivation. After collecting an adequate sample volume (>0.3 mL), and within 2–3 minutes of the start of sampling, the dog was given the treat. This facilitated easy acquisition of an adequate volume for analysis during the present study. On the day of the baseline collection, dogs remained in their home kennels and saliva was collected during a time when there were no husbandry activities occurring. Saliva samples were extracted and stored at –20°C until analysis using a high-sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics).

**Table 1**  
Open-field experimental protocol

Period	Day 1 Session 1	Day 2 Session 2	Day 3 Session 3	Day 4 Session 4	Day 5 Session 5
Pre-session	HR, BT				
A (3 min)	Control (ambient noise)				
B (3 min)	Control (ambient noise)	Thunderstorm sound	Control (ambient noise)	Gunfire sound	Control (ambient noise)
C (3 min)	Control (ambient noise)				
Post-session	HR, BT Salivary cortisol <sup>a</sup>				

HR, heart rate recorded as beats per minute; B, body temperature recorded, degree Fahrenheit.

Periods A, B, and C on days 1–5 were conducted in the open-field arena.

<sup>a</sup> Salivary cortisol refers to the saliva sample collected.

### Behavioral data

Behavioral data consisted of both distance traveled (activity) in the open field, and fear/anxiety scores calculated based on the dogs' postures and expressions in the open field.

### Activity data

Video recordings from the overhead camera were analyzed for distance traveled per time period using a dedicated behavioral analysis program (EthoVision XT 7.1). Because dogs could be visualized at all times by the 2 cameras, there were no "out-of-sight" time intervals. A sampling rate of 10 samples per second was used during video acquisition. After being started manually by 1 investigator, data acquisition in the open field was fully automated and synchronized with the audio recordings by the software system.

### Behavioral assessment of fear/anxiety behaviors

Video recordings from both camera views were used to evaluate noise-induced fear/anxiety responses. Video recordings of each dog's test sessions were uniquely identified, randomized, and then analyzed for behavioral signs without sound by a single trained observer (BCC) who was blinded to session and sound treatment. From these detailed observations, fear/anxiety scores were determined based on 3 general categories of behaviors associated with fear/anxiety (Table 2), namely inactive, active, and global (subjective score composite of inactive and active scores). The fear/anxiety scores were based on duration and intensity of specific fear/anxiety-associated behaviors observed over a given period of time (Landsberg et al., 2013). Inactive fear/anxiety behaviors included decreased activity, lowered body postures, and autonomic/conflict behaviors. Active fear/anxiety behaviors included startle, vigilance, and active responses. The global score was based on observation by the blinded observer based on determination of inactive and active behaviors. For each of these types of fear/anxiety, scores were based on a scale of 1–6 increasing stepwise by half points, where a score of 1 reflected no expression of fear/anxiety behaviors and a score of 6 reflected severe fear/anxiety behaviors exhibited most of the time (Table 3). There were 3 periods each of 5 days for a total of 15 three-minute periods per dog. Each dog had 3 fear/anxiety scores for each 3-minute period for a total of 9 fear/anxiety scores per day, and 45 fear/anxiety scores over the duration of OFT. This design allowed us to evaluate each dog's fear/anxiety response over the 3 time periods on each day and over the 5-day OFT period.

### Statistical analysis

Days were treated as a categorical covariate throughout. There were no significant differences for each measure on days 3 and 5, in which no sound stimuli were presented. Thus, values on these 2

**Table 2**  
Definitions of fear/anxiety score terminology

Fear/anxiety score types	Definition
Inactive	Passive behaviors, including decreased activity, such as freezing, hiding, position against wall, or at door; lowered body postures, such as crouching, tail tucking, and ears back; and autonomic/conflict behaviors, such as panting, shaking, salivating, yawning, lip licking, or elimination.
Active	Positive fear/anxiety behaviors included startle, bolting, vigilance, scanning, and active responses, such as pacing, aimless activity, stereotypic circling, retreat/escape attempts, digging, and climbing.
Global	Overall opinion of masked rater, based on observation of dog and determination of negative and positive fear/anxiety behaviors.

days were pooled and termed days 3 and 5. Because the group size for spayed versus intact female dogs was small, all female dogs were analyzed collectively irrespective of their reproductive status. For physiologic measures of heart rate and body temperature, post-session values (immediately after period C) were compared with pre-session values (immediately before period A). For salivary cortisol data, results were analyzed as change from baseline. Physiologic and motor data were analyzed by repeated measures models, with compound symmetry covariance structure, with day, period (for motor data), treatment, age (continuous), and sex evaluated as factors, and baseline values included as covariates where appropriate. Owing to the skewed distribution of motor data, the natural logarithm of the distance traveled was used in statistical models; however, raw data were used for Spearman partial correlations of distance with fear/anxiety scores. Fear/anxiety scores were analyzed using repeated measures models with compound symmetry covariance. When a factor was identified as not statistically significant, the data were pooled appropriately. Spearman partial correlations were used to investigate relationships between anxiety scores and physiologic and motor data. All statistical analyses were performed using SAS v9.2 (SAS, Cary, NC). The results were considered statistically significant if  $P$  value was 0.05 or lower. Results are presented for all dogs ( $n = 16$ ) unless otherwise specified.

## Results

One dog became destructive in the OFT arena on the first day of testing only (day 1). To prevent self-trauma, his OFT session on that day was terminated after 6 minutes. Therefore, for this dog, data from day 1 are available for periods A and B only and physiological data were collected after 6 minutes rather than 9 minutes.

### Physiologic measures

For all dogs on all days, heart rates, body temperatures, and salivary cortisol levels were within normal physiologic ranges.

**Table 3**  
The scoring rubric for generation of fear/anxiety scores

Anxiety score	Expression of anxiety behaviors
1	None; no anxiety behaviors
2	Occasional and mild
3	Some of the time and mild/Occasional and moderate
4	Most of the time and mild/Some of the time and moderate/Occasional and severe
5	Some of the time and severe/Most of the time and moderate
6	Most of the time and severe

### Heart rate

There were no statistically significant differences by sex ( $F = 0.1$ ,  $P = 0.923$ ) or by age ( $F = 0.50$ ,  $P = 0.8352$ ) for the change in heart rate over the session (post-pre) using a repeated measures model controlling for day and pre-session heart rate, so these data were pooled for further analyses. The final model analyzed change in heart rate as a function of pre-session heart rate ( $F = 17.61$ ,  $P < 0.0001$ ) and day ( $F = 5.34$ ,  $P = 0.0031$ ). The mean change in heart rate by day is shown in Figure 2. Heart rates did not change significantly between the start and end of the session on acclimation Day 1 ( $P = 0.5640$ ). Heart rates showed a statistically significant decrease by the end of the session on control days 3 and 5 ( $P = 0.0014$ ). This effect was not observed on treatment day 2 or 4 ( $P = 0.199$  and  $P = 0.0544$ , respectively).

### Body temperature

There were no statistically significant differences for sex ( $F = 0.84$ ,  $P = 0.375$ ) or age ( $F = 3.71$ ,  $P = 0.0802$ ) for day and pre-session temperature using a repeated measures model controlling for day and pre-session temperature, so these data were pooled for further analyses. The final model analyzed change in body temperature as a function of pre-session body temperature ( $F = 6.68$ ,  $P = 0.0008$ ) and day ( $F = 67.07$ ,  $P < 0.0001$ ). Body temperature showed a statistically significant increase by the end of the session on Day 1 ( $P = 0.022$ ), but did not change significantly between the start and end of the session on days 2, 4, or 3 and 5 ( $P = 0.273$ , 0.667, and 0.098, respectively). The change in body temperature on day 1 was significantly different from days 2, 4, and 3 and 5 ( $P = 0.001$ , 0.0080, and  $< 0.0001$ , respectively), but no other significant differences in the change in body temperature were found between other days (all  $P > 0.2$ ; Figure 3).

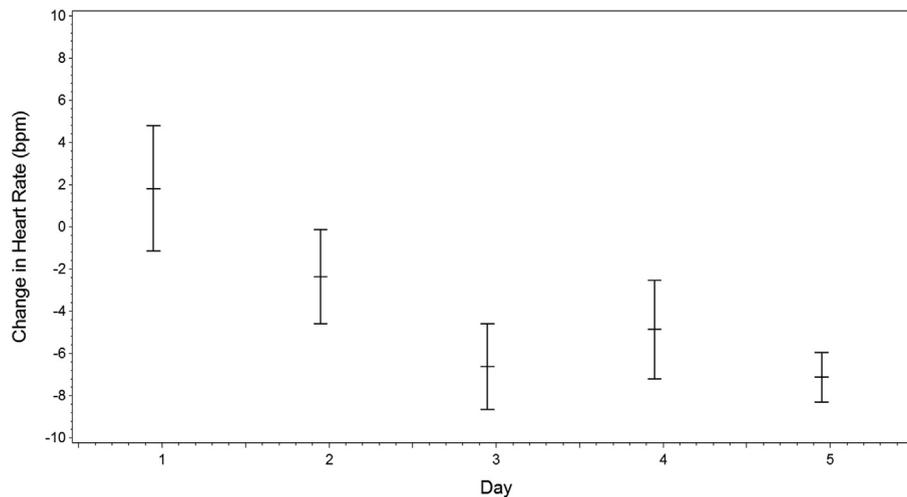
### Salivary cortisol concentration

There were no statistically significant differences for sex ( $F = 3.05$ ,  $P = 0.104$ ) or age ( $F = 0.00$ ,  $P = 0.981$ ) using a repeated measures model controlling for day and baseline cortisol levels, so these data were pooled for further analyses. A model analyzing change in salivary cortisol concentration as a function of baseline cortisol level and day was not significant ( $F = 0.64$ ,  $P = 0.5934$ ), so the final model included only baseline cortisol level ( $F = 7.63$ ,  $P = 0.015$ ). The mean change in salivary cortisol concentration between baseline and the ends of sessions was an increase of 0.093  $\mu\text{g/dL}$  (range: 0.191–0.398  $\mu\text{g/dL}$ ; Figure 4).

### Activity data

There were no statistically significant differences for sex ( $F = 1.09$ ,  $P = 0.3149$ ) or age ( $F = 0.40$ ,  $P = 0.5363$ ) for logged distance traveled using a repeated measures model with day and period, with compound symmetry covariance structure. There was a significant decrease in distance traveled over 5 days (Figure 5), based on a repeated measures model on logged distance with a compound symmetry covariance structure, including period and day ( $F = 26.72$ ,  $P < 0.0001$ ). Dogs were more active when first entering the enclosure, with significantly greater distance traveled during period A than period B ( $P < 0.0001$ ) and significantly greater distance traveled during the period B than C ( $P < 0.0001$ ). Dogs were also more active on day 1 than day 2 ( $P < 0.01$ ), day 2 than day 4 ( $P < 0.01$ ), and day 2 than days 3 and 5 ( $P < 0.01$ ).

Distance traveled during period B was further evaluated using similar repeated measures models, excluding acclimation data collected on day 1. Dogs traveled significantly longer distances during pooled treatment periods (2B and 4B) than equivalent pooled nontreatment periods (3B and 5B;  $F = 33.28$ ,  $P < 0.0001$ ; means 19.5 m vs. 14.0 m, respectively). A subsequent repeated



**Figure 2.** Mean ( $\pm$ SE) change in heart rate over days (post-session value – pre-session value) in the open-field test arena. SE, standard error; bpm, beats per minute.

measures model found that dogs traveled significantly longer during thunderstorm treatment (mean: 23.1 m) than gunfire treatment (mean: 16.0 m; [ $F = 8.41, P = 0.0110$ ]).

#### Fear/anxiety scores

There were no statistically significant differences for sex or age for active (sex:  $F = 0.47, P = 0.502$ ; age:  $F = 1.05, P = 0.510$ ), inactive (sex:  $F = 0.94, P = 0.348$ ; age:  $F = 0.48, P = 0.848$ ), or global (sex:  $F = 0.90, P = 0.360$ ; age:  $F = 0.94, P = 0.348$ ) fear/anxiety scores when evaluated with a repeated measures model with compound symmetry controlling for day and period.

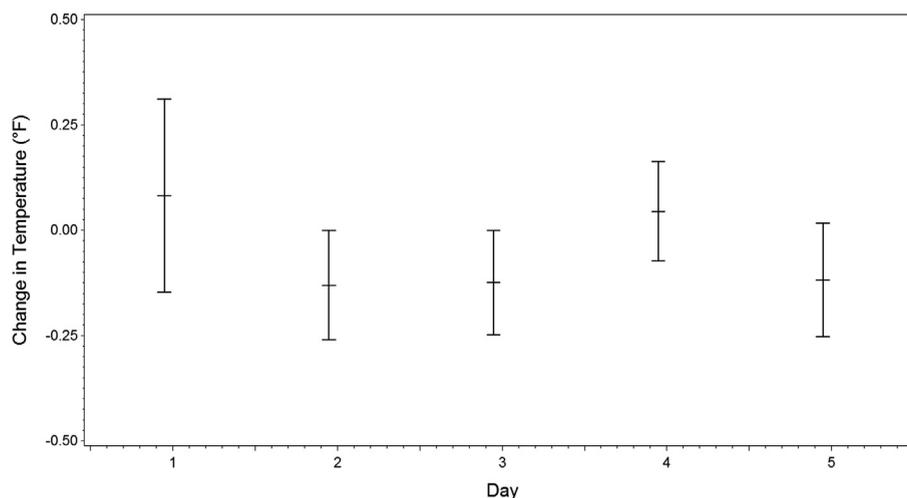
After controlling for day and period, active fear/anxiety scores, inactive fear/anxiety scores, and global fear/anxiety scores were significantly higher during treatment periods 2B and 4B ( $F = 55.59, P < 0.0001$ ;  $F = 20.65, P = 0.0004$ ;  $F = 36.45, P < 0.0001$ , respectively). For active and global fear/anxiety, but not for inactive global fear/anxiety, scores were significantly higher during period A than C ( $t = 3.54, P = 0.0013$ ;  $t = 2.37, P = 0.0242$ ;  $t = 0.78, P = 0.4392$ , respectively). An overall day effect was present in all 3 models ( $P < 0.0001$  for all, [Figure 6](#)). After controlling for day and period, global fear/anxiety was higher during period C on treatment days 2 and 4

( $F = 8.3, P = 0.0114$ ) showing a post-treatment effect on fear/anxiety.

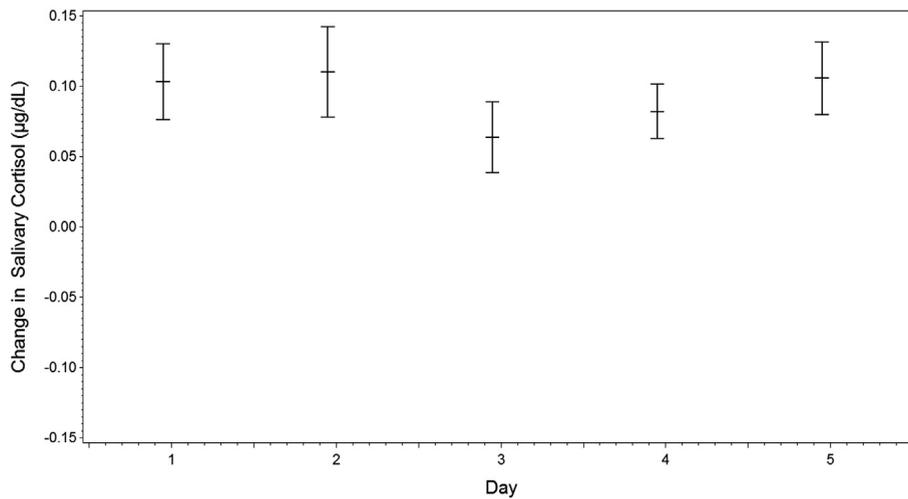
Partial Spearman correlations between fear/anxiety scores and distance (controlling for day and period) found that active, inactive, and global fear/anxiety scores were significantly correlated with distance traveled (active  $s = 0.71$ , inactive  $s = 0.37$ , global  $s = 0.65$ ;  $P < 0.0001$  for each). Fear/anxiety scores were also correlated with both pre-session heart rate ( $s = 0.391, P < 0.001$ ) and post-session heart rate ( $s = 0.525, P < 0.0001$ ).

#### Discussion

Our results highlight 2 key behavioral features of dog behavior in the OFT: habituation and response to sound stressors. In the present study, habituation occurred in dogs over 5 days in the OFT and within each day. Dogs traveled significantly farther on day 1 than on subsequent days and there was a significant decrease in distance traveled over 5 days. On all days, dogs traveled further during the first 3 minutes (period A) than during the last 3 minutes of each session. Our data suggest that motor activity might be a useful measure for evaluating habituation in the open field, as is frequently done in rodent studies ([Bolivar et al., 2000](#)). We also



**Figure 3.** Mean ( $\pm$ SE) change in body temperature over days (post-session value – pre-session value) in the open-field test arena. SE, standard error.



**Figure 4.** Mean ( $\pm$ SE) change in salivary cortisol (post-session value – baseline value) over days for the open-field sessions for males (gray) and females (black). SE, standard error.

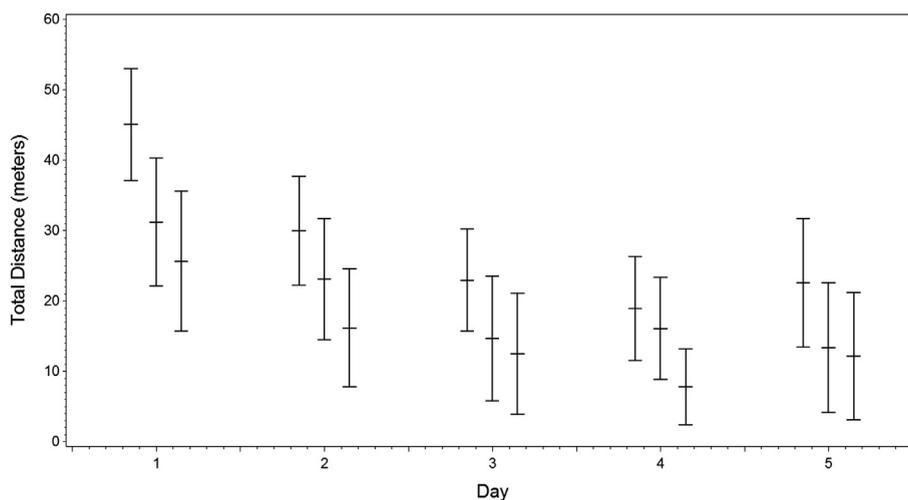
observed physiologic changes consistent with habituation. For example, post-session heart rates were higher on day 1 as compared with other days, and there was a decrease in heart rate over the 9-minute course of the session on control days 3 and 5. In general, exploratory behavior in an open field is decreased both within a session and between sessions as the environment loses its novelty. This change in exploratory behavior has been demonstrated in multiple species (Leussis and Bolivar, 2006; Matsunaga and Watanabe, 2010) including dogs (Head et al., 1997). Our results are consistent with other studies showing that habituation in an open field is an adaptive phenomenon characterized by decreased responses following a continuous or repeated stimulus over time (Head et al., 1997; Matsunaga and Watanabe, 2010).

Our study also demonstrated sound-induced behavioral and physiologic responses suggestive of sound aversion or fear. Fear/anxiety scores measured during thunderstorm and gunfire sounds on treatment days 2 and 4 were significantly elevated when compared with control periods. Elevations of active, inactive, and global fear/anxiety scores highlight the importance of training observers or handlers to attend to cues that clearly show agitation (active fear/anxiety signs) as well as those that might be more subtle and easier to dismiss (inactive fear/anxiety signs). Global

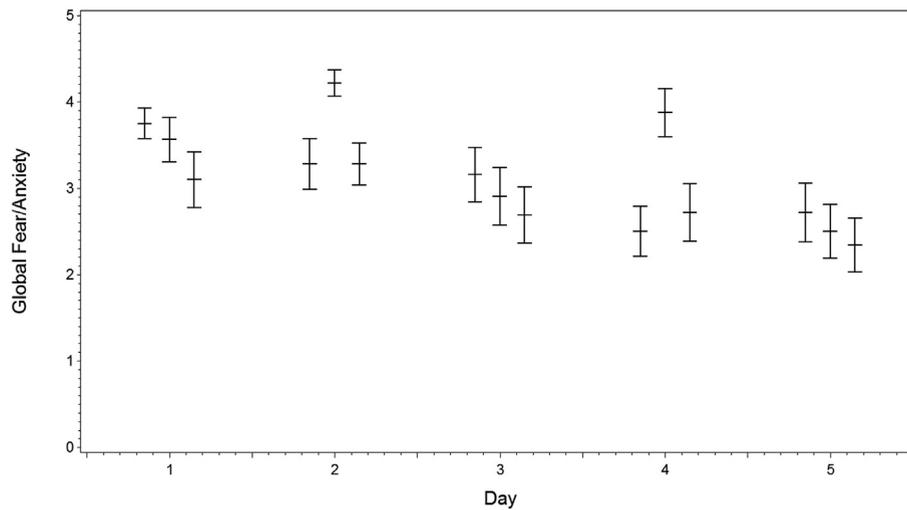
fear/anxiety reflects a composite representation of a range of behavioral responses, from minor to pronounced. We also observed sound-induced changes in motor activity. The mean distance traveled during Period B on study days 2 and 4 was significantly greater than the corresponding period during control days, and the distance traveled correlated with fear/anxiety scores.

The sound OFTs stratified dogs with respect to their fear/anxiety responses. We have shown that their fear/anxiety scores were correlated with these dogs' performance on an "Emotional Reactivity Test" (Sherman et al., 2014). This relationship suggests that response to relevant sounds may be a useful criterion for selection or rejection of dogs for IED training. Further performance testing is needed to determine if the OFT to relevant sound stimuli might predict dog responses to the loud sounds that occur in combat situations.

Testing in the open field also produced a significant increase in salivary cortisol concentration over baseline levels; however, our results did not reveal an effect of day or treatment consistent with conditioned sound-induced fear or anxiety. Salivary cortisol concentration has been used as a noninvasive surrogate measure for plasma cortisol concentration to detect the effects of stress in dogs (Beerda et al., 1996; Kirschbaum and Hellhammer, 1989). The lack of



**Figure 5.** Mean ( $\pm$ SE) distance traveled (in meters) in the open-field test arena by day and period (A, B, and C). Thunderstorm sound was presented on day 2, period B and gunfire sound was presented on day 4, period B. SE, standard error.



**Figure 6.** Global fear/anxiety scores ( $\pm$ SE) by day and period (A, B, and C). On days 2 and 4, global anxiety scores were higher in response to sound stimuli (period B) compared with periods A and C ( $P < 0.0001$ ). Patterns for inactive and active fear/anxiety scores were similar. SE, standard error.

significant correlative finding is supported by [Beerda et al. \(1998\)](#) in which there was a nonsignificant correlation between behavioral and physiological stress parameters in their tests of dogs exposed to different stimuli. Indeed, other efforts to establish the predictive validity of increased salivary cortisol levels in dogs have met with similar difficulty ([Hekman et al., 2012](#)). We also observed that dogs' heart rates had returned to baseline levels at the time of measurement (i.e., after Period C), but did not show the decrease, attributed to habituation, observed on control days. [Beerda et al. \(1998\)](#) found that recovery for heart rate increases after sound exposure in dogs was approximately 6 minutes from the onset of the noise stimulus. Rectal temperature was likewise an insensitive physiologic measurement in our study, showing insignificant changes over the 9-minute sessions and across days, with the exception of the initial acclimation session on day 1.

Another goal of our study was to assess sound-induced anxiety in candidate IDD's by comparing fear/anxiety scores during the period after the sound stimulus. Unlike previous studies using recorded thunderstorm sounds in a canine OFT model ([Araujo et al., 2013](#)), we observed a rapid return to baseline (period A) scores during period C on days 2 and 4. However, the scores did not fall below baseline scores as they did during period C on nontreatment days. This suggests a disruption in the pattern of habituation observed on control days. In fact, after statistically controlling for day and period, the global fear/anxiety score was higher during period C on treatment days 2 and 4 compared with nontreatment days. These observations suggest that we were able to show sound-induced fear during period B and persistence of sound-induced anxiety during period C. There was no increase in fear/anxiety scores during Period A on the days after the sounds stimulus (days 3 and 5).

The responses seen in the Labrador retrievers used in the present study appear to have lower sound-induced behavioral responses when compared with adult Beagles ([Araujo et al., 2013](#)). This observation suggests that different populations of dogs may demonstrate reduced response to a sound-induced stressor. Several studies have presented breed differences in responsiveness to sound stimuli, including gunfire, and suggested that Labrador retrievers may be more resilient to sound-induced fear/anxiety ([Blackwell et al., 2013](#); [Mehrkam and Wynn, 2014](#)). Even within the Labrador retriever breed, different lines of dogs ("field" Labradors vs. "conformation" Labradors) may show different levels of resilience, with field-type dogs showing decreased noise fear ([Lofgren et al., 2014](#)). The dogs used in the present study were bred as

field trial dogs and had been preselected as candidate IDD's. As such, they were not randomly selected as representatives of the Labrador breed. Another possibility is that more intense sound stressors may be needed with certain cohorts of dogs. However, our study used only a 3-minute sound stimulus meant to generate an acute stress effect without producing distress [i.e., an aversive, negative state in which an animal's coping and adaptation responses fail to return the animal to a state of normal physiological and/or psychological well-being ([National Academies Press, 2008](#))].

Several changes in our experimental design could be considered in future studies. For example, noninvasive methods for recording heart rate and body temperature over the course of the session may better characterize physiologic responses during the test session ([Bergamasco et al., 2010](#)). Likewise, saliva samples taken immediately pre- and post-session may reveal more contemporaneous changes.

Based on our results, we conclude that the OFT is a useful model for evaluating acute sound-induced aversion or fear reactions in candidate IDD's. As expected, dogs in this study showed habituation in the open field, as measured by physiologic and activity data and behavioral changes suggestive of sound aversion or fear. In a companion report ([Sherman et al., 2014](#)), results from the OFT fear/anxiety scores were well correlated with a screening test for emotional resilience (discussed in [Sherman et al., 2014](#)). Collectively, these features allow the OFT model, and the use of a standardized subjective scoring system, to be considered as a screening test for Labrador retrievers to be used as IDD's.

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### Conflict of interest

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