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Research Paper

Phenotypic determination of noise reactivity in 3 breeds of working dogs: A cautionary tale of age, breed, behavioral assessment, and genetics

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ABSTRACT

Noise reactivity is a common problem for dogs and may progress to true phobia. Survey studies report that some type of noise reaction occurs in up to half of all pet dogs throughout their lifetimes, indicating that noise reactivity and/or phobia is a welfare issue. Familial aggregations of affected dogs have been reported, and increased prevalence in certain breeds has been suggested. Reactivity to noise can severely compromise function in both pet and working dogs. Noise reactivity may be comorbid with many anxiety disorders for both canines and humans and is postulated to effect information processing in associated human, rodent, and dog conditions. Any putative effect of noise on information processing becomes a concern for problem solving and other aspects of cognition that are important to working dogs. Accordingly, we sought to phenotype 3 breeds of herding dogs commonly used for work as detection dogs, police and/or patrol dogs, search and rescue dogs, and/or service dogs: Australian shepherds (AUS), border collies (BOC), and German shepherds (GSD). We analyzed demographic information and behavioral responses to noises (guns, storms, and fireworks) known to provoke fearful or phobic responses for 59 AUS, 81 BOC, and 58 GSD, who were also included in a genetic analysis. Behaviors were compared using a metric constructed from information on type, frequency, and intensity of response, and the Anxiety Intensity Rank (AIR) score. Reactivity to noise was found to segregate in some family lines for the dogs in this study, although individuals expressed considerable variation in noise response. Such variation may be time and exposure dependent and presents a phenotyping challenge. In this study, the presence and intensity of reactivity as represented by AIR scores varied by breed but only slightly with age. The BOC studied were older, and BOC and AUS were more severely affected (higher AIR scores) than were GSD. Source and/or purpose of dog may also affect severity of affliction. Determination of crisp and accurate phenotypes is essential for understanding underlying genetic contributions. For noise reactivity and/or phobia, accurate phenotypes include age of onset and specific behavioral characterization. Standardized and objective assessments are essential for assessment of progression and comorbidity. Our data imply that accurate phenotypic assessment is possible at a relatively early age, providing for both humane treatment and accurate phenotyping that facilitates good genotyping.

Introduction

Noise reactivity and phobia are common pathologic behavioral conditions in pet dogs. Many surveys report that up to 50% of dogs may be affected by some extreme reaction to some noise during their lifetime (Blackshaw et al., 1990; Dale et al., 2010; Blackwell et al., 2013; Storengen and Lingaas, 2015; Tiira and Lohi, 2015, 2016). Reactions are most commonly reported for storms, fireworks, and guns, but noises associated with vehicles, machines, alarms, and others can also trigger fearful, anxious, or phobic responses in dogs (McCobb et al., 2001; King et al., 2003; Ley et al., 2007).

A number of other terms are often used to describe an adverse reactive, fearful, or phobic response, including noise aversion, noise

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fear, noise stress, storm or thunderstorm phobia, and noise sensitivity. Necessary and sufficient criteria for labeling a dog noise reactive or phobic or any of these other terms neither are usually included in most studies (but see, Overall et al., 2001; Dreschel and Granger, 2005) nor are the range of behaviors potentially displayed by the afflicted dog often noted (but see Overall et al., 2001; Crowell-Davis et al., 2003; Tiira and Lohi, 2015; Tiira et al., 2016). Without standardized and discrete diagnostic criteria as well as unbiased and quantifiable behavioral assays for such criteria, we greatly diminish the possibility of understanding and identifying true familial, breed, and population associations for any behaviors that are problematic for dogs because we cannot recognize dogs that are more similar or dissimilar (Overall, 2005; Overall et al., 2014; Tiira and Lohi, 2015; van Rooy et al., 2014).

Diagnostic criteria also permit discrimination of behavior patterns that are a manifestation of normal behavior from those displayed as a manifestation of abnormal and pathologic behavior. Some response to an acute stressor is normal and adaptive, and such responses are characterized by recovery with a return to the individual's baseline of behavior. Pathologic responses include those that are out of context to the stimulus where signs of acute stress are excessive in duration and/or intensity and become more so with each exposure until an extreme plateau is reached. Spontaneous recovery is absent in pathologic responses. This distinction between normal and abnormal or pathologic is essential because the misinterpretation of canine behavior by humans is common (Haverbeke et al., 2008; Tami and Gallagher, 2009; Kuhne et al., 2012a,b; Wan et al., 2012; Bloom and Friedman, 2013; Kuhne et al., 2014; Foyer et al., 2015), and owners do not recognize subtle signs of anxiety (Mariti et al., 2012). An owner judgment about undefined anxiety, fear, and aggression in a survey questionnaire, especially one using a Likert scale (Hsu and Serpell, 2003; Temesi et al., 2014), is impossible to validate (Diederich and Giffoy, 2006; van Rooy et al., 2014) and is void of information pertaining to behavioral heterogeneity that can allow us to study mechanism.

We propose that it is possible to create objective assessments of behavior that are void of judgment and use clear terminology (Overall et al., 2001; Crowell-Davis et al., 2003; Overall et al., 2006a,b; Tiira et al., 2014). It is possible to identify observable criteria associated with the dog's indication that he is reacting to a stressor (here, noise) and to evaluate those criteria in terms of intensity, frequency, duration, and specific response, as we have done here. Assessment of phenotypes can only elucidate genetic studies if the behavioral criteria used are clear, crisp, and accurately reflect and discriminate among the behaviors exhibited by the dogs.

The diagnostic criteria used here were validated in a clinical study of noise phobia (Overall et al., 2001) and require that noise-phobic dogs exhibit a profound, nongraded, and extreme response to noise manifest as intense avoidance, escape, or anxiety and associated. Such signs are associated with the sympathetic branch of the autonomic nervous system and triggered by reactivity in the locus ceruleus (LeDoux, 2000; Tully and Bolshakov, 2010). Dogs who are characteristically distressed when exposed to specified noises, including storms, but who do not meet the criteria for a phobia may be classified as reactive (Overall, 2013). We chose the term 'reactive' rather than 'sensitive' (Sherman and Mills, 2008; Tiira et al., 2016) because sensitive may imply, could be confounded with, and is commonly used to describe attributes of auditory capability, for which we have no data (but see Scheifele et al., 2016). The term 'reactive' implies no underlying mechanism, merely a lowering of threshold for the behavioral response.

The behavioral signs of distress associated with noise reactivity and phobia are nonspecific but can be benchmarked and quantified. These signs may include trembling, freezing, panting, social withdrawal, pacing, salivating, urinating, defecating, destruction (with or without self-injury), hiding and/or crouching (includes body lowering and tail tuck postures), and escape and/or running away behaviors (with or without self-injury) (Schull-Selcer and Stagg, 1991; Beerda et al., 1997, 1998; Overall et al., 2001; Crowell-Davis et al., 2003; Hydbring-Sandberg et al., 2004; Sherman and Mills, 2008; Cracknell and Mills, 2011), which are all classic responses to anxious states and represent an acute stress response. These are all signs of anxiety that owners can recognize and use to tell when their dog is distressed (Mariti et al., 2012).

Dogs exhibiting these anxious and panicky signs in response to a noise stimulus experience both physical and behavioral debility and compromise (Dykman et al., 1966; Murphree et al., 1967; Overall et al., 2001; Dreschel and Granger, 2005; Dreschel, 2010; Siniscalchi et al., 2013). Noise reactivity and phobia interferes with performance in working dogs (Tomkins et al., 2011, 2012; Gazzano et al., 2007; Batt et al., 2008; Asher et al., 2013; Burghardt, 2013; Arvelius et al., 2014; Sherman et al., 2014; Evans et al., 2015) and interferes routine patterns of daily life in pet dogs (Overall et al., 2001; Crowell-Davis et al., 2003; Gruen and Sherman, 2008; Cottam et al., 2013). Noise reactivity and phobia is associated with patterns of brain organization (Branson and Rogers, 2006; Francks et al., 2007; Siniscalchi et al., 2008; Foler et al., 2011), which may be one mechanism through which pathologic changes occur.

Similar patterns pertain in other species. In rats, performance in maze tests (number of errors, time to goal, and number of rearings) was impaired when the rats were exposed to loud noise (100 dB background noise level, the low end of noise estimates for the stimuli in this study; http://www.noisehelp.com/noise-level-chart.html), and neurons in the hypothalamic paraventricular nucleus, central nucleus, and basolateral nucleus of the amygdala, regions associated with stress, were activated (Amemiya et al., 2010). Chronic and acute noise stresses produced differential responses in the hippocampus but similar responses in the hypothalamus, suggesting that behavioral effects can be influenced by exposure (Eraslan et al., 2015). Noise stress (105 dB) has been shown to impair high-order, prefrontal cortex, and delayed-response performance in cognitive trials in monkeys (Arnsten and Goldman-Rakic, 1998; Arnsten, 2009). Babisch (2003) noted that noise activates sympathetic responses and stimulates epinephrine, norepinephrine, and cortisol, all hormones associated with stress. Acute noise stress in humans has been shown to impair cognitive control in the anterior cingulate cortex (Banis and Lorist, 2012).

Pathologic noise reactions in dogs worsen quickly with exposure, suggesting that adverse effects on mental and physical health are long term and may be more profound than usually appreciated. Many of these reactions may be modulated by changes in glucocorticoid receptor regional activity and subsequent molecular processes that adversely affect both cognitive ability and retrieval and use of memory (Popoli et al., 2011; Nasca et al., 2015; Jasnow et al., 2016; Rogerson et al., 2016). Noise phobia is considered a commonly comorbid condition (affecting both general fears (Tiira and Lohi, 2016)) and specific conditions like separation anxiety (Overall et al., 2001). When noise phobia is comorbid, the signs of each condition are worse than for canine patients with a single anxiety-related condition (Overall et al., 2001), suggesting that the noise pathology, itself, changes the underlying neurochemical or neuronal reactivity. Comorbid conditions in humans and other primates show similar patterns of effect where signaling in the amygdala, hippocampus, and frontal cortex can be impaired in response to repeated stress signaling (Arnsten and Goldman-Radic, 1998; Arnsten 2009).

Materials and methods

Dogs of the 3 breeds studied (59 Australian shepherds [AUS], 81 border collies [BOC], and 58 German shepherds [GSD]) were solicited from breed clubs during working dog trials at breed club shows. For all the dogs, trials involved herding and/or obedience, and for the GSD, many also were involved in Schutzhund (protection sport training and competition). All the AUS and BOC were solicited and sampled in the United States, although more than half of the BOC were from European origin or lines. About half of the GSD were solicited and sampled at a GSD show in Holland, and the overwhelming majority of the rest were detection dogs from various US government contractors. All these dogs likely originated in Europe, although we could not confirm country of origin for all dogs from the contractor records, and pedigrees were unreliable.

Owners of the dogs were asked to complete a short questionnaire (Overall et al., 2001, 2006a,b: see supplemental materials for questionnaire version used) that included demographic questions and that asked whether the dogs reacted to storms and/or thunderstorms, gunshots, fireworks, and other noises.

If the owners and/or handlers noted that their dog responded to other noises, they were asked to specify the noise and describe the reaction because not all reactions to all noises are associated with pathology (e.g., dogs who chased or played with vacuum cleaners were not considered phobic, but those who hid in response to the noise of the vacuum were considered phobic). These stimuli are likely not all perceived the same way for dogs. Storms have visual, auditory, barometric, and other atmospheric components (wind and rain), whereas the sound of a gunshot depends on the weapon, and the sound of fireworks depends on the pattern or the display and chemical formulation. Fireworks also have a visual component present for many but not all storms and absent for most guns. For the purposes of this study, we investigated the reaction to the situation, not individual stimuli (although see Scheifele et al., 2016).

Choices for each of the noise categories were (1) yes, (2) no, or (3) unknown. If the owners chose yes, they were asked to estimate with what frequency to the noise the dog reacted:

- 100% of the time,
- Less than 100% but more than 60% of the time,
- 40%-60% of the time, and
- More than 0% but less than 40% of the time.

Because frequency of reaction may not be independent of frequency of occurrence of the noise stimulus, clients were also asked how often the dog was exposed to each of the noises. Choices were never, occasionally/a few times per year, regularly/about once a month or so, and frequently/a few times a month or more in some seasons. This question ensured we were studying dogs for whom adequate information was available. For the purposes of the analysis, only dogs who experienced the noise regularly/about once a month or so or frequently/a few times a month or more in some seasons were included, but these were the vast majority of the dogs belonging to owners who decided to participate.

For each noise to which the dog reacted, clients were asked to specify the type(s) of response: salivate, defecate, tremble, urinate, vocalize, destroy, pace, escape, freeze, pant, and/or hide (which included crouching—the photo used in the solicitation showed an AUS crouched in a bathtub and a BOC hiding in a crate; see Figure 1). Not all owners will notice all signs equally (Mariti et al., 2012); therefore, there may be some false negatives in our data set, but false positives are minimized because there is no mistaking of these signs if the dog exhibits them when exposed to the triggering noise.

All dogs had a 5-mL cephalic vein blood sample taken using a 21-gauge butterfly catheter. Samples were chilled and sent overnight on ice for later genetic analysis. Preliminary genetic analyses were conducted using Affymetrix arrays (see Yokoyama, 2010 for specific details). Pedigrees were requested for all dogs.

Figure 1. Photos used in solicitation of dogs for the study. Upper *Photo*: Karen Overall. Lower *Photo*: Melanie Chang.

Questionnaires were reviewed by the researchers with the owners on site for completion, errors, and understanding. Owners were encouraged to ask questions so that they understood the importance of the information they were providing. For the Dutch dogs, questionnaires were provided in both English and Dutch, and the Dutch questionnaire was both forward and reverse translated. In addition, a native Dutch speaker aided in the solicitation of the owners and in the review of the questionnaires with both the owner and the researcher. The native Dutch speaker also helped with the discussion of the study and the dog's behaviors between the owner and researcher, if needed.

Data were entered into a Filemaker Pro database. Anxiety Intensity Rank (AIR) scores were calculated by multiplying the number of signs any dog showed by a weight determined by frequency of reaction, with the frequencies mentioned previously receiving a weight of 4, 2.5, 1.5, 1, and 0, respectively, and summed for all provocative stimuli (Overall, 2013). AIR scores can be calculated for a single inciting stimulus, a discrete subset of stimuli, or for a sum of all AIR scores from all trigger stimuli (global AIR score). As reported here, the AIR score refers to the sum of scores across all stimuli (i.e., global AIR score). The total score possible for the 3 main stimuli if all signs were evident all the time is 132. This extreme score represents an upper bound and would be exceptional because dogs would have to exhibit both freezing and pacing, which is possible, but appears to be rarely reported.

Data were subjected to routine parametric, where appropriate, and nonparametric analyses using R software (The R Project for

Statistical Computing; https://www.r-project.org/; R Core Team, 2016). The phenotyping results for dogs of each breed for which preliminary genome-wide association (GWA) analysis data were available are discussed here.

All aspects of this study were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC) as required by the US law. Additional approvals were obtained from the funding agencies' IACUC. All participating owners signed an informed consent statement.

Results

Only dogs who experienced noise regularly/about once a month or so or frequently/a few times a month or more in some seasons were included in this study. No one reported that their dog was never exposed to noises.

Most owners and/or handlers participating in this study indicated that their dog never reacted to noises, although fewer BOC (26 of 81) than AUS (32 of 59) and GSD (42 of 58) were reported to not react. Of those who reported the various behavioral reactions to noise stimuli, most AUS and BOC owners reported that the dog always reacts, regardless of noise stimulus, but more AUS were reported to react less than 100% but more than 60% of the time. Owners and/or handlers of GSD most commonly reported that dogs react less than 60% but more than 40% of the time for all stimuli, a different presentation than for the other breeds (Figure 2) (all comparisons, $P < 0.0001$).

Mean ages of dogs in this study were 58.00 months for AUS (N = 59), 79.95 for BOC (N = 81), and 42.85 (N = 58) for GSD (Figure 3; Table 1). In this study, BOC were older than AUS, and both AUS and BOC were older than GSD. This pattern was reflected in both those dogs reported to react and those dogs who did not react to noise. As reflected by the age distribution, young and immature dogs were a small part of this study population: no GSD, 2 BOC, and 4 AUS were 10 months of age or younger (Figure 3). Puppies and immature dogs are not common at trials where these dogs were solicited. Our first large pulse of dogs with noise reactivity and/or phobia for all breeds occurred at 20 months, concomitant with social maturity.

The nonzero global AIR score data (e.g., affected dogs only) were 18.02 for AUS (N = 27; range, 1-64), 25.25 for BOC (N = 55; range, 1-84), and 6.13 for GSD (N = 16; range, 1.5-16) (Table 2). The mean AIR scores for AUS and BOC are not significantly different, but both differ significantly from those of GSD, which are considerably lower. In the dogs studied here, GSD are less affected by noise reactivity than are AUS and BOC (AUS × BOC, not significant, $P = 0.322$; F test; AUS × GSD and BOC × GSD, $P = 0.00001$; F test).

No regression of AIR scores on age for any breed was significant except for that of GSD (Figure 4). The linear regressions of AIR score on age for affected dogs of each breed were as follows: AUS: $F = 1.315$; $df = 1.25$; $P > 0.262$; $r^2_{adj} = 0.12$; BOC: $F = 1.387$; $df = 1.47$; $P > 0.244$; $r^2_{adj} = 0.008$; and GSD: $F = 11.69$; $df = 1.13$; $P < 0.005$; $r^2_{adj} = 0.433$. In this study, older GSDs had lower scores than younger dogs.

There are no significant regressions for any breed when comparing age with number of signs shown by the dogs under any stimulus condition (Figure 5). For AUS, thunderstorms ($F = 0.184$; $df = 1.17$; $P > 0.67$), fireworks ($F = 0.850$; $df = 1.19$; $P > 0.369$), gunshots ($F = 0.203$; $df = 1.9$; $P > 0.66$). For BOC, thunderstorms ($F = 4.01$; $df = 1.44$; $P > 0.051$), fireworks ($F = 0.748$; $df = 1.40$; $P > 0.392$), gunshots ($F = 0.064$; $df = 1.30$; $P > 0.80$). Regression analysis on the GSD data cannot be done because of the lack of variation in the dependent variable. This finding suggests that the condition of noise phobia and/or reactivity was fully developed in the dogs in this study. The average age of any breed in our study was older than 40 months.

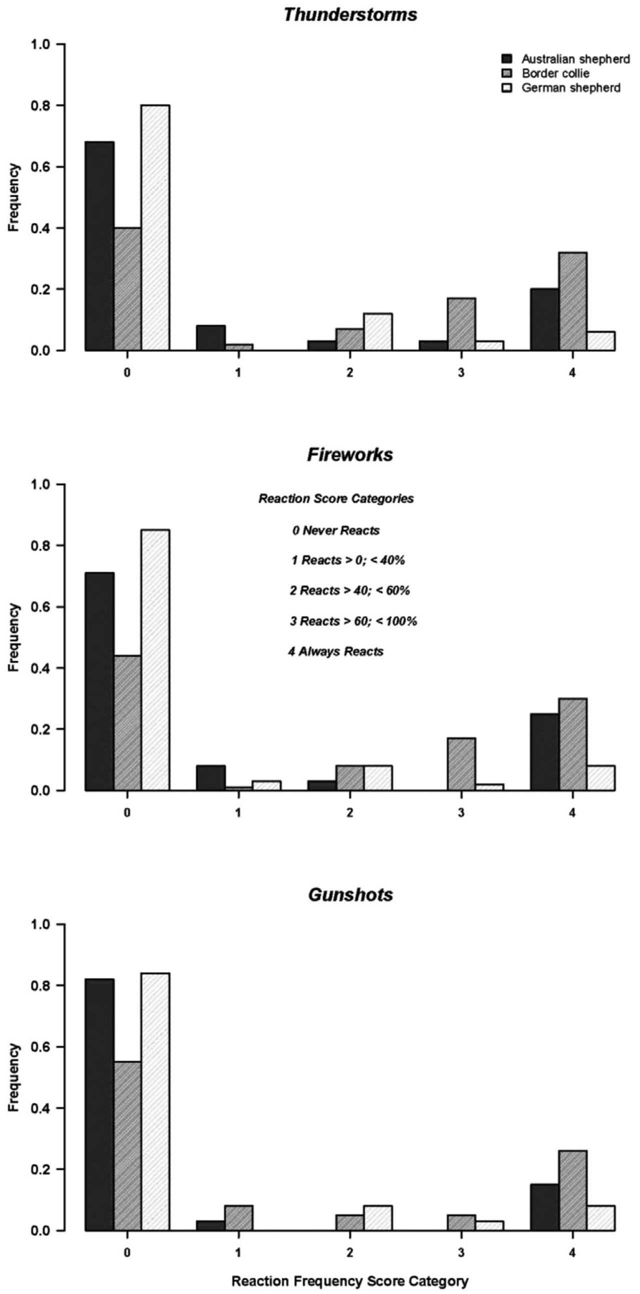

Figure 2. Frequency of reported reaction score categories by breed and stimulus. Border collie (N = 81), Australian shepherd (N = 59), and German shepherd (N = 58). Score distributions for all breeds are significantly different. Randomization tests, all $P < 0.05$.

For none of the breeds in this study was co-occurrence of signs independent, regardless of the type of provocative stimulus (Spearman rank correlation analysis with all $P < 0.01$; Table 3), suggesting that true comorbidity may be occurring. Our data show that (1) reactions for all breeds are not general noise responses but responses to specific stimuli and (2) reactions to different stimuli are highly comorbid. Pairwise permutation tests indicate that all conditional probabilities are significantly greater than 0.7 or higher with all $P < 0.001$ (Table 4).

Breeds from the populations used in this study differ in the signs and frequencies of signs that they show within and across provocative stimuli (Figure 6). In this study, GSD differed from AUS and BOC, 2 breeds that have GSD in their genetic ancestry

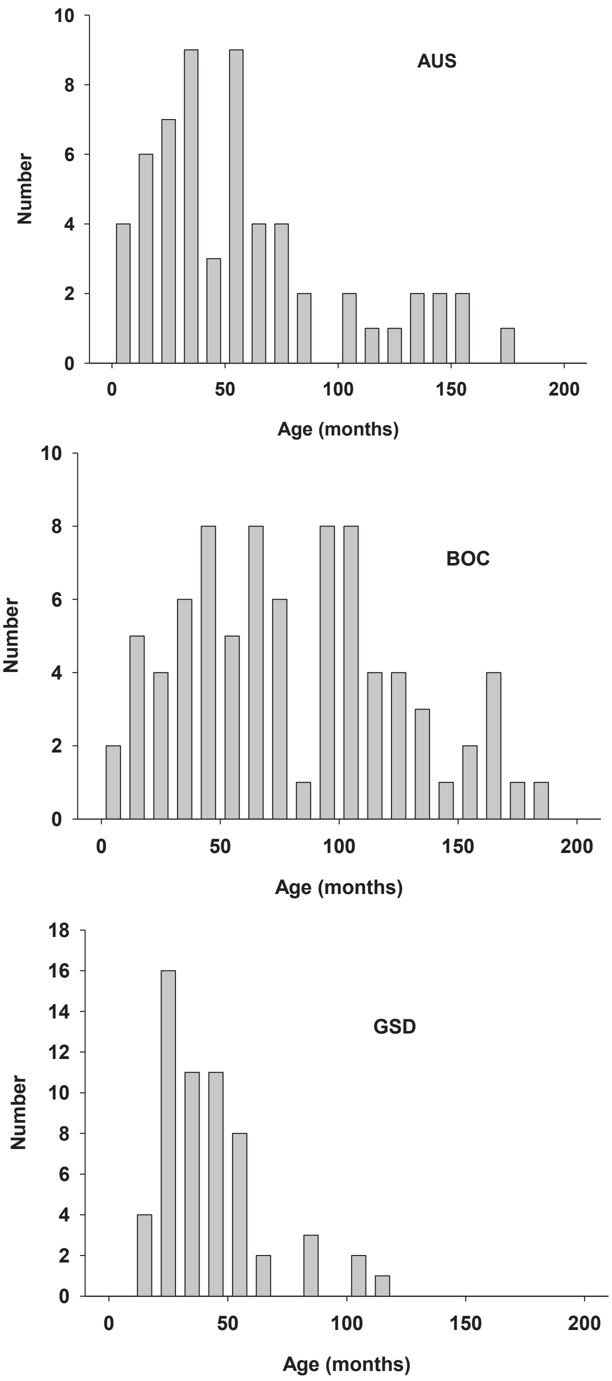

Figure 3. Age frequency distribution by breed. Note differences in Y-axis by breed. Mean ages differ significantly among breeds (analysis of variance, $F = 15.62$; $df = 2.195$; $P < 5.08\text{e-}7$). Post hoc comparison tests (Tukey honest significant difference test) reveal significant differences among all pairwise comparisons except AUS-GSD ($P_{adj} = 0.096$). BOC differ significantly from both AUS ($P_{adj} < 0.004$) and GSD ($P_{adj} = 0.04\text{e-}5$). AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.

(vonHoldt et al., 2010). Pairwise permutation tests indicate that the distribution of signs for GSD differs significantly from those of both AUS ($P < 0.0001$) and BOC ($P < 0.0001$). The distribution of signs of AUS and BOC does not differ significantly from each other ($P > 0.45$). Regardless of stimulus, GSD in this study paced, whereas BOC and AUS reacted by hiding and panting in a relatively constant way across stimuli. GSD in this study were never reported to salivate, escape, tremble, or freeze.

Table 1
Age data by breed

Breed	N	Mean (mo)	SE	95% CI	Median
AUS	59	58.00	5.60	11.21	50
BOC	81	79.95	5.01	9.98	72
GSD	58	42.85	3.07	6.14	37

SE, standard error; 95% CI, 95% confidence interval; AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.
All pairwise comparisons for means significantly different (Welch's t test; all $P < 0.0001$). Significance levels estimated by permutation tests using 10,000 permutations per test. All pairwise comparisons for medians are significantly different (Wilcoxon rank sum tests; all $P < 0.05$).

A GWA analysis revealed areas of interest on chromosomes 5, 8, and 10. No findings reached genome-wide significance (Yokoyama, 2010). Treating these dogs as affected (dogs reacted 60% and more of the time) or not and expanding the number of dogs genotyped (BOC, 189; AUS, 119; GSD, 93) allowed a population-based case-control comparison of BOC, AUS, and GSD and yielded some potential regions of interest on chromosomes 7, 10, 12, 23, 25, and 28. The strongest finding in the case-control comparison for an expanded subset of data was for chromosome 12 in an intron of the *KLHL32* gene, which was the top finding in the BOC only analysis, the combined BOC × AUS and BOC × AUS × GSD analyses (2×10^{-06}; odds ratio, 0.07; 95% confidence interval, 0.02-0.24) (Overall and Hamilton, unpublished, Defense Advance Research Projects Agency [DARPA] report).

Examination of population substructure revealed an average heterozygosity for AUS of 0.424 (standard deviation [SD], 0.019), greater than that for BOC, which was 0.407 (SD, 0.007) (Yokoyama, 2010). The SD for AUS was nearly 3 times that of BOC, suggesting that the BOC we studied were less variable. The underlying genetic substructure between BOC and AUS is shown in Figure 7. A pedigree for one of the BOC families in this study is shown in Figure 8.

Discussion

One of the challenges for phenotyping behaviors in dogs and of canine behavioral genetic studies, in general, is enrollment of a suitable number of dogs. The data presented here focus on 59 AUS, 81 BOC, and 58 GSD recruited over an 8-month period at trials and included in a GWA analysis. Over the course of 1.5 years, 189 BOC, 119 AUS, and 93 GSD were recruited, but the putative polygenic nature of most behavioral conditions requires even larger numbers of unrelated dogs (van Rooy et al., 2014). It is possible to recruit the appropriate dogs and engage in the type of due diligence required to view the dogs and discuss them with each owner, but the process is viewed as labor intensive and expensive.

Table 2
AIR score data for *affected dogs* (nonzero AIR score) by breed

Variable	AUS	BOC	GSD
N	27	55	16
Mean	18.02	25.25	6.13
SD	16.67	19.94	4.72
SE	3.21	2.69	1.18
Max	64	84	16
Min	1	1	1.5

AIR, Anxiety Intensity Rank; AUS, Australian shepherd; BOC, border collie; GSD, German shepherd; SD, standard deviation; SE, standard error; Max, maximum AIR score; Min, minimum AIR score.
Mean AIR scores for AUS and BOC are not significantly different ($t = 1.73$, $df = 60.87$, $P > 0.0839$), but both are significantly different from those of GSD (AUS-GSD: $t = 3.48$, $df = 32.48$, $P < 0.0015$; BOC-GSD: $t = 6.51$, $df = 67.76$, $P < 1\text{e-}08$).

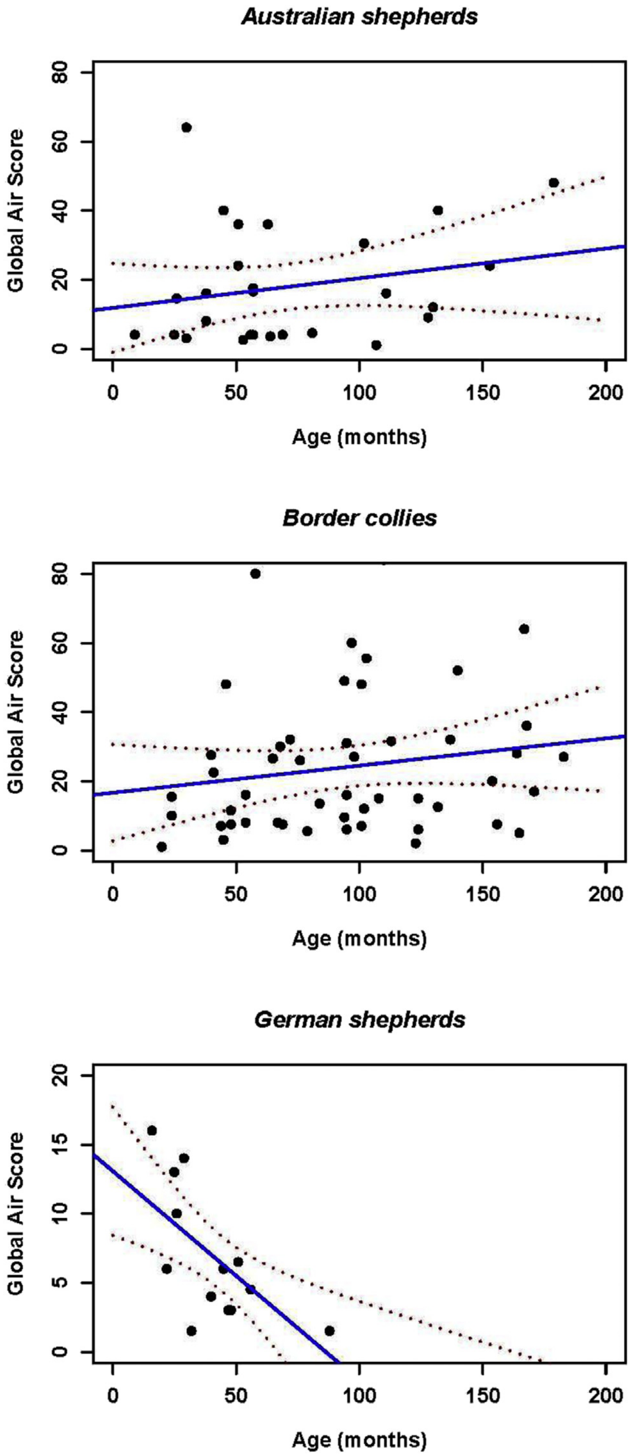

Figure 4. Linear regressions of Anxiety Intensity Rank score on age for affected dogs of each breed. Note differences in Y-axis for German shepherd. Australian shepherd: $F = 1.315$; $df = 1.25$; $P > 0.262$; $r^2_{adj} = 0.12$. Border collie: $F = 1.387$; $df = 1.47$; $P > 0.244$, $r^2_{adj} = 0.008$. German shepherd: $F = 11.69$; $df = 1.13$; $P < 0005$; $r^2_{adj} = 0.433$.

All dogs in this study on noise reactivity and/or phobia were exposed to the main classes of noises discussed (storms, fireworks, and gunshots) sufficiently often for owners to comment in an informed manner on whether the dogs were reactive and/or phobic. Although not all the signs of noise reactivity and/or phobia are equally obvious to all owners, all have been noted in clinical patients in various studies (Overall et al., 2001; Crowell-Davis et al., 2003; Cracknell and Mills, 2011). With the exception of 1 contractor who provided dogs, participating groups and individuals appeared extremely knowledgeable about and interested in their dogs.

Most AUS and GSD were reported to not react to exposure to these noises. Dogs for this study were solicited at trials, which may be one reason why so many of these dogs were unaffected. Although one might expect that dogs at trials are less seldom affected with behavioral conditions than are dogs in the population as a whole, this may not be the case. We do not know which owners are willing to share behavioral information with researchers, and without knowing this, no assumptions should be made. It is possible that people from trialing populations are happy to share information about their dogs when the dogs are unaffected by behavioral problems but are less willing to do so if their dogs are affected. We lack good prevalence data for this and all other conditions in behavioral medicine, and it is a problem.

Breeders, trainers, and owners may feel a stigma about behavioral pathologies. Owners seeking the help of specialists in behavioral medicine often report that they feel that they have contributed to the problem or feel guilty or responsible for some aspect of it; therefore, it is possible that others feel similarly. This unfortunate and usually incorrect attitude adversely affects studies like ours, but it also prevents dogs from getting needed and available specialist and researcher help in a timely manner. As a result, breeding of dogs and genetic lines that may be at enhanced risk for behavioral pathology continues without the benefit of genetic and behavioral counseling. Although dogs with behavioral conditions are excellent natural animal models for human psychiatric illnesses (Overall, 2000; Overall et al., 2001; Overall and Dunham, 2002; Dodman et al., 2010, 2016; Ogata et al., 2013; Cao et al., 2014), breed and individual health is primary and improved by establishing and participating in such studies (O'Neill et al., 2013; Overall et al., 2014; van Rooy et al., 2014).

Unfortunately, there are no population-wide epidemiologic data in this field that could allow us to learn whether participation tracks incidence or true prevalence, although Tiira et al. (2016) provide some survey data. Our study provides some of the first age- and breed-associated incidence data available not solely from long-distance surveys, and it is an inadequate base for conclusions other than those which are conservative. The lack of comparable and validated behavioral data across populations in the fields of canine behavior and canine behavioral medicine has been and remains an impediment to progress (Overall et al., 2014; van Rooy et al., 2014). In no small part, this deficiency is because of lack of standardized terminology, lack of validated assessment tools, and lack of validated ethograms (Overall, 2013a,b, 2014; Overall et al., 2006a,b, 2014; van Rooy et al., 2014; O'Neill and Packer, 2016; Stone et al., 2016). An understanding of underlying mechanism for any behavior—normal or pathologic—at any level, including the genetic one, requires a rule to cluster increasingly more similar patterns in groups separate from those which are increasingly less similar, in a manner that acknowledges the role of context and that will facilitate objective tests of underlying mechanism (Overall, 2005).

Both the ages of dogs in this study and the lack of any significant regression of AIR scores on age for BOC and AUS supports that the condition was fully developed by the time these dogs were assessed and that young and immature dogs were not a large part of this study population. As noted, our first large pulse of dogs with noise reactivity and/or phobia for all breeds occurred at 20 months, concomitant with social maturity. The implication is that by 20 months of age, noise reactivity and/or phobia is fully developed. Social maturity, when neurochemical liability is thought to develop or become characteristic as has been suggested in rodents and humans (see Kerestes et al., 2014 for a review of imaging studies;

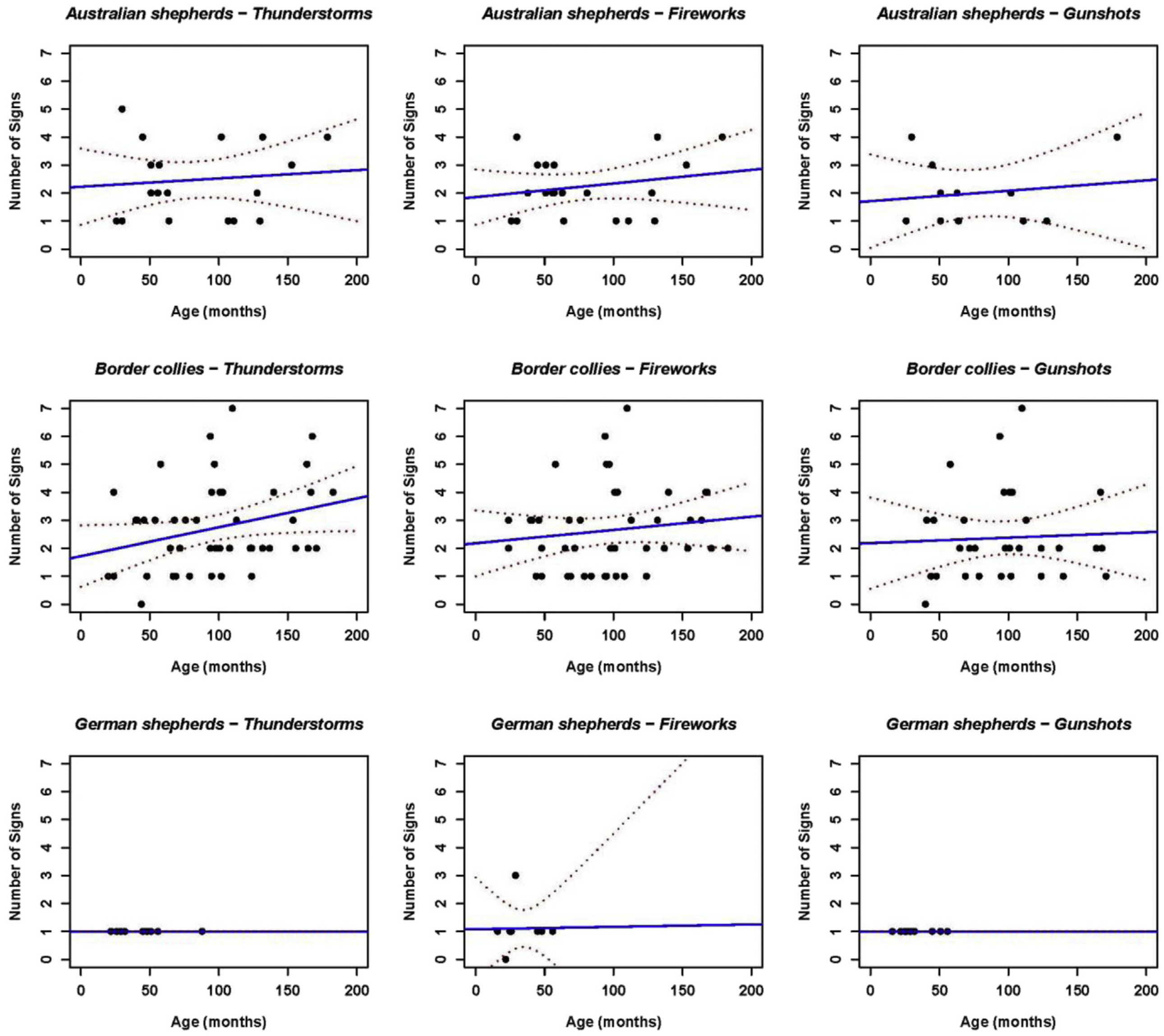

Figure 5. Number of signs as a function of stimulus × age. Note that the scales for the X- and Y-axes vary by breed and condition. For AUS, thunderstorms ($F = 0.184$; $df = 1.17$; $P > 0.67$), fireworks ($F = 0.850$; $df = 1.19$; $P > 0.369$), gunshots ($F = 0.203$; $df = 1.9$; $P > 0.66$). For BOC, thunderstorms ($F = 4.01$; $df = 1.44$; $P > 0.051$), fireworks ($F = 0.748$; $df = 1.40$; $P > 0.392$), gunshots ($F = 0.064$; $df = 1.30$; $P > 0.80$). Regression analysis on the GSD data cannot be done because of lack of variation in the dependent variable. AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.

Whittaker et al., 2016), appears to occur between ~10 and 36 months, with the most common manifestations being apparent at 12-18 months (Overall, 2013), the time when behavioral tests become reliable (Asher et al., 2013; Foyer et al., 2014; Evans et al., 2015; Harvey et al., 2015; 2016). That GSD who were older had lower AIR scores than those who were younger may suggest that as dogs age, those GSD who react to noise were excluded from the trialing population. Given the source of our GSD and the testing to which they are exposed, this hypothesis may have merit. These GSD may have come from more than one population of European dogs.

It is important to remember that our data are a snapshot in time. No dogs were followed through time so no time-penetrant developmental patterns should be assumed, although they are reported in clinical populations (Overall et al., 2001; Crowell-Davis et al., 2003; Dreschel, 2010; Overall, 2013). Regardless, this study strongly supports the published recommendation and need for frequent (at least 3), presocial maturity veterinary evaluations of all dogs, with routine behavioral screening, including for reactions to noise (Hammerle et al., 2015). Relatively short questionnaires that can be used at each visit to track development of behavioral pathologies, including noise reactivity and/or phobia, are in the public domain (Overall, 2013).

The mean AIR scores for AUS and BOC did not differ from each other, but both differed from those of GSD, which also displayed different reactive behaviors when exposed to noise. Regardless of stimulus, GSD in this study most commonly paced, whereas BOC and AUS reacted by hiding and panting in a relatively constant way across stimuli. All 3 of these signs were reported in more than 50% of dogs of various breeds enrolled in a clinical study, which found that 94% of the afflicted dogs exhibiting panting, 88% exhibiting trembling, 88% becoming clingy or seeking atypical closeness, 86% pacing, and 81% hiding (Crowell-Davis et al., 2003). These breed-associated behavioral differences are important because in rats, crouching behaviors may be indicators of fear (Blanchard and Blanchard, 1969), and increasing numbers of canine studies are using lowered body postures as similar

Table 3
Co-occurrence of signs related to noise reactivity

Breed/stimulus	Thunderstorms	Fireworks	Gunshots
AUS (N)			
Thunderstorms	1.000	0.896^{****} (59)	0.685^{****} (59)
Fireworks	0.759^{***} (21)	1.000	0.753^{****} (59)
Gunshots	0.678^{***} (21)	0.478^{*} (21)	1.000
BOC (N)			
Thunderstorms	1.000	0.885^{****} (87)	0.717^{****} (87)
Fireworks	0.608^{***} (53)	1.000	0.842^{****} (87)
Gunshots	0.394^{**} (53)	0.678^{***} (53)	1.000
GSD (N)			
Thunderstorms	1.000	0.860^{****} (59)	0.697^{****} (59)
Fireworks	0.460^{**} (14)	1.000	0.805^{****} (59)
Gunshots	—	—	1.000

AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.
$^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$.
This table presents the Spearman rank correlation of individuals that react to the stimulus in the first column to reaction to the other stimuli (listed in the other 3 columns).

indicators (Schilder and van der Borg, 2004; Haverbeke et al., 2008; De Meester et al., 2011; Tiira et al., 2016).

In the present study, GSD were never reported to salivate, escape, tremble, or freeze, signs commonly reported in both clinical (Overall et al., 2001) and nonclinical (Tiira et al., 2016) studies. It is tempting to make an argument about shared breed derivations (VonHoldt et al., 2010), but only a subset of the dogs in this study (BOC > AUS > GSD) was severely affected by exposure to noises that triggered their distress and none of the GSD were. No dogs in this study urinated, defecated, or destroyed, nonspecific signs routinely reported in clinical noise-phobic patients (Overall et al., 2001; Crowell-Davis et al., 2003). The lack of reports of salivating, escaping, trembling, and freezing for GSD in this study suggested that the GSDs surveyed were mildly affected, a conclusion supported by AIR score patterns. In clinical situations, GSD are reported to exhibit the range of behaviors associated with greater distress. It is possible that there are multiple subpopulations of dogs within any breed that react to noise in different ways for a given stimulus. If so, this would suggest underlying population genetic and mechanistic variability.

Knowledge of nonspecific signs exhibited is essential for behavioral genetics studies. Our data suggest that although breeds appear to share genetic regions that likely increase their liability risk for noise reactivity and/or phobia, there are also heritable differences within breeds that affect the manifestation of the reactivity (e.g., specific behavioral response) within that breed. Indeed, different regions of the brain, in general, and the amygdala, specifically, affect the behaviors exhibited by noise reactive and/or

Table 4
Comorbidity data by breed and provocative stimulus showing conditional probabilities of having comorbid conditions

Given that you react to this stimulus ↓	Conditional probability of reacting to these other stimuli		
	Thunderstorms	Fireworks	Gunshots
AUS			
Thunderstorms	—	0.95 (19/20)	0.75 (12/20)
Fireworks	0.86 (19/22)	—	0.71 (12/17)
Guns	1.00 (12/12)	1.00 (12/12)	—
BOC			
Thunderstorms	—	0.96 (48/50)	0.84 (38/45)
Fireworks	0.98 (48/49)	—	0.91 (41/45)
Guns	0.97 (37/38)	1.00 (38/38)	—
GSD			
Thunderstorms	—	0.69 (9/13)	0.69 (9/13)
Fireworks	1.00 (9/9)	—	0.89 (8/9)
Guns	0.89 (8/9)	0.78 (7/9)	—

AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.
Pairwise permutation tests indicate that all conditional probabilities are significantly greater than 0.7 or higher with all $P < 0.001$.

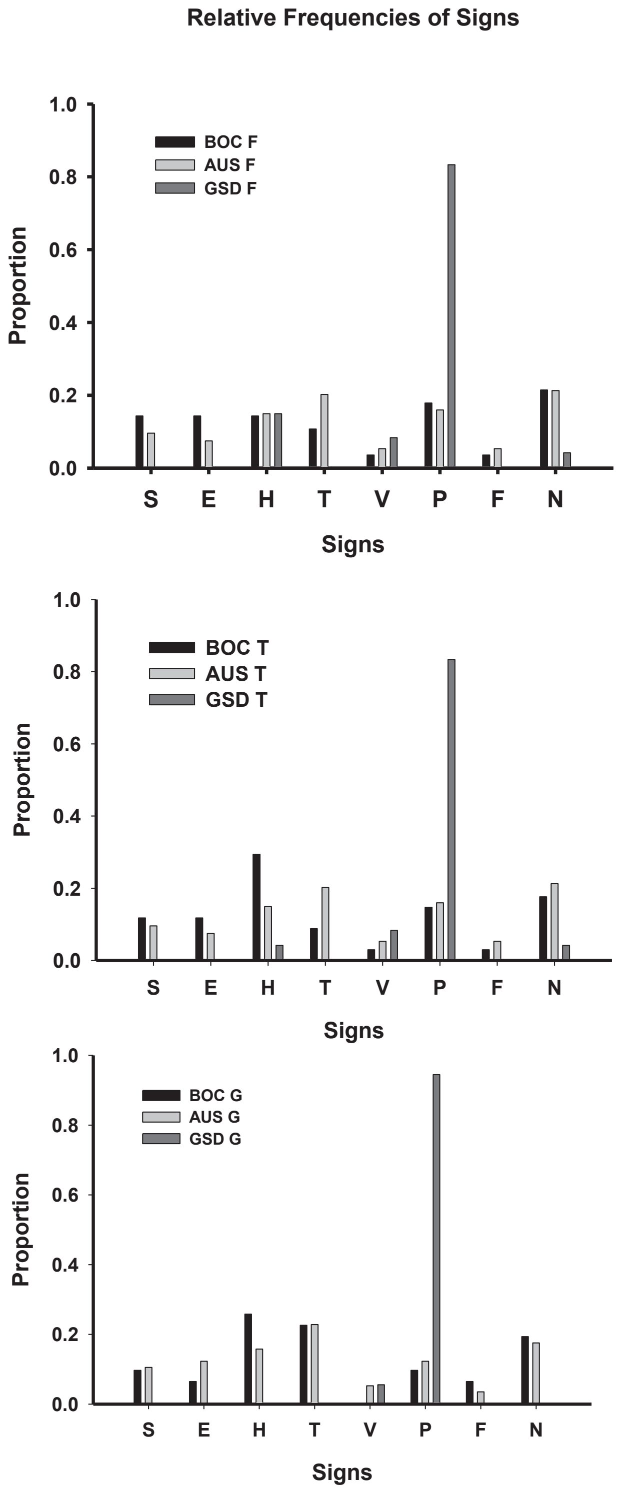

Figure 6. Relative frequencies of signs, given breed, and provocative stimulus. *Conditions*: F = fireworks, T = thunderstorms, and G = guns and *Signs*: S = salivate, E = escape, H = hide, T = tremble, V = vocalize, P = pace, F = freeze, and N = pant. Behaviors not engaged in are not represented. Pairwise permutation tests indicate that the distribution of signs for GSD differs significantly from those of both AUS ($P < 0.0001$) and BOC ($P < 0.0001$). The distribution of signs of AUS and BOC does not differ significantly from each other ($P > 0.45$). AUS, Australian shepherd; BOC, border collie; GSD, German shepherd.

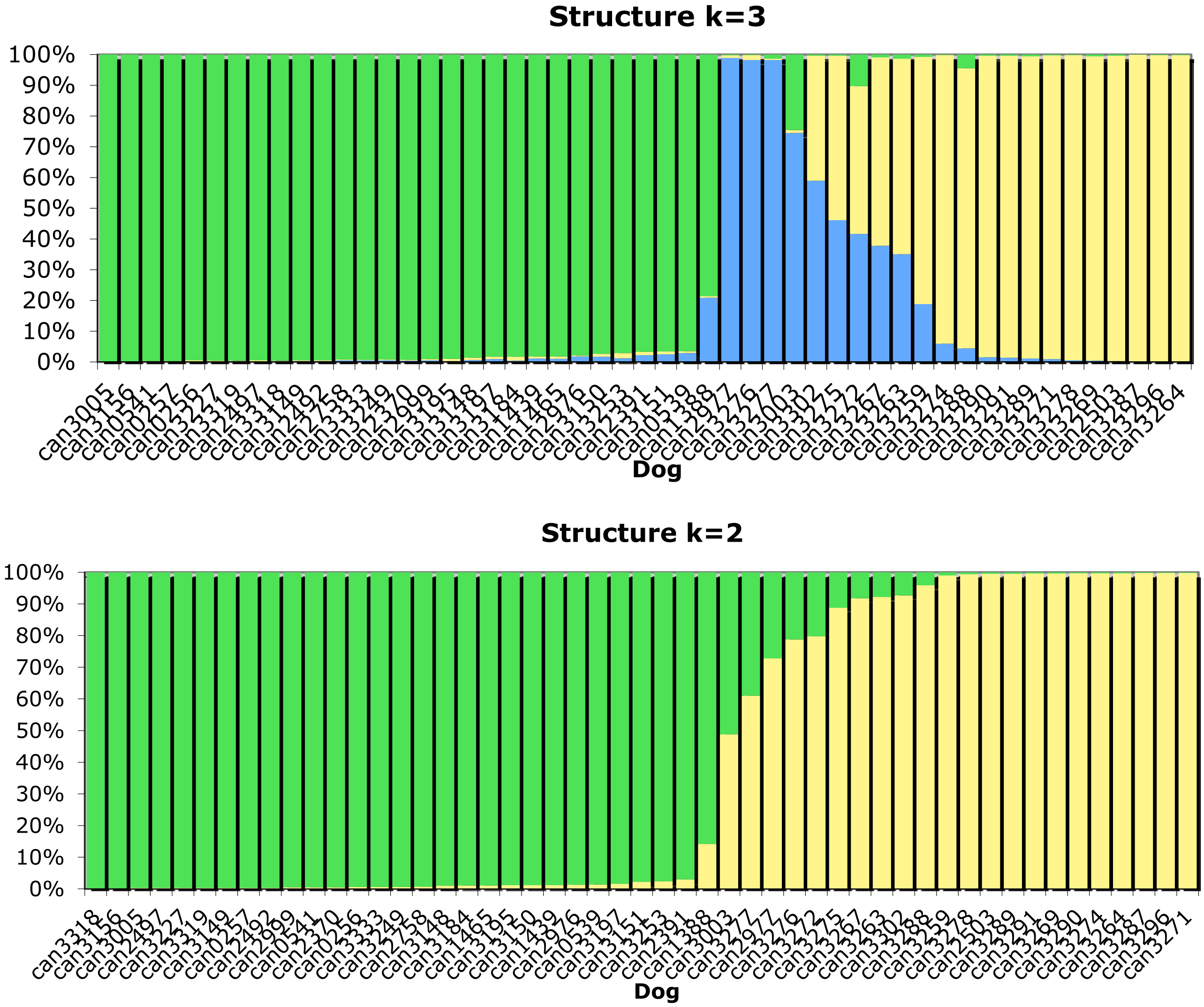

Figure 7. Underlying genetic substructure between border collie (predominantly in green) and Australian shepherd (predominantly yellow and/or blue) with k = 2 and 3. Each dog is represented as one bar; k represents the number of putative number of underlying assumed groups within the entire sample group (Overall and Hamilton, unpublished). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

phobic AUS and BOC compared with GSD (Davis, 1997). Accordingly, noise reactivity and/or phobia is likely a polygenic condition or set of conditions, a conclusion supported by our preliminary genetic results. Large numbers of affected dogs are needed to define the phenotypes and genetic basis of such conditions, although new sequencing methodologies make extremely effective use of data from stratified populations (van Rooy et al., 2014).

The breed pattern of the behavioral responses is likely important for determination of a phenotype. Diagnoses are not phenotypes, but when carefully defined using restrictive criteria and noting specific patterns of behaviors, they may group dogs together in ways that ensure that they are more similar than less, diagnoses can inform phenotypes (Overall, 2000; Overall, 2005) and allow breeds to be compared. Overall and Dunham (2002) noted that breed influenced the specific manifestation of canine obsessive-compulsive disorder (OCD) in 1 clinical study. This observation has been made by others, and different breed groups are associated with different OCD manifestations. Bull terriers and Staffordshire terriers spin and chase their tails (Moon-Fanelli et al., 1998; Tiira et al., 2012), GSD chase their tails (Overall and Dunham, 2002; Tiira et al., 2012), and Doberman pinschers flank and fabric suck (Moon-Fanelli et al., 2007; Dodman et al., 2010, 2016). It is entirely possible that in the course of selecting for breed-related behaviors or physical manifestations, we have inadvertently selected for co-varying liability genes. It is also possible that by selecting for some attribute, an extreme variant of some behavioral association was selected for and expression varied depending on the dog's environment (Tiira et al., 2012) or utility, resulting in a series of liability genes contributing to various behavioral expressions. Cao et al. (2014) proposed the latter process for Belgian malinois, where circling and tail chasing is frequently seen in working dogs, resulting in balancing selection for a genomic block of the *CDH2* gene. It is possible that a similar process has occurred for noise reactivity and/or phobias in any of these breeds, were one to select for some degree of heightened responsivity. The risk may be higher for BOC because owners of dogs in this study reported that many of their affected dogs could or were forced to work throughout their reactions to noise.

A GWA analysis for the dogs reported here revealed areas of interest on chromosomes 5, 8, and 10, but none of our findings reached genome-wide significance. Although our data were largely distributed in a way that could support a case-control analysis

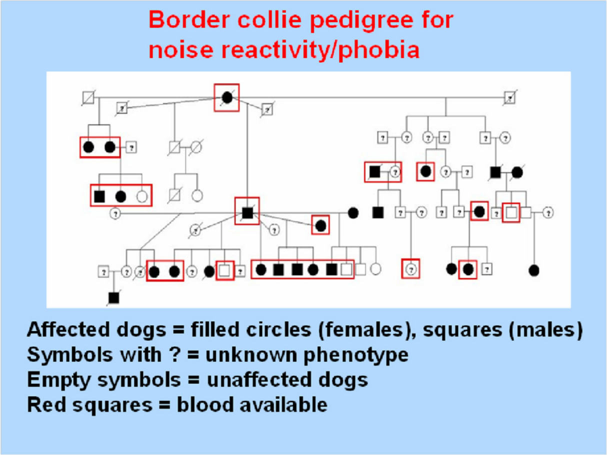

Figure 8. Five-generation border collie pedigree that provided 24 genetic samples and 22 histories for the study. Pedigree courtesy of Melanie Chang (Overall et al., 2014).

(reacts 0% of the time vs. 60% plus, a more conservative standard than Tiira et al., 2016), liability for noise reactivity and/or phobia varies considerably and is likely polygenic, as suggested by the breed differences noted here. Examination of population substructure revealed an average heterozygosity for AUS that was greater than that for BOC, and an SD for heterozygosity for AUS that was nearly 3 times that of BOC. This lower heterozygosity may also play a role in the extent to which BOC were affected with noise reactivity and/or phobia because there is a likelihood that some risk liability genes may be identical by descent.

Our study demonstrates that noise reactivity and/or phobia—which is only 1 clinical type of pathologic fear—does not have one manifestation. Fear has long been treated in the canine heritability and genetic literature as a unitary condition. It is not, and an approach to phenotypes that encompasses context and specific behavior is long overdue (Overall, 2005). Regardless, it would be unusual if these varied manifestations were not driven by different genes because they represent different system responses and different brain regions (LeDoux, 1988; Davis, 1997). The ability of breed to inform such differences adds another layer of complexity to mechanisms that may be important.

Number and intensity of signs may also suggest that different phenotypes of any condition may be driven by different mechanisms. The importance of such patterns has become apparent in the evolving research on flank sucking in Doberman pinschers. Dobermans with flank and blanket sucking expressed a higher frequency of an allele in the N-cadherin/*CDH2* gene on canine chromosome 7 (CFA 7) than did unaffected dogs (Dodman et al., 2010). Number and intensity of signs may be reflected in ancillary gene frequencies (Dodman et al., 2016). Brains of affected dogs also differed with affected dogs showing higher total brain and gray matter volumes in lower dorsal anterior cingulate cortex and right anterior insula gray matter density (Ogata et al., 2013). Interestingly, the fractional changes in corpus callosum communication correlated with the severity of the condition. Other studies have shown lower 5-HT_{2A} (serotonin receptor 2A) receptor binding in the frontal and temporal cortex of dogs with OCD (Vermeire et al., 2012), but it is not known whether levels vary with severity. Number and intensity of signs are likely relevant for both phenotyping and genotyping because severity of condition of OCD has been reported to correlate with treatment success in both dogs (Overall and Dunham, 2002) and humans (Haghihi et al., 2013).

It should be noted that what made it possible to obtain Doberman pinschers in sufficient numbers to phenotype and genotype were the very patterns that we have discussed earlier, which are not the norm for most behavioral conditions: the behavior was easily recognizable by owners and could not be mistaken for anything else, the behavior was widespread in family lines in a breed with other, historical interests in genetic health, the behavior does not occur in other breeds, the dogs either engaged in the behavior or did not, and time penetrance may have affected the severity of the condition (number and intensity of signs) (Dodman et al., 2016). If the latter is reflected in ancillary gene frequencies, the patterns of how pathologies may develop become an important consideration for phenotype. In addition, in contrast to the situation for noise reactivity and/or phobia, flank-sucking behavior in Dobermans is not injurious to the dog, and so no blame could be attendant with owner or breeder practices, a situation appealing to dog fanciers. The time has come for owners and breeders to work closely with behavioral medicine specialists and researchers so that we can uncover important phenotypes and their underlying genetic risks and mechanisms.

Our data suggest that true comorbidity of responses to multiple stimuli is occurring and that it may worsen the presentation of the behavioral condition studied here, noise reactivity and phobia. Furthermore, reactions for all 3 breeds that we studied are not general noise responses but responses to specific stimuli. That reactions to the classes of noise stimuli studied are most highly comorbid in the breed with the highest global AIR scores (BOC) and less comorbid in the breed with the lowest global AIR scores (GSD) may be important and have considerable implications for mechanism. A similar pattern was reported for a general patient population of dogs affected with comorbid separation anxiety and noise reactivity and/or phobia (Overall et al., 2001). In that clinical population, dogs who reacted to storms, which were more unpredictable in time and characterization than other noises to which the patients were reported to react, were more likely to react to other noises (conditional probability of reacting to other noises given that you react to storms = 0.8974), than when the pattern was reversed (conditional probability of reacting to storms given that you reacted to other noises = 0.7609). Lack of control and unpredictability are often associated with stress responses, including those related to noise. Breier et al. (1987) similarly found that stress associated with unpredictable noise was associated with more pronounced behavioral and hormonal responses in humans.

Whether the comorbidity between noise responses and triggers is because of shared neurochemical mechanisms or changes in underlying neurochemical substrate caused by 1 stimulus is not known but may be important. For owners who need to treat the distress experienced by these dogs, knowledge that these conditions are likely to be comorbid, that the condition is time penetrant, and that the number of signs exhibited matters is essential, especially in light of cognitive effects, if treatment is to be successful (Overall et al., 2001; Tiira et al., 2016).

Conclusion

Our data show that clear definitional criteria and specific behavioral evaluations lead to crisp phenotypes that permit further epidemiologic and mechanistic investigations. Furthermore, breed may inform phenotype, emphasizing the importance of determining the epidemiology and discrete pattern of behavioral responses. The mental health and welfare of dogs benefits from such approaches.

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Ethical considerations

Parts of this article were presented at the Canine Behaviour and Genetics meeting in London, United Kingdom, in June 2015. Dr. Overall is the Editor-in-Chief of the *Journal of Veterinary Behavior: Clinical Applications and Research.* A guest editor was appointed for this special volume. This study was conceived by Karen Overall and Art Dunham. The study was conducted by Karen Overall, Art Dunham, and Soraya V. Juarbe-Diaz with technical help from Donna Dyer and support from Melanie Chang and Jennifer Yokoyama from Steve Hamilton's laboratory at the University of California San Francisco (UCSF). All parts of the study, including the GWA analysis, were funded by Karen Overall. The genetic analyses were performed by Melanie Chang (who was a postdoctoral paid by the grant) and Jennifer Yokoyama. Soraya Juarbe-Diaz created the AIR score system. The statistical analysis was performed by Art Dunham. All authors contributed to the writing of the article. This research was approved by the IACUC at the University of Pennsylvania, and the US Department of Defense equivalent. All owners signed informed consent statements.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jveb.2016.09.007.

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