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Anxiety and impulsivity: Factors associated with premature graying in young dogs

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ABSTRACT

The present study examined the association of anxiety and impulsivity with premature muzzle grayness among young dogs. A sample of 400 dogs, ages 1–4 years was obtained at dog parks, shows, veterinary clinics, and other venues. Each dog was photographed and the degree of muzzle grayness was rated on an ordinal scale ranging from "no gray" to "full gray." White or pale colored dogs were dropped from the study because it was impossible to determine degree of grayness. Each owner filled out a questionnaire assessing the constructs of anxiety and impulsivity, as well as other behaviors and characteristics. To prevent response bias, owners were told that the purpose of the study involved dog lifestyle. Distractor items were added to the survey to prevent the owner from guessing the purpose of the survey. Examples of survey items indicating anxiety included: destruction when left alone; hair loss on vet exam or being in a new place; and cringes/cowers in response to groups of people. Examples of survey items indicating impulsivity included: jumping on people, inability to calm, loss of focus, hyperactivity after exercise. In our sample of young dogs, latent variable regression showed that the extent of muzzle grayness was significantly and positively predicted by anxiety ($p = 0.005$) and impulsivity ($p < 0.001$). Dog size, spay/neuter status, or medical problems did not predict extent of muzzle grayness. Fear responses to loud noise, unfamiliar animals and people were associated with increased grayness. Ordinal regression analysis showed that muzzle grayness was significantly predicted by fear of loud noises ($p = 0.001$), unfamiliar animals ($p = 0.031$), and unfamiliar people ($p < 0.001$). Premature graying in young dogs may be a possible indicator of anxiety, fear or impulsivity issues in dogs under four years of age.

1. Introduction

As dogs mature, it is not uncommon to see facial hairs begin to lose pigment, resulting in graying of the muzzle line hair coat. In reviewing case studies in an animal behavior practice along the Front Range of Colorado, USA, it was noted anecdotally that there were numerous observed cases in which young dogs (four years or less) showed premature graying in the muzzle line. Informal examination of the cases showed many of the dogs identified as displaying premature graying, had anxiety or impulse control issues. A review of the scientific literature revealed no prior work researching premature graying in canines.

Premature graying in humans has received a fair amount of inquiry. An examination of "before and after" photographs of United States presidents, shows an increase in the amount of gray hair by the end of their four year term. Scientists debate whether this graying among presidents is related to stress or genetics (Wolchover, 2011; Willingham, 2013). The scientific literature assesses associative factors for premature graying in humans. The literature regarding such evidence may be classified into four categories: (1) physiological oxidative stress at the cellular level (Kauser et al., 2011), (2) genetics (Trueb and Tobin, 2010), (3) disease-based premature graying (Kocaman et al., 2012), and (4) emotional or work-related stress (Tenibiaje, 2013). At this time, no single causal factor has been determined definitively to predict premature graying in humans. Additionally, there is minimal extant research on premature graying in mammals, specifically dogs.

Disease-based premature graying suggests a lifestyle of stress that influences the hair follicles and melanocytes to show low-

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ered resistance to stress (Westgate et al., 2013). Genetic propensity to premature graying may be compounded by environmental factors, inflammation, or psychological stress (Brownlee, 2004). The literature review shows evidence of other factors associated with premature graying in humans such as: lifestyle influences (Westgate et al., 2013); personality (Tucker and Friedman, 1996; Hampson and Friedman, 2008); pain (Hassett et al., 2012); and anxiety (Samarakoon et al., 2011).

In addition to cellular stress, genetics and disease, research suggests that there are emotional associations to premature graying in humans. Samarakoon et al. (2011) conducted a study examining how biological and social lifestyle of individuals related to aging. In this study, the Hamilton Anxiety and Depression Rating Scales—which included such indicators as insomnia, depression, anxious mood, and tension in lifestyles—were completed. The data showed that these indicators accelerated aging in young individuals. Similarly, certain behavioral and psychosocial mechanisms such as personality and emotional response patterns have been shown to have an effect on health (Jelinek, 1972; Tucker and Freidman, 1996). Mental and emotional changes such as depression, feelings of loneliness, emotional instability and anxiety in humans can be associated with aging and premature hair graying (Ballantyne, 2007; Rosch, 2009). Further research from Tenibiaje (2013) details how work-related stress relates to premature aging and graying of hair in humans. Heavy work demands can exert pressures on the body ranging from chronic mental stress to exhaustion. These demands alter bodily production of adrenaline, which is associated with hair color changes, and can have other adverse effects on the body. Just as in humans, stress from anxiety or fear can have a negative impact on health in canines (Dreschel, 2010).

In contrast to the multiple studies related to aging/graying in humans and mice, a single study was identified that potentially relates to premature graying in canines. Siniscalchi et al. (2013) used a sample of dogs to show that behavioral measures such as hiding, running away, seeking attention from the tester, panting, and lowering of the body posture were correlated with higher measured levels of cortisol in the hair. The levels of cortisol in hair reflected the dog's chronic state of emotional reactivity or temperament.

Research with humans and mice consistently demonstrates that stress is associated with premature aging (Tobin and Paus, 2001; Sanders, 2009). The primary research question for the present study was: (RQ1) "Do owner reported levels of anxiety and impulsivity levels predict premature graying in young dogs?" Our hypothesis was that, among dogs of a young age (1–4 years), anxiety and impulsivity would show a positive association with the extent of premature graying at the muzzle line. We additionally asked, (RQ2) "Do responses to specific fear stimuli (thunderstorms/loud noises, and unfamiliar places/animals/people) predict premature graying in young dogs?" Finally, we posed an exploratory research question (RQ3), "Does the presence of other dogs in the household, the presence of cats in the household, participation in organized or competitive activities, and the amount of time the dog spent unsupervised outdoors predict premature muzzle graying in young dogs?"

2. Methods

Permission for this study was granted through the Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC) at Northern Illinois University (Dekalb, Illinois).

2.1. Animals and marketing

A sample of $N = 400$ dogs (198 females, 202 males) was used in this study. Initially 443 dogs were screened, but 43 dogs were eliminated from the study due to failure to meet criteria. These criteria included age (1–4 years) and breed-specific hair color that allowed for the researchers to discern presence or absence of gray muzzle hair. Dogs were eliminated from the study if it was too difficult or impossible for the researchers to determine if the dog had a gray-colored muzzle. Dogs excluded from the study included breeds that presented with muzzle hair coats that were white (e.g., Great Pyrenees, Samoyeds, Eskimo dogs), pale cream-colored (e.g., Golden Retrievers, Shiba Inus), gray (e.g., Bedlington terriers, Poodles), or merled (e.g., Australian cattle dogs, Australian Shepherds). Any pure-breed or mix-breed dog was allowed in the study as long as age and color requirements were met.

Dogs were obtained from multiple venues. Flyers were placed in veterinary clinics, pet stores, dog parks, and at dog shows across the Front Range of Colorado, USA. Anonymity of human participants was maintained throughout data collection. The flyers were placed in these heavily-concentrated dog areas specifying dates and times the researchers would be present to collect data, should the dog owner want to participate in the study. The researchers also presented at dog parks during the heaviest dog traffic times-–usually weekends and before and after work hours on week-days. Researchers also attended local dog shows that were advertised online. Permission from the dog show hosts was obtained prior to data collection. Dog owners were very willing to have their dog participate in data collection for this study. Although much time was spent organizing calendar days/times for data collection at veterinary clinics and pet stores, more than 65% of the dogs participating in the study were found at dog parks and dog shows.

2.2. Data collection

Dog owners were asked to participate in a research study on dog behavior lifestyles that included completing a short questionnaire and allowing a photograph of their dog. Dog owners were blinded to the purpose of the study to avoid biasing the results. Written informed consent was provided by all dog owners who permitted their dog to participate in the study.

2.3. Questionnaire

The 42-item questionnaire was developed by the authors. Demographic data were collected as well as information on the dog's participation in organized or competitive activities (e.g. agility, search and rescue, service dog work); knowledge of basic obedience commands; the amount of time the dog spent outside unsupervised; presence of other animals (dogs or cats) in the dog's place of residence; medical history; current medications taken, and pain issues. The questionnaire also included two specific multi-item scales intended to assess levels of (1) fear/anxiety, and (2) impulsivity/arousal in the dogs (Table 1).

The researcher reviewed each completed questionnaire immediately after submission for completeness, or to clarify responses. The questionnaire also included several non-relevant distractor items (e.g., "Does your dog have hind limb dewclaws?") so that participants would be less likely to guess the purpose of the study.

2.4. Definitions

Anxiety is defined as a reaction to a prospective or imagined danger and includes physiological and behavioral signs (p. 1081, Sherman and Mills, 2008). Symptoms may include restlessness, stress whining, barking, closeness in proximity to an owner, and avoidance to name a few (Rooney et al., 2007; Sherman and Mills, 2008). Shedding hair coats in dogs (Klein, 2012) and hair loss in humans can also be related to anxiety and emotional distress (Sinclair, 1999; Trueb, 2008).

Table 1
Anxiety and Impulsivity Scale Items.

My dog. . .	Measured Construct
1. Is typically fearful.	Anxiety
2. Tends to shed or lose hair during unusual times such as a veterinary exam or being in a new place.	Anxiety
3. Either initiates or experiences hostility, dominance, threats, or fighting with other dogs.	Anxiety
4. Whines or barks when left alone at home.	Anxiety
5. Scratches, chews, or causes destruction when left alone at home.	Anxiety
6. Urinates or defecates in the home when left alone.	Anxiety
7. Whines or barks when at the vet.	Anxiety
8. Cringes, cowers, hides, or tries to avoid handling when at the vet.	Anxiety
9. Responds to groups of people by cringing, cowering, hiding, or not interacting.	Anxiety
10. Responds to groups of people by barking or growling.	Anxiety
11. Is calm when I return home.	Impulsivity
12. Jumps up on me when I return home.	Impulsivity
13. Jumps up on other people when greeting them.	Impulsivity
14. Can maintain a "sit-stay" calmly without agitation.	Impulsivity
15. Pulls on the leash when going on walks.	Impulsivity
16. Seeks attention by persistent barking.	Impulsivity
17. Would benefit from learning "leave it!" as a command.	Impulsivity
18. Loses focus and ignores me when there is a lot of activity occurring in the environment.	Impulsivity
19. Chases after small animals.	Impulsivity
20. Is hyperactive and wanting more exercise after 30–60 min of walking.	Impulsivity
21. Is calm and relaxed after 30–60 min of vigorous exercise (e.g., running, playing Frisbee, chasing/retrieving balls).	Impulsivity

Note: Response options for each item were 0 = *strongly disagree*, 1 = *disagree*, 2 = *neither agree, nor disagree*, 3 = *agree*, 4 = *strongly agree*.

Impulsivity is defined as a trait related to inhibitory control and is expressed in a range of behaviors (p.676, Wright et al., 2012). In humans, anxiety is associated with impulsivity (Taylor et al., 2008). In dogs, a loss of concentration with difficulty to focus, challenges to maintain a stay position, endless barking, and hyperactivity are some of the indicators for impulsivity (Lit et al., 2010).

2.5. Photographs

Two photographs were taken of each dog at the same time the questionnaire was completed by the owner to provide evidence of the degree of premature graying the dog possessed. One photograph provided a side view of the dog's head, the other photograph provided a frontal view of the head, specifically the mouth and chin. Photographs were taken by a digital camera approximately three to five feet (1 m–1.75 m) away from the dog.

2.6. Ratings

Two raters, who were not involved in data collection and did not have access to the questionnaire data, each independently rated the photographs for each dog using an ordinal rating scored as 0 = *No gray*, 1 = *Frontal gray*, 2 = *Half gray*, and 3 = *Full gray* (See Figs. 1 and 2). (Score card directions—Appendix A). Raters' scores based on the photographs were entered into an Excel data file together with questionnaire responses. Statistical analysis was carried out.

2.7. Analyses

Descriptive statistics and frequencies for characteristics of the dogs were first computed. To address the association of anxiety and impulsivity with the extent of graying in dogs, a latent variable regression analysis (Bollen, 1989; Everitt, 1984) was carried out, where the mean grayness ratings across the two raters served as an ordinally-coded dependent variable, and owner responses to the relevant anxiety and impulsivity items on questionnaire served as indicators for two latent predictors—anxiety and impulsivity. Modeling anxiety and impulsivity as latent variables were used to account for potential measurement error in these scales. To facilitate model parsimony, minimize residual correlation, and reduce sources of sampling variability, item parceling (Cattell, 1956; Cattell and Burdsal, 1975; Yang et al., 2010) using random item-to-parcel assignment (Little et al., 2002) was employed to construct four item parcels for each of the two latent constructs. Due to observed non-normality in responses to the anxiety and impulsivity scale items, robust maximum likelihood estimation was used for this model.

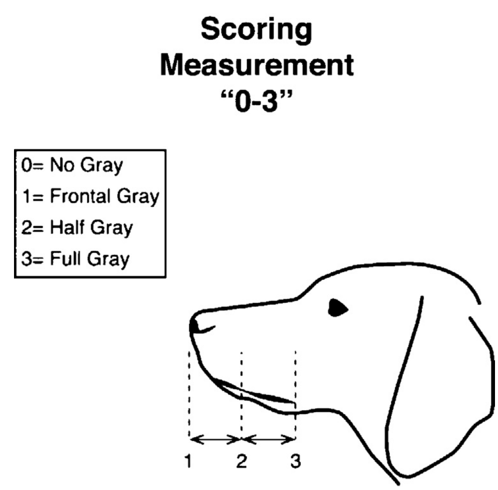

Fig. 1. Scoring Measurement for Muzzle Line.

To address how reactions to specific fear stimuli (thunderstorms, loud noises, unfamiliar places, unfamiliar animals, and unfamiliar people) were related to ratings of muzzle grayness (i.e., RQ2), ordinal regression was carried out using each of these predictors. Similarly, to address how the presence of other animals (dogs or cats) in the household, participation in organized or competitive activities, and time spent unsupervised outdoors was related to muzzle grayness (i.e. RQ3), ordinal regression was carried out with these variables as predictors.

Control variables in each of the regression models described above included the age in years, sex, spay/neuter status, presence of medical problems, and dog size. Although we didn't weigh or measure height of the dogs, it was relatively easy to distinguish tiny breeds (e.g., Chihuahua, Yorkshire Terrier, Toy Poodle, etc.) from giant breeds (e.g., Great Dane, English Mastiff, St. Bernard) on the basis of owner report and photographs. To categorize the remainder of the dogs, we developed two additional size groups–small-

Fig. 2. Examples of dogs in the study rated as No gray, Frontal gray, Half gray, or Full gray.

medium dogs (e.g., Pug, Beagle, Spaniel, Corgi, Sheltie, Whippet) and large-medium dogs (e.g., Boxer, German Shepherd, Airedale, Golden Retriever). Thus, dog size was ordinally coded as 1 = *tiny*, 2 = *small-medium*, 3 = *large-medium*, and 4 = *giant*). All inferential analyses were carried out using Mplus v6.12 (Muthén and Muthén, 2010), and an a priori alpha level of 0.05 was used for all inferential tests.

3. Results

Table 2 provides descriptive information about the sample (N = 400) of dogs. As can be seen, dogs were uniformly represented by sex, relatively few had medical problems or were on behavioral medication. Most were spayed/neutered, were medium-sized, and knew basic obedience commands (e.g., "sit," or "stay"). The mean age of dogs was 2.54 years (SD = 1.04). Table 3 shows the joint distribution of the grayness ratings by rater. For ordinal data, an appropriate index of inter-rater reliability/agreement is weighted Kappa (Cohen, 1968), which emphasizes large differences in ratings more than small differences. Based on Landis and Koch (1977) guidelines for interpretation of Kappa, inter-rater agreement for these ordinal ratings was strong (weighted Kappa = 0.855). Slightly less than half of the sample of dogs (46%) showed no muzzle graying.

Messick (1995), in his influential unified construct-based model of validity, describes how validity is properly viewed as a property of inferences made from data, rather than a property of instruments per se. As such, evidence for the validity of inferences is an ongoing process occurring over multiple studies, with this evidence potentially coming from a variety of sources. Within this framework, a method that has become widely-used for providing evidence of construct validity is confirmatory factor analysis (CFA), which posits an a priori structure for the data that is consistent with the constructs being assessed, and assesses whether the data support this structure (see Kelly et al., 2005). In the present study, a two correlated factor CFA measurement model of the latent anxiety and impulsivity constructs fitted to the obtained data indicated good model-to-data fit, exceeding established criteria with goodness-of-fit indices CFI = 0.967 and TLI = 0.951, and residual fit RMSEA = 0.043 and SRMR = 0.040. Additionally, and as expected, anxiety showed

Table 2
Distribution of Owner-Reported Characteristics of Dogs (N = 400).

Characteristic	*n*	%
Sex		
Female	202	50.5
Male	198	49.5
Spayed/neutered		
Yes	315	78.8
No	85	21.3
Size		
Toy	29	7.3
Medium-small	41	10.3
Medium-large	304	76.0
Giant	26	6.5
Medical problems		
Yes	31	7.8
No	369	92.3
Behavioral medication		
Yes	6	1.5
No	394	98.5
Knows basic obedience commands		
Yes	364	91
No	36	9.0
Number of other dogs in household		
None	189	47.3
1–2	149	37.3
3–4	32	8.0
5 or more	30	7.5
Number of cats in household		
None	386	71.5
1–2	89	22.3
3–4	21	5.3
5 or more	4	1.0
Age in years		
1	113	28.3
2	101	25.2
3	104	26.0
4	82	20.5

significant correlations with fear of loud noises, and unfamiliar places/animals/people. Conversely, as expected impulsivity was not associated with these variables, except for fear of unfamiliar animals (a situation in which impulsivity might reasonably be expected), providing some evidence of the convergent and discriminant validity of these the anxiety and impulsivity constructs. The correlation between the two latent constructs (anxiety and impulsivity) was, as expected, moderate ($r = 0.44$). Evidence for the stability or reliability of these constructs also can be provided within the CFA (latent variable) framework using McDonald (1999) Omega. Based on this statistic, construct reliability was determined to be good for both anxiety ($\omega = 0.795$) and impulsivity ($\omega = 0.755$).

Table 4 shows the results of the latent ordinal regression model predicting extent of grayness from anxiety and impulsivity, including regression coefficients (b), associated standard errors ($SE(b)$), test statistics (z), and adjusted odds-ratios (AOR). As these results indicate, when controlling for the dogs' age, sex, spay/neuter status, size, and presence of medical problems, the extent of muzzle grayness was significantly and positively predicted by both anxiety ($b = 0.339$, $p = 0.005$) and impulsivity ($b = 0.503$, $p < 0.001$). Specifically, examination of adjusted odds-ratios (AORs) showed that increased levels of anxiety and impulsivity were associated with increased extent of muzzle grayness (AOR = 1.403 and AOR = 1.654, respectively). Among the control variables in the model, extent of grayness was significantly and positively associated with age of the dog ($b = 0.877$, $p < 0.001$, AOR = 2.404), and female dogs showed higher levels of grayness than males ($b = 0.537$, $p = 0.011$, AOR = 1.711). Dog size, spay/neuter status, and presence of medical problems did not significantly predict extent of muzzle grayness.

Table 4
Results for Latent Regression of Muzzle Grayness on Anxiety, Impulsivity, and Control Variables.

Effect	*b*	*SE*(*b*)	*z*	*AOR*	*p*
Size	0.097	0.189	0.517	1.102	0.605
Age	0.877	0.108	8.107	2.404	<0.001***
Sex	0.537	0.211	2.542	1.711	0.011*
Neuter/spay	−0.040	0.301	−0.134	0.960	0.893
Medical problems	−0.374	0.391	−0.957	0.688	0.338
Anxiety	0.339	0.120	2.829	1.403	0.005**
Impulsivity	0.503	0.122	4.142	1.654	<0.001***

Note: AOR = adjusted odds-ratio.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

In a similar manner, to address the effects of reported responses to fear stimuli on muzzle graying, ordinal regression analysis was carried out, regressing the ordinal grayness ratings on dichotomous (yes/no) owner reports of dogs' fear response to five potential fear-inducing stimuli (thunderstorms, loud noises, and unfamiliar places/animals/people). The dogs' age, sex, spay/neuter status, size, and presence of medical problems again served as control variables. Results (Table 5) showed that among these five stimuli, dogs' fear responses to three significantly predicted muzzle grayness. These included loud noises ($b = 0.494$, $p = 0.001$, AOR = 1.639), unfamiliar

Table 5
Results for Ordinal Regression of Muzzle Grayness on Fear Stimuli and Control Variables.

Effect	*b*	*SE*(*b*)	*z*	*AOR*	*p*
Size	0.030	0.094	0.318	1.030	0.751
Age	0.422	0.062	6.795	1.525	<0.001***
Sex	0.200	0.121	1.648	1.221	0.099
Neuter/spay	−0.125	0.143	−0.872	0.882	0.383
Medical problems	−0.066	0.201	−0.327	0.936	0.744
Thunderstorms	0.086	0.205	0.421	1.090	0.674
Loud noises	0.494	0.148	3.347	1.639	0.001**
Unfamiliar places	0.294	0.181	1.629	1.342	0.103
Unfamiliar animals	0.377	0.175	2.154	1.458	0.031*
Unfamiliar people	0.651	0.146	4.447	1.917	<0.001***

Note: AOR = adjusted odds-ratio.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Joint Distribution by Rater of Muzzle Grayness Ratings.

		Muzzle score (Rater 2)				
		No gray	Frontal gray	Half gray	Full gray	Total
Muzzle score (Rater 1)	No gray	185	0	0	0	185
	Frontal gray	0	115	12	0	127
	Half gray	0	5	64	0	69
	Full gray	0	0	0	19	19
Total		185	120	76	19	400

Table 6
Results for Ordinal Regression of Muzzle Grayness on Organized Activity, Number of Dogs/Cats in Household, Hours Spent Outside Unsupervised, and Control Variables.

Effect	*b*	*SE*(*b*)	*z*	*AOR*	*p*
Size	0.085	0.092	0.922	1.089	0.356
Age	0.465	0.060	7.681	1.592	<0.001***
Sex	0.290	0.115	2.515	1.336	0.012*
Neuter/spay	0.169	0.170	0.997	1.184	0.319
Medical problems	−0.242	0.200	−1.207	0.785	0.227
Organized activity	0.143	0.157	0.908	1.154	0.364
Number of other dogs in HH	−0.005	0.076	−0.071	0.995	0.944
Number of cats in HH	−0.057	0.087	−0.659	0.945	0.510
Hours spent outside unsupervised	−0.013	0.038	−0.344	0.987	0.731

Note: $^{**}p < 0.01$, AOR = Adjusted odds-ratio.
* $p < 0.05$.
*** $p < 0.001$.

animals ($b = 0.377$, $p = 0.031$, AOR = 1.458), and unfamiliar people ($b = 0.651$, $p < 0.001$, AOR = 1.917). Dogs whose owners reported fear responses to these stimuli showed increased levels of muzzle grayness compared to dogs not exhibiting such responses.

Lastly, we examined whether the presence of other dogs in the household, the presence of cats in the household, participation in organized or competitive activities, and the amount of time the dog spent unsupervised outdoors was associated with muzzle grayness. When controlling age, sex, spay/neuter status, size, and presence of medical problems, the results (Table 6) showed that none of these variables was significantly related to muzzle grayness (each $p > 0.05$). Follow-up analysis, however, introducing anxiety as a potential moderator of this relationship, showed that a dog's level of anxiety significantly moderated the effects of participation in organized or competitive activities on muzzle grayness, with dogs with higher anxiety showing a significantly stronger positive relationship between participation in these activities and muzzle grayness than dogs with low levels of anxiety ($b = 1.539$, $p < 0.001$, AOR = 4.658).

As a follow-up analysis, we additionally examined whether the observed effect of anxiety and impulsivity on muzzle grayness was moderated by either the dog's age or the dog's sex. Here, a latent ordinal regression model incorporating latent age × anxiety and age × impulsivity interaction terms showed no statistically significant moderating effects of either age or sex on the effects of anxiety ($b = 0.076$, $p = 0.563$ and $b = -0.102$, $p = 0.337$ for age and sex, respectively) or impulsivity ($b = 0.090$, $p = 0.698$ and $b = -0.322$, $p = 0.112$ for age and sex, respectively). That is, the positive observed effects of anxiety and impulsiveness on muzzle grayness were similar for female and male dogs, and were the same regardless of the dog's age.

4. Discussion

The primary purpose of this study was to examine the relationship between owner-reported anxiety and impulsiveness in dogs and premature muzzle graying. Results from the sample of 400 dogs showed that, when controlling for the dogs' age, sex, spay/neuter status, size, and presence of medical problems, both anxiety and impulsivity were significantly associated with premature graying in dogs between 2 and 4 years of age. Specifically, dogs presenting a greater extent of anxiety and impulsivity were more likely to present premature muzzle grayness than dogs showing less anxiety and impulsivity. Similarly, dogs' fear responses to three specific stimuli—loud noises, and unfamiliar animals, and unfamiliar people were significantly associated with increased muzzle grayness. Among the other predictors that were examined, only the dogs' age and sex significantly predicted premature grayness.

The significant deleterious effects of anxiety and impulsivity on muzzle grayness are consistent with prior research using mice. Viveros et al. (2007), for example, hypothesized that chronic hyper-reactivity to stress, usually anxiety-based, led to immune system disorder and premature aging in mice. Their research showed that mice with higher levels of emotionality and anxiety ran a maze more slowly than mice with lower levels of emotionality and anxiety. Similarly, a prior study by Viveros et al. (2001) that compared these "fast" and "slow" mice using three behavioral tests ("hole-board," the "open field," and the "plus-maze") showed that the hyper-emotional mice had impaired immune and neurobehavioral functions, consistent with premature aging. Arck et al. (2003) found parallel results in which psycho-emotional stress in mice was associated with premature graying and hair loss.

Psycho-emotional stress in mice can affect hair growth via neuro-endocrine and or neuro-immunological signaling pathways (Peters et al., 2006). Vida et al. (2014) observed that anxiety is associated with chronic oxidative stress and inflammation in brain cells, and accelerates the rate of aging. Wu et al. (2014) posited a model of premature aging in mice, in which animals show a poor response to stress and high levels of anxiety, resulting in oxidative stress in their immune cells and tissues. Wu et al.'s study showed that stress can induce alterations of skin pigmentary response, whereby acute stress is associated with an increased turnover of serotonin (5-Hydroxytriptamine; 5-HT) and chronic stress, which then can cause a decrease in 5-HT in pigment cells. Thus, the serotoninergic system appears to play a role in the regulation of stress-induced depigmentation in mice.

The observed results in this study also are consistent with studies involving humans. Hassett et al. (2012) observed that chronic pain can cause increased stress and premature cellular aging, including premature hair graying. Similarly, Suresh and Yashvant (2015) found auto-immune diseases, vitamin deficiencies, and chronic stress to be associated with premature graying. In a study of over 6000 adult human males, premature graying was significantly associated with environmental stressors in lifestyle such as smoking and obesity (Shin et al., 2015).

In the present study, no significant moderating effects for dog age or sex were observed on the relationship between anxiety/impulsivity and muzzle grayness. That is, although older dogs were significantly more likely to present graying than younger dogs, and female dogs were more likely to present graying than male dogs, the relationship between anxiety/impulsivity and muzzle grayness appears to be consistent across the varied ages of the dogs, and also consistent for both female and male dogs. For age, in particular, this is notable, as it suggests the deleterious effects of anxiety/impulsivity are just as strong among younger dogs as among older dogs.

Because it is generally known that participation in leisure activities and sports can reduce stress and anxiety in humans (Penedo and Dahn, 2005), we anticipated that participation in organized or competitive activities among dogs might decrease propensity towards muzzle grayness. The results, however, failed to discern any association between such participation and muzzle grayness. Perhaps there are further distinctions between specific types of activities that might result in predictive relationships. The sample sizes for specific activities in the present study, however, were too small to facilitate such analyses.

A notable auxiliary finding of the present study was that female dogs were significantly more likely than male dogs to present premature muzzle graying. This is a finding not observed (or even investigated) in prior studies of graying.

Surprisingly, only about 20% of the dogs in various settings met the criteria for participation. Many dogs either had a white/cream/gray muzzle or were under one year old, and thus were excluded from participation. Although the exclusion of older dogs was an explicit delimitation of this study—as the purpose was to examine premature muzzle graying, rather than age-related graying—the exclusion of dogs with light-colored muzzles was an unfortunate (but necessary) limitation. The results of this study can be generalized only to populations of dogs without naturally light-colored muzzles. Additionally, the instrument used to assess anxiety and impulsivity was a new, researcher-created tool. Although construct validity evidence was observed with the fit of the measurement model and with correlations with other variables, and the construct also showed good internal consistency reliability, further studies using this instrument are necessary to obtain additional evidence for reliability and validity. Similarly, although good inter-rater reliability of the individuals assessing muzzle grayness was demonstrated, additional variability may have occurred if other raters had been used.

One practical implication of the findings of this study involves the possibility of using observations of muzzle grayness in a diagnostic manner to address anxiety, impulsivity, or fear issues. That is, if dog professionals (veterinarians, applied behaviorists, dog trainers, etc.) are able to note premature graying in their assessments and or training, then these dogs might be assessed more thoroughly for anxiety/impulsivity/fear problems and, if necessary, started on behavior modification programs earlier in their developmental life stages. Additionally, owners could be educated to monitor for premature graying in young dogs.

When premature graying is evident, early intervention programs then could be initiated, whereby owners are assisted with teaching their dogs coping skills/default behavior, providing their dogs with mental stimulation and facilitating their dogs' ability to read body language to help alleviate some of the anxiety/impulsivity/fear problems their dogs may experience and thereby promote a healthy, high-quality life. Working in tandem with veterinarians, applied animal behaviorists and professional dog trainers may find increased success in addressing some of these problems in dogs.

Future research may include replicating this study using a standardized tool for anxiety and impulsivity. Temperament testing of the dogs may also be of value.

5. Conclusion

One practical implication of the findings of this study involves the possibility of using observations of muzzle grayness (assessed in dogs without white, cream, gray or merle-colored muzzles) in a diagnostic manner to address anxiety, impulsivity, or fear issues. Young dogs with owner-reported anxiety and impulsivity issues showed significant values related to premature graying in the muzzle line. There is a need for future research studies to develop behavioral interventions for dogs with anxiety and impulsivity problems.

Conflict of interest

None.

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Appendix A. : Score Card Directions

0 **NO GRAY** Assess the picture of the dog. Note any silver or gray hair along the muzzle line. If there is **no silver or gray hair** in the dog's muzzle, then the score is 0.

1 **FRONTAL GRAY ONLY** Assess the frontal picture of the dog. Note any silver or gray hair along the top or bottom lip. If there is **any amount of silver or gray hair with a frontal view on either the top or bottom lip,** then the score is 1.

2 **HALF GRAY** Assess the side view of the dog's muzzle. If the dog has silver or gray hair on the upper or lower lip when visualizing the length of the dog's muzzle from the nose leather to the end of the lip junction (commissure), and the amount of gray is **50% or less**, then the score is 2. (Note: If the dog was scored at 1 because the frontal view of the muzzle shows silver or gray hair and there is silver or gray anywhere on the length of the dog's muzzle, then rescore that dog to a 2 or 3).

3 **FULL GRAY** Assess the side view of the dog's muzzle. If the dog has silver or gray hair noted (upper or lower lip) when visualizing the length of the dog's muzzle from the nose leather to the end of the lip junction (commissure), and the gray is **greater than 50%,** then the score is 3.

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